The ninth annual

q-bio Summer Conference

at Virginia Tech

August 5th - 8th, 2015

VirginiaTech

Invent the Future

Welcome to Blacksburg, VA!

The q-bio Conference is an annual event intended to advance predictive modeling of cellular regulation. The q-bio community emphasizes the integration of modeling and quantitative experimentation to understand and predict behaviors of specific cell regulatory systems, to interpret manifestations of complex biological phenomena, and to elucidate general principles of cellular information processing and related biological phenomena. The single-track program includes invited talks from leading experimental and theoretical researchers, as well as contributed talks, poster presentations, and invited and contributed tutorials selected from abstract submissions.

This short printed program provides a brief overview, including the program schedule, titles of presented work, and a local map. Please refer to the primary conference website for the most complete and recent information:
http://q-bio.org/wiki/The_Ninth_q-bio_Conference

Virginia Tech is proud to be the first host of the q-bio summer conference outside of its New Mexico home. It is our sincerest hope to continue the q-bio tradition of excellence. If you have questions or concerns, please contact CPE (Holly McClung, hmcclung@exchange.vt.edu) or any of the local organizers:

William Mather,	wmather@vt.edu
John Tyson,	tyson@vt.edu
Jianhua Xing,	xing1@pitt.edu
Yang Cao,	ycao@cs.vt.edu
Silke Hauf,	silke@vt.edu

Continuity and success of the q-bio conference has been ensured by the efforts of the Program Committee:

Jim Faeder, University of Pittsburgh, School of Medicine (Chair)
Aleksandra Walczak, Ecole Normale Superieure (co-Chair)
Rosalind Allen, University of Edinburgh
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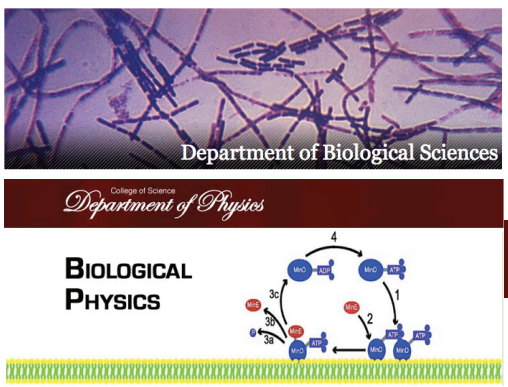
Finally, we would like to thank our corporate sponsors (Nikon and Science Signaling), our local sponsors (Dept. of Biological Sciences, Dept. of Physics, VBI, Fralin Institute, and VTCRI), and NSF (award number 1542329) for generous support.



Program Schedule

Wednesday, August 5

Registration (12:00 - 13:30, Squires Student Center and 18:00 - 19:00, The Inn at Virginia Tech)

Session 1: Tutorial Session (13:00 - 18:00, Squires Student Center, Various Rooms)

13:00-14:30 John Sekar, Robert Sheehan, and Jose Juan Tapia, University of Pittsburgh, Modeling Complex Biochemical Systems in Time and Space Using BioNetGen and MCell

13:00-14:30 William Mather, Virginia Tech, COPASI for Biochemical Network Simulation

14:45-16:15 Steven S. Andrews, Fred Hutchinson Cancer Research Center, Smoldyn: Spatial Cell Biology Simulation

14:45-16:15 Emek Demir, Memorial Sloan Kettering Cancer Center, Pathway Commons/BioPAX

14:45-16:15 Ilya Nemenman, Emory University, Information Theory for Cells

16:30-18:00 Michael Blinov, University of Connecticut Health Center, VCell: Spatial Modeling, Reactions, and Rules

16:30-18:00 Alexander L. R. Lubbock and Oscar O. Ortega, Vanderbilt University, PySB, a programming environment to generate algorithmic representations of biological systems

Session 2: Opening Banquet (18:30 - 20:30, The Inn at Virginia Tech)

18:30-18:35 Welcome to q-bio, Will Mather, Virginia Tech

18:35-18:45 Viva q-bio! Jim Faeder, University of Pittsburgh

18:45-18:50 q-bio Summer School Recap, Brian Munsky, Colorado State University

18:50-18:55 q-bio Special Issue, Ilya Nemenman, Emory University

18:55-19:30 Dinner

19:30-19:35 Banquet Speaker Introduction

19:35-20:30, John Tyson, Virginia Tech, Network Dynamics and Cell Physiology

Thursday, August 6

7:30-9:00 Breakfast (on own or Dining Hall)

Registration (8:00 - 10:00, Squires Student Center)

Session 3 (9:00-12:20, Squires Student Center, Colonial Hall)

Chair: James Faeder, University of Pittsburgh

9:00-9:30 Johan Elf, Uppsala University, SMeagol Simulated Microscopy - a tool against inverse crimes

9:30-9:50 Tatiana Marquez-Lago, Okinawa Institute of Science and Technology, Cell-cycle synchronized oscillations of a negatively self-regulated gene in E. coli

9:50-10:20 Hang Lu, Georgia Tech, Automation and Microfluidic Tools for Q-Bio

10:20-10:50 Coffee Break

10:50-11:10 Poster Spotlights 1

Deborah Striegel, NIH, Morphology of Pancreatic Islet Cytoarchitecture with Type 2 Diabetes

Jae Kyoung Kim, Ohio State, Determining the validity of Hill functions in stochastic simulations

Ryan Nikin-Beers, Virginia Tech, The role of antibody in dengue viral infection

Ji Hyun Bak, Princeton, Characterizing the statistical properties of protein surfaces

11:10-11:40 Oskar Hallatschek, UC Berkeley, Microbes Under Pressure

11:40-12:00 Lily Chylek, Cornell, IgE receptor signaling encodes dynamic memories of antigen exposure

12:00-12:20 Ryan Suderman, University of Kansas, The Noise is the Signal: Information Flow in Single Cells and Cellular Populations

12:20-14:30 Lunch (Dining Hall)

Session 4 (14:30-17:30, Squires Student Center, Colonial Hall)

Chair: William Mather, Virginia Tech

14:30-15:00 Alejandro Colman-Lerner, University of Buenos Aires, Use of information far from steady-state by signal transduction systems.

15:00-15:20 Thomas Ouldridge, Imperial College, On the connection between computational and biochemical measurement

15:20-15:40 Jeremy Purvis, UNC-Chapel Hill, Single-cell dynamics reflect underlying signaling mechanisms

15:40-16:10 Coffee Break

16:10-16:30 Poster Spotlights 2

Abed Alnaif, UC Irvine, Pattern formation and morphogen gradients: A causality dilemma

Rory Donovan, University of Pittsburgh, Enhanced Sampling in Spatial Stochastic Systems Biology Models Using a Weighted Ensemble of Trajectories

Mark Transtrum, BYU, Effective models of emergent behavior from the manifold boundary approximation method

Vipul Periwal, NIH, The Universality of Cancer

16:30-16:50 Vernita Gordon, UT-Austin, The bacterial population's spatial structure non-monotonically impacts bacterial growth

16:50-17:10 Nicholas C. Butzin, Virginia Tech, Entrainment of synthetic gene oscillators by a noisy stimulus

17:10-17:30 Arolyn Conwill, MIT, Seasonality gives rise to population oscillations in a bacterial cross-protection mutualism

17:30-19:00 Dinner (on own)

Session 5 (19:00-20:00, Squires Student Center, Colonial Hall)

19:00-19:30 Sudhakaran Prabakaran, Science Signaling, How to Get Published in a Science Journal

19:30-20:00 Craig Giroux, NIH, Update on NIH Peer Review and Research Initiatives for Quantitative Biologists

Session 6 (20:00-23:00, Poster Session 1, Squires Student Center, Commonwealth Ballroom)

20:00-23:00 Poster Session 1 (snacks and drinks served)

Friday, August 7

7:30-9:00 Breakfast (on own or Dining Hall)

Session 7 (9:00-12:20, Squires Student Center, Colonial Hall)

Chair: Yi Jiang, Georgia State

9:00-9:30 Arup Chakraborty, MIT, How to hit HIV where it hurts

9:30-9:50 Robin Lee, University of Pittsburgh, Cell fate decisions in response to a short pulse of TNF

9:50-10:20 John Hancock, UT Health Center, Ras nanoclusters: lipid-based assemblies for signal processing

10:20-10:50 Coffee Break

10:50-11:10 Poster Spotlights 3

Philip Hochendoner, Virginia Tech, Queueing Entrainment – Downstream control of a synthetic oscillator

Sargis Karapetyan, Duke, Redox rhythms reinforce the plant circadian clock: New insights into coupled biological oscillators

Xiuxiu He, Georgia State, How Adhesion Regulates Cell Migration Plasticity: A Computational Study

David Wooten, Vanderbilt, Transcription factor network supports phenotypic heterogeneity in cancer

11:10-11:40 Tamar Schlick, NYU, Simulating Large-Scale Chromatin Fibers

11:40-12:00 Allison Lopatkin, Duke, Microbial growth dynamics are the primary contributor to observed increase in resistance exchange following antibiotic treatment

12:00-12:20 Xiling Shen, Cornell, MicroRNA and protein cell fate determinants synergize in asymmetric division as safeguard against stem cell proliferation

12:20-14:30 Lunch

Session 8 (14:30-17:20, Squires Student Center, Colonial Hall)

Chair: Orna Resnekov, Molecular Sciences Institute

14:30-15:00 Martin Howard, John Innes Center, How to control the size of a fission yeast cell

15:00-15:20 Robert Sheehan, University of Pittsburgh, Novel positive feedback loop sets antigen dose-dependent threshold for T cell differentiation

15:20-15:40 Megan McClean, University of Wisconsin, Robust network structure of the Sln1-Ypd1-Ssk1

three-component phosphorelay prevents unintended activation of the HOG MAPK pathway in Saccharomyces cerevisiae

15:40-16:10 Coffee Break

16:10-16:30 Poster Spotlights 4

Byoungkoo Lee, Georgia State, Cancer cell invasion analysis in ECM using in vitro models

Xiao-jun Tian, University of Pittsburgh, Molecular Cooperativity Leads to Monoallelic Olfactory Receptor Expression

Faisal Reza, Yale, Modeling Genomic Recombination Potentials Regulated by Synthetic Donor DNA and Triplex-forming Molecules

Fei Li, Virginia Tech, Stochastic Model of the Histidine Kinase Switch in the Caulobacter Cell Cycle

16:30-17:00 Zhaoping Li, UCL/Tsinghua, A theory of the primary visual cortex, its zero-parameter quantitative prediction, and its experimental tests

17:00-17:20 Xiao Wang, Arizona State, Quorum-sensing crosstalk driven synthetic circuits: from unimodality to trimodality

17:20-19:00 Dinner (on own)

Session 9 (19:00-20:00, Squires Student Center, Colonial Hall)

19:00-20:00 Special session, Paul Cohen, DARPA, Machines that Assemble Signaling Pathways by Reading the Literature: Progress in DARPA's Big Mechanism Program

Session 10 (20:00-23:00, Poster Session 2, Squires Student Center, Commonwealth Ballroom)

20:00-23:00 Poster Session 2 (snacks and drinks served)

Saturday, August 8

7:30-9:00 Breakfast (on own or Dining Hall)

Session 11 (9:00-12:30, Squires Student Center, Colonial Hall)

Chair: Yang Cao, Virginia Tech

9:00-9:30 Karsten Weis, ETH, Global Changes In Chromosome Conformation In Budding Yeast In Different Physiological Conditions

9:30-9:50 Ilya Nemenman, Emory, Sensing Multiple Ligands with a Single Receptor

9:50-10:10 Eric Batchelor, NCI, Functional Roles of p53 Dynamics in Regulating Target Gene Expression

10:10-10:30 David Bruce Borenstein, Princeton, Established microbial colonies can survive Type VI secretion assault

10:30-11:00 Coffee Break

11:00-11:30 Linda Broadbelt, Northwestern, Discovery and Analysis of Novel Biochemical Transformations

11:30-11:50 Rhys Adams, ENS, Measuring the sequence-affinity landscape of antibodies

11:50-12:10 Heungwon Park, Duke, A synthetic oscillator couples to the cell division cycle in budding yeast

12:10-12:30 David Fange, Uppsala, Growth rate variations establish distributions of generation times and division sizes in E. coli

12:30-14:30 Lunch

Session 12 (14:30-18:00, Squires Student Center, Colonial Hall)

Chair: John Tyson, Virginia Tech

14:30-15:00 Carla Finkielstein, Virginia Tech, A systems-driven experimental approach reveals the complex regulatory distribution of p53 by circadian factors

15:00-15:20 Daniel Lewis, UC Davis, Unraveling dynamics of reconfigurable network motifs using a synthetic biology approach

15:20-15:40 Alan L. Hutchison, University of Chicago, Stochastic modeling of variability in circadian rhythms utilizing measured variance

15:40-16:00 Ertugrul Ozbudak, Albert Einstein, Stochasticity and the Mechanism of Precision in the Vertebrate Segmentation Clock

16:00-16:30 Coffee Break

16:30-17:00 Jeff Hasty, UCSD, Engineered Gene Circuits: From Clocks and Biopixels to Stealth Delivery

17:00-17:20 Neil Adames, Virginia Tech, Testing predictions of a new model for the budding yeast START transition using novel cell cycle mutants

17:20-17:40 Andre Leier, Okinawa Institute of Science and Technology, Hierarchical membrane compartmentalization stabilizes IFN receptor dynamics

17:40-18:00 Erel Levine, Harvard, Coordinated heat-shock response in C. elegans

Session 13: Closing Banquet (18:30-20:45, The Inn at Virginia Tech)

18:30-18:35 Acknowledgments, Will Mather, Virginia Tech

18:35-18:40 Closing remarks

18:40-18:45 Long live q-bio!, Jim Faeder, University of Pittsburgh

18:45-18:50 Introduction of q-bio 2016

18:30-19:45 Dinner

19:45-19:50 Banquet Speaker Introduction

19:50-20:45 Phil Nelson, University of Pennsylvania, Old and new news about single-photon sensitivity in human vision

The Ninth q-bio Conference: Poster Session 1

1. Deborah A Striegel (spotlight), Laboratory of Biological Modeling, NIDDK, NIH, Morphology of Pancreatic Islet Cytoarchitecture with Type 2 Diabetes
2. Jae Kyoung Kim (spotlight), Mathematical Biosciences Institute, The Ohio State Univ., Determining the validity of Hill functions in stochastic simulations
3. Ryan Nikin-Beers (spotlight), Department of Mathematics, Virginia Tech, The role of antibody in dengue viral infection
4. Ji Hyun Bak (spotlight), Princeton University, Characterizing the statistical properties of protein surfaces
5. Abed Alnaif (spotlight), University of California, Irvine, Pattern formation and morphogen gradients: A causality dilemma
6. Rory Donovan (spotlight), CMU-Pitt Ph.D. Program in Computational Biology, Enhanced Sampling in Spatial Stochastic Systems Biology Models Using a Weighted Ensemble of Trajectories
7. Mark Transtrum (spotlight), Brigham Young University, Effective models of emergent behavior from the manifold boundary approximation method
8. Vipul Periwal (spotlight), LBM, NIDDK, NIH, The Universality of Cancer
9. Steve Andrews, Fred Hutchinson Cancer Research Center, A Model of Lipid A Biosynthesis in E. coli
10. Jeanette Baran-Gale, University of North Carolina at Chapel Hill, Dynamics of estrogen stimulated regulatory networks in breast cancer
11. Madeleine Bonsma, University of Toronto, Building bacteria-phage interaction networks using the CRISPR locus
12. Anthony Burnetti, Duke University, The yeast metabolic cycle is coupled to cell division cycle Start across diverse strains
13. Minghan Chen, Computer Science Department, Virginia Tech, Two-dimensional Modeling on PopZ Bipolarization in Caulobacter Cell Cycle
14. Matthias Chung, Virginia Tech, Optimal Experimental Design for Biological Systems
15. Nicola Coker Gordon, Oxford University, Regulation of T Cell Receptor Phosphorylation
16. Jayajit Das, Battelle Center for Mathematical Medicine, the Research Institute at the Nationwide Children's Hospital and the Ohio State University, Restricted energy dissipation induces glass-like kinetics in high precision responses
17. Andrey Dovzhenok, University of Cincinnati, Comprehensive Modeling and Validation of Glucose and Temperature Compensation of the Neurospora Circadian Clock
18. Cemal Erdem, University of Pittsburgh, Characterization of differences in IGF1 and insulin induced proteomic signaling cascades
19. Chad Glen, Georgia Institute of Technology, Dynamic Intercellular Communication within Pluripotency Networks
20. Emine Guven, West Virginia University, Toward a Predictive Model of Spontaneous Clustering of VEGF Receptors
21. Silke Hauf, Virginia Tech, Abrupt sister chromatid splitting in anaphase without obligatory positive feedback
22. Noriko Hiroi, Keio University, Self-organization mechanism affects the time dependency of molecular diffusion
24. Jiayin Hong, Peking University, Role for Clockwork Orange Gene in Drosophila Circadian Clock
25. Chieh Hsu, School of Biosciences, University of Kent, Mapping of deterministic versus stochastic network activity by feedback splitting
26. Shuqiang Huang, Duke, Dynamic control and quantification of bacterial population dynamics in droplets
27. Sergey Klimov, Georgia State University, From Clinical Slides to Mathematical Prediction, a Twofold Approach
28. Justin Krueger, Virginia Tech, Robust Parameter Estimation for Biological Systems
29. Kyung Suk Lee, Harvard University, Serotonin-dependent Pulse-Width-Modulation control of food uptake
30. Melissa Lever, William Dunn School of Pathology, Oxford University, Phenotypic models of T cell activation
31. Xiang Liu, Peking University, A Model for β-cell's death during ER stress
32. Mathias Løvgreen Engel, Linding Lab, Deep Learning of Cell Morphologies for Kinome Wide Screening
33. Sarah Lueck, Institute of Theoretical Biology, Humboldt University Berlin, Rhythmic Degradation Explains and Unifies Circadian Transcriptome and Proteome Data
34. Mariana Martínez Sánchez, Universidad Nacional Autónoma de México, Minimal Regulatory Network of Extrinsic and Intrinsic Factors Recovers Patterns of CD4+ T Cell Differentiation and Plasticity
35. Patrick McCarter, University of North Carolina at Chapel Hill, Ranking proposed yeast feedback networks through Approximate Bayesian Computation parameter estimation and model selection.
36. Sina Mirzaeifard, University of Tennessee, Knoxville, Actin-Membrane Interactions in Membrane Nanotubes
37. Michele Monti, FOM institute Amolf, Time perception in cyanobacteria
38. Manikandan Narayanan, NIH, Robust inference of expression heterogeneity from simultaneous single- and k-cell profiling
39. Curtis Ogle, Virginia Tech, Multi-Protease Queueing
40. Libertad Pantoja-Hernández, Departamento de Control Automático, CINVESTAV, The TF binding mechanism role in the retroactivity impact
41. Joshua Porter, National Cancer Institute, p53 pulses diversify and coordinate target gene expression
42. Robert Pullen, University of Tennessee, Receptor binding, membrane deformations, and signal propagation at the cell membrane
43. Shanshan Qin, Center for Quantitative Biology,Peking University, Correlation of Gene Expression Noise During Cell Fate Transition
44. Nicholas Rossi, University of Vermont, Propagation of

signals from the transcription factor MarA to downstream genes

45. Warren Ruder, Virginia Tech, Simulating Emergent Behavior in Host-Microbiome Systems using Robots and Synthetic Biology
46. John Sekar, University of Pittsburgh, Visualizing Regulation in Rule-based Models
47. Rati Sharma, Johns Hopkins University, Directional Accuracy in a Model of Gradient Signaling during Yeast Mating
48. Christopher Short, West Virginia University, VEGF binding with high affinity domains
49. Emrah Simsek, Emory University, Phenotypic heterogeneity of nutrient-starved E. coli cells
50. Edward Stites, Washington University in St. Louis, Analysis of Ras as a tumor suppressor
51. Bradford Taylor, Georgia Institute of Technology, Frequency of multiply infecting bacteriophage in natural environments exposed by spatial models
52. Xiao-jun Tian, University of Pittsburgh, Ultrasensitivity and Bistability arising from miRNA-mRNA Reciprocal Interaction
53. Lior Vered, Department of Chemistry, University of North Carolina - Chapel Hill, Bistability and Hysteresis in the Pheromone Response Pathway
54. Margaritis Voliotis, University of Bristol, Stochastic Simulation of Biomolecular Networks in Dynamic Environments
55. Qiuyue Wang, Peking University, Using NF-κB modules and DNA elements to engineer combinatorial and dynamic gene regulation
56. Andrew White, Brigham Young University, The limitations of model-based experimental design in sloppy systems
57. Martin Wong, University of Sydney, Mathematical Modelling Reveals Missing Mechanism in AKT Activation
58. Peipei Yin, Peking University, Quantitative Analysis on Mitochondrial Apoptosis Pathway
59. Ruoshi Yuan, Shanghai Center for Systems Biomedicine Shanghai Jiao Tong University, Construction of Potential Landscape Uncovers Robust Dynamical Structure in Prostate Carcinogenesis
60. Jingyu Zhang, University of Pittsburgh, Crosstalk among TGF-β, Hedgehog and Wnt signaling pathway during EMT
61. Charles Zhao, Georgia Institute of Technology, Examining Genetic Background and Synaptic Morphology with Heterozygotes

The Ninth q-bio Conference: Poster Session 2

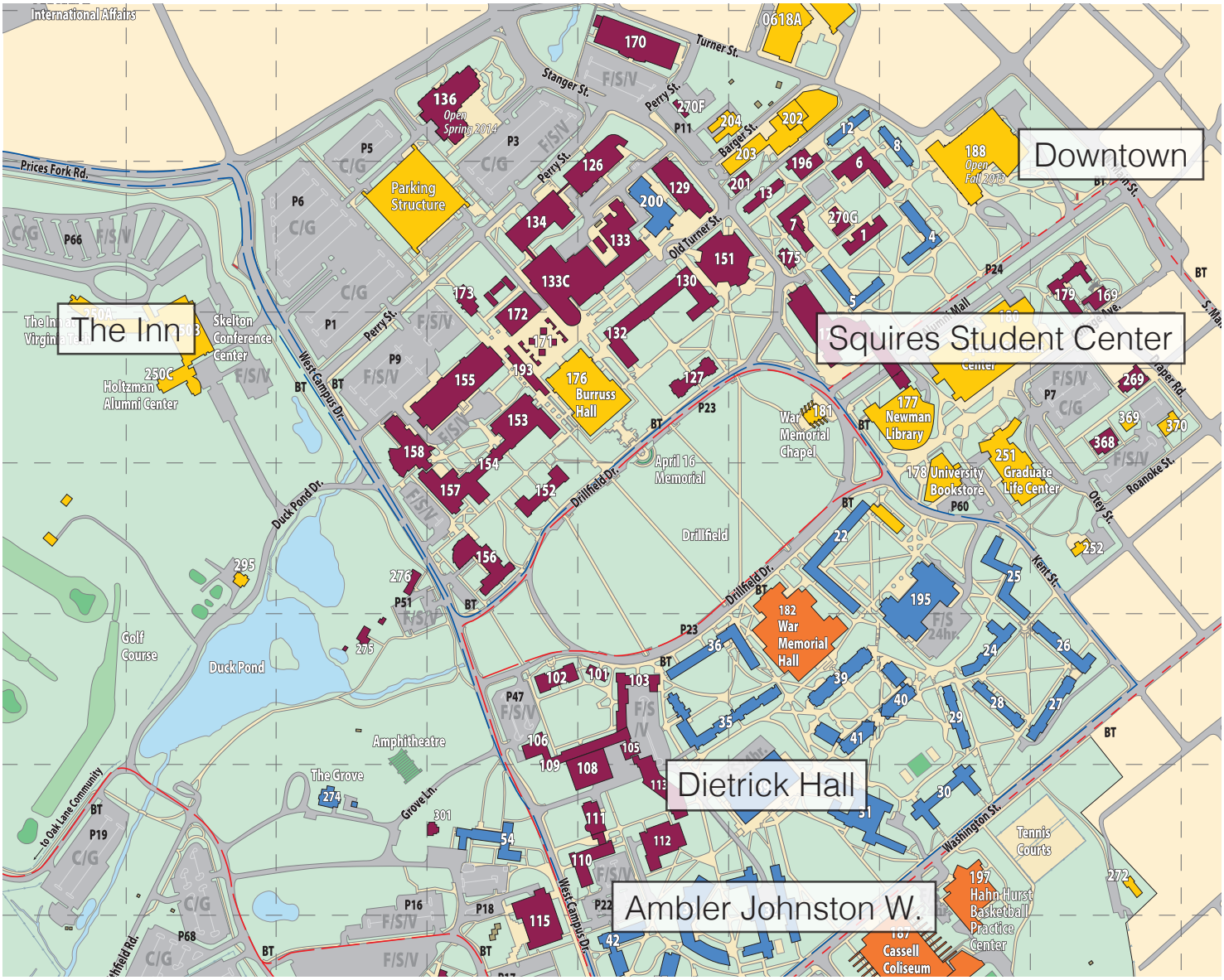
1. Philip Hochendoner (spotlight), Virginia Tech, Queueing Entrainment Downstream control of a synthetic oscillator
2. Sargis Karapetyan (spotlight), Duke University, Redox rhythms reinforce the plant circadian clock: New insights into coupled biological oscillators
3. Xiuxiu He (spotlight), Georgia State University, How Adhesion Regulates Cell Migration Plasticity: A Computational Study
4. David Wooten (spotlight), Vanderbilt University, Transcription factor network supports phenotypic heterogeneity in cancer
5. Byoungkoo Lee (spotlight), Georgia State University, Cancer cell invasion analysis in ECM using in vitro models
6. Xiao-Jun Tian (spotlight), University of Pittsburgh, Molecular Cooperativity Leads to Monoallelic Olfactory Receptor Expression
7. Faisal Reza (spotlight), Yale University, Modeling Genomic Recombination Potentials Regulated by Synthetic Donor DNA and Triplex-forming Molecules.
8. Fei Li (spotlight), Computer Science Department, Virginia Tech, Stochastic Model of the Histidine Kinase Switch in the Caulobacter Cell Cycle
9. Ariel Aptekmann, Biological Chemistry Institute, Exact Sciences Faculty, University of Buenos Aires., Archean core promoter region information content and its relation with optimal growth temperature.
10. Dorjsuren Battogtokh, Virginia Tech, Comparison of Domain Nucleation Mechanisms in a Minimal Model of Shoot Apical Meristem
11. Kevin Brown, University of Connecticut, Ensemble Learning for Correlated Substitution Analysis
13. Sheng Chen, Virginia Tech, physics department, Non-equilibrium relaxation in the vicinity of the extinction critical point in a stochastic lattice Lotka-Volterra model
14. Daniela Cimini, Virginia Tech, Global effects from errors at single kinetochores
15. Bryan Daniels, University of Wisconsin Madison, Accumulation versus propagation: coding dynamics in motion discrimination
16. Khanh Dinh, University of Alabama, Analysis of Inexact Krylov subspace methods for approximating the Matrix Exponential
17. Huijing Du, University of California, Irvine, Regulation of intestinal crypt homoeostasis: A balance between Wnt mediated expansion and proliferation inhibition
18. Song Feng, School of Life Sciences, University of Warwick, Explore design principles of signaling networks with in silico evolution of rule-based models
19. Vernita Gordon, The University of Texas at Austin, Distinct mechanical roles for bacterial biopolymers in sensing and strength
20. Rachel Haggerty, University of North Carolina at Chapel Hill, Mechanism Inference from Single Cells (MISC)
21. Teng He, Peking University, Control of inflammatory gene expression at the step of transcription elongation
22. Nora Hlavac, Virginia Tech, Markers of Astrocyte Reactivity in in vitro Models of Blast-Induced Neurotrauma
23. David Holland, Johns Hopkins School of Medicine, Optimizing protein expression levels as a function of network topology minimizes nonfunctional complex formation
24. Tian Hong, University of California, Irvine, Semi-adaptive

response and noise attenuation in BMP signaling

25. Haitao Huang, Georgia State University, Spatial Quantification of Morphological Changes in Retinal Pigment Epithelium
26. Sukanya Iyer, Emory University, Characterizing gene expression kinetics in nutrient-starved E.coli cells
27. Pavel Kraikivski, Virginia Polytechnic Institute and State University, From START to FINISH: Computational Analysis of Cell Cycle Control in Budding Yeast
28. Anuva Kulkarni, Carnegie Mellon University, Automation of Model Design and Analysis for Big Mechanisms
29. Kevin Leslie, Virginia Commonwealth University, Applications of Precision, Real-time Cell Biomass Measurements in Cell Physiology and Drug Development
30. Feng Liu, Nanjing University, Transient DNA looping bridged on low-affinity sequences substantially promotes gene transcription
31. Wolfgang Losert, University of Maryland, Adhesive forces play key roll in pattern formation and stability in chemotaxing cells
32. Alexander Lubbock, Vanderbilt University, Gabi: Network inference from antibody-based proteomics data
33. Lan Ma, University of Texas at Dallas, Regulation of P53 Oscillations by MircoRNA-mediated Positive Feedback Loops
34. Shibin Mathew, University of Pittsburgh, Mechanistic Insights into Early Endoderm Differentiation of Human Embryonic Stem Cells using Systems Analysis of Signaling Interactions
35. Callie Miller, University of North Carolina, Chapel Hill, Computational Model of Cortical Actomyosin
36. Paul Mlynarczyk, The University of Tennessee, Dept. of Chemical and Biomolecular Engineering, Diffusion- and Geometry-Influenced Stochastic Switching in a Reaction Network with Positive Feedback
37. Sayak Mukherjee, Ohio State University, Reverse Engineering Signaling Cascade from High Throughput Data
38. Philip Nelson, University of Pennsylvania, Light, Imaging, Vision: An interdisciplinary undergraduate course
39. Oscar Ortega, Vanderbilt University, Automated tropical algebra reduction and hybridization of biological models
40. Vipul Periwal, LBM/NIDDK/NIH, Mitochondrial Energetic Homeostasis and Parallel Activation
41. Aaron Prescott, University of Tennessee, Department of Chemical and Bimolecular Engineering, Redesigning the response of T cell signaling networks using in silico evolution
42. Hong Qin, Spelman College, A network model of cellular aging and its applications
43. Samuel Ramirez, Duke University, Modeling the spatio-temporal dynamics of Cdc42 activity at dendritic spines accounting for membrane geometry
44. Julia Roth, Virginia Tech, A Synthetic Biology Approach to Ribosome Collisions and Stalling
45. Monika Scholz, University of Chicago, Optimal feeding regulation in noisy environmental conditions
46. Leili Shahriyari, Mathematical Biosciences Institute, Ohio State University, Role of Stem Cell Niche Structure in Cancer
47. Wenjia Shi, Peking University, How to Achieve Perfect Adaptation
48. Alex Shumway, Brigham Young University, Classifying and quantifying parameter nonlinearity in biological models
49. Minjun Son, Department of Physics, University of Florida, Microfluidic Study of a Stochastic Genetic Circuit Carefully Modulated by Environmental Inputs
50. Jose-Juan Tapia, University of Pittsburgh, Mechanistic analysis of reaction network models
51. Marcus Thomas, Carnegie Mellon University, Improved Model Fitting for Complex Self-Assembly Reaction Networks
52. Michael Trogdon, University of California - Santa Barbara, Simulating Yeast Polarization in the Cloud
53. Huy Vo, The University of Alabama, A projection method for solving the chemical master equation
54. Margaritis Voliotis, University of Bristol, Dynamic environments reveal specialized roles in the HOG network
55. Xujing Wang, The National Heart, Lung, and Blood Institute (NHLBI), Mathematical modeling of insulin secretion from a network of coupled islet beta-cells via glucose-induced changes in membrane potential, intracellular calcium, and insulin granule dynamics
56. Martin Wong, Univesity of Sydney, Improved Enzyme Kinetics Model for Simulating Complex Biochemical Networks
57. Guang Yao, Univ. of Arizona, Controlling the Heterogeneous Quiescent State by an Rb-E2F Bistable Switch
58. Osman Yogurtcu, Johns Hopkins U, Novel Single-Molecule Resolution Method for Spatio-Temporal Simulations of Protein Binding and Recruitment on the Membrane
59. Hang Zhang, Virginia Tech, Integrative Proteomic and MicroRNA Approaches Reveal a Novel Post-Transcriptional Motif Regulating Human Definitive Endoderm Differentiation
60. Zining Zhang, Computational & System biology, University of Pittsburgh, The F-ATP synthase: what advantages might the rotary mechanism confer?
61. Riccardo Ziraldo, The University of Texas at Dallas, Mechanistic dissection of Drosophila apoptotic switch

Map of Key Locations

http://www.maps.vt.edu/interactive/
http://www.maps.vt.edu/PDF/campus-map-highres.pdf



Squires Student Center: primary conference location, including tutorials, invited and contributed talks, and poster sessions.

The Inn: opening and closing banquets.

Dietrick Hall: award winning dining hall for lunch.

Downtown: excellent dining options for lunch and dinner.

Ambler Johnston West: dorm housing.

The remaining pages include abstracts from the following:

- Talks from the main program, including poster spotlights
- Poster Session 1
- Poster Session 2

Abstracts are presented in the order in which they appear in this program.

(please continue to next page)

Network Dynamic and Cell Physiology

John J. Tyson

Department of Biological Sciences,
Virginia Polytechnic Institute & State University,
Blacksburg, VA

THE PHYSIOLOGICAL capabilities of a living cell (metabolism, reproduction, signaling, motility, etc.) are controlled by complex networks of interacting biochemicals (genes, RNAs, proteins and metabolites). Although intuitive reasoning about these networks may be sufficient to guide the next experiment, detailed computational models are required to codify the results of hundreds of observations, and a sophisticated theoretical framework is necessary to understand the "molecular logic" of the rich dynamical repertoire of cellular control systems. Over the past 40 years, in collaboration with splendid colleagues such as Art Winfree, Albert Goldbeter, Jim Keener, Bela Novak and Kathy Chen, I have been developing methods to predict the dynamical properties of biochemical networks and relate these properties to the life-sustaining behaviors of cells.

SMeagol Simulated Microscopy - a tool against inverse crimes

Martin Lindén, Johan Elf

SINGLE MOLECULE tracking in living cells using fluorescence microscopy is a powerful method to study otherwise inaccessible aspects of intracellular kinetics. The development of accurate analysis methods for such data is however limited by the lack of ground truth reference data. I will present a computational tool that extends the capability of reaction diffusion simulation softwares to make physics based simulation of single molecule tracking experiments in living cells. Stochastic reaction diffusion models makes it possible to account for the influence of geometry, randomness and diffusion limitations in intracellular kinetics. In addition the new tool makes it possible to consider the photo-physics of the labeled molecules, the optics of the microscopy system and electronic properties of the camera. In combination this allows for optimization of experimental parameters and to testing and improving the data analysis methods.

Cell-cycle synchronized oscillations of a negatively self-regulated gene in E. coli

Zach Hensel[1] and Tatiana T. Marquez-Lago[1]

***Short Abstract* — Robust, tailored behaviours such as genetic oscillations have been successfully implemented in prokaryotic and eukaryotic organisms. However, periodic processes such as gene doubling and cell division are rarely accounted for. Accordingly, we studied a chromosome-integrated, negative-feedback circuit based upon the bacteriophage λ transcriptional repressor Cro and observed strong, feedback-dependent oscillations in single-cell time traces [1]. This finding was surprising due to a lack of cooperativity, long delays or fast protein degradation [2]. Moreover, we found feedback-dependent oscillations to be synchronized to the cell cycle by gene duplication, with phase shifts predictably correlating with estimated gene doubling times.**

***Keywords* — Oscillations, self-regulation, gene-doubling, timelapse imaging, λ lysis/lysogeny, synthetic biology, stochastic modelling.**

I. Background

ADVANCED applications in synthetic biology require the design of genetic networks that are both *predictable* and *robust*. However, many critical aspects of cell and molecular biology are still poorly characterized and, as a consequence, engineering even simple genetic circuits remains challenging. This is further accentuated by the requirement that synthetic circuits must reliably operate within noisy and heterogeneous environments. In fact, identical challenges apply to developing predictive models of natural biological systems. Thus, among many uses, simple circuits can be studied to refine complex gene-regulation models, providing powerful insights into natural organisms.

There are surprisingly few studies of negative feedback networks with the single-cell, time-lapse resolution required to study gene expression dynamics. Some recent studies show how repressor-binding strength impacts protein expression, and others how negative feedback increases the bandwidth of expression noise [4]. Notably, oscillations in Lac repressor expression have been attributed to reaction delays combined with fast repressor degradation [5]. However, all known experiments utilized genetic constructs with multiple high-affinity binding sites and, in some cases, additional degrees of cooperativity in autoregulation.

[1] Integrative Systems Biology Unit, Okinawa Institute of Science and Technology, Onna-son, Okinawa, Japan. Email: tatiana.marquez@oist.jp

II. Methods

To facilitate comparison with recent theoretical and stochastic simulation results, we sought to create a simpler circuit in which the bacteriophage λ transcriptional repressor Cro represses its own expression. We then tracked and analysed single-cell expression dynamics over several hours for tens of individual microcolonies.

Timelapse imaging revealed oscillations in Cro expression that were in phase with the cell cycle, while strong oscillations did not occur in the absence of negative feedback. The circuit was then integrated at different genomic loci to shift the gene doubling time; this produced phase shifts with Cro expression peaks tending to lag estimated doubling times by ~20 minutes. Consistent with our theoretical predictions and simulations [2-3], as well as previous experiments, negative feedback increased the coefficient of variation and reduced the Fano factor. Aside, the size and resolution of our single-cell data sets made it possible to calculate the power spectral density of Cro expression time series, which showed that negative feedback increased the noise bandwidth of Cro expression.

III. Conclusion

Our results suggest that accurate models of bacteriophage λ lysis/lysogeny must necessarily include cell-cycle dependence and genome-location effects, and suggest a molecular and evolutionary basis for biases in chromosome integration sites of lamboid prophages. Moreover, these factors should also be accounted for in the rational design of chromosome-integrated synthetic genetic networks, especially in light of the recent development of simplified recombineering techniques.

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Automation and Microfluidic Tools for Q Bio

Hang Lu

Georgia Institute of Technology
311 Ferst Dr. NW
Atlanta, GA 30332-0100

IN this talk, I will focus on how my lab develops and uses a set of automation, microfluidics, and image-based data mining technologies to address questions in quantitative biology. In one example, I will show how we take advantage of simple hydrodynamics to design microfluidic systems for large throughput and spatially and temporally well-controlled experiments in embryonic development as well as in immunology. In another example, I will show how we combine the power of experimental tools and computational tools to study problems in development neurobiology and behavior in intact animals. The power of these engineered systems lies in that the throughput that can be achieved by using automation and microfluidics is 100-1,000 times that of conventional methods, and furthermore, we can obtain information unattainable or at least not easily attainable by conventional tools. For example, quantitative analysis and unbiased image data mining allowed the discovery of phenotypes (and subsequently genes) that are difficult for human users.

Morphology of Pancreatic Islet Cytoarchitecture with Type 2 Diabetes

Deborah A. Striegel[1], Manami Hara[2], Vipul Periwal[3]

***Short Abstract* — Blood glucose levels are maintained by hormones secreted by endocrine cells in pancreatic islets of Langerhans. Cell-cell contacts between these cells regulate the oscillatory production of insulin and glucagon. Thus appropriate cellular arrangement is necessary for normal function. Graph theory provides a framework for quantifying cytoarchitectural features. Here, using large-scale imaging data for ~15,000 islets containing 100,000+ cells in human organ donor pancreata, we show that quantitative graph characteristics differ between control and type 2 diabetic islets. We then modeled islet rearrangement to determine processes that leave observed islet graph measures invariant, and compared these processes between normal and T2D islets.**

***Keywords* — diabetes, pancreatic islets, cytoarchitecture, graphs, β cells**

I. Purpose

PANCREATIC islet cells play a major role in blood glucose homeostasis by secreting a number of hormones: glucagon by α cells, insulin by β cells, and somatostatin by δ cells. For normal insulin secretion, these endocrine cells need functional patterns of cell-cell contacts [1]. Defining the correct anatomical arrangement is difficult since normal cellular architecture varies among species and, in humans, varies by size of the islet [2]. Recent studies showed that α-cell locations observed in human islets are not random, but instead create a characteristic structure [2]. β-cell mass is dynamic due to cell reorganization, death, and replication, in individual islets. Type 2 diabetes (T2D) is characterized by lack of glucose homeostasis and β-cell mass loss [3]. The T2D morphological and physiological changes should have a marked effect on this characteristic structure. Here, we capture the cellular arrangement of each islet by utilizing a graph, use measures from graph theory to quantify islet architecture and changes observed with T2D, and study the effects of β cell reorganization on these graphs.

II. Methods

Data from high resolution large-scale automated imaging of islets in human organ donor pancreas sections [2] was used to first compute graphs consisting of α, β, and/or δ cells as nodes and cell-cell contacts as edges for each individual islet. This allowed for a comprehensive capture of their characteristic structure. The graphs were quantified using measures, such as mean degree (the mean number of edges per node) and components. We examined architectural changes under different rearrangement processes. We numerically modeled stochastic processes simulating cellular reorganization to find degree- and component-specific parameter combinations that leave the quantitative graph-theoretical measures of islet cytoarchitecture invariant. We then compared the parameter combinations that leave normal islet architecture invariant to those associated with T2D islets.

III. Results

We found that T2D islets have a higher mean degree than control islets. Furthermore, large control islets have more components but fewer cells per component than large T2D islets. To maintain an equilibrium population, we found that cells with a large degree and respective component size are removed independent of the placement of newly added cells. Furthermore, if cells are added preferentially to large degree cells then the degree and component-size for removing a cell is increased to maintain equilibrium.

IV. Conclusions

Cell-cell interaction graphs give a new quantitative perspective on islet endocrine cell interactions in the pathophysiology of T2D, and may allow for a better understanding of the changes in islet architecture that accompany diabetes. We speculate that the higher mean degree of T2D islets is due to the increase in demand for insulin secretion in T2D that can only be met by β cells that have a higher mean degree compared to control. This may result in the death of less connected β cells and the elimination of components that have lower than average degree. Large degree cell loss may maintain optimal contact with capillaries for oxygen uptake and glucose sensing.

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[1]Laboratory of Biological Modeling, National Institute of Diabetes Digestive and Kidney Disorders, NIH. E-mail: deborah.striegel@nih.gov

[2]Kovler Diabetes Center, The University of Chicago. Email: mhara@uchicago.edu

[3]Laboratory of Biological Modeling, National Institute of Diabetes Digestive and Kidney Disorders, NIH. E-mail: vipulp@mail.nih.gov

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Determining the validity of Hill functions in stochastic simulations

Jae Kyoung Kim[1], Krešimir Josić [2], and Matthew R. Bennett[3]

The non-elementary reaction functions (e.g. Michaelis-Menten or Hill functions) are used to reduce the model of biochemical network. Such deterministic reductions are frequently a basis for heuristic stochastic models in which non-elementary reaction functions are used as propensities of Gillespie algorithm. Despite their popularity, it remains unclear when such stochastic reductions are valid. Here, we first identify the validity condition for using non-elementary reaction functions for the stochastic simulations. This provides a simple and computationally inexpensive way to test the accuracy of reduced stochastic model.

Keywords **– multi-scale stochastic system, stochastic simulation, Hill-function, Michaelis-Menten function**

I. INTRODUCTION

BIOCHEMICAL systems are often regulated by processes that evolve on widely differing timescales. Simulating the fastest of these processes is computationally expensive and often not of direct interest. Thus, to replace the fast processes, non-elementary reaction functions (e.g. the Michaelis-Menten or Hill function) have been used. This approach is simple, computationally inexpensive, and has been used widely in both deterministic and stochastic simulations [1-3].

While the deterministic reductions have been theoretically justified [4], it is not clear when their stochastic counterparts will be accurate. Many previous modeling results rely on the assumption that the results of stochastic simulations can be accepted if their deterministic counterparts are valid. However, a number of recent examples show that this is not necessarily the case [5-7].

In this study [8], we show that the validity of these approximations is closely related, but in a more subtle way than previously assumed. This insight provides a simple and concrete method for testing the validity of using non-elementary functions for the propensity functions in stochastic simulations.

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[1]Mathematical Biosciences Institute, The Ohio State University, Columbus, OH 43210. E-mail: kim.5052@mbi.osu.edu

[2]Department of Mathematics, University of Houston, Houston, TX 77204. E-mail: josic@uh.edu

[3]Institute of Biosciences and Bioengineering, Rice University, Houston, TX, 77005 E-mail: Matthew.Bennett@rice.edu

II. RESULTS

We find that discrepancies between the stochastic and the deterministic reduction stem from the fact that, due to the random fluctuations, the stochastic system explores a wider range of states than its deterministic counterpart. Our analysis and simulations show that the stochastic reduction is accurate only when the corresponding deterministic reduction is accurate over a range that covers the most likely states of the stochastic system.

This finding implies that, for testing the validity of stochastic reduction, it is sufficient to examine the validity of deterministic reduction *post facto* -- after the corresponding stochastic simulations of the reduced model reveal the range of status that need to be tested.

III. CONCLUSION

Our work first develops a simple and general method to test the validity of stochastic models that include non-elementary propensity functions. If the validity condition is satisfied, we can perform accurate and computationally inexpensive stochastic simulation without converting the non-elementary functions to the elementary functions (e.g. mass action kinetics). Considering the popularity of Michaelis-Menten or Hill functions in various biological models, our results will provide a useful tool to a large modeling community.

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The Role of Antibody in Dengue Viral Infection

Ryan P. Nikin-Beers[1] and Stanca M. Ciupe[2]

Short Abstract — **Dengue virus has four distinct serotypes whose cross-reactive immune responses contribute to increased disease severity following heterologous infections. Since cross-reactive antibodies may play a role in disease enhancement, we develop a mathematical model of host-virus interaction and predict the mechanisms responsible for virus expansion and loss during primary and secondary dengue infections. We use the model to determine the role of cross-reactive antibodies during dengue fever and dengue hemorrhagic fever-inducing secondary infections, and compare the model to patient data. We predict that the cross-reactive antibodies interfere with the non-neutralizing antibody effects by reducing the phagocyte-mediated removal of antibody-virus immune complexes.**

I. Background

IN recent years, dengue viral infection has become one of the most widely-spread mosquito-borne diseases in the world, with an estimated 50-100 million cases annually, resulting in 500,000 hospitalizations [1]. Dengue viruses (DENV) cause mild dengue fever (DF) and severe dengue hemorrhagic fever (DHF). Dengue virus has four distinct serotypes, DENV 1-4, whose cross-reactive immune responses contribute to increased disease severity following heterologous infections. One current hypothesis postulates that cross-reactive antibodies are responsible for the enhancement of the infection, in a mechanism known as "antibody-dependent enhancement" (ADE) [1,2]. When a patient is first infected with one dengue strain, the host produces neutralizing antibodies specific to that strain. After the primary infection is eliminated, long-lived antibody-producing plasma cells specific to the first strain persist in the body. When infection with a second dengue serotype occurs, antibodies from the primary infection bind the second virus but do not neutralize it. Instead, phagocytes recruited to clear the virus antibody immune complexes internalize non-neutralized virus and become infected in the process [1,2]. The result is higher levels of viremia, which in turn is associated with more severe infection [3].

II. Results

We first model both the neutralizing and non-neutralizing antibody effects in dengue primary infection. Due to observed host-virus characteristics such as high level viremia followed by virus clearance and delayed antibody responses which become detectable after virus resolution [4], we are able to determine unknown parameters in our model. We find that the neutralizing rate has the strongest effect on viral reduction in primary infection.

We next develop a model for secondary infection of a heterologous serotype, taking into account that both strain-specific and cross-reactive antibodies are produced during secondary dengue infection [5]. We then fit the model to published patient data [6] in order to determine the role of the cross-reactive antibodies in both secondary DF and secondary DHF. We find that if the neutralizing rate of the antibody in secondary infection is enhanced as described in ADE, the model gives results which contradict clinical reports [4,6]. However, we are able to fit the known biological data when the cross-reactive antibody results in the decrease of the overall heterologous virus clearance. This suggests that the non-neutralizing antibody effects have more of a role in explaining the difference between secondary DF and secondary DHF. One biological explanation for this result may be that by binding heterologous virus, cross-reactive antibodies render it unavailable for binding and subsequent removal by strain-specific antibodies.

III. Conclusion

We developed mathematical models of antibody response (including both neutralizing and non-neutralizing effects) to model both primary dengue infection and secondary dengue infection. In primary dengue infection, the neutralizing antibody effect was shown to have the strongest effect on viral reduction. In secondary dengue infection, the difference between mild and severe disease can be attributed to the non-neutralizing antibody effect.

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[1]Department of Mathematics, Virginia Tech, 460 McBryde Hall, Blacksburg, VA 24060. E-mail: rnikinb@vt.edu

[2] Department of Mathematics, Virginia Tech, 460 McBryde Hall, Blacksburg, VA 24060. E-mail: stanca@math.vt.edu

Characterizing the statistical properties of protein surfaces

Ji Hyun Bak[1,2], Anne-Florence Bitbol[1,2] and William Bialek[1,2]

***Short Abstract* — In order to ensure the accuracy as well as the specificity of biological signaling, it is crucial that proteins recognize their correct interaction partners. An important ingredient of recognition is shape complementarity. Not only does shape complementarity allow the short-ranged chemical attractions to be at work, but it also provides additional degrees of freedom in the space of interactions. Here we aim to characterize the statistical properties of the ensemble of protein surface shapes. Specifically, we evaluate the intrinsic dimensionality of the space of protein surfaces, and show how it is related to the characteristic length scale of the surfaces.**

***Keywords* — protein-protein interaction, specificity, shape complementarity, space of protein surfaces**

I. Introduction

PROTEINS and their interactions form the body of the signaling transduction pathways in many living systems. In order to ensure the accuracy as well as the specificity of signaling, it is crucial that proteins recognize their correct interaction partners. How difficult, then, is it for a protein to discriminate its correct interaction partner(s) from the possibly large set of other proteins it may encounter in the cell?

An important ingredient of recognition is shape complementarity. While there has been much attention to the determinants of protein-protein recognition [1], most efforts were directed to the role of chemical compositions, and we are still a long way from a system-level understanding of the role of shape. In fact, shape complementarity is a prerequisite for the recognition process, because of the short-ranged nature of chemical interactions.

The ensemble of protein shapes should be constrained by the need for maintaining functional interactions while avoiding spurious ones. There must be enough degrees of freedom to accommodate the whole proteome while maintaining the specificity of interactions, while too many effective degrees of freedom would make the recognition problem difficult. To address this aspect of protein recognition, we start by investigating the dimensionality of the space of protein shapes.

II. Methods

We consider the ensemble of proteins in terms of their three-dimensional shapes, more precisely in terms of their solvent-excluded surfaces. We take into account all complete high-resolution X-ray crystalized structures from *E. coli* non-DNA-binding cytoplasmic proteins that can be retrieved from the Protein Data Bank, resulting in a database of 397 proteins.

[1]Joseph Henry Laboratory of Physics, [2]Lewis-Sigler Institute of for Integrative Gemonics, Princeton University, Princeton, NJ 08544 USA
Email: jhbak@princeton.edu

In order to measure the intrinsic dimension of the dataset, we apply a statistics that was first developed in chaotic theory, called the correlation dimension [2], to the high-dimensional space where each point corresponds to a shape object. The space of surfaces is the set of geodesic-disk patches with a fixed surface area, sampled from the protein surfaces in the dataset. The space of curves is the set of geodesic curves with a fixed length, also sampled from the surfaces.

III. Results

The dimension D_2 of the space of surface patches turns out to be high, about $D_2 = 30$ for patches with area 1000 $\mathring{A}^2$, typical size of reported interfaces [3]. However, it is known that the effect of systematic error in calculating the correlation dimension of a finite dataset aggravates as the true dimension increases [4]. On the other hand, if we consider the dimension D_1 of the space of geodesic curves sampled from the surfaces, generically we can expect the dimensions to scale as $D_2 \sim (D_1)^2$. Our statistics can therefore be more reliable with this lower dimensional dataset.

We find that D_1 grows with the length of the curves, and that there is a clear transition between two regimes of growth: there is an initial steep growth, followed by a less steep and linear growth regime at larger curve lengths. We argue that this pattern of growth can be explained by a single length scale, which corresponds to the characteristic scale of the protein surface (that can actually be measured). Beyond the characteristic scale, there is roughly one extra dimension per characteristic scale, representing an extra degree of freedom; below this scale the steep growth reflects the smaller-scale "noisy" features, such as the roughness of the surface at the atomic scale. We test this idea by generating synthetic curves characterized by a single correlation length and calculating the dimensionality of the generated dataset.

Taking this argument further, we also discuss how these results may be connected back to the question of interaction specificity through a simple model with harmonic interactions, where each surface is modeled as a set of independent points.

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Microbes Under Pressure

Oskar Hallatschek

University of California, Berkeley
Berkeley, California
USA

When cells grow in crowded environments, such as biofilms, organs or tumors, they need to push their surroundings to accommodate space for new cells. The magnitude of the resulting mechanical forces and their bio-physical consequences have remained difficult to study using common in vitro techniques that lack mechanical control. Using a novel microfluidic chemostat, we show that populations of budding yeast can develop highly fluctuating mechanical pressures in the MPa range when they are growing in leaky cavities. The growth-induced compressive stresses strongly modify the shape and dynamic arrangement of the cells and slow down their growth rates, correlated with a cell cycle arrest in the G1 phase. By using the cell shape deformation as an indication for locally acting mechanical stresses, we show that dense cell packings are mechanically stabilized by heterogenous force networks with 'force-chains' spanning numerous cells. These force networks are interrupted and reformed by sudden collective rearrangement events with a broad distribution of magnitudes that allow cells to flow out of the leaky cavity. These features strikingly resemble those of driven granular materials and can be reproduced in overdamped simulations of proliferating soft particles. In both experiments and simulations, cell-cell cohesion strongly promotes jamming-induced clogging of confined spaces. The self-driven jamming and build-up of significant mechanical pressures could thus be a natural tendency of microbes growing in confined spaces, and possibly contribute to microbial pathogenesis, biofilm formation, and biofouling.

IgE receptor signaling encodes dynamic memories of antigen exposure

Lily A. Chylek*[1], Yanli Liu*[2], Brooke Harmon[2], William S. Hlavacek[3], and Anup K. Singh[2]

***Short Abstract* — Mast cells drive allergic reactions in response to allergen-mediated crosslinking of the high-affinity IgE receptor. The relationship between patterns of allergen exposure and cellular responses is unclear. To investigate this issue, we used a microfluidics platform to expose cells to pulses of stimulatory multivalent antigen interrupted by intervals of inhibitory, excess monovalent antigen. We measured secretion of inflammatory mediators. Results were analyzed with the aid of a mathematical model. The secretory response to a repeated antigen pulse was diminished when the inhibitory interval was <1 hr, which we were able to attribute to a desensitization process involving the lipid phosphatase Ship1. After an interval of ~1 hr, response to repeated stimulation became similar to the initial response. Hyperactive responses were then observed for longer intervals of up to 4 hrs, suggesting that Ship1 is slowly deactivated after termination of signaling and eventually (on an even slower time scale) becomes refractory to (re)activation. Hyperactivity is linked to proteosomal degradation of a Ship1 co-factor, the adaptor protein Shc1.**

***Keywords* — cell signaling, microfluidics, modeling**

I. Introduction

ALLERGIES afflict ~25% of people in the developed world. Central players in allergic reactions include mast cells and basophils, which release histamine and other mediators of inflammation upon allergen interaction with cell-surface IgE-FcεRI complexes. Aggregation of FcεRI leads to activation of several kinases, including the protein tyrosine kinase Syk. Like most receptor-initiated signaling cascades, signaling by FcεRI is held in check by desensitization processes. The dynamic interplay between these positive and negative signaling axes likely govern how a cell responds to complex inputs.

Study of cellular outputs in response to complex inputs has been accelerated by the advent of microfluidics devices, which allow for precise manipulation of fluids at small length scales from micrometers to millimeters. These new and distinctive capabilities enable the study of fundamental cellular behaviors, including cellular information processing and decision-making. Microfluidics facilitates controlled exposure profiles to single cells including pulsatile, ramp, square-wave and oscillatory signals. In this work, we used a microfluidic platform to examine desensitization linked to pulsed stimulation of FcεRI. This work represents an investigation of the system's frequency response properties.

II. Results

A. Short- and long-term molecular memory

Two surprising phenomena were observed in experiments: rapid desensitization, or short-term molecular memory, and hypersensitization after a delay, or long-term molecular memory. A model reproducing the observed responses to pulsed stimulation and capturing (fast) positive signaling by Syk and (slow) negative signaling by Ship1 was developed and used to guide investigation of short- and long-term memory. Enzyme activity assays confirmed that Syk signals decay quickly, whereas Ship1 signals decay slowly, after IgE-FcεRI aggregate breakup is induced by excess monovalent antigen.

B. Ship1 and the proteasome regulate memory

The model, as initially formulated, predicted that inhibition of Ship1 phosphatase activity would abrogate short-term memory, whereas inhibition of Ship1 degradation would abrogate long-term memory. The former prediction was confirmed experimentally but not the latter. Although proteosome inhibition eliminated long-term memory, Ship1 levels stayed constant over time. The model was able to explain the available observations when it was modified to include a Ship1 co-factor that is degraded in response to multivalent antigen inputs.

C. Shc1 plays a role in delayed hypersensitivity

We hypothesized that the Ship1 co-factor in the model might correspond to one of the several known Ship1 binding partners, such as Shc1. The model predicted that co-factor knockdown would result in accelerated hyperdegranulation. This prediction was confirmed experimentally in cells with reduced levels of Shc1 after siRNA treatment.

III. Conclusion

We leveraged a microfluidic platform enabling precisely controlled complex waveform inputs and mathematical modeling to elucidate how signaling processes operating on distinct time scales (quickly induced and rapidly reversed Syk activation, slowly induced and slowly reversed Ship1 activation, and very slow decay of the Ship1 co-factor Shc1) can give rise to short- and long-term molecular memories of antigen exposure.

* These authors contributed equally.
[1]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY
[2]Department of Biotechnology and Bioengineering, Sandia National Laboratories, Livermore, CA
[3]Theoretical Division, Los Alamos National Lab, Los Alamos, NM

The Noise is the Signal: Information Flow in Single Cells and Cellular Populations

Ryan Suderman[1*], John Bachman[2*], Adam Smith[1], Peter Sorger[2], and Eric J. Deeds[1,3,4]

***Short Abstract* — Intracellular signaling networks controlling critical cell-fate decisions (*e.g.* apoptosis) have been shown to exhibit exceedingly high levels of noise, preventing the reliable transmission of information. Our results reveal that this poor information transfer in individual cells is required to increase the information available to cellular populations, so that a single extracellular stimulus can induce graded behavior among an isogenic population of cells. We also show that responses relevant to individual cells (*e.g.* chemotaxis) exhibit more reliable information transfer. Thus, noisy signaling is not necessarily a consequence of some inherent physical limitation. Our work provides an explanation for the observed high levels of noise prevalent in certain metazoan signaling networks, and how noise might be exploited by evolution.**

***Keywords* — Information Theory, Cellular Heterogeneity**

I. Introduction

SIGNALING networks allow cells to make fate-altering decisions based on changing environmental factors. Apoptosis and commitment to cell division are typical binary responses to a signal, whereas chemotaxis and gene expression are examples of continuously variable responses. Traditionally, increasing the information available to the cell via these signaling networks has been viewed as beneficial, spurring investigation of the reliability of dose-response behavior given certain network motifs (*1*). However, the observed heterogeneity among certain isogenic cellular populations confounds this perspective and has become an object of considerable interest in recent years (*2*). Recently, the application of information theory to intracellular signaling has provided a means to quantify the impact of variability (*3*).

Levchenko and coworkers employed this strategy to characterize maximum information transduction (*i.e.* the channel capacity) in the TNF-α signaling network and found that the network transferred less than 1 bit of information from the extracellular stimulus to the transcription factor, NF-κB (*4*). Due to stochasticity in gene expression, the channel capacity between stimulus and phenotypic response is likely even lower.

[*]These authors contributed equally to this work.
[1]Center for Computational Biology, University of Kansas, Lawrence, KS. E-mail: ryants@ku.edu;
[2]Department of Systems Biology, Harvard Medical School, Boston, MA. E-mail: bachmanjohn@gmail.com, peter_sorger@hms.harvard.edu
[3]Department of Molecular Biosciences, University of Kansas, Lawrence, KS.
[4]External Faculty, Santa Fe Institute, Santa Fe, NM. Email: deeds@ku.edu

II. Results

To explore information transfer in the context of a system with a clear phenotypic output (i.e. cell death), we examined the channel capacity of extrinsic apoptosis signaling (*5*). Measuring individual cells' intracellular response to signal resulted in low channel capacities (<0.4 bits), however, if population-level response (fraction dead) is considered, the channel capacity increases dramatically (>3 bits, dependent on population size). Using a simple theoretical model, we showed that an increase in population-level channel capacity generally involves a corresponding decrease in channel capacity in single cells.

One possible explanation of these findings is that noise in individual cells arises from some kind of biophysical limit on information transfer, and organisms might simply take advantage of that limit to control cellular populations. To test this possibility, we considered two cases where the response of individual cells is paramount: eukaryotic chemotaxis and mating in yeast cells. In each case, we found single-cell channel capacities much higher than those previously observed (>2 bits). This implies that signaling networks are *capable* of transmitting relatively high levels of information despite the inherently stochastic nature of biochemical signaling.

III. Conclusion

Our findings strongly suggest that the low channel capacities previously observed at a single cell level does not reflect an inherent physical limit, but rather a natural trade off between information transfer at the single cell vs. population levels. This implies that the level of noise in individual signaling networks and cells can be regulated to produce reliable information at the level of individuals or populations, depending on the phenotypic requirements of the organism. Ultimately, our work provides a framework for understanding the high levels of noise observed in a wide variety of growth factor signaling networks in metazoan cells (*2, 5*).

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Use of information far from steady-state by signal transduction systems.

Alejandro Colman-Lerner

Departamento de Fisiología, Biología Molecular y Celular
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
Buenos Aires, Argentina

We present a mechanism that enables signaling systems to discriminate between different levels of extracellular signals that saturate receptors at equilibrium. This mechanism, which we called PRESS, for Pre Equilibrium Sensing and Signaling, operates when the kinetics of ligand receptor binding is slower than a transient downstream signaling step. This dynamic coupling between slow receptor binding and fast downstream transduction enables sensing systems to use information about the initial rate of receptor occupation, which is proportional to ligand concentration, even at concentrations that yield almost complete and practically indistinguishable levels of occupied receptors. We provide experimental and modeling evidence that PRESS is involved in the directional budding of yeast cells in gradients of mating pheromone. We further show by mathematical modeling that PRES is not limited to plasma membrane receptor signaling: it can also operate at other levels, such as cycles of phosphorylation and dephosphorylation, provided that a slow activation step couples to a faster downstream step.

PRESS expands and shifts the dynamic range of sensing systems, allowing cells to generate distinct responses to ligands or signaling components concentrations so high that cannot be distinguished by the levels of binding site occupation at steady-state. Published kinetic data for receptor ligand interactions in mammalian systems suggests that PRESS operates in many cell-signaling systems throughout biology.

On the Connection between Computational and Biochemical Measurement

Thomas E. Ouldridge[1], Christopher C. Govern[2], and Pieter Rein ten Wolde[2]

Short Abstract — **Cells use readout molecules to record the state of cell-surface receptors, apparently analogously to computational measurements. But at what level does this analogy hold? Do living cells operate at the thermodynamic limits of efficiency? We consider a canonical biochemical network for receptor readout and map it onto a typical idealized computational measurement protocol. The biochemical network does not achieve thermodynamic limits of efficiency, facing a tradeoff between dissipation and accuracy of measurement that is more severe than and qualitatively distinct from that required thermodynamically. Biomolecules can, however, perform optimal measurements when the concentrations of ATP and ADP are externally manipulated.**

Keywords — **Cellular sensing, computation, biochemical networks, non-equilibrium thermodynamics.**

I. Introduction

From the literature on computation developed in the 20th century, and particularly in the wake of Maxwell's demon, much is known about the thermodynamics of taking a measurement or copying a system's state into a memory device. If it were possible to perform many measurements using a single bit of memory without putting in work, Maxwell's demon would be able to violate the second law of thermodynamics. It has been argued, however, that this is impossible, and the necessity of work in the measurement cycle has been demonstrated in a range of physical models [1-3]. The Landauer bound of kTln2 sets the minimum amount of work that is required to perform a copy if it is perfectly accurate and has a 50/50 outcome [1,3].

At least superficially, many biological processes appear to perform computational copies [1]. Perhaps the most tantalising analogy is in the context of cellular sensing of external ligand concentrations. In 1977, Berg and Purcell suggested remarkable accuracy can be achieved in spite of the noisy signal from a single receptor by averaging the receptor signal over time [4]. It has since been argued that downstream signaling networks can achieve this by taking multiple measurements of the same receptor, essentially copying the receptor's state into memory [5-7]. Currently, however, the analogy between computational and biological systems is qualitative, rather than quantitative. How efficient are biological networks at performing copies, and can they reach the Landauer bound? Can the action of biological networks be understood in terms of typical idealised computational protocols?

In this work we formally describe a steady-state receptor-readout network as a process that performs copies at a certain rate and with a certain accuracy. We relate the network directly to typical idealized protocols from the computational literature, highlighting the limitations placed on the biochemical network. We find that the biochemical network does not reach the limits of thermodynamic efficiency, with a cost per copy that diverges logarithmically as the system approaches 100% accuracy. This deviation is qualitative as well as quantitative, and optimal behaviour cannot be achieved simply by reducing copying speed. The biochemical network, however, is more adaptive than standard thermodynamically optimal protocols. Biased measurement outcomes (i.e., not 50:50) have a lower minimal cost per measurement [3], but achieving this limit requires a distinct ideal protocol for each bias. By contrast, the biochemical network automatically adapts to dissipate less when the measurement outcome is biased.

Fundamentally, the biochemical network has a constant thermodynamic drive set by the free energy stored in fuel molecules such as ATP, whereas optimally efficient computational protocols involve quasistatic manipulation of biasing potentials. We conclude by demonstrating that this difference can be overcome through exogeneous manipulation of ATP and ADP concentrations. This approach enables the design of biochemical protocols that reach the optimal Landauer bound. Our proposed system suggests a novel setting for the experimental investigation of non-equilibrium and computational thermodynamics.

[1]Department of Mathematics, Imperial College, Queen's Gate, London, SW7 2AZ, UK. E-mail: t.ouldridge@imperial.ac.uk

[2]FOM Institute AMOLF, Science Park 104, 1098 XE Amsterdam, The Netherlands. E-mail: tenwolde@amolf.nl

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Single-cell dynamics reflect underlying signaling mechanisms

Rachel A. Haggerty[1], Jeanette Baran-Gale[1] and Jeremy E. Purvis[1,2,3]

Short Abstract — **Individual cells show differences in their signaling dynamics that can lead to alternate cellular fates. We exploited this phenomenon by developing a computer algorithm that searches for a common signaling mechanism that can explain the individual responses of each cell in a population. With no prior knowledge of the pathway, the algorithm successfully identified network motifs that were consistent with known pathway architectures. Predictions became better as more cells were added to the analysis, while random pairing of single-cell measurements responses led to poorer network predictions. Out results show that averaged measurements obscure mechanistic information that is naturally embedded in single-cell dynamics.**

Keywords — **cell signaling, single-cell dynamics, network inference.**

I. Background

Cells use molecular signaling networks to respond to complex and changing environmental cues [1,2]. The components of signaling networks are often organized into specialized structures, or motifs, that allow the network to carry out a specific signal-processing goal [3,4]. For example, a network may contain strong negative feedback that allows it to adapt to different levels of an input signal. Understanding the functional roles of different network motifs is a major goal of systems biology because it provides a mechanistic description of how cellular systems work.

A major advance in our understanding of cell signaling has come from the ability to visualize signaling events in single, living cells. A collective observation from single-cell studies is that individual cells show considerable heterogeneity in their dynamic responses—even when exposed to the same stimulus. This observation suggests an exciting but untested possibility: if differences between individual cells have predictable effects on downstream responses, then it is possible that all cells share a common signaling mechanism that consistently interprets each cell's unique signaling dynamics. We reasoned that it may be possible to infer this mechanism given enough examples of the signaling response among individual cells.

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[1]Bioinformatics and Computational Biology Graduate Program, [2]Department of Genetics, [3]Lineberger Comprehensive Cancer Center. University of North Carolina at Chapel Hill. E-mail: jeremy_purvis@med.unc.edu

II. Results

We first considered how a single decoding mechanism could convert a set of varying input signals, representing individual cells, to a corresponding set of output signals. We chose a simple input signal representing a step function corresponding to a 2-fold increase. We then simulated this input signal in individual cells by allows the step function to vary in its delay, amplitude, and noise. For simpler network architectures, instantaneous measurements of input signal correlated well with the output signal. However, for more complicated signaling mechanisms, slices through time showed no correlation between input and output signal.

We next asked whether a common decoding mechanism could be inferred from single-cell traces with no prior knowledge of the system. To do this, we developed an algorithm, Mechanism Inference from Single Cells (MISC), which analyzes pairs of input and output signals among individual cells to infer a common signaling mechanism that produced them. An implicit feature of MISC is the ability to exploit heterogeneous time-series data by detecting mechanistic relationships between input and output signals. When applied to single-cell data describing the yeast stress response, we identified a small subset of networks that explained, in mechanistic terms, how these upstream signals may be integrated to produce the downstream responses. Network predictions became better as more cells were added to the analysis, providing a benchmark for the number of single cells needed to describe the underlying network.

III. Conclusions

Here, we show that single-cell measurements contain information that constrains the prediction of signaling mechanisms. Our results also demonstrate that more than one network may achieve the same functional goal but that the correct network is robust to topological perturbations. Thus, single-cell dynamics contains information that reflects underlying signaling mechanisms.

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Pattern formation and morphogen gradients: A causality dilemma

Abed E. Alnaif[1,3] and Arthur D. Lander[1,2,3]

***Short Abstract* — The development of multicellular organisms depends on pattern formation – the spatial organization of cell types. This, in turn, depends on the ability of cells to acquire positional information. A longstanding paradigm for the establishment of positional information in tissues is through the concentration gradients of "morphogens". Our studies of the *Drosophila* wing suggest that patterns feed back onto the shape of the morphogen gradients that produce them. This suggests a degree of self-organization in morphogen-dependent pattern formation that departs from the common view that patterns are formed strictly "downstream" of morphogens.**

***Keywords* — pattern formation, morphogen gradients, Dpp,**

I. Background

MORPHOGENS are signaling factors that are secreted from specific regions of a developing tissue, and whose decreasing concentrations over space provide positional information to cells; i.e., a cell can use the local concentration of a morphogen in order to determine its distance from the morphogen's source.

The shape of a morphogen concentration gradient is important in determining how positional information is communicated in a tissue, as well as how that information is affected by perturbations. The concentration gradient of a morphogen that experiences no consumption, except at a boundary far from its source, will be linear. Such "source-sink" gradients have desirable adaptive properties (e.g., automatic scaling in differently sized tissues), and were originally proposed to underlie pattern formation [1]. However, it is now known that most morphogens are consumed throughout the tissues in which they diffuse, usually through receptor-mediated endocytosis (via the same cell-surface receptors used to sense morphogen concentration). A morphogen experiencing uniform consumption will exhibit a decaying exponential gradient, the steepness of which can be described by a constant decay length (which depends on the consumption rate and diffusivity of the morphogen).

II. Results

One of the best-understood morphogen gradient systems is the Decapentaplegic (Dpp; an orthologue of the vertebrate BMP2/4) gradient that patterns the wing primordium (wing disc) of the fruit fly *Drosophila melanogaster* (reviewed in [2]). Many studies have shown that the overall shape of the Dpp gradient is well-fit by a decaying exponential. Our measurements support this view broadly, but when we examine sub-regions of the gradient more carefully, we conclude that the gradient's shape is closer to linear up to the boundary of a specific pattern element, the location where the Dpp target gene *Spalt* switches from being on to off (this boundary is important in determining the placement of adult wing veins [2]). This conclusion is supported by the presence of reproducible trends in the residuals from exponential fits across many wing discs. From solutions to reaction diffusion equations with spatially varying decay lengths, we know that a necessary and sufficient condition for the observed trends in fitting-residuals is for the morphogen gradient to have a long (intrinsic) decay length proximal to the pattern boundary, and a short one distal to it (this would make this distal region act as a "sink" for the proximal region, thus causing the gradient to fall linearly in the proximal region). By "intrinsic" decay length, here, we mean the decay length that would be observed without any distal "sink".

The possibility that *Spalt* itself is responsible for the change in decay length at the *Spalt* boundary is supported by the observation that the Dpp gradient is much steeper in *Spalt*-mutant wing discs. One possible explanation for these results is that *Spalt* downregulates the expression of Dpp's cell-surface receptor, a phenomenon we confirm using clonal loss-of-function experiments.

III. Conclusions

Although many feedback loops have been identified in morphogen gradient systems, this is, to our knowledge, the first example where what has been identified as a downstream pattern element feeds back onto the shape of a morphogen gradient. This suggests that morphogen gradients do not just passively provide positional information, that their shapes may be modified by patterns in the course of pattern formation.

[1]Department of Biomedical Engineering, University of California, Irvine. Email: aalnaif@uci.edu

[2]Department of Developmental and Cell Biology, University of California, Irvine. Email: adlander@uci.edu

[3]Center for Complex Biological Systems, University of California, Irvine

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Enhanced Sampling in Spatial Stochastic Systems Biology Models Using a Weighted Ensemble of Trajectories

Rory M. Donovan[1], Jose Juan Tapia[1], Devin P. Sullivan[1], James R. Faeder[2], Robert F. Murphy[3], Markus T. Dittrich[4], and Daniel M. Zuckerman[2]

***Short Abstract* — We demonstrate that the weighted ensemble (WE) sampling strategy, initially developed for molecular simulations, can be effectively employed for spatial cell-scale simulations. Here, we use WE to orchestrate kinetic Monte Carlo simulations of the MCell platform, which include spatial geometry (e.g. organelles, membranes) and biochemical interactions among mobile molecular species. We study a series of models exhibiting spatial, temporal, and biochemical complexity and show that WE can achieve performance significantly exceeding standard parallel simulation by orders of magnitude for measuring certain observables.**

***Keywords* — Stochastic Dynamics, Monte Carlo, Signaling Networks, Enhanced Sampling, Weighted Ensemble.**

I. Introduction

STOCHASTIC effects are of crucial importance in many biological processes, from protein dynamics, to gene expression, to population-level phenotypic heterogeneity [1,2]. Unfortunately, due to the high computational cost of simulating complex stochastic systems, the effects of stochasticity on system response remain under-studied in complex, realistic biological models.

Spatial models of stochastic reaction-diffusion processes have found widespread use as tools in understanding the mechanics of biological processes on the cellular level and beyond [3]. Regrettably, exhaustively simulating large, realistic models and extracting well-sampled values of experimentally relevant quantities is often beyond the current realm of computational feasibility.

Enhanced sampling algorithms offer an attractive resolution to the dilemma of sampling complex systems: instead of compromising on model complexity in order to achieve well sampled results, rather use one's simulation resources more effectively and extract more information given the same resources. There has been significant interest in sampling algorithms in the field of protein simulation; arguably, such approaches have transformed the field of molecular simulation [4].

[1]Joint CMU-Pitt Ph.D. Program in Computational Biology
[2]Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh
[3]Department of Computational Biology, School of Computer Science, Carnegie Mellon University
[4]Pittsburgh Supercomputing Center
E-mail: donovanr@pitt.edu

II. Results

Here, we demonstrate a method to drastically decrease the cost of simulating spatial models of stochastic cellular systems, by applying the weighted ensemble sampling procedure [5]. The WE approach runs an ensemble of parallel trajectories and uses a statistical strategy of replicating and pruning trajectories to focus computational effort on difficult-to-sample regions, which it uses to generate unbiased estimates of non-equilibrium and equilibrium observables.

We present initial results for a toy diffusive binding system, as well as two more complex systems: a cross-compartmental signal transduction model in a realistic cellular geometry, and a model of an active zone in a frog neuromuscular junction. We demonstrate speedups of many orders of magnitude in sampling these models of cellular behavior with spatial dependence.

III. Conclusion

We are able to sample the rare events and full probability distributions for stochastic systems biology models over a wide range of complexity. We demonstrate speed-ups over brute-force sampling that are dramatic enough to encourage the design of more complex, more realistic models. Long time-scale behavior can be extrapolated from short simulations, providing a bridge between dynamics over multiple time-scales. Weighted ensemble is an ideal approach to employ in addressing the issue of difficult-to-sample stochastic systems, and we anticipate further applications to more realistic systems.

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Effective models of emergent behavior from the manifold boundary approximation method

Dane Bjork[1], Merrill Asp[1], Mark K. Transtrum[1]

Understanding the collective behavior (e.g, signaling or gene regulation) of complex biological systems in terms of their fundamental components (e.g., proteins, genes, reaction rates), is a fundamental problem in systems biology. While a coarse, macroscopic model can describe system level behavior it often comes at the cost of a microscopic, causal explanation for that behavior. Connecting the microscopic to the macroscopic descriptions is particularly important for predicting the outcome of new experiments that directly manipulate the microscopic components. We present a new model reduction method called the Manifold Boundary Approximation Method (MBAM) that derives simple macroscopic models of collective behavior directly from a microscopic description. The result is an effective model of the system that retains the causal explanation in terms of the microscopic components. We illustrate the method with three systems: EGFR signaling, Wnt signaling, and a fully connected network of Michaelis-Menten reactions fit to adaptation behavior. The resulting models have only a few parameters that are statistically identifiable from experimental data (i.e., not sloppy), dramatically highlight the effective control mechanisms, and remain expressed in terms of the microscopic components.

MODELS of complex biological phenomena, are often built by combining several microscopic elements together. This constructionist approach to modeling is a powerful tool, finding widespread use in many fields. Nevertheless, it is not without its pitfalls, most of which arise as models grow in scale and complexity. Overly complex models can be problematic if they are computationally expensive, numerically unstable, or difficult to fit to data [1]. These problems, however, are only manifestations of a more fundamental issue. Specifically, although reductionism implies that the system behavior ultimately derives from the same fundamental laws as its basic components, this does not imply that the collective behavior can easily be understood in terms of these laws [2]. The collective behavior of the system is typically compressed into a few key parameter combinations while most other combinations remain irrelevant [3].

The Manifold Boundary Approximation Method (MBAM) has recently been proposed as a general purpose approximation method for connecting microscopic models with macroscopic theories in complex systems [4]. It is particularly promising for understanding complex biological systems because it identifies the combinations of microscopic components, i.e., those components that are directly manipulated by experiment or evolution, that control a particular collective behavior.

The idea behind the method is that a model can be interpreted as a manifold of predictions embedded in data space. It has been observed that complex models with many parameters often exhibit a low-effective dimensionality: all but a few of the dimensions of this manifold are very thin [1]. The MBAM operates by approximating this long, narrow manifold by its boundary. Boundaries are identified using computational differential geometry. Geodesics (analogs of straight lines) are constructed numerically along the least-important parameter combination (identified by the eigenvalues of the Fisher Information Matrix). From the geodesic, a limiting approximation is identified that can be analytically evaluated in the functional form of the model.

We illustrate the method with three examples from systems biology: a model of EGFR signaling [5], Wnt signaling [6], and a fully-connected network of Michaelis-Menten reactions fit to adaptation data [7]. In each case, the MBAM identifies a simple effective model of the complex system. Through the sequence of limiting approximations, the parameters of the effective model are identified with nonlinear combinations of parameters in the original model. By connecting the macroscopic and microscopic descriptions in this way, the MBAM characterizes the equivalence class of microscopic systems that are statistically indistinguishable from their collective behavior and predicts which microscopic control knobs govern the system behavior.

[1]Department of Physics & Astronomy, Brigham Young University, Provo Utah. E-mail: mktranstrum@byu.edu

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The Universality of Cancer

Carson C Chow, Yanjun Li, Vipul Periwal

***Short Abstract* —** Cancer is a constellation of diseases differing in underlying mutations and on cellular environments. The stochastic process of carcinogenesis has been studied for sixty years, but there is no accepted model. We show that the hazard rates of all cancers are characterized by a simple dynamic stochastic process on a half-line, with a universal linear restoring force balancing a universal simple Brownian motion starting from a universal initial distribution. Only a critical radius defining the transition from normal to tumorigenic genomes distinguishes between different cancer types when time is measured in cell-cycle units. Reparametrizing to chronological time units introduces two additional parameters: the onset of cellular senescence with age and the time interval over which senescence takes place. Thus, there may exist a finite separation between normal cells and tumorigenic cells in all tissue types that may be a viable target for both early detection and preventive therapy.

***Keywords* — cancer, DNA replication, DNA damage, DNA repair, senescence**

I. Purpose

CANCER is part of life for multi-cellular organisms when individual fitness in propagation overcomes the checks and balances required for collective fitness. It is a multifaceted disease where the phenotypic similarities of tumor progression are a veneer over a multitude of possible underlying genetic alterations[1]. 75-80 % of all cancers are sporadic. As an organism ages, the accumulation of mutations increases the likelihood of alteration in an oncogene or in a tumor suppressor gene, which in turn can lead to an accumulation of mutations and other alterations allowing for unchecked proliferation. The process of carcinogenesis has been modeled for over 60 years[2]. Tomasetti and Vogelstein[3] showed that the lifetime risk of cancers of many different types is correlated with the total number of divisions of the normal self-renewing cells maintaining each tissue's homeostasis. Thus most cancer is due to random mutations arising during DNA replication in normal, noncancerous stem cells. This motivates the existence of a simple universal quantitative physical model for this stochastic process of tumorigenesis. We posited that DNA replication could be described by a continuous diffusive process on a mean mutational distance, or error, coordinate from an initial genome. Following the Ornstein-Uhlenback process[4], we hypothesized that DNA replication error correction could be modeled by the simplest possible restoring force, just Hooke's law with a universal spring constant in this coordinate.

II. Methods

Harding, Pompei and Wilson[2] have analyzed the Surveillance, Epidemiology and End Results (SEER, specifically SEER 9) cancer registries to compile age-specific incidence rates, with particular care accorded to the data on the very elderly (ages > 80 years). [2] suggested that tissue and cellular senescence are the likely biology mechanisms for the observed drop off in cancer incidence in the very elderly. These incidence rate curves provide a test for our model. We defined cancer incidence as the likelihood that the diffusing DNA has moved beyond a cancer-type independent critical threshold radius, R, in this coordinate, with the cancer incidence rate defined as the derivative of this likelihood. As the number of replicating stem cells differs from tissue to tissue[3], we explicitly set the maximum value of the incidence rate to the maximum value of the incidence rate of the data for each cancer type[2].

III. Results

We could fit the age-specific incidence rates for all cancers with our models. The best model selected by the Bayes Information Criterion has a width of the initial distribution about 0.16 for all tissues in units where the equilibrium Ornstein-Uhlenbeck distribution has width 1.

IV. Conclusions

The space of mutational histories has a natural diffusion away from the initial starting distribution. The rate of moving beyond a relatively sharp tissue-specific threshold is the incidence rate for all cancers. An interval between normal cells and tumors that could serve as a target for early detection, and a relatively sharp demarcation between tumors and normal cells, are concrete predictions of our model of tumorigenesis. Combining cancer mutation data and epidemiology to find an appropriately weighted cell-type specific mutational burden that could serve as the tissue-specific error coordinate would be of great value.

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Laboratory of Biological Modeling, National Institute of Diabetes Digestive and Kidney Disorders, NIH. E-mail: carsonc@niddk.nih.gov, yanjun.li@nih.gov, vipulp@mail.nih.gov

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The bacterial population's spatial structure non-monotonically impacts bacterial growth

Karishma S. Kaushik[1], Nalin Ratnayeke[1], Jaime Hutchison[1], Kasper Kragh[2], Gavin Melaugh[3], Parag Katira[1], Thomas Bjarnsholt[2], Rosalind Allen[3], and Vernita D. Gordon[1]

***Short Abstract* — In most natural settings, most bacteria are found in spatially-structured, pluralistic communities. The spatial structure of these communities governs interactions within and between species, and with the environment. Here, we present two cases in which cooperative and competitive inter-bacterial interactions impact growth fitness. (1) The per-cell likelihood of growth for antibiotic-resistant mutants in an antibiotic environment depends non-monotonically on the density of the surrounding, antibiotic-susceptible wild-type cells. (2) The relative growth fitness of large, multicellular aggregates, compared with single cells, depends on the density of competition, which is set by the concentration of single cells.**

***Keywords* — *Pseudomonas aeruginosa*, cooperation, competition, antibiotic, aminoglycoside, spatial structure, growth substrate, modeling, Poisson distribution.**

I. Spatial structure in bacterial populations

Most microbial communities consist of interacting, multispecies populations with inter- and intra-species interactions governed by the spatial structure of the microbial population and the environment. Recent work has shown that heterogeneity in the spatial distribution of antibiotic in the environment can accelerate the evolution of genetically-based antibiotic resistance [1,2]. Here, we examine the impact of ecological changes resulting from the spatial distribution of the microbial population on the growth of *genotypic* antibiotic resistance.

Biofilms are three-dimensional, sessile communities that promote *phenotypic* antibiotic resistance and differentiated patterns of gene expression and growth [3]. Differentiation is often linked to the positioning of cells in the biofilm structure, which helps control resource transport. Three-dimensional, multicellular aggregates can slough off to seed new biofilms, yet their role in seeding new biofilms is unknown.

Here, we examine how the spatial structure of the microbial population impacts growth fitness as the result of ecological interactions of bacteria with their environment. Our primary model organism is *Pseudomonas aeruginosa*, an opportunistic human pathogen that notoriously forms biofilm infections.

II. Growth of antibiotic-resistant mutants

In the presence of aminoglycoside antibiotic, we find that the per-cell likelihood of growth for antibiotic-resistant mutants depends non-monotonically on the overall cell density, which is primarily antibiotic-susceptible, wild-type (WT) bacteria. Two effects compete: mutants are inhibited by an alkaline, diffusible catabolic by-product, and protected when the local concentration of WT cells is sufficiently high to reduce the per-cell concentration of antibiotic+inhibitory factor below an effective threshold. We use the Poisson distribution to describe local fluctuations in cell density as a function of overall cell density and show that the resulting model describes our experimental data well [4].

III. Relative fitness of biofilm structures

When the overall cell density, and therefore the competition for growth resources, is low, we experimentally find that single cells have a growth advantage over multicellular aggregates. However, when competition is high, multicellular aggregates have a growth advantage over single cells. Agent-based modeling shows that cells in the aggregate interior have restricted access to growth substrate and therefore produce fewer progeny than do exterior cells. When competition is low, single cells have unfettered access to growth substrate and therefore have an overall growth advantage over the aggregate. However, when competition is high, the height of multicellular aggregates gives cells at the top better access to growth substrate, so that aggregates are at an overall growth advantage [5].

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[1]Center for Nonlinear Dynamics, The University of Texas at Austin, Austin TX 78731. E-mail: gordon@chaos.utexas.edu

[2]Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark. E-mail: tbjarnsholt@sund.ku.dk

[3]School of Physics and Astronomy, University of Edinburgh, Edinburgh, Scotland. E-mail: rallen2@ph.ed.ac.uk

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Entrainment of synthetic gene oscillators by a noisy stimulus

Nicholas C. Butzin[1], Philip L. Hochendoner[1], Curtis T. Ogle[1], Paul Hill[1], William Mather[1,2]

***Short Abstract* —We investigate experimentally and theoretically the entrainment of an ensemble of synthetic gene oscillators by a noisy stimulus. Stochastic simulations suggested that a synthetic gene oscillator would be strongly entrained by two aperiodic signals: telegraph noise and phase noise. This simulation-based prediction was tested by a combination of microfluidic and microscopy using a real synthetic circuit in *Escherichia coli*. We use delayed feedback models to analyze these cells. We show that cells are entrained by two noisy signals: telegraph and phase noise. Cells are entrained when either signal period or amplitudes are varied.**

***Keywords* — Entrainment, oscillators, synthetic and systems biology, aperiodic and periodic noise.**

I. Purpose

Most organisms (bacteria to humans) regulate processes using molecular clocks to synchronize their physiology and behavior to stimuli from their environment [1]. When individual components that oscillate on their own (self-sustaining) are forced to synchronize to an outside signal (matching their period and phase to the external signal) they are said to be entrained (the signal is independent of the oscillator). The rhythm of gene expression can be entrained where by the expression of the gene correlates with an environmental signal e.g. our natural circadian rhythm oscillator is entrained to a 24 hour period by the daily cycle of sunlight and darkness [1,2]. Periodic signals have been intensively studied in this regard, but most natural signals contain a strong aperiodic (noisy) component, and it has been long known that aperiodic signals can entrain systems to common a behavior, e.g. in the stimulation of independent neurons [3], or even in the random forcing of material particles [4]. Synchronized cells may produce an amplified response by activating in unison. Entrainment of neurons by aperiodic signal is well documented [3,5,6]; however, we failed to identify other biological examples in the literature with experimental support. This is likely due to the difficulty in doing such experiments and the underestimation of the importance of this phenomenon in the biological community.

A synthetic biology approach has already proven useful in understanding the entrainment of oscillators by periodic signaling [7,8], which can mimic the response of a number of noisy oscillating systems: cell cycles [9], and NFkB response [10], for example. We used stochastic simulations to predict that a synthetic gene oscillator would be strongly entrained by two aperiodic signals: telegraph noise and phase noise. We tested our *in silico* prediction with *in vivo* experiments using the model organism *Escherichia coli* and showed that cells can be entrained by such signals. Oscillator entrainment via aperiodic signaling has been well documented and simulated in physics journals [11-13], but this phenomenon has rarely been studied in living cells. It is likely that oscillator entrainment of cells via aperiodic signaling happens all the time. Many natural signals contain a strong aperiodic component, but it is difficult to study this in a natural ecosystem (biofilm, eukaryotic cells, etc.). Here we demonstrate that an aperiodic signal can drive entrain a synthetic oscillator; however, this work may have broader impact on future studies of other organisms and natural ecology's.

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[1]Department of Physics, Virginia Tech. ncb@vt.edu, plh5012@vt.edu, cogle@vt.edu, metta@vt.edu, wmather@vt.edu.

[2]Department of Biology, Virginia Tech. wmather@vt.edu.

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Seasonality gives rise to population oscillations in a bacterial cross-protection mutualism

Arolyn Conwill[1], Eugene A. Yurtsev[1], and Jeff Gore[1]

***Short Abstract* — Cooperative behavior plays a vital role in ecological communities and, when reciprocal, can result in mutualistic relationships. Here, we use an experimental microbial system to study the dynamics of a cross-protection mutualism in which two bacterial strains cooperate to survive in a multidrug environment. When subject to seasonal antibiotic dosing, the populations of the two mutualistic partners exhibit strong limit-cycle oscillations, even when there is long-term coexistence above the concentrations at which the individual strains can survive on their own. Our results provide insight into the ecological stability of mutualisms and the evolution of cooperative antibiotic resistance.**

***Keywords* — mutualism, seasonality, antibiotic resistance, oscillations, evolutionary dynamics, model system**

I. Purpose

A mutualism arises between two species when each increases the fitness of its partner. Since mutualistic interactions between species are thought to be fundamental to the establishment and maintenance of ecological communities [1], much research has focused on studying mutualisms in their natural settings. Such studies suggest that the nature of the interaction between mutualistic partners is context dependent: while two species can be mutualistic in a harsh environment, they can nonetheless become direct competitors in a more benign environment [2]. Laboratory experiments with model systems (e.g., [3]), which are more readily controllable than their natural counterparts, can provide insight into the population and evolutionary dynamics that govern mutualistic relationships.

II. Methods

Our model system consists of a bacterial mutualism that arises from cross-protection against antibiotics. Each strain produces an enzyme that deactivates one of the two antibiotics—ampicillin or chloramphenicol—thereby protecting cells from the second strain that would otherwise be sensitive to the drug [4]. Serial dilution experiments result in a seasonal environment: we dilute the bacterial population periodically by a fixed amount into new medium supplemented with the two antibiotics. The dilution strength, the time between dilutions, and the concentrations of the two antibiotics determine the strength of this periodic change in the environment. We experimentally track the population dynamics of the mutualism at the end of each growth cycle by measuring the density of each subpopulation using a combination of spectrophotometry and flow cytometry.

III. Results

As the concentrations of the antibiotics in the environment increase, the two strains become increasingly dependent on each other for survival, with their interaction changing from primarily competitive to primarily mutualistic. Together the two strains can grow in antibiotic concentrations that inhibit growth of either one of the strains alone, thus forming an obligatory mutualism and enabling long-term coexistence.

In a seasonal environment (i.e. increased dilution strength, longer time between dilutions, and higher antibiotic concentrations), we observe strong limit-cycle oscillations between the subpopulations of the two mutualistic partners. Even so, the size of the total population exhibits little variation. In comparison, in the absence of seasonality (i.e. in the limit of a continuous environment), the population dynamics settle to an apparent equilibrium.

This obligatory mutualism persists until even stronger seasons give rise to oscillations so large that the population ultimately collapses. In particular, at one extreme of the oscillation, the population of one partner in the mutualism becomes so small that it can no longer protect its partner from the antibiotic it deactivates. Interestingly, often times the mutualism can successfully survive one dilution cycle before collapse, suggesting that the oscillatory dynamics cause the subpopulations to become too imbalanced to survive indefinitely in extreme environmental conditions.

We also investigate the evolutionary stability of the mutualism in the presence of potential invaders that employ different strategies, such as strains that are either sensitive or resistant to both antibiotics present in the environment. The outcomes provide insight about the evolutionary origins and resilience of such mutualisms.

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[1]Department of Physics, Massachusetts Institute of Technology, Cambridge, MA. E-mail: gore@mit.edu

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How to hit HIV where it hurts

Arup K. Chakraborty

MIT

HIV continues to wreak havoc around the world, especially in developing countries. It is a highly mutable virus which can evade natural or vaccine induced immune responses by mutating at multiple sites linked by compensatory interactions. If one wishes to define the mutational vulnerabilities of HIV, these collective compensatory pathways need to be identified so as to not target the involved sites by a vaccine induced immune response. Moreover, the combinations of mutations that the virus cannot make and still maintain viability need to be determined, so as to target the pertinent sites by vaccination. Thus, knowledge of the fitness landscape of HIV could enable rational design of vaccines that can confront this scourge. We developed models to translate data on HIV protein sequences to knowledge of the prevalence landscape of the circulating HIV population. Theoretical analyses and biological reasoning led us to surmise that, unlike many other viruses, the relationship between the prevalence and fitness landscapes of HIV may be simple. I will show that this surmise is supported by positive correlations between predictions emerging from our inferred fitness landscape and in vitro experiments and clinical data obtained from patients. Based on these results, a therapeutic T cell-based vaccine was designed, which is now being advanced to pre-clinical studies in monkeys. I will also describe how scaling laws describe the HIV population and discuss an interesting analogy with Hopfield neural networks.

Cell fate decisions in response to a short pulse of TNF

Robin E. C. Lee[1,2,5,*], Mohammad A. Qasaimeh[1,2,3,6,*], Xianfang Xia[1,2], David Junker[3,4] and Suzanne Gaudet[1,2]

***Short Abstract* — Tumor necrosis factor (TNF) cytokines regulate survival and death signaling pathways in the same cell. Although exposure to TNF can be of short duration *in vivo*, in experiments cells are often exposed to TNF continuously. To understand how survival and death signals respond to transient TNF exposure, we monitored live cells expressing fluorescent reporter proteins in a microfluidic flow device. We find that a TNF pulse of the order of seconds can provoke both pathways. Strikingly, a short pulse can be more effective at killing than a longer pulse, suggesting that TNF concentration and duration together coordinate cell fate decisions.**

***Keywords* — NF-kB transcription factor, apoptosis, caspase protease, microfluidics, laminar flow**

I. Introduction

TNF is a pro-inflammatory cytokine that modulates cellular behaviors including proliferation, differentiation and apoptotic cell death. While TNF is important for the normal development and function of immune cells, chronically elevated TNF is associated with autoimmune diseases and linked with tumor progression in some cancers.

TNF regulates many cellular behaviors by sequentially activating intracellular signals [1]. Binding of TNF to its receptor at the plasma membrane rapidly induces nuclear accumulation of the NF-κB transcription factor, driving transcription of anti-apoptotic genes to promote cell survival. This is followed by internalization of TNF-bound receptors, a process that initiates signals for caspase protease-dependent apoptotic death in the same cell.

To regulate diverse cell fates in a healthy tissue, TNF exposure is strictly controlled and likely to be transient. However, little is known about the duration of TNF exposure required to activate NF-κB–driven transcription or induce apoptosis. Here, we set out to determine the minimum TNF pulse duration required for activation of NF-κB, and to study TNF-induced apoptosis, comparing cell fates in continuous versus transient TNF exposures.

[1]Department of Cancer Biology and CCSB, DFCI, USA
[2]Department of Genetics, Harvard Medical School, USA
[3]Biomedical Engineering Department, McGill University, Canada
[4]McGill University and Genome Québec Innovation Centre, Canada
[5]Department of Computational and Systems Biology, University of Pittsburgh School of Medicine, USA
[6]Division of Engineering, New York University Abu Dhabi, UAE
*Equal contribution; E-mail: robinlee@pitt.edu

II. Results

We designed and built a microfluidic system that uses laminar fluid streams to provide spatiotemporal control over TNF delivery to cell cultures. The device was seeded with HeLa cells stably expressing EGFP-fused NF-κB, and its nuclear fluorescence was quantified from time-lapse microscopy. Using our recently collected dataset of same-cell NF-κB localization dynamics and target transcript numbers [2], we established the threshold of nuclear NF-κB accumulation required to induce gene transcription in single cells. For a high TNF concentration, a short pulse of 10s elicits significant NF-κB translocation in a fraction of cells, although a 30s pulse or longer is required to approximate continuous exposure. We also find that the minimal TNF pulse is dose dependent, with lower concentrations requiring a longer pulse for comparable NF-κB activation.

To monitor caspase activity in single cells exposed to a pulse of TNF, we imaged HeLa cells stably expressing a FRET-based initiator caspase reporter (IC-RP; [3]) by time-lapse microscopy and quantified IC-RP cleavage in single cells. Consistently we observed a non-monotonic relationship between TNF pulse duration and both the timing and extent of caspase-dependent cell death. There is a relative maximum of cell killing in response to a 1-minute pulse when compared to a pulse of shorter or longer duration.

III. Conclusion

Our data show that a short pulse of TNF is sufficient to induce substantial activation of pro-survival and pro-death signaling, and that the pulse duration in turn affects efficacy and timing of cell death. These data also suggest that the condition for highest fractional kill may not require sustained exposure to the pro-death stimulus. Overall, our study complements a growing body of work showing that signaling dynamics as well as the timing and sequence of drug addition together influence cell fate decisions.

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Ras nanoclusters: lipid-based assemblies for signal processing

John F Hancock

University of Texas Health Science Center at Houston, Medical School, Texas

My lab studies the plasma membrane nanoscale organization of Ras proteins using quantitative electron microscopy and FLIM-FRET microscopy. We have shown that the ubiquitously expressed Ras isoforms, H-Ras, K-Ras and N-Ras operate in spatially non-overlapping, transient nanoclusters. Approximately 40% of each Ras protein assembles into nanoclusters of ~6 proteins, with radii of ~9nm and lifetimes of <1s. Furthermore H-, K- and N-Ras all undergo GTP-regulated segregation, such that GTP- and GDP-nanoclusters of each isoform are also spatially segregated. Since Ras effector activation is restricted to Ras-GTP nanoclusters interesting emergent properties flow from the imposition of nanocluster spatiotemporal dynamics on Ras signal transmission. On one level the system operates as an analog-digital-analog converter to deliver high fidelity signal transmission in the Raf-MAPK circuit with signal gain being controlled by the Ras nanocluster fraction. Lipid mapping experiments also show that different Ras nanoclusters have distinct compositions revealing isoform-selective lipid sorting. The anionic phospholipid phosphatidylserine (PS) is an obligate structural component of K-Ras nanoclusters. Our most recent experiments now reveal that PS spatial organization, and thereby K-Ras nanoclustering, are sensitive to transmembrane potential, revealing a previously unsuspected mechanism whereby electrical signals can control the gain in K-Ras signaling circuits.

Queueing Entrainment – Downstream control of a synthetic oscillator

Philip Hochendoner[1], Nicholas C. Butzin[1], Curtis T. Ogle[1], and William Mather[1,2]

***Short Abstract* — Using microfluidic experiments, stochastic simulations, and analytical theory, we investigate how a synthetic oscillator in *E. coli* can be entrained via modulation of its protein degradation pathway. The interaction occurs primarily through "queueing" of components for degradation, where proteins compete for the oscillator's primary protease, ClpXP, which effectively acts as a queueing server with a finite bandwidth. We find that periodically varying the production rate of an otherwise independent protein targeted to ClpXP can lead to entrainment, which we understand analytically using a degrade-and-fire formalism.**

Keywords — **Entrainment, queueing theory, oscillators, synthetic biology, systems biology**

I. Introduction

Biological oscillators permeate our daily life, ranging from circadian rhythms, to cell cycles, to our very heartbeats. Control over these systems is often done through entrainment [1], but detangling the mechanism of entrainment tends to be difficult in natural oscillators due to their complex web of interactions.

A complementary strategy to understanding biological entrainment is the synthetic biology approach, where genetically encoded circuits are constructed using known parts with (mostly) known interactions. Previously, investigators successfully leveraged a synthetic oscillator in *E. coli* as a model for transcriptional regulation-based entrainment [2]. In the following, we seek to extend this investigation to explore a particular form of post-translational entrainment, where competition of components for proteolytic machinery leads to the coupling of environment to oscillator. This entrainment mechanism may arise in a number of natural oscillators, since many natural oscillators include analogous proteolytic pathways as one of their essential components.

II. A Synthetic Oscillator and ClpXP Queueing

Our model synthetic oscillator in *E. coli* functions based on two primary ingredients: delayed negative feedback and enzymatic degradation [3]. Focusing on the latter, the oscillator depends on the cell's natural degradation pathways to remove proteins from the system quickly. This degradation is due to the processive protease ClpXP targeting genetically encoded tags on oscillator proteins.

Recent work has revealed that the finite bandwidth of ClpXP naturally leads to a queueing interpretation of protein degradation [4,5], whereby the protease acts as a server for proteins. A consequence is that the protease exhibits classical queueing regimes, such as underloaded and overloaded regimes where competition for the protease is low and high, respectively [6]. These regimes can be experimentally realized using synthetic means [4].

III. Queueing Entrainment

We utilize queueing to couple two sets of tagged proteins: the oscillator proteins and a protein controlled by an externally controlled inducer. Competition for the protease is the primary source of the interaction between the two sets of proteins. This coupling allows us to entrain the oscillator with a wide array of external signals with variable strengths and periods. Entrainment is demonstrated experimentally using a microfluidic platform, which allows for tightly controlled and highly repeatable experiments. The theoretical basis for entrainment stems from the ability for queueing coupling to either dilate or contract the oscillatory period, depending on oscillatory phase. This conclusion is supported by both stochastic simulations and analytic arguments.

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[1]Department of Physics, Virginia Tech. plh5012@vt.edu, ncb@vt.edu, cogle@vt.edu, wmather@vt.edu.

[2]Department of Biological Sciences, Virginia Tech. wmather@vt.edu.

Redox rhythms reinforce the plant circadian clock: New insights into coupled biological oscillators

Sargis Karapetyan[1], Mian Zhou[2, 3], Wei Wang[2, 3], Musoki Mwimba[2, 3], Jorge Marques[2, 3], Xinnian Dong[2,3] and Nicolas E. Buchler[1, 2]

***Short Abstract* — Multiple oscillators often co-exist within the same cell. Are there mechanisms and regulatory principles to ensure functional harmony between these oscillators? Here, we show that salicylic acid, a plant immune signaling hormone, uniquely perturbs the cellular circadian redox rhythm to reinforce the expression of core circadian clock genes through the master immune regulator NPR1. Mathematical modeling and subsequent experiments demonstrated that NPR1 targets both morning and evening genes of the circadian clock. This balanced network architecture ensures the maintenance of period and reinforcement of the circadian clock amplitude by simultaneous regulation of differently phased components.**

***Keywords* — Circadian Clock, Redox Rhythm, Immunity, Arabidopsis.**

I. Introduction

RECENT studies have shown that many organisms, including *Arabidopsis,* have a circadian redox rhythm driven by the organism's metabolic activities [1,2]. It has been hypothesized that the redox rhythm in plants is linked to the circadian clock, but the mechanism of this link remains largely unknown. Our experimental work shows that the master immune regulator NPR1 of *Arabidopsis* is a sensor of the plant's redox state and regulates transcription of core circadian clock gene *TOC1* through TGA transcription factors. Strikingly, acute perturbation in the redox status triggered by the immune hormone salicylic acid (SA) leads to the reinforcement rather than perturbation of oscillations in *TOC1* expression in an NPR1-dependent manner. Mutation of NPR1 resulted in lower *TOC1* expression with the same period of oscillations. Because the levels of TOC1 are known to regulate the period of the circadian clock [3], our results suggest that NPR1 couples to other clock genes, in addition to the evening-phased *TOC1*.

II. Quantitative Model of Plant Circadian Clock

To systematically search for other possible clock components that are regulated by redox rhythms, we explored the effect of adding NPR1 regulation to a mathematical model of the plant circadian clock that includes most of the known components of the *Arabidopsis* circadian clock [4].

[1]Department of Physics, Duke University, Durham, NC, USA
[2]Department of Biology, Duke University, Durham, NC, USA
[3]Howard Hughes Medical Institute-Gordon and Betty Moore Foundation

A. Fitting procedure

Based on our data, we made the assumption that NPR1 is a transcriptional activator of other clock genes. We systematically coupled NPR1 to *TOC1* and two other circadian clock genes, X and Y. For each X, Y pair, we used nonlinear least squares fitting to find NPR1 parameters that best fit our *TOC1p:LUC* time-series. We repeated this procedure for all X, Y pairwise combinations of the circadian clock genes.

B. Results

Our modeling showed that NPR1 must also activate the expression of the morning-phased *PRR7* and *LHY* genes. We experimentally confirmed these new regulatory links using qPCR of *LHY* and *PRR7* transcripts in *npr1* mutants and under SA-induction. We show how a balanced network architecture converts a redox perturbation into reinforcement of the circadian clock with no change in period.

III. Biological Significance

We further showed that TOC1 is a repressor of plant immunity. Because morning phased LHY positively regulates plant immunity [5], we hypothesized that the reinforced circadian clock helps gate plant immunity to be more responsive to induction in the morning and less responsive at night to avoid diverting scarce resources from plant growth at night. Our microarray analysis revealed that, indeed, plants have a greater immune response in the morning upon SA induction, and suffer a larger penalty on growth at night upon SA induction. Last, we showed that the expression of catalase *CAT3* (*CATALASE3*) is also upregulated by SA. This may help the circadian clock restore a circadian redox rhythm after pathogen challenge and SA induction.

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How Adhesion Regulates Cell Migration Plasticity: A Computational Study

X He[1], BH Sung[2], K Riching[2], P Keeley[3], K Eliceri[4], A Weaver[2], Y Jiang[1]

Short Abstract — Cell migration is important for development, wound healing and cancer invasion. It is a complex process that involves multi-scale interactions between cells and the extracellular matrix (ECM). Empirical evidence of cell migration showed that the cell substrate interaction through focal adhesion is a key mechanism to regulate cell migration plasticity. How the cell integrates the biomechanical properties of microenvironment with cytoskeleton remodeling to initiate polarity, adhesion and regulate migration modes is still not clear. Increasing experimental evidence suggests that migration behaviors differ and transit over physical parameters, including substrate rigidity, topography, and cell property. We built a 3-D cell model with cell motility signaling pathway and explicit cell membrane, cytoskeleton, nucleus. We simulated cell migration in 1-D and 2-D substrates with varied distribution and intensity. The model provides a flexible platform for investigating cell migration plasticity with complex microenvironments through biomechanical cell-substrate interactions.

***Keywords* — 3D cell migration, amoeboid, mesenchymal, cancer invasion, signal pathway.**

I. Introduction

Cell migration is a fundamental process that regulates numerous physiological functions of biological system [1]. Migrating cells exhibit distinct motility modes and can switch between mesenchymal and various amoeboid motilities [2]. The formation of integrin-mediated adhesion breaks the cell symmetry, followed by signal transduction and generations of interruptions of lamellipodial extension [3]. Contraction is the main part of the motility process during which the cells explore micro-environment, together with activated cell motility signal pathway, regulate actin and actomyosin spatial intensity and membrane deformation including lamellipodia, filopodia, stable and unstable blebs [2]. To characterize how the adhesion site dimensionality and adhesion intensity regulates cell migration plasticity, we developed a computational model of 3D cell migration in micro-patterned substrate, which reproduces the experimental measurements and provides new insights into the cell migration plasticity.

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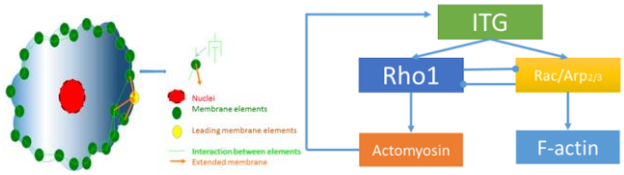
[1] Dept of Mathematics and Statistics, Georgia State University. E-mail: xhe9@student.gsu.edu

[2] Dept of Cancer Biology, Vanderbilt University Medical Center.

[3] Dept of Pharmacology and [4] Biomedical Engineering, University of Wisconsin-Madison.

II. Results

We model cell migration using a subcellular element model [4], which represent a cell as interacting membrane elements together with a deformable cell nucleus through cytoskeletal dynamics. The intracellular reaction-diffusion dynamics of F-actin and actomyosin determine the protrusion *vs*. contractile forces on each membrane element. The cell deforms and moves as a result of force calculation of every membrane element.



The key features of the model are 1) the substrate and integrin distribution regulate focal adhesion formation and adhesion strength, 2) the cell morphological adaption and integrin transportation are solved using 3-D moving boundary diffusion-reaction method, 3) the duration for the cell and substrate binding depends on the number of focal adhesion [5].

From *In vitro* experiments of single cell migration in 3D collagen, we quantify the cell protrusion number and velocity. With our 3-D cell migration model, we simulate the detailed morphological evolution and molecule diffusion-reaction and investigate the cell migration plasticity as a function of binding set width and adhesion intensity. In particular, we focus on studying the resulting cell deformation and migration directionality, cell diffusion coefficient. The results resemble those observed in 3D cell traction experiments as well as 3D cell migration assays.

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Transcription factor network supports phenotypic heterogeneity cancer

David J. Wooten[1], Akshata Udyavar[2], Vito Quaranta[1]

***Short Abstract* — Cancer is recognized as a phenotypically heterogeneous disease, however the underlying causes of this heterogeneity are not well understood. Genomic instability in cancer has generally cited as the mechanism by which tumors acquire a phenotypically diverse profile, however recent results suggest the existence of epigenetically mediated heterogeneity as well. We hypothesized that epigenetic heterogeneity in cancer is supported by transcription factor regulatory networks and adopt the view that stable cell phenotypes can be described as attractors of this system. We test this hypothesis by building a Boolean network model of transcriptional regulation in small cell lung cancer, and conclude that experimentally observed heterogeneity is captured by the model.**

***Keywords* — Cancer heterogeneity, epigenetic landscape, attractors, transcription factor dynamics, small cell lung cancer**

I. Introduction

UNDERSTANDING the origins and roles of inter- and intra-tumor heterogeneity remains a significant challenge facing cancer researchers. Molecular and genetic subtyping has introduced the promise of personalized therapies, however success has been limited in practice by a lack of well classified subtypes and the emergence of treatment resistant tumors. Both genetic and epigenetic intra-tumor heterogeneity has been implicated in the emergence of resistance in multiple tumor types [1,2], and overcoming this will be critical to the future development of more effective therapies.

A popular framework in theoretical systems biology suggests that a cell's phenotype may be understood as an attractor of the dynamical gene regulatory process [3-5]. This view reflects Waddington's epigenetic landscape in which undifferentiated cells roll "downhill" as they adopt distinct and differentiated identities. In this work, we test the hypothesis that heterogeneous cell states in small cell lung cancer (SCLC) can be explained as attractors of a transcription factor (TF) regulatory network.

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[1]Department of Cancer Biology, Vanderbilt University, 2201 West End Ave, Nashville, TN. E-mail: david.j.wooten@vanderbilt.edu

[2]Department of Chemical and Physical Biology, Vanderbitl University, 2201 West End Ave, Nashville, TN.

II. Methods and Results

Using 53 SCLC cell lines from the Cancer Cell Line Encyclopedia we applied weighted gene co-expression network analysis (WGCNA) [6] to identify clusters of co-regulated genes. Two clusters were found whose expression patterns were significantly anti-correlated, and determined to be enriched for neuroendocrine/epithelial (NE) and mesenchymal (ML) phenotypes, respectively.

To derive a set of TFs which regulate the expression of these genes we cross-referenced results from ARACNE [7] with TF-DNA binding databases [8]. The resulting TF network was simulated as a Boolean network to identify stable attractors. These theoretically predicted attractors were found to correlate significantly with the observed expression profiles for both the NE and ML phenotypes. Western blots verify the differential expression of key transcription factors, and single cell flow cytometry reveals the heterogeneous presence of both cell types across multiple cell lines.

III. Conclusion

We have derived a transcription factor regulatory network which is capable of capturing observed phenotypic heterogeneity in SCLC. This work provides a foundation for future studies exploring the controllability of heterogeneity in cancer.

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Simulating Large-Scale Chromatin Fibers

Tamar Schlick

Department of Chemistry and Courant Institute of Mathematical Science, New York University

Understanding chromosome tertiary organization and its role in control of gene expression represents one of the most fundamental open biological challenges. Chromatin structure and gene expression are intimately related because the complex nature and dynamics of protein-bound DNA folding in the living cell regulates gene activity at a large range of spatial and temporal scales. Recent advances in experimental studies of chromatin using nucleosome structure determination, ultra-structural techniques, single-force extension studies, and analysis of chromosomal interactions have revealed important chromatin characteristics under various internal and external conditions. Modeling studies, anchored to high-resolution nucleosome models, have explored many related questions systematically. In this talk, I will describe recent findings regarding chromatin structure and function using a combination of coarse-grained modeling and large-scale all-atom molecular dynamics simulations of chromatin fibers. In particular, I will describe how such multiscale modeling can successfully address questions regarding the effects of epigenetic chemical modifications and the structure of condensed chromosomes in the metaphase cell cycle.

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Microbial growth dynamics govern conjugation efficiency in the presence of antibiotics

Allison J. Lopatkin[1], Shuqiang Huang[1], Robert P. Smith[3], Jaydeep K. Srimani[1], & Lingchong You[1, 2,+]

Short Abstract — **It is generally assumed that antibiotics can promote horizontal gene transfer (HGT). Due to a variety of confounding factors that complicate the interpretation of previous studies, however, how antibiotics modulate HGT remains poorly understood. In particular, it is unclear whether antibiotics directly regulate the efficiency of HGT, serve as a selection force to modulate the population dynamics after HGT has occurred, or both. Here we address this question by quantifying conjugation dynamics in the presence and absence of antibiotic-mediated selection. Surprisingly, we find that antibiotics from all major classes, when dosed at sub-lethal concentrations, do not significantly affect the conjugation efficiency. Instead, our modeling and experimental results demonstrate that conjugation dynamics are dictated by antibiotic-mediated selection. In contrast to conventional wisdom, we further show that antibiotics do not always promote conjugation but instead cause diverse dynamics, depending on how the growth rates of parental strains are influenced by the antibiotics. Our findings may explain the apparently paradoxical observation that HGT appears to be happening to a lesser extent than expected.**

Keywords — **Horizontal gene transfer, antibiotic resistance, synthetic biology**

I. Introduction

HORIZONTAL gene transfer (HGT) has given rise to the rapid, widespread dissemination of antibiotic resistance genes within and among bacterial species [1,2]. It has been speculated that antibiotics can promote HGT based on the observed increase in resistance following antibiotic treatment [3-5]. However, these theories are confounded by the inability to distinguish antibiotic selection dynamics from antibiotic-induced resistance exchange [6,7]. Thus, whether, and to what extent antibiotics specifically modulate conjugation efficiency is still unknown.

[1]Department of Biomedical Engineering, Duke University, Durham, North Carolina, USA. E-mail: ajl49@duke.edu
[2]Center for Genomic and Computational Biology, Duke University, Durham, North Carolina, USA.
[3]Department of Biology, Halmos College of Natural Sciences and Oceanography, Nova Southeastern University, Fort Lauderdale FL, USA.

II. Results

To determine whether antibiotics modulate conjugation efficiency, we first decoupled bacterial growth from HGT to quantify the rate of conjugation for increasing concentrations of 10 antibiotics. We show there is no statistically significant effect on the conjugation efficiency up to the highest concentration tested for each antibiotic. We then built a simple mathematical model describing HGT to investigate how antibiotic selection influences population dynamics of conjugants with a fixed rate of conjugation. We identified four main growth scenarios that demonstrate signature dynamics of the relative amount of transconjugants present. In particular, the relative growth rates of the donor and recipient are primarily responsible for the observed increase in transconjugants, and depending on the selection environment, can generate a diverse range of conjugation dynamics. Surprisingly, our results demonstrate that higher concentrations of antibiotics do not always promote conjugation.

We tested these predictions using *E. coli* engineered to undergo HGT in a microfluidic device. We show that experimental results validate modeling predictions, suggesting that antibiotic selection dynamics alone are an accurate predictor of conjugation dynamics.

III. Conclusion

The results from this study demonstrate that antibiotic influence on conjugation efficiency is negligible, but instead selection dynamics are the dominant contributor to observed population dynamics following conjugation. Counter-intuitively, higher concentrations of antibiotics do not necessarily promote conjugation. These findings help elucidate the apparently paradoxical observation that HGT appears to be happening to a lesser extent than expected.

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MicroRNA and protein cell fate determinants synergize in asymmetric division as safeguard against stem cell proliferation

Pengcheng Bu[1], Kai-Yuan Chen[1], Preetish Murthy[2], and Xiling Shen[1]

***Short Abstract* — The microRNA miR-34a regulates the decision of colon cancer stem cells to undergo either symmetric or asymmetric division. However, how does miR-34a manage to achieve this seemingly complex task reliably? Here we report that miR-34a employs several mechanisms for robust spatiotemporal regulation. First, miR-34a forms an incoherent feedforward loop with a canonical cell fate determinant to enhance bimodality and adaptivity. Second, this spatial microRNA switch is enforced by an epigenetic mechanism. Third, miR-34a selectively form bimodal switches with cell fate decision genes. Collectively, microRNA-mediated cell fate decisions involve multiple layers of regulatory strategies in a context-dependent manner for decision-making.**

***Keywords* — microRNA, cancer stem cell, asymmetric division, feedforward loop, robustness.**

I. Purpose

Many stem cells can perform asymmetric division to accomplish self-renewal and differentiation simultaneously [1,2]. There have also been reports that cancer stem cells of various cancer types undergo both symmetric and asymmetric division [3-5]. Altering the ratio between symmetric and asymmetric division can change the balance between self-renewal and differentiation, which impact tumor growth.

Asymmetric cell division usually relies on imbalance of cell fate determinant proteins in the two cellular compartments to break symmetry, resulting in daughter cells with distinct cell fates. Recently, emerging evidence suggests that asymmetric distribution of microRNAs can also give rise to asymmetric cell fates [6,7]. For example, we have reported that miR-34a directly targets Notch to form a cell fate determination switch in colon cancer stem cells (CCSCs) [6]. These CCSCs then form xenograft tumors with the heterogeneous histopathology observed in human cancer [8].

However, this raises the question as to whether microRNA and protein cell fate determinants act independently or coordinate with each other to determine cell fate. The relationship between miR-34a and Numb is intriguing, because both target Notch in CCSCs.

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[1]School of Electrical and Computer Engineering, Department of Biomedical Engineering, Cornell University, E-mail: xs66@cornell.edu

[2]School of Mechanical and Aerospace Engineering. Cornel University.

II. Results

Here we show that miR-34a directly suppresses the canonical cell fate determinant protein Numb in early-stage colon cancer stem cell (CCSC), although both target Notch to promote differentiation. Computational modeling and quantitative analysis revealed that this incoherent feedforward loop (IFFL) synergizes the two cell fate determinants to produce a sharper and more robust switch. This switch enforces strict bifurcation of cell fates and generates a well-separated bimodal distribution in the population. Perturbation to the IFFL leads to a new population of cells with more plastic and ambiguous identity between stem and differentiated cells. The IFFL is also active in normal intestinal stem cells (ISCs). Knockout of miR-34 in ISCs does not generate any phenotype in mice, but causes excessive proliferation of ISCs in organoids to form CCSC-like spheres upon TNF-a treatment.

III. Conclusion

Collectively, our data indicate that microRNA and protein cell fate determinants form regulatory motif to enhance robustness of cell fate decision, and they provide a safeguard mechanism against stem cell proliferation under stress conditions. This mechanism is still active in early-stage tumors but eventually subverted by progression of cancer.

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How to control the size of a fission yeast cell

Martin Howard

John Innes Centre
Norwich, UK

In this talk I will discuss how fission yeast cells control their size[1]. It has previously been proposed that fission yeast implements a "sizer" mechanism, where cells actively monitor their size and divide upon reaching a critical size. However, which measure of size is monitored and how has been unknown. Here, we propose a theory that explains how size control is implemented via an effective measurement of the plasma membrane surface area through the cortical dynamics of the protein Cdr2. Predictions from this theory are then successfully tested in the lab. I will also briefly point out that a similar control mechanism may be implemented in a completely different problem, namely equal spacing of low copy number plasmids in bacteria[2].

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Novel positive feedback loop sets antigen dose-dependent threshold for T cell differentiation

Robert P. Sheehan[1], William F. Hawse[2], Robin E. C. Lee,[1] Penelope A. Morel[2], and James R. Faeder[1]

Antigen stimulation of the T cell receptor (TCR) in a naïve T cell triggers a complex network of signaling pathways that determine whether the cell becomes a tolerogenic T regulatory (Treg) or immunogenic T helper (T_H) cell. We have recently discovered a potential positive feedback loop involving Akt-mediated transcriptional downregulation of PTEN, a lipid phosphatase that opposes activation of Akt. To explore the effects of this feedback we developed a model of signaling downstream of the TCR, with a focus on the regulation of PTEN and Akt. This model recapitulates dose-dependent dynamics of PTEN and Akt activity and predicts a dose- and time-dependent threshold for TCR stimulation to drive the sustained Akt activity required for the differentiation and proliferation of T_H cells. The model also shows that sub-threshold signals lead to transient Akt activation, potentially leading to a Treg phenotype.

Keywords **— T cell differentiation, rule-based modeling, bistability, parameter estimation, PTEN**

I. Background

THE proper differentiation of naïve CD4+ T cells into Treg and T_H populations is critical to immune function. Maintenance of T_H populations is needed to fight infection, while Treg populations are necessary to prevent autoimmune disorders. It has been shown that Treg induction can prevent the onset of type 1 diabetes in mice [1].

TCR signaling is an important regulator of differentiation outcome. Akt activation downstream of the TCR has been shown to correlate with T_H development [2,3]. One key regulator of Akt is the phosphatase PTEN, which inhibits Akt activity through upstream dephosphorylation of PIP3 [4], a phospholipid that recruits numerous signaling proteins to the plasma membrane. Regulation of PTEN involves both post-translational modifications [5] and a recently-identified transcriptional control circuit involving the transcription factor Foxo1, which is inactivated through phosphorylation by Akt, forming a positive feedback loop for Akt activation [6]. Mathematical modeling allows us to predict TCR-dependent regulation of PTEN activity and its effects on CD4+ T cell differentiation.

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[1]Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA, 15260. E-mail: rps32@pitt.edu, robinlee@pitt.edu, faeder@pitt.edu

[2]Department of Immunology, University of Pittsburgh, Pittsburgh, PA, 15260. E-mail: whawse@pitt.edu, morel@pitt.edu

II. Results

A. A detailed model of Akt activation dynamics recapitulates experiments results and reveals bistability

We have developed a rule-based model of Akt activation downstream of the TCR and calibrated it using Bayesian parameter estimation [7] augmented by parallel tempering [8]. The resulting parameterization reveals bistability in the system, with high-dose antigen stimulation leading to a sustained drop in PTEN levels, and a corresponding increase in Akt activity, which lead to T_H cell differentiation. We predict a bistable switch in the system, resulting in two stable states with high and low levels of PTEN respectively. The threshold of the switch is controlled by the strength and duration of TCR activation.

B. A second signal activating PI3K is necessary for full commitment and sustained PTEN suppression

Activation of PI3K through CD28 is required for commitment to the T_H phenotype following antigen removal. Commitment requires maintenance of high levels of Akt activity and suppression of PTEN. Varying levels of CD28 can change the level of antigen stimulation required to cross the threshold for T_H differentiation.

III. Conclusion

Using a combination of mathematical modeling and experiments in primary cells, we have identified PTEN opposition of Akt as a critical circuit in the differentiation of naïve CD4+ T Cells. TCR stimulation-dependent regulation of PTEN leads to a bistable switch influencing the differentiation commitment of these cells. We have also made predictions on the duration of antigen stimulation needed to induce commitment to an Akt-high state, which we are currently in the process of testing experimentally.

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Robust network structure of the Sln1-Ypd1-Ssk1 three-component phosphorelay prevents unintended activation of the HOG MAPK pathway in *Saccharomyces cerevisiae*

Joseph P. Dexter[1], Ping Xu[2], Jeremy Gunawardena[3] and Megan N. McClean[4]

***Short Abstract* —Under normal growth conditions a three-component (Sln1-Ypd1-Ssk1) phosphorelay represses high-osmolarity glycerol (HOG) pathway activity in the yeast *Saccharomyces cerevisiae*. This inhibition is essential for cellular fitness in normal osmolarity. We established that the phosphorelay is robust and maintains inhibition of the HOG pathway even after significant changes in the levels of its three components. A biochemically realistic mathematical model of the phosphorelay suggested that robustness is due to buffering by a large excess pool of Ypd1. We confirmed this result experimentally. Buffering by an intermediate component in excess represents a novel mechanism through which a phosphorelay can achieve robustness.**

***Keywords* — robustness, HOG pathway, osmotic stress, histidine kinase, mathematical modeling, invariants.**

I. Overview

DESPITE its importance during periods of increased osmolarity, unintended activation of the high-osmolarity glycerol (HOG) pathway during growth in normal osmolarity conditions is severely deleterious to the budding yeast *Saccharomyces cerevisiae* [1]. The Sln1-Ypd1-Ssk1 three-component phosphorelay is responsible for maintaining inactivation of the HOG pathway. Under normal osmolarity the phosphorelay is active and maintains Ssk1 in its phosphorylated form. In response to osmotic shock, the phosphorelay is inactivated, and Ssk1 is rapidly dephosphorylated. Unphosphorylated Ssk1 then activates downstream HOG pathway components. It is thus the essential controller of HOG pathway activity.

We undertook a comprehensive characterization of the sensitivity of HOG pathway activation to changes in the expression levels of the phosphorelay proteins Sln1, Ypd1, and Ssk1. We systematically under- and overexpressed the three proteins using an artificial induction system and found that phosphorelay activity is robust to changes in the concentrations of its components. We developed a detailed, biochemically realistic mathematical model of the HOG pathway three-component phosphorelay to elucidate the mechanism underlying this robustness. Our model incorporates extensive structural and mechanistic information about the phosphorelay and considers nearly all possible interactions between the three relay proteins. We used mass-action kinetics and algebraic calculations to characterize the steady-state behavior of the model. Steady-state algebraic models are a useful alternative to existing computational models of the HOG pathway for understanding robust behavior. Algebraic manipulations can be done without ever assigning special values to the parameters (i.e., the rate constants in the reaction network), many of which are difficult or impossible to measure experimentally [2].

Our steady-state analysis predicted that relative levels of dephosphorylated Ssk1 depend solely on Ypd1 levels and that robustness is achieved by maintaining Ypd1 in large excess. We experimentally tested this prediction by perturbing protein expression levels so as to deplete this buffering pool of Ypd1. All such perturbations compromised the ability of the phosphorelay to inhibit the HOG pathway, leading to hyperactivation in normal osmolarity conditions. The presence of a large buffering pool of an intermediate phosphorelay component is a previously underappreciated mechanism for robustness and suggests a possible advantage of a three-component relay over a two-component system.

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[1]Department of Systems Biology, Harvard Medical School, Boston, MA, USA E-mail: jdexter@fas.harvard.edu

[2] Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA E-mail: pxu@princeton.edu

[3] Department of Systems Biology, Harvard Medical School, Boston, MA, USA E-mail: jeremy@hms.harvard.edu

[3]Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI, USA E-mail: mmcclean@wisc.edu

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Cancer cell invasion analysis in ECM using *in vitro* models

Byoungkoo Lee[1], Jessica Konen[2], Songli Xu[3], Adam I. Marcus[4], Yi Jiang[5]

***Short Abstract* — Invasive cancer cells actively interact with the surrounding extracellular matrix fibers. Properly examining the invasive cancer cell behavior with ECM is necessary to more comprehensively understand cancer. We developed *in vitro* model to more quantitatively test the cancer cell behavior in ECM environments, using glioblastoma cultured in Matrigel and human non-small cell lung cancer cells cultured in collagen. We analyzed cell and fiber-bound bead motility as well as fiber alignment for various invasion conditions modifying key players along the signaling pathway: cdc42, Rho, FAK, LKB1. Active invasion conditions showed strong fiber pulling into the tumor and fiber realignment.**

I. Introduction

ONE main difficulty to treat cancer disease is due to invasion, in which cancer cells spread from their primary sites. *In vitro* tumor spheroid studies and transgenic mice studies have shown that invasive cancer cells actively remodel the surrounding ECM, and ECM alignment significantly influences cancer cell invasion [1]. Instead of focusing on cancer cells, we analyze the cells and ECM simultaneously, focusing on their interactions.

II. Results

To examine both cancer cell invasion and ECM dynamics together, we first cultured glioblastoma spheroids in Matrigel seeded with fluorescent beads, and tracked both cell and bead motions. Using the persistent random walk model [2], we analyzed cell and bead motility for different mechanotransduction signals: active cdc42, inactive cdc42, Rho inhibitor, and FAK inhibitor.

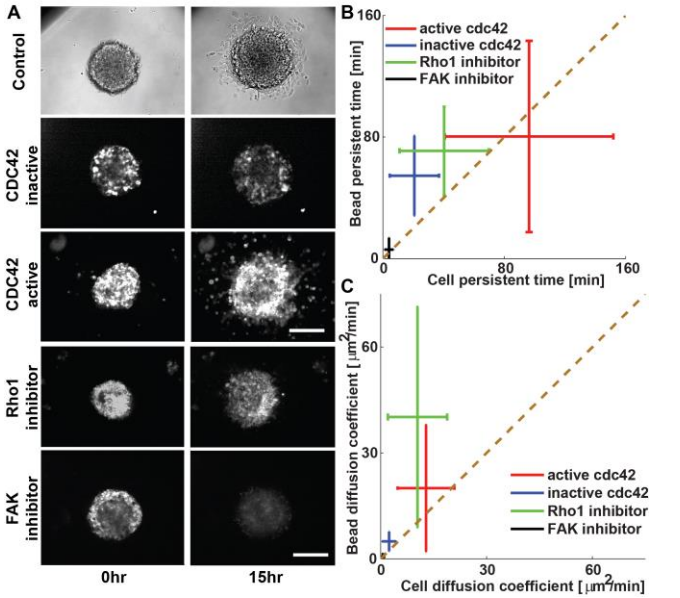

Figure 1: Glioblastoma spheroids in Matrigel. (A) Control and four different test cases (cdc42 inactive, cdc42 active, Rho inhibitor, FAK inhibitor) at 0 and 15 hours. Persistent time (B) and diffusion coefficient (C) for cell and bead motility data of four different test cases using a persistent random walk model.

In our experiments, Matrigel fiber-bound beads were pulled into the tumor spheroid before cancer cells actively invaded out. Active cdc42 cells (Fig.1) showed aggressive cell invasion and strong fiber pulling movement, while FAK inhibitor case showed neither cell migration nor fiber activity. Inactive cdc42 and Rho inhibitor cases showed more interesting cell-ECM traction in the absence of cell migration, and the rescue of migration in time.

To better visualize the ECM structural change as a result of cancer invasion, we cultured human non-small cell lung cancer (NSCLC) spheroids in collagen, and analyzed collagen fiber alignment for both LKB1+ and LKB1- spheroids. Using CT-FIRE (curvelet transform fiber extraction) [3] and an orientational order parameter [4], we developed a novel method to analyze fiber local alignment. LKB1- cells invade, resulting in increased fiber alignment. LKB+ cells do not invade, resulting in slightly decreased local alignment.

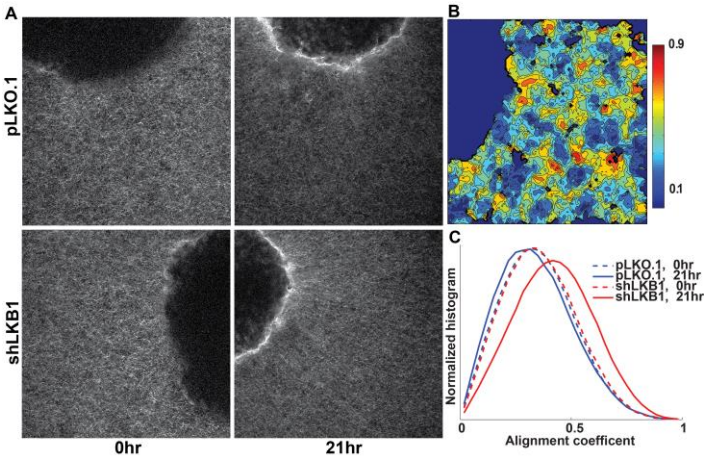

Figure 2: Human non-small cell lung cancer spheroids (H1299) in collagen gel. Control case (pLKO.1, LKB1+) and shRNA knocked down LKB1 case (shLKB1, LKB1-). (A) Confocal microscopy image at 0 and 21 hours. (B) Fiber alignment contour plot for the collagen image of shLKB1 at 21 hours. (C) Normalized fiber alignment histogram.

III. Conclusion

Glioblastoma cell and Matrigel-bound bead motility showed that invasive cells (active cdc42) "pull" the ECM into the tumor, as suggested by the strong correlation between cell and bead motility. Inhibiting different cell molecular signals altered both cell and bead motility. Inhibiting FAK showed almost block the cell and bead movement, while inactive cdc42 and Rho1 inhibitor showed moderate motility, which suggest that cell has multiple redundant signaling pathway via these signals. LKB1 inhibits FAK, and thus LKB1+ NSCLC did not show collagen fiber realignment, while LKB- NSCLC increased the fiber alignment. Our *in vitro* models provide alternative view on cancer invasion and help to better understand metastatic cancer in various ECM conditions.

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[1,5]Department of Mathematics and Statistics, Georgia State University, [1]E-mail: blee37@gsu.edu, [5]E-mail: yjiang12@gsu.edu, [2]Caner Biology Graduate Program, Emory University, E-mail: jtepe@emory.edu, [3]Ceners for Disease Control and Prevention, [4]Department of Hematology and Oncology, Emory University, E-mail: aimarcu@emory.edu

Molecular Cooperativity Leads to Monoallelic Olfactory Receptor Expression

Xiao-Jun Tian[1,†], Hang Zhang[2,†] , and Jianhua Xing[1,3]

Short Abstract — **Each mammalian olfactory sensory neuron expresses only one out of thousands of olfactory receptor alleles and the molecular mechanism remains as one of the biggest puzzles in neurobiology. We constructed a mathematical model and identified a three-layer regulation mechanism that robustly generates single-allele expression: zonal separation, epigenetic activation and subsequent allele competition for a limited number of enhancers. Model analyses conclude that the regulatory system has been evolutionarily optimized to minimize multiple allele activation and alleles trapped in incomplete epigenetic activation states. The identified design principles demonstrate the importance of molecular cooperativity in selecting and maintaining monoallelic olfactory receptor expression.**

I. INTRODUCTION

Olfaction is essential for the proliferation and survival of an organism. One of the most intriguing puzzles in neurobiology that remains elusive after several decades of intensive investigations is: how is a single allele selected for activation from a large number of possible OR genes and maintained throughout the lifespan of the neuron? Proposals on the selection mechanism can be divided into two categories: individual-allele centered selection, and enhancer-regulated selection. Each of the two proposed mechanisms has experimental supports and complications. The individual-allele epigenetic competition model reveals a natural feedback mechanism that expression of the winning allele causes endoplasmic reticulum stress and expression of Adcy3 enzyme, which then down-regulates LSD1, leading to an epigenetic trap that stabilizes the OR choice [1]. Multiple enhancers bind to the active OR alleles, but not the silenced ones, and form a dense interaction network [2]. The present work aims to reconcile the above two models and provides a mechanistic explanation on single-allele OR expression.

II. MODEL AND RESULTS

We formulated a mathematical model for the OR activation problem. First, zonal segregation reduces the number of OR alleles competing for single allele expression from thousands to hundreds within a zone. We therefore modeled a cell with 100 alleles to recapitulate the selection process from within a single zone of olfactory epithelium. Each OR allele consists of a linear array of 41 nucleosomes, and each nucleosome can bear repressive H3K9, no, or active H3K4 methylations [3]. Transition between these states is governed by enzyme concentration dependent rates.

We first examined the model under conditions prior to and after OSN differentiation. We found that maintaining high levels of methyltransferases and low level of demethylases forces an allele to be kinetically trapped at one of the two possible epigenetic states throughout the life time of an OSN.

Second, we found that elevation of bifunctional demethylase level leads to a barrier-crossing like dynamics and most of the OSNs with one allele epigenetically activated while a small fraction has two and rarely 3 alleles epigenetically activated. A prominent feature of this barrier-crossing-like dynamics is that throughout the process the probability of having an allele with hybrid pattern of epigenetic marks is low, and most alleles only fluctuate around the H3K9me3 dominated state.

Third, we found that the epigenetic conversion mechanism is insufficient to explain the experimental results on inhibiting methyltransferases/demethylases unless we added another layer with cooperative enhancer competition. We predict a loss of diversity of OR expression when the level of H3K9 methyltransferases is reduced, which is consistent with what observed experimentally [4].

III. DISCUSSION & CONCLUSION

Our theoretical studies suggest that single allele activation may be achieved through a series of selection processes functioning synergistically. A subset of the alleles is selected by the zonal segregation. Then they are randomly chosen to be epigenetically activated though elevation of bifunctional LSD1. Most of the cells only have one epigenetically active and thus transcriptional active allele. If more than one allele are epigenetically activated, they compete for the enhancers to be transcriptionally active, resulting in only one epigenetically and transcriptional active allele. If the activated allele is not pseudo gene, it triggers the feedback to prevent further epigenetic state change.

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[1]Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, 15260, USA
[2]Genetics, Bioinformatics, and Computational Biology Program, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24060, USA
[3]Email: xing1@pitt.edu
† Equal contribution.

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Modeling Genomic Recombination Potentials Regulated by Synthetic Donor DNA and Triplex-forming Molecules

Faisal Reza[1] and Peter M. Glazer[2]

***Short Abstract* — Endogenous genome targeting and editing in an efficient and specific manner are technological challenges, particularly in development and translational settings, with significant foreseeable impacts. To address these challenges, quantitative modeling genomic recombination potentials of synthetic donor DNA and triplex-forming molecules from sequence content and structural conformation perspectives are developed. These designed sequence-specific and structure-reshaping molecules are explored for their non-covalent intramolecular self and intermolecular genomic interactions. Findings indicate contraints and nuances for the design of the donor DNA molecule particular to a genomic editing site and, analogously, of the triplex-forming molecule particular to a genomic targeting site.**

***Keywords* — recombinagenic donor DNA, mutagenic triplex-forming molecule, genome engineering, precision medicine**

I. Purpose

SYNTHETIC oligo- and peptide- nucleic acid mutagenic molecules have been more predictably designed and externally delivered into the intracellular milieu. These deployed technologies interact with, and influence, the cytoplasmic and nuclear molecular machinery in order to regulate potentials involved in genomic targeting and editing. By regulating these potentials, silenced yet functional genes can be reactivated, as well as exquisitely controlled by external and environmental stimuli, thus modulating the cellular regulatory hematopoiesis system [1].

It is demonstrated that synthetic nucleic acid nanostructures composed of various nucleobase and backbone modifications can regulate the genomic recombination rate, the sequence-specific restriction of a locus, and the endogenous repair pathways. The formation of a triplex nanostructure (Fig. 1), by exogenously introduced PNA molecules with the duplex chromosomal and episomal DNA, is shown to elevate the cell's targeted recombination potential [2].

Recombinagenic donor DNA molecules co-opt these elevated recombination or initiated restriction potentials to form competing nanostructures that act as homology-dependent templates, sans edits to be introduced, thus potentiating repair [3]. Safety and efficacy of these nanostructures is achieved by leveraging the performance profile of the cell's own endogenous recombination, restriction, and repair machineries in concert with these sequence-specific and localizing-in-tandem molecules. Progenitor cells drugged with designed molecules, and primed with chemical cell modulators, safely and effectively redesign the genome, which are then propagated to cellular progeny.

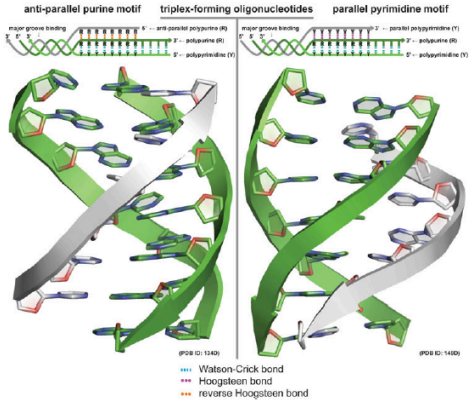

Figure 1. Triplex-forming molecule genomic motifs.

These molecular technologies are developed to remediate the underlying genomic causes of monogenic human diseases such as hemaglobinopathies, engineer living genetic codes, improve crop characteristics, and defend against outbreaks, through quantitative modeling and elucidation of cellular genomic recombination, and thus has well-positioned technology profiles for healthcare, biotechnology, agrotechnology, and national security.

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[1]Department of Therapeutic Radiology, Yale University, School of Medicine, New Haven, CT, USA. E-mail: faisal.reza@yale.edu

[2]Department of Therapeutic Radiology, Yale University, School of Medicine, New Haven, CT, USA. E-mail: peter,glazer@yale.edu

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Stochastic Model of the Histidine Kinase Switch in the *Caulobacter* Cell Cycle

F Li[1], K Subramanian[2], JJ Tyson[2] and Y Cao[1]

***Short Abstract* — The morphological change of *Caulobacter crescentus* from swarmer to stalked cell is a result of elaborate regulations. The two histidine kinase PleC and CckA controls the physiology development and the cell cycle development. Here we present a stochastic model that reveals the states of the histidine kinase at different cell stage. With the simulation result, we believe the kinase form of PleC in the swarmer pole is essential for the cell cycle development.**

***Keywords* — *Cauoubacter* cell cycle, histidine kinase, stochastic simulation.**

I. Introduction

The asymmetric division of *Caulobacter* requires elaborate regulations that control the chromosome segregation, polar differentiation and regulator localization [1]. Experiments have identified CtrA as one of the master transcription regulators in the *Caulobacter* cell cycle [2]. In swarmer cells the CtrA response regulator binds to the chromosome origin of replication and inhibits the initiation of chromosome replication. During the swarmer-to-stalk transition the active CtrA~p, gets dephosphorylated and degraded, and the cell initiates the chromosome replication.

In the physiology level, the flagella pole development in *Caulobacter* is controlled by the response regulator DivK, with the histidine kinase DivJ and PleC [3]. The freely diffusing DivK is phosphorylated by DivJ kinase, localized at the stalked pole and dephosphorylated by PleC phosphatase at the flagella pole [4]. After cytokinesis, the activities of PleC and DivJ are physically separates. As a consequence, DivK~p level drops dramatically in the swarmer cell, and permits flagella development. In addition, DivK~p is as an allosteric regulator that turns the PleC phosphatase into the kinase form, which phosphorylates DivK in return.

Furthermore, the DivK~p pole indirectly inactivates the CtrA through a non-canonical histidine kinase DivL [4]. In the swarmer to stalk cell transition, the DivK~p binds to DivL and inhibits CckA kinase activity, which in turn inactivates CtrA. In this abstract we present our stochastic simulation on the reaction-diffusion model for the DivJ-DivK-PleC and DivL-CckA-CtrA regulation network.

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[1]Department of Computer Science, Virginia Tech, Blacksburg, VA 24061. E-mail: felix@vt.edu, ycao@vt.edu.

[2]Department of Biological Science, Virginia Tech, Blacksburg, VA 24061. Email: skartik@vt.edu, tyson@vt.edu.

II. Mathematical Model

Our stochastic model is built based on the bistable switch model [5] in coordinate with the gene localization and mRNA. To demonstrate the spatiotemporal localization of regulator species, we deliberately enforce the localization of the regulatory species as model events. In the swarmer cell, PleC is localized to the old pole. At 30min, swarmer-to-stalk transition begins and DivJ is forced to the old pole. At 50min, PleC is cleared from the old pole and reappeared in the new pole at 90min. At 120min, cell is divided into two pre-division cells. Figure 1 shows the spatiotemporal population evolution of PleC kinase and CtrAp in the stochastic simulation. Our model reveals that PleC in the new pole stays in the kinase form and sequesters DivKp from binding to DivL. Hence, in the swarmer pole, CtrA is phosphorylated and active.

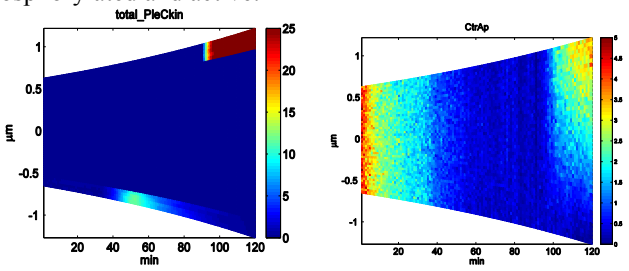

Figure 1: The spatiotemporal population of PleC kinase (left) and CtrAp (right), the plotted population is calculated from the average of 500 stochastic runs.

III. Conclusion

We developed a stochastic model for the regulatory network in the Caulobacter cell cycle. Our model favors the explanation that PleC in the swarmer pole stays in the kinase form and sequesters DivKp from binding to DivL. Hence, CtrA in the swarmer pole is active and inhibits the initiation of chromosome replication.

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A theory of the primary visual cortex, its zero-parameter quantitative prediction, and its experimental tests

Li Zhaoping

University College London
www.cs.ucl.ac.uk/staff/Zhaoping.Li/

A monkeys brain, a good model of human brain, devotes about half of its area to visual processing, and the primary visual cortex, also called V1, is the largest brain area devoted to vision. There has been a great amount of experimental data on the neurobiology of V1, including those by Hubel and Wiesel who won Nobel prize in 1981. However, the role of V1 for the perceptual and cognitive function of vision was unclear for many years. I will introduce a recent theory of V1 proposing that V1 serves to guide visual attention using external visual inputs so that the brain can devote its resources to process a tiny fraction of visual inputs in the attentional spotlight. This theory can be described by a simple equation, which combined with physiology of V1 can easily derive another simple equation relating several measurable quantities about visual behavior without free parameters. This prediction is experimentally confirmed. In addition, surprising insights about our brain are revealed by an "impossible" qualitative prediction of this theory, and its experimental confirmation.

Quorum-sensing crosstalk driven synthetic circuits: from unimodality to trimodality

Fuqing Wu[1], David Menn[1], and Xiao Wang[1]

***Short Abstract* — Quorum-sensing (QS) enables bacteria to communicate and plays a critical role in controlling bacterial virulence. However, effects of promiscuous QS crosstalk remain unexplored. Here we systematically studied the crosstalk between LuxR/LuxI and LasR/LasI systems and found that QS crosstalk can be dissected into signal crosstalk and promoter crosstalk. Investigations using synthetic positive feedback circuits revealed that signal crosstalk decreases circuit's bistable potential. Promoter crosstalk, however, reproducibly generates complex trimodal responses resulting from noise-induced state transitions and host-circuit interactions. A mathematical model that integrates nonlinearity, stochasticity, and host-circuit interactions was developed, and its predictions of conditions for trimodality were verified experimentally.**

***Keywords* — Quorum-sensing, synthetic circuits, noise, host-circuit interactions.**

Quorum-sensing (QS) is a ubiquitous mechanism in nature, and its regulator-autoinducer pairs, such as LuxR/LuxI and LasR/LasI, have been used in synthetic biology for a wide range of applications [1]–[3]. However, evolutionary pressures from limited resources in a competitive environment promote promiscuous bacterial communication, which takes the form of either different genera of bacteria producing the same types of autoinducers or non-specific regulator-autoinducer binding [4]. As a result, QS regulator-autoinducer pairs are not orthogonal, and there is crosstalk between them. Dissecting the crosstalk is critical for unraveling the underlying principles of bacterial decision-making and survival strategies for both natural and synthetic systems.

In this work [5], we used synthetic biology approaches to dissect QS crosstalk between LuxR/I and LasR/I. By applying engineering principles to construct modular gene networks, we were able to characterize and categorize QS crosstalk into signal crosstalk, where LuxR binds with the non-naturally paired C12 to activate pLux, and promoter crosstalk, where LasR binds with C12 to activate non-naturally paired pLux.

When signal crosstalk is constructed and tested in the context of positive feedback, our results showed a significant shrinkage of the bistable region. On the other hand, promoter crosstalk caused complex ***trimodal*** responses when embedded within a positive feedback circuit. This can only be explained when network bistability, gene expression stochasticity, and genetic mutations are all taken into consideration. We computationally predicted and experimentally verified that the C12-LasR-pLux positive feedback circuit could drive the formation of three subpopulations from an isogenic initial culture: one population expressing high GFP expression, the second showing basal GFP expression, and the third population with no GFP expression. The high and low GFP states are the result of positive feedback enabled bistability and gene expression stochasticity-induced random state transitions.

The third non-GFP population is the result of genetic mutation from IS10 insertion. From an engineer's perspective, the mutation stands in contrast to previously reported host-circuit interactions, which are primarily related to resource limitation and resulting growth defects [6]. Here we were able to illustrate that both the components used and the topology of the network constructed could contribute to resource independent host-circuit interactions. This concept of combining nonlinear dynamics and host-circuit interactions to enrich population diversity expands our understanding of mechanisms contributing to cell-cell variability, and suggests new directions in engineering gene networks to utilize hybrid factors.

Taken together, our studies not only showcase living cells' amazing complexity and the difficulty in the refining of engineered biological systems, but they also reveal an overlooked mechanism by which multimodality arises from the combination of an engineered gene circuit and host-circuit interactions.

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[1]School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ. E-mail: xiaowang@asu.edu

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Machines that Assemble Signaling Pathways by Reading the Literature: Progress in DARPA's Big Mechanism Program

Paul Cohen

DARPA'S BIG Mechanism program is developing technology to help humans build mechanistic models of very complicated systems. The test case for the program is Ras signaling, as Ras is implicated in roughly 30% of cancers and is the focus of the NCI's recent Ras Initiative. The idea of Big Mechanism is that computers will read journal articles and extract fragments of systems -- such as the Ras pathway -- and will assemble these fragments into executable models of the systems. A range of issues arise, from the challenges of machine reading, to the ways humans hedge their conclusions, to the diversity of model organisms and tissue types that are represented in articles, to the lack of essential information such as rate constants, to the difficulty of assembling results into models. And yet, a year into the Big Mechanism program, machines are reading large numbers of articles and the technologies for assembling models show promise.

GLOBAL CHANGES IN CHROMOSOME CONFORMATION IN BUDDING YEAST IN DIFFERENT PHYSIOLOGICAL CONDITIONS

Karsten Weis

ETH Zurich
Department of Biology
Zurich, Switzerland

A long-standing problem in eukaryotic cell biology is to understand how the genetic information is organized and folded to fit into the interphase nucleus. The organization of the genome is non-random and was shown to be important for the correct genome function. For example, the nuclear envelope plays a critical role in gene regulation and interactions between genes and the nuclear periphery can lead to gene repression. However, several genes, including the *GAL* gene locus in budding yeast, are recruited to the nuclear periphery upon activation. We have asked how the association of the single gene locus with the nuclear envelope influences the surrounding chromosome architecture. Using modeling and light microscopy assays we follow the movement of an entire chromosome in yeast demonstrating that peripheral recruitment of the *GAL* locus upon carbon source change is not an isolated event but part of a large scale rearrangement that shifts many chromosomal regions closer to the nuclear envelope. This process is likely due to the presence of independent anchoring points along the chromosome and depends on the activity of histone modifying enzymes.

Sensing Multiple Ligands with a Single Receptor

Vijay Singh[1] and Ilya Nemenman[1, 2]

***Short Abstract* — Cells use surface receptors to estimate the concentration of external ligands. Limits on the accuracy of such estimations have been well studied for cases of single ligand-receptor species. However, cell surface contains many species of receptors that measure the concentration of several external ligands, and non-cognate ligands can bind to receptors, resulting in the phenomenon of cross-talk. We show that the cross-talk does not interfere substantially with determination of ligand concentrations if one is allowed to use the entire temporal sequence of receptor binding-unbinding instead of only the receptor's average occupancy. In fact, concentrations of two different chemical ligands can be measured with just one receptor with an accuracy approaching the limit set by basic statistical considerations. We argue that a high-accuracy approximation to such inference of multiple chemical concentrations can be done using the kinetic proofreading mechanism that is abundant in real cells.**

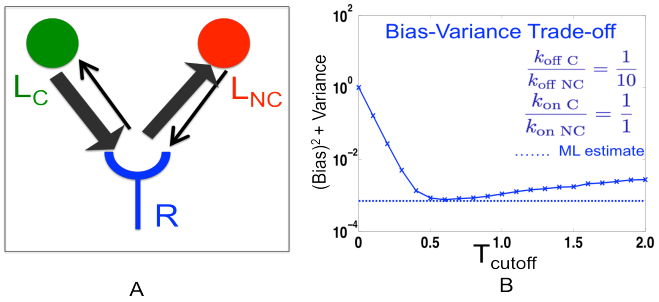
***Keywords* — ligand concentration estimation, maximum-likelihood, kinetic proofreading.**

I. Motivation

Cells estimate the concentration of external ligands by capturing the ligand molecules with cell-surface receptors. Limit on the accuracy of such estimation has been a subject of interest since the seminal work of Berg and Purcell [1], with several substantial improvements found in the recent years [2-5]. All these estimates assume one ligand species coupled to a single receptor species. However, realistically, there are many species of ligands present in the vicinity of a cell. Similarly, the cell surface contains several types of receptors. In principle, each ligand can bind to each receptor, albeit with different affinities. Does this cross-talk affect the accuracy of estimation of ligand concentrations by the cell? Is it always detrimental, or can it be used to improve the estimation?

II. Model

We answer these questions in the context of a simplified model of a single receptor estimating concentration of two chemical ligands (cognate and non-cognate) (Fig. A). The on-rates for both ligands are assumed to be diffusion-limited and hence are nearly the same. However, the cognate ligand has a smaller off-rate and hence stays bound to the receptor for longer periods of time, generally. Writing down the master equation for the system allows us to calculate the probability of each particular sequence of binding-unbinding events for the receptor, which we then use for estimation of ligand concentrations.



A B

III. Results and Conclusions

We observe that the time series of binding-unbinding events carries information about both ligand concentrations, where the number of long binding events carries information about cognate ligand, and short binding events can be used to estimate the concentration of the non-cognate ones. We write down the maximum-likelihood (ML) solution [6] for this estimation, and an approximate solution that assumes that *all* long binding duration events come from cognate receptors. This results in a simplified estimation that can be implemented by cells using the kinetic-proofreading mechanism, which is an abundant motif in protein signaling. We use analytical and stochastic simulation methods to investigate the bias and the variance of the approximate estimator as a function of the cutoff time above which all binding events are considered cognate. The minimum of the bias-variance tradeoff curve is very close to the perfect estimation (see Fig. B). This shows that the cross-talk allows to estimate concentrations of two ligands simultaneously and efficiently by one type of receptors. Multiple ligand concentrations can be estimated similarly by setting up multiple kinetic proofreading cascades. Finally we argue that cross-talk can be beneficial in concentration estimation problems allowing to extend the dynamic range of the system by measuring concentration of a ligand on a non-cognate receptor when the cognate one is saturated.

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[1]Department of Physics, Emory University, Atlanta, GA 30322. E-mail: vijay.singh@emory.edu

[2] Department of Physics and Biology, Emory University, Atlanta, GA 30322. E-mail: ilya.nemenman@emory.edu

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Functional Roles of p53 Dynamics in Regulating Target Gene Expression

Joshua Porter[1], Brian Fisher[1], Amie Moody[1] and Eric Batchelor[1]

***Short Abstract* — The tumor suppressor p53 is among a growing list of transcription factors that show complex pulsatile dynamics in response to stimuli. While p53 pulses have been shown to impact cell fate decisions, the mechanisms by which this regulation occurs remain poorly understood. Here, we describe our recent computational and experimental efforts in which we identify specific functions of p53 dynamics in the regulation of its numerous target genes. Our findings point to general principles that will likely be important for understanding a variety of pulsatile systems.**

***Keywords* — dynamical systems, pulsatile transcription factors, p53, live-cell imaging, single-cell transcriptional profiling**

I. Purpose

CELLS use complex signaling pathways to detect environmental stimuli and execute appropriate responses. As methods for quantifying intracellular signaling have improved, several signaling pathways have been found to transmit information using signals that pulse in time [1, 2]. The transcription factor p53 is a key stress-response regulator that exhibits pulsatile dynamics [3, 4]. In response to DNA double-strand breaks, p53 levels in the nucleus increase in pulses with a fixed amplitude, duration, and period; the mean number of pulses increases with DNA damage [3].

p53 regulates the expression of over 100 target genes involved in a range of cellular stress responses including apoptosis, cell cycle arrest, senescence, DNA repair, and changes in metabolism [5]. p53 pulsing directly impacts p53 function: altering p53 dynamics by pharmacologically inhibiting p53 degradation changes patterns of target gene expression and cell fate [6]. While p53 pulsing serves an important signaling function, it is less clear what it accomplishes mechanistically.

Here we will describe our recent efforts to determine the impact of p53 pulsing on the dynamics and coordination of target gene expression.

II. Results

We used a combination of experimental approaches, both at the population level and at the single-cell level, informed by computational and mathematical modeling efforts, to identify functional consequences of p53 dynamics on target gene expression.

A. p53 pulses diversify target gene dynamics

Using quantitative PCR, we measured the expression of the majority of p53 target genes in response to DNA damage. From these measurements, we determined several distinct classes of target gene dynamics, including pulsatile, rising, and step-like dynamics. Using mathematical modeling, we identified mRNA half-life as an important parameter in determining expression dynamics.

B. Subnetwork architecture in the p53 network

Using single-cell transcriptional profiling at key time points following damage, we identified specific subnetworks of co-regulated p53 target genes. The majority of target genes composing the major subnetwork were genes with pulsatile expression dynamics.

C. Tools to control p53 dynamics

We used pharmacological and novel synthetic biological approaches to control the dynamics and localization of p53 in individual single cells. Using these tools, we are identifying the roles of specific characteristics (amplitude, duration, and period) of p53 dynamics on the activation of p53 target genes.

III. Conclusion

Our results give new insight into the function of a growing number of pulsatile signaling pathways and may inform chemotherapeutic strategies based on manipulation of p53 dynamics.

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[1]Systems Biology Unit, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health. E-mail: batchelore@mail.nih.gov

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Established microbial colonies can survive Type VI secretion assault

David Bruce Borenstein[1], Peter Ringel[2], Marek Basler[2], and Ned S. Wingreen[1,3]

***Short Abstract* — Type VI secretion (T6S) is a cell-to-cell injection system that can be used as a weapon and is present in ~ 25% of sequenced Gram-negative bacteria. To examine the ecological role of T6S, we competed self-immune T6S+ cells and T6S-sensitive cells in simulated range expansions. As killing takes place only at the interface between sensitive and T6S+ strains, while growth takes place everywhere, sufficiently large domains of sensitive cells can achieve net growth in the face of attack. We validated these findings through *in vivo* competition experiments between T6S+ *Vibrio cholerae* and T6S-sensitive *Escherichia coli*. We found that *E. coli* can survive and even dominate so long as they have an adequate opportunity to form microcolonies. Finally, in simulated competitions between two equivalent T6S+ strains, the more numerous strain has an advantage that increases with the T6S attack rate.**

***Keywords* — Type VI secretion, microbial competition, evolutionary dynamics, simulation, agent-based modeling**

I. Introduction

Microbes employ a staggering range of extracellular tools to engineer their immediate environment [1]. Very often that environment is defined by the multitude of other cells in close proximity. The Type VI secretion system (T6SS) is a mechanism for direct cell-to-cell manipulation of these neighbors through the translocation of effector proteins [2]. By far the most commonly observed function of T6S is attack [4]. Specialized T6SSs can directly damage both prokaryotic and eukaryotic target cells through the translocation of toxic proteins directly across the membrane. T6SSs are present in approximately 25% of the Gram-negative genomes studied by Boyer and colleagues [3]. Antibacterial T6SSs appear to be found with cognate immunity proteins in every case [4]. Given this tactical advantage, one might expect T6SS to be even more widespread. Why is T6S not universal?

II. Methods and Results

To address the question of T6S's utility, we focused on the case of cell-to-cell killing between bacteria. We explored this scenario through the use of individual-based models (IBMs). We first developed an IBM that competes self-immune T6S+ and sensitive individuals in a range expansion, analogous to a surface colony (2D) or a biofilm (3D). We find that cell growth from the inside of an established domain can offset cell death at the interface between a T6S-sensitive strain and a self-immune T6S attacker. Consequently, given a sufficiently large domain, T6S-sensitive strains can survive T6S attack. The sensitive strain does not require a growth advantage to survive. Given even a small growth advantage, the T6S-sensitive strain can outcompete a self-immune T6S+ competitor.

We validated these findings through *in vivo* competition experiments between T6S+ *V. cholerae* and T6S-sensitive *E. coli* (Fig. 1). In these 2D plate assays, *E. coli* can form persistent microcolonies that survive, provided the initial local density of *V. cholerae* is not too high. Along similar lines, simulated competitions between self-immune T6S+ strains reveal that the initially more numerous strain benefits most from higher attack rates.

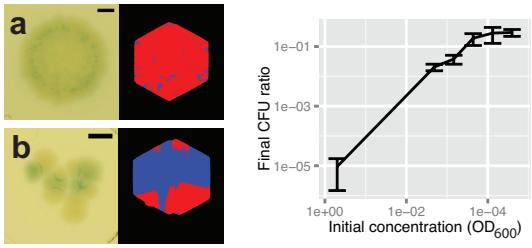

Fig. 1 Domain size predicts T6S-sensitive survival. Comparisons of experimental to simulation outcomes. (a) Left, overnight growth on x-gal media from an inoculum consisting of *V. cholerae* str. 2740-80 (LacZ-) and *E. coli* MG1655 (LacZ+), starting from equal amounts of OD600 = $2x10^{-4}$ culture from each species. Right, simulated range expansion from 729 T6S+ individuals and an equal number of sensitive individuals. (b) 9-fold dilution of simulated and experimental conditions, showing increased survival of sensitive *E. coli*. (c) Final ratio of sensitive *E. coli* to T6S+ *V. cholerae* as a function of initial inoculum concentration.

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[1] Princeton University, Lewis-Sigler Institute for Integrative Genomics.
Email: dbborens@princeton.edu
[2] Universitat Basel, Biozentrum, Basel, Switzerland.
Email: marek.basler@unibas.ch, peter.ringel@unibas.ch
[3] Princeton University, Department of Molecular Biology.
Email: wingreen@princeton.edu

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Discovery and Analysis of Novel Biochemical Transformations

Linda J. Broadbelt

Northwestern University
Department of Chemical and Biological Engineering, 2145 Sheridan Road
Evanston, IL 60208

We have developed a computational discovery platform for identifying and analyzing novel biochemical pathways to target chemicals. Automated network generation that defines and implements the chemistry of what we have coined "generalized enzyme functions" based on knowledge compiled in existing biochemical databases is employed. The output is a set of compounds and the pathways connecting them, both known and novel. To identify the most promising of the thousands of different pathways generated, we link the automated network generation algorithms with pathway evaluation tools. The simplest screening metrics to rank pathways are pathway length and number of known reactions. More sophisticated screening tools include thermodynamic feasibility and potential of known enzymes for carrying out novel reactions. Our method for automated generation of pathways creates *novel compounds and pathways* that have not been reported in biochemical or chemical databases. Thus, our method goes beyond a survey of existing compounds and reactions and provides an alternative to the conventional approaches practiced to develop novel biochemical processes. This presentation will focus on the components of this computational discovery platform and its application.

Measuring the sequence-affinity landscape of antibodies

Rhys M. Adams[1], Justin Kinney[†2], Thierry Mora[†1], Aleksandra M. Walczak[†1]

To ensure a fast and reliable immune response upon infection, B-cells rapidly mutate the sequence of their antibodies to improve their binding affinity to invading pathogens. However, little is known quantitatively about the precise relationship between the antibody sequence and its binding properties. To study this question, we developed a high-throughput method combining experiments with advanced statistical analysis to measure antibody affinity with high precision. We used this method to measure the effects of a wide variety of mutations in complementary determining regions (CDR) 1 and 3 of the antibody heavy chain (H). We find that the CDR1H domain tends to have fewer severe mutations than CDR3H, but has a higher tendency to affect antibody expression for non-severe mutations.

Keywords **— yeast display, protein affinity landscape, immunology, antibody, statistical analysis.**

I. Introduction

A successful immune response is based on the recognition of antigens by binding them to antibodies.

Antibody binding affinity is largely determined by 6 domains called complementarity determining regions (CDR). Mutating these CDRs affect antibody stability and affinity. Since measuring antibody stability and affinity is a labor intensive and expensive process it is not know how they quantitatively depend on the CDR region sequences. To overcome these limitations, we developed a method to quantitatively measure the landscape of functional antibody properties directly from their sequences.

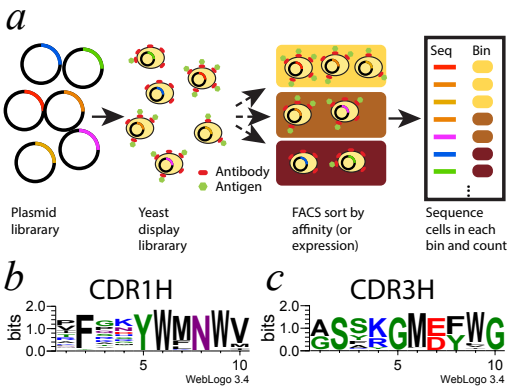
Our method combines a novel high throughput sequencing approach with advanced statistical analysis to quantitatively map out the binding landscape. It combines a sequencing based method for simultaneously measuring functional protein-protein interactions in a large sample, called Sort-seq [1], with yeast display [2]. Sort-seq uses FACS armed with a statistical analysis to en masse sort cells based on their binding affinity and expression. Yeast display has the advantage that it allows for disentangling protein expression and affinity. Yeast display expression levels also correlate highly with protein thermostability.

II. Methods and Results

We designed yeast plasmid libraries of CDR1H and CDR3H mutants for the fluorescein binding 4-4-20 scFv antibody using microarray oligonucleotides. Each CDR library consisted of 1950 codon mutations including synonymous mutation controls. We FACS separated the mutants and counted the frequencies of mutants in each bin. Using statistical techniques and experimental validation, we mapped mutant frequencies to affinity and expression measurements.

Because affinity measurement is tightly related to both antigen and antibody expression, we show that explicitly deconvolving expression from affinity is essential for accurately measuring affinity. This deconvolution, along with the set of synonymous mutations in our library, allow us to precisely measure antibody properties with an estimated (50:1) signal to noise ratio.

Our initial results clearly show different effects of mutations in CDR1H and CDR3H. CDR3H mutants are more likely to destroy antibody affinity. CDR1H mutants affect expression independently of affinity, while CDR3H mutations tend to affect expression only if affinity was abolished.



a) Schematic for sort-seq yeast display experiment. Weblogos are generated from the effects of single amino acid mutations of the 4-4-20 antibody on b) CDR1H and c) CDR3H affinity to fluorescein.

III. Conclusion

By designing libraries and measuring both affinity and expression, we can precisely and accurately simultaneously measure the biophysical properties of thousands of antibodies. This work gives us a basis for studying the effects of mutations on immunological recognition.

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[1] Departament de Physique, École Normale Supérieure. Paris, France, Primary author e-mail: rhysm.adams@gmail.com

[2]Department of Quantitative Biology, Cold Spring Harbor Laboratory, NY, USA

[†]These authors contributed equally to this work.

A synthetic oscillator couples to the cell division cycle in budding yeast

Heungwon Park[1,2,3], Sargis Karapetyan[1,3], Shuqiang Huang[1,4], and Nicolas E. Buchler[1,2,3]

***Short Abstract* — Living systems have evolved different natural oscillations, such as the cell division cycle, metabolic rhythms and circadian clocks. These oscillators play important biological roles in the survival and the function of cells. These different oscillations often co-exist in the same cell with variable periods, yet are expected to affect and synchronize with each other even with weak coupling. Here we developed a synthetic oscillator in budding yeast using negative feedback loop based on protein sequestration, a key mechanism of natural oscillators. We show that our synthetic oscillator is autonomous but strongly coupled to the cell cycle in proliferating cells.**

***Keywords* — Synthetic oscillator, budding yeast, protein sequestration, cell cycle coupling and mixed feedback loop.**

I. INTRODUCTION

Living systems have different oscillations such as the cell division cycle, metabolic rhythms and circadian clocks [1,2]. Organisms have evolved several genetic oscillators, many of which use protein sequestration as a key mechanism to generate negative feedback. For example, a common architecture is the mixed feedback loop (MFL) [3], which is a two-gene circuit that consists of a constitutive activator and an inhibitor driven by activator homo-dimer binding. The negative feedback occurs when the activator produces high levels of inhibitor that eventually sequester the activator into an inactive hetero-dimer complex. To better understand the design principles of sequestration-based oscillators, we have built a synthetic MFL circuit in budding yeast.

Strikingly, our synthetic oscillator was strongly locked to the budding yeast cell cycle. We verified that the synthetic oscillator was autonomous by blocking the yeast cell cycle with nocodazole. The blocked MFL circuit exhibited autonomous oscillatory dynamics with a period similar to wild-type cell cycle. We could also modulate the MFL oscillator period by changing the inhibitor degradation rate via a tunable degron [4]. However, the autonomous period in blocked cells did not change much relative to the cell cycle period, which explained why the MFL continued to exhibit 1:1 locking with cell cycle in non-blocked cells over a range of degradation rates. These results are different from those with bacterial synthetic oscillators [5,6], which did not couple to the underlying cell cycle. We are currently testing whether this arises from fundamental differences in cell cycle biology and/or synthetic oscillator design.

II. CONCLUSION & DISCUSSION

We have constructed a genetic oscillator in budding yeast that uses the mechanism and the topology frequently found in natural oscillators, i.e. mixed feedback loop (MFL) based on the protein sequestration. Our synthetic oscillator was capable of exhibiting autonomous oscillations with varying periods via a tunable degron of the inhibitor in cells with a blocked cell cycle. However, the MFL would always couple to the cell division cycle in proliferating cells.

Our work shows that yeast cell cycle can have a strong coupling with endogenous oscillators. That, in turn, raises questions as to whether cell cycle coupling is a universal feature across all eukaryote organisms and whether the cell cycle can impact the evolution of other natural oscillators and vice versa.

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[1]Duke Center for Genomic and Computational Biology, Duke University, Durham NC 27710.

[2]Department of Biology, Duke University, Durham NC 27710.

[3]Department of Physics, Duke University, Durham NC 27710.

[4]Department of Biomedical Engineering, Duke University, Durham, NC 27710.

E-mail: hp56@duke.edu (HP), sk197@duke.edu (SK), sh269@duke.edu (SH) and nb69@duke.edu (NEB).

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Growth rate variations establish distributions of generation times and division sizes in *E. coli*

Mats Wallden[1], David Fange[1], Gustaf Ullman[1,2], Erik Marklund[1,3] and Johan Elf[1,4]

Escherichia coli **cells growing in a constant environment vary considerably in growth rates, generation times and division sizes. In spite of fluctuations, cells have to complete one round of replication in each cycle in order to retain its chromosome and it is unclear how this is accomplished. Using fluorescence microscopy to localize the replisome we find that initiation of replication occurs at a fixed origin to volume ratio independently of the time from division. In addition, a model of the bacterial cell cycle, where division occurs at a constant time after replication initiation, reproduces the variations in timing and sizes at division.**

I. BACKGROUND

For *E. coli* cells the time between two consecutive division events can, during fast growth, be substantially shorter than the time required to replicate the genome[1]. This is achieved by having parallel ongoing replication processes, which were initiated during the cell cycle of an individuals' mother or even grandmother[2]. In the classical description, initiation of DNA replication occurs at constant ratio of cell volume to number of origins independent of growth conditions[3]; a notion, which recently has been both questioned[4] and affirmed[5]. Isogenic *E. coli* cells living under constant growth conditions will vary considerably in their growth rates, cell cycle times and division sizes[6-7]. In spite of these fluctuations, a cell has to initiate and terminate one round of replication during each cycle in order to maintain its chromosome content. It has recently been suggested that, on average, adding a constant volume following cell division regulates the cell size at division[8-9]. It is, however, still unclear how chromosome replication is connected to cell division and how cells uphold one initiation and termination per generation given that Eukaryote-like cell cycle checkpoints are incompatible with overlapping rounds of replication.

II. EXPERIMENTAL METHODS

E. coli cells grow exponentially in a constant environment using a microfluidic device. Individual cells in the device can be localized and tracked over multiple generations using fully automated analysis methods. In addition, using single-molecule widefield fluorescence microscopy, we can detect and localize individual replisomes (DnaQ-Ypet) within individual cells.

III. RESULTS

We find that when the intracellular replisome localizations are aligned based on cell volume, the distribution of replisome localization for all cells is better defined than the corresponding distribution for cells aligned by time after division. This suggests that initiation of replication is size- rather than time-dependent. For two different growth rates we find a striking similarity in the ratio of number of origins to volume at initiation of replication. Based on these observations we construct and test a model where initiation of replication occurs at a constant ratio of number of origins per cell volume and that cell division occurs after a constant time-delay following initiation of replication. The model is parameterized based on our experimentally observed data. Using our observation of the variation in growth rates we can predict the variance in cell size at division, generation times and the correlation between these two parameters.

IV. CONCLUSIONS

We find that initiation of DNA replication is based on the ratio of origin numbers to cell volume rather than time after cell division and that the cell-to-cell variability in growth-rate accurately accounts for the variability in generation times, cell sizes and the correlations between the two.

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[1]Dept. of Cell and Molecular Biology, Uppsala University.
[2]Current affiliation: Savantic AB, Stockholm, Sweden.
[3]Current affiliation: Dept. of Chemistry, University of Oxford.
[4]E-mail: johan.elf@icm.uu.se

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A systems-driven experimental approach reveals the complex regulatory distribution of p53 by circadian factors

Jae Kyoung Kim[1,2], Tetsuya Gotoh[3], Jingjing Liu[3], John Tyson[4], Carla V. Finkielstein[3]

Unlike single-cell organisms with self-contained timekeeping systems, multicellular organisms partition their oscillators among different cell types and depend on more complex molecular networks to sense signals and coordinate effective responses. We found that the core circadian clock protein Period 2 (Per2) directly interacts with the checkpoint regulatory component p53, promoting its stabilization and controlling p53 transcriptional activity. Remarkably, circadian phases of Per2 and p53 are anti-phase in the cytoplasm and in-phase in the nucleus, posing new questions about the extent to which Per2 association modulates p53 distribution. Therefore, we focused our efforts on investigating what simulated conditions better relate to the experimental data using mathematical models. Specifically, the model predicted that the phase of the Per2:p53 interaction strongly depends on the binding mechanisms between Per2 and p53 mediated by ubiquitin, as determined by evolving the interaction types between Per2 and p53 in the model during the fitting process. As a result, the ubiquitilation state of p53 impacts Per2 binding and subcellular distribution. All predictions were confirmed experimentally.

Keywords **– circadian rhythms, p53, Period 2 (Per2), reverse engineering technique, protein shuttling.**

I. INTRODUCTION

A KEY ASPECT of cell homeostasis in multicellular systems involves synchronizing cells to changes in environmental conditions, which results in coordinated responses that influence cell proliferation and death. Our previous findings indicate that the circadian sensor factor Per2 directly acts at the p53 node of the checkpoint response influencing various levels of regulation that impact cellular metabolism and bioenergetics ultimately supporting growth and proliferation. In this study, we unveil the time-dependent regulatory mechanisms that modulate p53's oscillatory behavior, stability, and cellular distribution through its association to Per2 using, initially, mathematical models. Predictions were validated and expanded by experimental data.

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[1]Department of Mathematical Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea
[2]Mathematical Biosciences Institute, The Ohio State University, Columbus, OH 43210
[3] Integrated Cellular Responses Laboratory, Virginia Tech, Blacksburg, VA, 24061, USA. E-mail: finkielc@vt.edu and
[4] Computational Cell Biology Laboratory, Virginia Tech

II. RESULTS

Our recent study finds that Per2 forms a trimeric complex with p53 and its negative oncogenic regulator Mdm2 [1]. In unstressed cells, this association leads to increased p53 stability through blocking Mdm2-dependent ubiquitination and transcription of p53 target genes. Despite these findings, when levels of these proteins were monitored in total extracts, circadian phases of p53 and Per2 were anti-phase. Subcellular fractionation provided a more comprehensive picture of their distribution and revealed that p53 and Per2 were anti-phase in the cytoplasm but in-phase in the nuclear fraction. To investigate the mechanisms underlying these unexpected phase relationships, we initially used mathematical modeling, where the interaction types between p53 and Per2 stochastically evolved during the parameter fitting process. Using this approach and timecourse data, we inferred that *i*) the half-life of p53 in the nucleus should be greater than that of the cytosolic-localized protein and that *ii*) p53 nuclear entry should be mediated by Per2. These predictions were confirmed experimentally. Overall, our data supports a model in which time-dependent phase shift accumulation of Per2 and p53 proteins results from a delay in post-translational modification events that take place in separate cellular compartments.

III. CONCLUSION

Our ongoing hypothesis is that Per2 helps to maintain basal levels of p53 in unstressed cells to "prime" the signaling pathway to rapidly respond to a stress condition (*i.e.*, metabolic, genotoxic). Our new data expand the current model to include regulation of their interaction by post-translational mechanisms and cellular compartmentalization as evidenced by modeling, and was proven experimentally. In fact, while previous studies have focused on using oscillating timecourse data to infer the presence of interactions among components of biochemical networks [2], our mathematical approach allows the use of timecourse data to further predict additional signature-types needed for molecular interactions to occur in specific cellular compartments.

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Unraveling dynamics of reconfigurable network motifs using a synthetic biology approach

Daniel D. Lewis[1,2], and Cheemeng Tan[2]

Short Abstract — **Gene networks are commonly studied using the classical paradigm of fixed network topology. In contrast, we create and investigate a new class of reconfigurable gene networks that can be switched between different topology without genetic changes. We show that a feedforward motif can be reconfigured to produce three distinct expression patterns. Furthermore, we demonstrate the motif's capacity to control the ratio between three proteins with only two inducers. Our work will impact the understanding of dynamical genetic networks and create a new class of synthetic systems for biotechnological applications.**

Keywords — **reconfigurable, network motifs, synthetic gene circuits**

I. Introduction

RECONFIGURABILITY refers to the ability of natural systems to dynamically change their properties, including spatial distribution of cells, cellular structures, and organization of cellular networks. While cells achieve such reconfigurability with relative ease, synthetic biological systems are primarily created and studied using the classical paradigm of engineered systems, in which circuit components are connected through static biochemical wiring [1]. However, natural gene networks are fundamentally reconfigurable and could potentially give rise to versatile, emergent dynamics. Can we take advantage of reconfiguration mechanisms of natural cells to create a new class of reconfigurable synthetic systems? What are the tradeoffs between versatility and fidelity of reconfigurable gene networks?

II. Results and Discussion

A. *Motif Search and Mathematical Modeling of A Reconfigurable Network Motif*

To start, we used a library of three node networks with interactions represented by Michaelis-Menten kinetics to identify network motifs [2] capable of producing multiple dynamics given a fixed range of inducer concentrations. We found the most robust reconfigurable architectures, and derived the analytical solution of one motif composed of a feedforward loop with competing positive and negative regulations at one of the nodes. The analytical solutions guide our subsequent experimental perturbations, as well as interpretation of experimental results.

B. *Experimental Validation and Perturbations of the Reconfigurable Motif*

To assemble the motif, we first characterized dynamics of each individual component, incorporating their respective kinetic parameters into our model. Next, based on the mathematical model, we assembled a reconfigurable circuit and quantified its dynamics using both population and single-cell assays. We demonstrated that the circuit is capable of reconfiguring its topology to produce three distinct dynamics: linear, band-pass, and inverter. We further perturbed the circuit by changing promoter strengths, copy number of genes, and levels of catabolite repression. Through the perturbations, we showed that network perturbations control the range of circuit reconfigurability.

C. *Exploiting Reconfigurability of the Motif to Control Concentration Ratios of Proteins*

The mathematical model predicts an intriguing, novel feature of the reconfigurable circuit, which controls the ratio between three proteins using only two inducers. We first showcased the ratio-control mechanism of the reconfigurable circuit using fluorescent reporters, then enzymes of a metabolic pathway to illustrate the modularity of the system.

III. Conclusion

We present a generic mechanism that can reconfigure dynamic behavior of a feedforward loop in response to an inducer. This motif can be applied to study reconfiguration of natural gene networks, and could shed light on mechanisms of cellular decision making. Our results represent a fundamental shift in how biological networks are understood, moving from a fixed-topology to a flexible-topology paradigm.

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[1]Integrative Genetics and Genomics, UC Davis. E-mail: aegodwin@ucdavis.edu

[2]Department of Biomedical Engineering, UC Davis. E-mail: cdwinter@ucdavis.edu

Stochastic modeling of variability in circadian rhythms utilizing measured variance

Alan L. Hutchison, Elzbieta Kula-Eversole, Fangke Xu, Ravi Allada, and Aaron R. Dinner

Circadian rhythms exist in all kingdoms of life. Computational models of circadian clocks incorporate molecular and behavioral period measurements of phenotypes of clock mutations. Stochastic models of clocks exist to understand the role of noise, yet none attempt to explain experimental variances in period. We develop an improved method for detecting rhythmicity in genome-wide time series, which we apply to a rich RNA-Seq dataset in *Drosophila.* We identify novel rhythmic genes and test knockouts to identify circadian phenotypes. We use these results to explore the effects of stochasticity on predicting period variance and compare our simulation to prior results.

Background

Circadian rhythms are endogenous rhythms with approximately 24-hour periods. In *Drosophila,* a series of transcriptional feedback loops creates the rhythm-generating core clock. Mutations in the core clock can lead to changes in the period of oscillations and strength of rhythmicity, which can be measured by an activity profile (actogram) of organismal behavior over time.

Many methods exist for detecting rhythms in time series data. When data are noisy, sparse, and contain many false positives, as genome-wide time series data does, successfully detecting rhythms becomes more difficult. One leading method is JTK_CYCLE [1], which uses non-parametric correlations with reference waveforms to detect rhythmicity. This method is limited by an overly conservative multiple hypothesis test correction and by only using symmetric reference waveforms.

Many models for the core circadian clock exist that attempt to replicate measured mean protein, transcript, and behavioral dynamics in wild type and mutant phenotypes. Most of these models are deterministic [2-4]. However, measurements produce distributions of these different dynamics, which can only be simulated via stochastic models. Stochastic models of differing complexity do exist [5,6], but no known detailed stochastic models attempt to match the variance observed in the behavioral period.

Results

We develop an improved method for detecting rhythmicity in circadian genome-wide data by using asymmetric reference waveforms to identify asymmetric rhythmic time series and using Monte Carlo simulations to empirically correct the p-values for multiple hypothesis testing [7]. We show that this gives greater sensitivity and specificity for rhythm detection in comparison to six other methods, including the original JTK_CYCLE method.

We apply our method to *Drosophila melanogaster* RNA-Seq data to identify novel cycling genes. We test knockouts of newly identified circadian genes for changes in circadian behavior. We compare these phenotypes to our stochastic models of the core clock network, with particular attention to the variance in period as well as the mean value. Reflecting a previously unappreciated asymmetry in period variance in actogram experiments, we find that the period distribution in our simulations tends to skew below the mean value in the deterministic models. We find that care must be taken to accurately assign the period of oscillation in a stochastic simulation while taking into account circadian arrhythmia, which affects a sizeable fraction of core clock mutants.

To inform our understanding of the role of noise in our models, we add noise selectively to each species to observe how it propagates through the whole clock. We compare these results to clock mutants as well as mutants of the newly identified circadian genes to verify our predictions of the mechanism by which these genes affect the core clock.

Conclusion

We conclude that empirically correcting for multiple hypothesis testing and searching for asymmetric waveforms provides improved rhythm detection over other methods. We find that using stochastic simulations to explicitly model the distribution of the period in organismal circadian activity provides a useful means of understanding the effects of circadian mutants on the core clock. Future work includes applying our methods to better understand the temperature-independence (temperature compensation) of circadian rhythms and phenotypic mutants.

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Stochasticity and the Mechanism of Precision in the Vertebrate Segmentation Clock

G. Sheela Devakanmalai[1] and Ertuğrul M. Özbudak[1]

***Short Abstract* — Oscillations are prevalent in biological systems. The vertebrate segmentation clock governs the rhythmic segmental patterning of the vertebral column during embryonic development. The period of the segmentation clock dictates the number and sizes of vertebrae. Stochastic gene expression imposes a great challenge to precise embryonic development. To address this issue, we counted single RNA transcripts and determined, for the first time, the amplitude and variability of clock gene expression in an intact tissue. In contrast to previously published computational models, our results unraveled low amplitudes and high variability in oscillatory gene expression, and suggested the presence of sharp transcriptional bursts.**

***Keywords* — Vertebrate Segmentation Clock, Cell-To-Cell Signaling, Ultradian Oscillations, Stochastic Gene Expression, Single Molecule Microscopy, Time-Delayed Feedback Loops.**

I. Background

THE embryonic development relies on precise spatiotemporal patterning. Rhythmic segmentation of the precursors of vertebral column, the somites, during development is one of the most intriguing examples of spatiotemporal patterning [1-3]. Periodic segmentation of somites is controlled by the oscillatory expression of Hes/Her gene family, which is called the vertebrate segmentation clock. Several groups including ours have demonstrated that disrupting oscillations results in vertebral defects [1-2]. The segmentation clock ticks rapidly with a period of 30 minutes in zebrafish. Upon completion of each oscillation cycle, a cohort of 200 cells collectively generate a new segment in zebrafish. The rapidity of oscillations in each cell and the entrainment of oscillations at the tissue augment the challenges in achieving precision in this fascinating developmental patterning. To elucidate the underlying mechanism of precision of the vertebrate segmentation clock, we combined quantitative experimentation with computational analysis.

II. Results

Despite the unavoidable gene expression fluctuations, embryos display robust outcomes. Stochastic fluctuations in gene expression must be buffered under wild-type conditions. The amplitude of oscillations should be tightly controlled but there is no knowledge about the amplitude of oscillations and its variability in any vertebrate species. To fill this critical gap in knowledge, we quantified RNA molecules transcribed by two master duplicated segmentation clock genes (*her1* and *her7*). Previously published computational simulations reported high amplitudes and low fluctuations in oscillatory gene expression. In contrast, our results unraveled low amplitudes and high variability in oscillatory gene expression, and suggested their transcription to occur in sharp bursts. Our results further demonstrate that the intrinsic (or extrinsic) factors dominate gene expression noise at low (or high) expression levels. We propose that two extrinsic factors underlie random and sharp transcriptional bursts: 1) polymerase pausing at the proximal promoters of clock genes, and 2) fluctuations in the levels of transcriptional activators of the clock genes. We further *hypothesize* that stochastic fluctuations in gene expression must be buffered under wild-type conditions by mechanisms of redundancy, cross-regulatory feedback loops and local and long-distance cell-to-cell communication.

III. Conclusion

Oscillations of Hes/Her proteins control the temporal switch from proliferation to differentiation in various tissues [4]. Their gain-of-function correlates with cancer, while inhibition restores differentiation. Elucidating the underlying mechanism of precision in their oscillations is significant for understanding and potentially preventing vertebral malformations, for enhancing stem cell proliferation and developing therapies against cancer, and for advancing predictive modeling of cellular regulatory systems.

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[1] Laboratory of Systems Developmental Biology, Department of Genetics, Albert Einstein College of Medicine, Bronx, NY. E-mail: ertugrul.ozbudak@einstein.yu.edu

Engineered Gene Circuits: From Clocks and Biopixels to Stealth Delivery

Jeff Hasty
University of California, San Diego

Intracellular variability is a major obstacle to the fidelity required for a genetic circuit to execute a series of "pre-programmed" instructions. Over the past five years we have explored how determinism can arise from the synchronization of a large number of cells; in other words, synchronize genetic circuits between individual cells and view the colony as the primary design element. This approach led to three studies describing (i) how quorum sensing can be used to couple clocks between cells (http://biodynamics.ucsd.edu/Intercellular.mov), (ii) how redox signaling can synergistically combine with quorum sensing to couple *colonies* at centimeter length scales (http://biodynamics.ucsd.edu/Intercolony.mov), and
(iii) how intra- and inter-cellular genetic circuits can be rapidly coupled and used to encode information (http://biodynamics.ucsd.edu/Multiplexing.mp4). We are currently using our understanding of these processes to engineer bacteria for the safe production and delivery of anti-tumor toxins. The long-held monolithic view of bacteria as pathogens has given way to an appreciation of the widespread prevalence of functional microbes within the human body. Given this vast milieu, it is perhaps inevitable that certain bacteria would evolve to preferentially grow within tumors and thus provide a natural platform for the development of engineered therapies. We have engineered a clinically tested bacterium to lyse at a threshold population density and release a genetically en-coded anti-tumor therapeutic. Upon lysis, a small number of surviving bacteria reseed the population, thus leading to pulsatile lysis and delivery cycles with a stealth *in vivo* footprint. We have demonstrated the therapeutic potential of the bacteria in a syngeneic colorectal mouse model of cancer using luciferase to monitor the delivery dynamics. This work represents our early progress in transversing the scales of Synthetic Biology from the level of mathematically designed circuitry to therapeutically relevant animal models.

Testing Predictions of a New Model for the Budding Yeast START Transition Using Novel Cell Cycle Mutants

Neil R. Adames[1], Kathy C. Chen[2], P. Logan Schuck[1], John J. Tyson[1,2], Jean Peccoud[1,3]

The cell cycle is the process by which a growing cell replicates its genome and partitions the two copies of each chromosome to two daughter cells. Many of the molecular details of the budding yeast G_1-S transition (START) have recently been elucidated, leading us to expand a previous yeast cell cycle model [1] to include this new information. We tested the accuracy of the new model by performing simulations of various mutants not described in the literature, generating these new mutants, and comparing simulated to observed phenotypes. This approach allowed us to modify the new model to fit nearly all experimental data.

***Keywords* — Cell Cycle, Deterministic Model, Cell Size, Mutant Phenotype, *Saccharomyces cerevisiae*.**

I. Introduction

The eukaryotic cell division cycle is regulated by cyclin-dependent protein kinases (CDKs) that phosphorylate many cellular proteins controlling DNA replication, chromosome segregation, and cell division. In the budding yeast, *Saccharomyces cerevisiae*, the sole CDK is Cdc28. Cdc28 activity and substrate specificity is governed by its obligatory binding partners, cyclins Cln1-3 and Clb1-6 [2]. The transitions between each stage of the cell cycle – G1, S, G2 and M – are controlled by bistable and irreversible biochemical switches involving positive feedback mechanisms [3]. In the case of G1-S transition, the mass of the cell must reach a critical threshold to ensure cell size homeostasis [4].

The molecular mechanisms involved in the G1-S transition – also known as START in yeast – have been well-characterized. In early G1, the only available cyclin is Cln3 and its synthesis is proportional to cell mass [5]. Moreover, *CLN3* mRNA and protein are sequestered at the endoplasmic reticulum (ER) by Whi3 and released into the nucleus to activate two transcription factors, SBF and MBF [6]. Activation of SBF occurs by phosphorylation of Whi5 - a stoichiometric repressor of SBF - by Cln3-Cdc28 [7]. SBF and MBF induce transcription of the partially redundant cyclin pairs, Cln1/Cln2 and Clb5/Clb6, respectively. Cln1/2-Cdc28 induces budding, transcription of S-phase genes and inactivates a stoichiometric inhibitor of Clb5/Clb6-Cdc28 called Sic1 [8]. Clb5/6-Cdc28 then activates numerous DNA replication proteins and the transcription of genes involved in replication.

The switch behavior of G1-S occurs because of the positive feedback from Cln1/2-Cdc28 to fully inhibit Whi5 and activate Swi6 [9].

II. Results and Conclusions

We have formulated a budding yeast START model incorporating most of the new experimental data since Chen et al. (2004). The START model recapitulates the phenotypes of 214/228 yeast cell cycle mutants (among them, 137/145 START mutants and 77/83 FINISH – M-G1 - mutants) described in the literature.

We generated and determined the viability and cell size phenotypes of 15 new cell cycle mutants not described in the literature, and compared the observed phenotypes to the phenotypes predicted by the START model.

The START model correctly predicted the cell size phenotypes of 10 new mutants. The new model also correctly predicted the viability of 2 mutants that were previously described as inviable. For 4 new mutants, the differences in the predicted versus observed cell sizes could be resolved by adjusting model parameters. Changes in model assumptions and architecture were required for the model to correctly predict the viability of other mutants. The current budding yeast START model now simulates the phenotypes of 149/153 START mutants and 80/84 FINISH mutants.

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[1]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg VA 24061
[2]Department of Biological Sciences, Virginia Tech, Blacksburg VA 24061
[3]ICTAS Center for Systems Biology of Engineered Tissues, Virginia Tech, Blacksburg, VA 24061

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Hierarchical membrane compartmentalization stabilizes IFN receptor dynamics

Andre Leier[1], Changjiang You[2], Tatiana Marquez-Lago[3], and Jacob Piehler[2]

Short Abstract — **Nanoscale compartmentalization of the plasma membrane caused by the actin meshwork or membrane microdomains has been speculated to play an important role for the assembly and stability of signaling complexes. Here we present results from a recent experimental and computational study quantifying the role of membrane compartmentalization in regulating the stability and the dynamics of type I interferon receptor complexes. Re-association of individual receptor dimers is promoted in a highly specific manner, ensuring maintenance of signaling complexes beyond their molecular lifetime.**

Keywords — **Receptor dynamics, plasma membrane compartmentalization, spatial-stochastic model.**

I. Introduction

Transport and communication across the plasma membrane frequently involves the association of transmembrane proteins into dimeric or oligomeric complexes. Two-dimensional association and dissociation rate constants determine the interaction dynamics within these complexes. Nano-scale confinement of the interaction partners by the actin meshwork and micro-domains in the plasma membrane has been speculated to play an important role for the dynamics of such protein complexes [1-6]. We recently combined experimental and computational methods to quantify the effects of plasma membrane compartmentalization on the assembly dynamics of type I interferon receptor complexes [7]. Such complexes are relatively long-lived, which makes them ideally suited to study compartmentalization effects.

II. Results

A detailed computer model of the membrane compartmentalization was developed, which was built entirely on experimentally obtained parameters. Simulation results of receptor dynamics were compared with those obtained from single molecule fluorescence microscopy experiments employing dual-color quantum-dot (QD) labeling of receptor subunits. The integration of model building and experiments let to the discovery that a two-tiered compartmentalization was involved in regulating receptor stability.

High-resolution spatial stochastic simulations of receptor hop diffusion in our model membrane further confirmed that confinement enables rapid re-association of dissociated signaling complexes in time frames similar to those of QD experiments. Our computer simulations also reproduced key control experiments.

III. Conclusions

Receptor dimers in the plasma membrane are stabilized beyond the molecular ligand-receptor interactions. Our spatial-stochastic model of a two-tiered MSK faithfully reproduces diffusion and interaction properties in the plasma membrane. The hierarchical organization was found to be critical for explaining the experimentally observed signaling complex stability. Moreover, our spatial-stochastic model enabled us to identify a crucial role of the association rate constant in complex stabilization. We found that efficient stabilization is achieved only beyond a threshold, which corresponds to typical 'on-rates' of protein complexes. Thus, specificity towards stabilization of protein complexes with high 'on-rates' is ensured.

In summary, our findings reveal the important role of plasma membrane compartmentalization for the assembly and stability of the signaling complex.

[1]Okinawa Institute of Science and Technology Graduate University, Onna-son, Japan.. E-mail: andre.leier@oist.jp

[2]Department of Biophysics, University of Osnabrück, Osnabrück, Germany. E-mails: Jacob.Piehler@biologie.uni-osnabrueck.de and Changjiang.You@biologie.uni-osnabrueck.de

[3]Integrative Systems Biology Unit, Okinawa Institute of Science and Technology Graduate University, Onna-son, Japan. E-mail: tatiana.marquez@oist.jp

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Coordinated heat-shock response in *C. elegans*

Ronen B Kopito, Kyung-Suk Lee, Erin Dahlstorm, Wei-Yin Chiang, and Erel Levine[1]

Exposure to high temperatures has an adverse effect on cellular processes and results in activation of the cellular heat shock response (HSR), a highly conserved program of inducible genes to maintain protein homeostasis. Quantitative studies of the HSR in singe cell organisms have been instrumental to our understanding of the principles of control and adaptation, and exemplify the utility of tools from control theory, dynamical systems, and formal models in molecular systems biology. HSR in multicellular organisms, however, adds another layer of complexity: while different cells may be exposed to different environmental cues and different stability requirements, organismic adaptation requires coordination and corporation among cells and tissues. Here we use time-resolved longitudinal imaging of HSR in *C. elegans* to study its dynamics and coordination. By applying precise spatiotemporal perturbation we show that somatic cells integrate local sensation with systemic signals to control the time and level of response. We describe a robust dynamical pattern of activation and deactivation, and implicate sensory neurons in initiating these dynamics. A distributed modeling approach assigns distinct functionalities to the presumed coupling modes in driving specialized but coordinated response.

***Keywords* — Stress response. Spatiotemporal perturbations. Microfluidics. Time-lapse imaging.**

THE heat-shock response is a highly conserved molecular response to environmental conditions that disrupt protein homeostasis [1,2]. Its major role is to prevent protein misfolding and aggregation, both under normal conditions and under stress. In a multi-cellular organism, this is a major challenge, as the proteome of different cells can be markedly different [3]. Heat-shock response (HSR) therefore provides an opportunity to address a fundamental question about signals and regulation in a multi-cellular organism: How does a regulatory network control a coordinated response while at the same time allowing for different levels of activation which meet the specific needs of individual cells?

The control of HSR at the cellular level is highly similar across organisms, from bacteria to human. Multiple control loops link temperature and load of misfolded proteins with activation of HSR and synthesis of protein chaperones that stabilize the proteome. The impacts of these regulatory modules on the robustness of the HSR and on its dynamics have been studied theoretically in multiple organisms, including bacteria [4], fungi [5] and mammalian cells [6], using tools *e.g.* from control theory and dynamical systems.

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[1]Department of Physics and Center for Systems Biology, Harvard University, Cambridge, MA 02138. E-mail: elevine@fas.harvard.edu

Recent results demonstrate that in *C. elegans* activation of HSR is not cell-autonomous, and involves both local interactions and systemic activation [7,8]. Here we study the activation of the insulin/IGF-1 pathway in *C. elegans* as part of HSR [9,10]. Upon activation, the central regulator of this pathway, the FOXO transcription factor DAF-16, is translocated to cell nuclei. A functional DAF-16::GFP fusion allows us to track this HSR in real time at the single cell level. This is done in a custom microfluidic device [11], which permits durable longitudinal imaging of up to 64 worms at a time as well as precise control of the spatiotemporal temperature profile.

By measuring the dynamics of response to spatiotemporal perturbations we show that information on the overall temperature load across the organism is integrated and transmitted systemically. This signal is necessary but not sufficient for cellular activation of HSR, which required in addition local sensation of heat stress. We find a robust dynamical pattern of activation and deactivation of HSR, and implicate sensory neurons in initiating these dynamics. Together, the integration of systemic and local signals balances between the need for coordination and for fine-tuning the response to the needs of individual cells.

Building on established models of single-cell HSR, we investigate the impact of multiple layers of couplings among cells. We characterize the multi-cellular HSR as a network-of-networks, where cellular HSR networks are coupled at different hierarchical levels to form the organismic network. Our results suggest that different forms of coupling serve different functional role, from synchronization and coordination to local fine-tuning. These results can be used to interpret our recent data, delineating the activation pattern of heat-shock proteins.

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Old and new news about single-photon sensitivity in human vision

Philip Nelson.[1]

University of Pennsylvania
Philadelphia PA
USA

ONE often hears that human vision is "sensitive to single photons," when in fact the faintest flash of light that can reliably be reported by human subjects is closer to 100 photons. Nevertheless, there is a sense in which the familiar claim is true. Experiments conducted long after the seminal work of Hecht, Shlaer, and Pirenne now admit a more precise, and in some ways even more remarkable, conclusion to be drawn about our visual apparatus.

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A Model of Lipid A Biosynthesis in *E. coli*

Akintunde Emiola[1], John George[1], and Steven S. Andrews[2]

***Short Abstract* — Lipid A is a highly conserved component of lipopolysaccharide, itself a major component of the Gram-negative bacterial outer membrane. We modeled the nine enzyme-catalyzed steps its biosynthesis in *E. coli*, focusing particularly on biosynthesis regulation, which occurs through regulated degradation of the LpxC and WaaA enzymes. The model agrees with many experimental findings, including the lipid A production rate and the behaviors of several LpxA mutants. Flux control is dominated by LpxC if pathway regulation is ignored, but by LpxK if regulation is present. These results suggest that LpxK may be a useful drug target.**

I. Introduction

LIPOPOLYSACCHARIDE (LPS) is a glycolipid that forms the major component of the outer leaflet of the outer membrane of most Gram-negative bacteria, covering about 75% of the *E. coli* cell surface area. LPS helps stabilize these membranes, protects them from chemical attack, and promotes cell adhesion to surfaces. It elicits a strong immune response in humans and other animals [1].

LPS comprises lipid A, core oligosaccharide, and O-antigen, of which the lipid A component is of particular interest because it is essential for cell viability and highly conserved. These also make its biosynthetic pathway an attractive target for new antibiotics. The lipid A biosynthesis pathway has been investigated thoroughly through several decades of experimentation [1] but has received remarkably little quantitative analysis.

II. Results and Discussion

E. coli lipid A biosynthesis proceeds through nine enzyme catalyzed steps (black arrows in Figure 1). These are well established from careful experimentation, largely by the Raetz group [1]. Lipid A synthesis is regulated (red arrows in Figure 1), at least in part, through controlled degradation of LpxC and WaaA, both performed by FtsH. We assume that FtsH reversibly converts between an inactive state, an active state for degrading LpxC, and a different active state for degrading WaaA [2]. Regulation that directs FtsH to degrade LpxC appears to arise from the lipid A disaccharide concentration, based on published experimental results and on our own experiments in which we overexpressed LpxK, finding that this increased LpxC levels [2]. Regulation that directs WaaA degradation appears to arise from mature lipid A, before it has been transported to the outer membrane.

[1]School of Health, Sports and Bioscience, University of East London, London E15 4LZ, UK. E-mails: A.Emiola@uel.ac.uk, j.t.george@uel.ac.uk.

[2]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109. E-mail: steven.s.andrews@gmail.com.

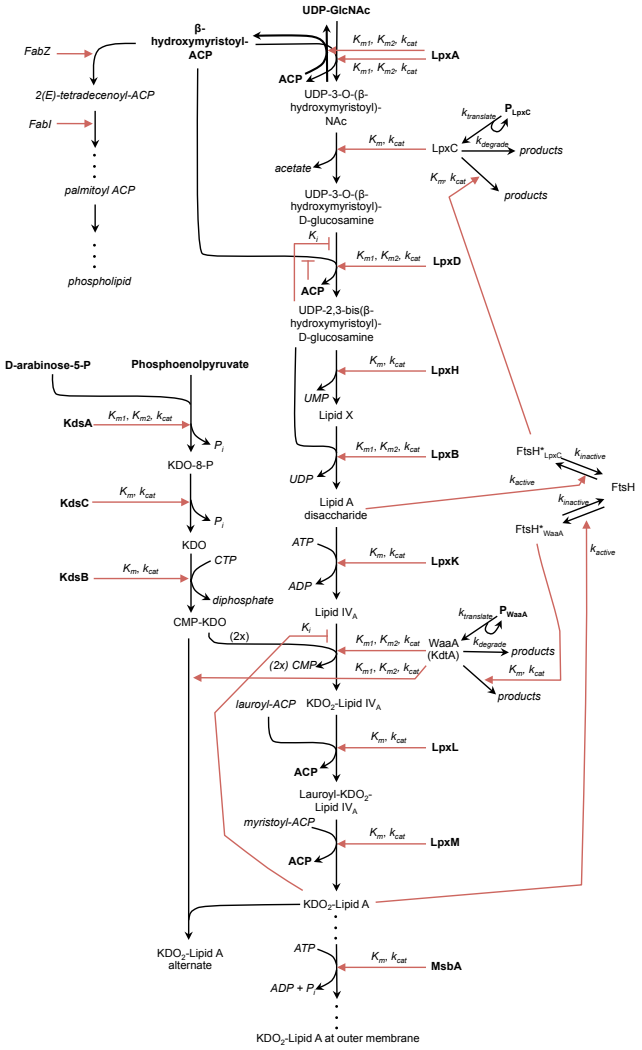

Figure 1. Model of *E. coli* lipid A biosynthesis pathway.

This model agrees with observed lipid A production rates, the behaviors of LpxA mutants, and correlations between LpxC half-lives and cell generation times. It predicts that LpxD can replace LpxA and that there may be metabolic channeling between LpxH and LpxB. It also showed that LpxC is only rate-limiting if pathway regulation is ignored, but that LpxK has the most control if not. Although LpxC has been pursued most often as a drug target, this suggests that LpxK may be a better target.

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Dynamics of estrogen stimulated regulatory networks in breast cancer

Jeanette Baran-Gale[1], Jeremy Purvis[2] and Praveen Sethupathy[3]

***Short Abstract* — Expression of the estrogen receptor α (ERα) is the most significant predictor of breast cancer severity and survival. ERα is overexpressed in the majority of breast tumors, and acts as a transcriptional regulator by cyclically binding to promoter regions and controlling both mitogenic and anti-metastatic pathways. microRNAs and alternative polyadenylation also play a role in the ERα regulatory network, further enriching the potential dynamics by introducing post-transcriptional regulation. Using a network modeling approach, we are examining the dynamics of coding and non-coding genes in the MCF7 estrogen response by integrating mRNA, microRNA, and 3'UTR expression profiles.**

***Keywords* — Estrogen response, microRNA, alternative polyadenylation**

I. Background

DESPITE advances in screening and treatment, breast cancer remains a leading cause of cancer and mortality in women worldwide [1]. The estrogen receptor plays a key role in breast cancer both as a biomarker of cancer severity, and as a therapeutic target to reduce tumor mass [1]. However, recent studies have demonstrated that estrogen signaling also protects tumors against metastatic transformation, suggesting that current therapeutics (ERα antagonists) have the potential to lead to tumor transformation [2].

ERα binds to estrogen and transcriptionally regulates expression of its targets [1]. In addition to ERα-responsive genes, several ERα-stimulated microRNAs (miRNAs) have been identified that post-transcriptionally regulate both ERα and its targets [1]. Also, alternative polyadenlyation (APA) alters the length of the 3' untranslated region (UTR) of mRNAs; thus gaining or eliminating miRNA target sites. While studies have shown that APA differs across cell types and tissues [3], it has not yet been globally investigated in response to a stimulus. It is known that ERα interacts with numerous co-factors to regulate transcription, and stimulates expression of several 3'-end processing proteins [4]. Therefore, we hypothesize that miRNA networks exert temporal control of ERα signaling, thereby regulating the timing and extent of specific cellular responses to estrogen.

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[1]jtbaran@email.unc.edu [2]purvisj@email.unc.edu
[3]praveen_sethupathy@med.unc.edu
Curriculum in Bioinformatics and Computational Biology, Department of Genetics & Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, NC, USA

II. Methods

To identify both coding and non-coding components in ERα regulatory networks, we examine a time course of RNA-seq and small RNA-seq data from MCF7 cells exposed to 10mM estradiol. Analysis of RNA-seq data using the DaPars algorithm [5] combined with 3'-end sequencing [3] allows us to infer APA sites and 3'-UTR usage. Together these data allow us to construct a regulatory map detailing the genes and miRNAs that play a role in estrogen signaling. By studying this map we can: (1) identify temporally regulated genes, miRNAs, and 3'-UTRs; (2) predict miRNA master regulators of ERα-stimulated pathways; and (3) identify candidate therapeutic targets that will interfere with the ERα's mitogenic pathways without eliminating its anti-metastatic functions.

III. Conclusions

Numerous genes and miRNAs respond to estrogen-stimulation, and detailed analysis of ERα occupancy at known targets has shown that the receptor cyclically binds to promoters and initiates bursts of transcriptional activity. ERα-targets such as *TFF1* and miRNA-21 both cycle following estrogen stimulation. Genome-wide profiling of both coding and non-coding transcripts will provide an unprecedented systems-level understanding of the temporal contribution of miRNAs to the estrogen response in breast cancer cells. Finally, these data will provide a dynamic map of predicted novel regulators of the estrogen response that will guide future therapeutic strategies for breast cancer.

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Building bacteria-phage interaction networks using the CRISPR locus

Madeleine Bonsma[1] and Sidhartha Goyal[1]

Bacteria-phage interaction networks provide an important window into the functioning and ecology of microbiomes. Here we utilize the CRISPR locus to build and analyze the structure of such networks. We find that a CRISPR-derived network in Human Microbiome Project data is both nested and modular, and a network constructed from the CRISPRdb database is modular.

Prokaryotes and their phage predators are abundant in many environments and can strongly impact their environments. Importantly, recent evidence links bacteria in the human microbiome to such phenomena as obesity[1], cancer[2], and immune disorders[3]. Phages influence their hosts in turn, producing population-level effects such as gene transfer[4] and mediation of pathogenic bacteria outbreaks[5], an effect exploited in phage therapy[6]. The interpretation of phage-bacteria interaction networks has wide-ranging implications for understanding the role of microbiomes in their environments.

Recently discovered prokaryotic adaptive immune system CRISPR-Cas[7–10] provides another window into bacteria-phage networks. Bacteria and archaea that possess a CRISPR-Cas system can develop a memory of past phage infections by incorporating small samples of phage DNA, called *spacers*, into a specific CRISPR locus. Many spacers can be stored in a CRISPR locus: up to 587 spacers have been documented in *Haliangium ochraceum*, but most CRISPR loci contain fewer than 50 spacers. Unlike previous which largely consisted of direct experiments with cultured bacteria and phages (Ref [[11]] compiles 38 such studies). This method, while yielding detailed and accurate results, is time-consuming to the point of being unfeasible for large networks. As well, only a small fraction of the microorganisms in natural environments can be cultured in a laboratory at all[12], meaning that a significant portion of microbial ecosystems remains inaccessible by this technique.

In this work, we propose and demonstrate both large-scale and small-scale phage-bacteria interaction networks constructed using the information contained in the CRISPR locus. Many species of bacteria and archaea possess the CRISPR system: according to the database CRISPRdb[13], 84% of archaeal genomes analyzed (126/150) and 45% of bacterial genomes analyzed (1176/2612) possess at least one CRISPR region. To the extent that CRISPR-Cas is utilized in a bacterial strain, the CRISPR locus provides a detailed snapshot of phage interaction history, which can be used to construct an interaction map. Displaying bacteria-phage relationships in this way facilitates comparison to previous experimental infection studies and opens the door to ecological analysis of microbiomes using existing network analysis metrics such as modularity (how well a network can be divided into subgroups) and nestedness (to what extent the interaction ranges of members are subsets of other interaction ranges)[11,14–16].

We construct CRISPR-based networks by aligning spacers using BLAST to a compilation of virus and phage databases and recording high-scoring matches. The results are subjected to the same analysis metrics for nestedness and modularity as the traditional experimental infection matrices. The CRISPR networks constructed here exhibit modularity on large scales, consistent with previous work[11]. Clustering between sub-groups of bacteria and phage is potentially indicative of ecologically distinct groups of interacting bacteria and phages.

CRISPR-based networks require much less experimental effort to construct than experimental infection studies. Additionally, CRISPR data can be extracted from metagenomic data with existing approaches[13,17–19] and used to build networks that more accurately capture interactions between bacteria and phages that cannot be lab-cultured. We explore this approach with samples from the Human Microbiome Project[20] using Crass[17] to extract candidate repeats and spacers.

Our analysis shows that a bacteria-phage interaction network in Human Microbiome Project data is nested and modular to a greater degree than two null model datasets, one with random interactions sampled from a Gaussian distribution, and one with the same number of interactions shuffled into random positions. We also find modularity in a large network constructed using CRISPR locus data from the database CRISPRdb[13]. This work shows promise as a method of investigating bacteria-phage interaction networks.

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[1]Department of Physics, University of Toronto, Toronto, Canada.

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The yeast metabolic cycle is coupled to cell division cycle Start across diverse strains

Anthony J. Burnetti[1,2,3] Mert Aydin[3], and Nicolas E. Buchler[3,4]

The yeast metabolic cycle is a synchronous rhythm observed in *Saccharomyces cerevisiae* grown under slow-growth aerobic chemostat conditions. It is known to couple to the cell division cycle, despite both cycles having different frequencies. Multiple interpretations have been proposed for the nature and purpose of this coupling. By quantitatively measuring the metabolic and cell cycle oscillations of multiple strains, we demonstrate strain-specific coupling between metabolic shifts and DNA replication. These data support a model in which metabolic shifts couple to cell cycle Start and the ratio of time spent in different metabolic cycle phases is proportional to the growth rate.

***Keywords* — Saccharomyces cerevisiae, yeast metabolic cycle, cell cycle, coupled oscillations, systems biology**

I. Introduction

The yeast metabolic cycle has been observed since 1969 [1] and consists of a synchronous oscillation in which yeasts growing aerobically alternate between building storage carbohydrates during a low-oxygen-consumption phase (LOC) and their rapid consumption in a high-oxygen-consumption phase (HOC), with large oscillations in the transcriptome content [2,3]. The cell division cycle couples to this oscillation despite having a different period from the yeast metabolic cycle. Specifically, a subpopulation of cells passes through cell cycle Start once per metabolic cycle [4]. The nature of this coupling has been a matter of debate in the scientific literature with DNA replication observed in both HOC and LOC depending on the strain examined and growth conditions used [4]. Understanding the nature of and reasons for this coupling stands to shed light on the nature of coupled oscillations in biological systems.

II. Methods & Results

A. Metabolic Cycle Analysis

Previous research on the yeast metabolic cycle was performed in different strains under different chemostat conditions. We wished to determine which behaviors were invariant across strains and which showed strain-specific variation. We selected two previous lab strains, as well as two wild isolates, to measure and analyze yeast metabolic cycle and cell division cycle events. These strains were grown under identical conditions at a set of varied growth rates. We measured yeast metabolic cycles via quantitative measurements of dissolved oxygen concentration.

We found that the HOC phase length changed little with decreasing growth rate, whereas LOC phase length extends asymptotically as the growth rate slows. This quantitative relationship was invariant across all strains, although the period and dissolved oxygen profile of the metabolic cycle differed between strains. Closer examination revealed that the fraction of time spent in HOC has a positive relationship with growth rate, projecting to ~100% at the growth rate associated with the switch from respiration to fermentation.

B. Cell Cycle Analysis

The cell division cycle was analyzed across strains and growth rates by sampling and fixing cells at 10-minute intervals over the course of several yeast metabolic cycles. We stained for DNA content with SYTOX Green to identify populations of cells in G1 (before DNA replication) and S/G2 (after replication) at each time-point. We found DNA replication could occur in LOC or HOC depending on the strain and growth rate. Each strain, however, has a characteristic delay between entry into HOC phase and initiation of DNA replication. This delay changes very little with differences in HOC length or growth rate.

III. Conclusions

All tested yeast strains exhibited a metabolic cycle, which primarily varies with growth via changes in the length of LOC and exhibits a pulse of DNA replication once per cycle. This pulse comes after a strain-specific delay following HOC entry and can occur in either LOC or HOC.

[1]Program in Cellular & Molecular Biology, Duke University, Durham NC, USA
[2]University Program in Genetics & Genomics, Duke University, Durham NC, USA
[3]Department of Biology, Duke University, Durham NC, USA
[4]Department of Physics, Duke University, Durham NC, USA

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Two-dimensional Modeling on PopZ Bipolarization in Caulobacter Cell Cycle

Minghan Chen[1], and Yang Cao[2]

***Short Abstract* — The asymmetric location of proteins is crucial to the *Caulobacter* cell cycle. The landmark protein PopZ determines the location of the key cell cycle regulators and tethers the replicated chromosome. Experiments demonstrate a self-assembly mechanism for the PopZ polarization. Here, we proposed a two-dimensional model based on Turing mechanism to explain PopZ bipolarization. We explore the parameter set and cell shapes that generate patterns with polar activator. Both deterministic and stochastic simulations capture the observed variations in cell length and time when PopZ becomes bipolar.**

***Keywords* — *Cauoubacter* cell cycle, PopZ polarization, Turing pattern.**

I. INTRODUCTION

Experiments on the bacterium *Caulobacter crescentus* reveal that the bacterial cytoplasm is elaborately organized on space and evolves during the cell cycle [1]. The localizations of proteins determine the cell shape, chromosome segregation event and differentiation [1]. In *Caulobacter crescentus*, the protein PopZ was identified as a potential landmark protein [2]. PopZ locates at the old pole of the swarmer cell and begins to accumulate at the new pole when the gene segregation is initiated in the stalked cell.

While the dynamic localization pattern of PopZ is clearly observed, the mechanism behind PopZ localization is still being revised and debated. This abstract demonstrates our two-dimensional model to explain the reaction mechanism behind the PopZ localization and illustrates the spatiotemporal properties of the cell cycle.

II. MATHEMATICAL MODEL

Experiments show that overexpression of PopZ can lead to cell division defects [3]. PopZ is able to maintain its population level by forming polymers under a self-organization mechanism. The PopZ polymerization is responsible for the PopZ polarization [3]. In order to explain the mechanism behind the polarization, we proposed a Turing pattern mechanism in coordination with the chromosome segregation [4]. Our model can reproduce the bipolarization behavior of PopZ in two-dimensional cell shapes, as well as the stochastic variation on the bipolar time. Figure 1 shows the deterministic PopZ distribution at the end of cell cycle. Figure 2 is a snapshot of an animation that shows the stochastic results during the cell cycle.

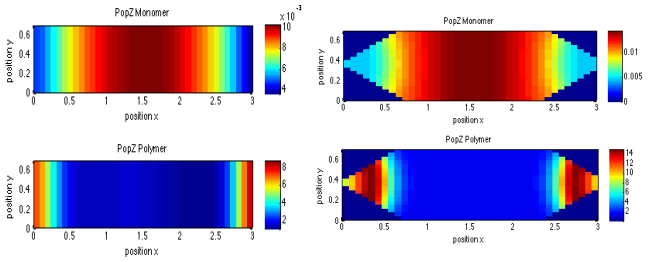

Figure 1: The distribution of PopZ with rectangular cell shape (left) and triangle-end shape (right).

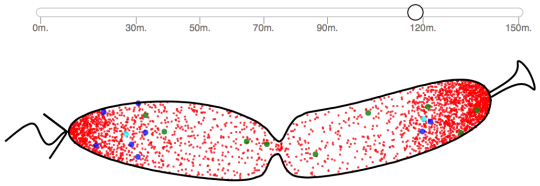

Figure 2: A snapshot of an animation that showing the stochastic results during the cell cycle. PopZ Polymer (red), Monomer (dark green), Gene (light green), mRNA (blue)

III. CONCLUSION

We propose a two-dimensional model for the PopZ localization based on Turing pattern. Under this mechanism, PopZ drives its own spatiotemporal distribution by a self-assembly process. Furthermore, the statistics shows the variant of the timing for chromosome segregation as well as the timing for the PopZ becomes bipolar.

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[1]Department of Computer Science, Virginia Tech, Blacksburg, VA 24061. E-mail: cmhshirl@vt.edu
[2]Department of Computer Science, Virginia Tech, Blacksburg, VA 24061. Email: ycao@vt.edu.

Optimal Experimental Design for Biological Systems

Matthias Chung[1]

***Short Abstract* — How often, when and where should a phenomenon of interest be observed to receive reliable results? Generally, experimentalists face the dilemma between accuracy and costs of an experiment. Each experiment has its own specific challenges. However, optimization methods form the basic computational tool to address eminent questions of optimal experimental design. We will present a general optimization framework for dynamical systems and will illustrate its performance on some key biological models and discuss its relevance to a glucose model for diabetes treatment.**

***Keywords* — Optimal experimental design, optimization, dynamical systems, glucose metabolism.**

I. Background

OPTIMAL experimental design is a growing topic that spans books [1,2] and review papers [3,4]. In particular, optimal experimental designs for biological and medical experiments have grown in importance [5,6,7]. The major idea behind experimental design is to minimize a utility function that reduces the errors or uncertainty in the recovered model parameters. For example, in the A-optimal design the mean square error is minimized, while in the D-design the ellipsoid of uncertainty is reduced. Although adequate for linear problems, these designs based on Fisher information can be grossly inaccurate for nonlinear problems [1,2].

II. New Optimal Experimental Design Framework

Most biological models are based on nonlinear systems of ordinary differential equations. Due to this end, we rather than basing the design criteria on linearization, tackle the full nonlinear problem head-on. The optimal design problem naturally lead to bi-level optimization problems where the nonlinear parameter estimation serves as the inner optimization problem, i.e.,

$$\min_{w} \mathbf{E}_{p_\mathrm{t}} \|p_\mathrm{opt}(w) - p_\mathrm{t}\|$$

$$\text{subject to}$$

$$w \geq 0 \quad \text{and}$$

$$p_\mathrm{opt}(w) = \arg\min_p \|m(y(p)) - d\| \ \text{ s.t. } y' = f(t, y, p).$$

Here, $\mathbf{E}$ is the expected value over the true parameter p_t, p_opt is the recovered parameter given design w, where d are observations and m the projection of the state solution y of the differential equation onto the obervations d, see [7]. We developed method to solve this challenging bi-level optimization efficiently.

III. Results

We will illustrate our optimal experimental design framework on basic biological systems such as logistic growth and Lotka-Volterra systems. Moreover, we present new optimal experimental designs for the intravenous glucose tolerance based on the Minimal Model. Our theoretical investigations also provided surprising results: For some experiments it can be advantageous to rather measure twice at the same time than at different time points.

IV. Conclusion

We developed a robust experimental design framework for problems that evolve from biological applications. The design problem lead to a bi-level stochastic optimization problem where the underlying problem is the nonlinear regression model used to evaluate the parameters. The flexibility of our framework to various types of biological models and design objectives makes it a powerful tool for designing biological experiments.

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[1]Department of Mathematics, Virginia Tech. E-mail: mcchung@vt.edu

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Regulation of T Cell Receptor Phosphorylation

Nicola Coker Gordon[1], Omer Dushek[1]

Abstract — **T cells play a central role in mobilising the adaptive immune system. They perform this function by using their T cell antigen receptor (TCR) to detect antigen, in the form of short peptides bound to MHC (pMHC). Binding of pMHC to the TCR results in TCR phosphorylation, which ultimately determines whether T cells become activated. The phosphorylation of the TCR is regulated by the tyrosine kinases Lck and ZAP and by the tyrosine phosphatases CD45 and SHP-1 but precisely how these molecules regulate TCR phosphorylation remains an open and important problem. In this work, we develop a mechanistic model based on known interactions in the literature. We find that the TCR can produce inhibitory signalling. This has implications for the rational design of therapies for pathologies including HIV and cancer.**

Keywords — **TCR, SHP-1, ZAP70, ITAM phosphorylation, (tandem) SH2 domain, Protein Tyrosine Kinase (PTK), Protein Tyrosine Phosphatase (PTP), Phosphotyrosine (pY).**

I. Introduction

THE TCR is a multi-protein complex comprising CD3 dimers δε, εγ and ζζ, alongside the ligand-interacting αβ heterodimer. The TCR contains 20 phosphorylatable tyrosine which reside as pairs within Immuno-receptor Tyrosine-based Activation Motifs (ITAMs). ITAM phosphorylation is reciprocally regulated by membrane-tethered Src-family kinases (SFKs) Lck and Fyn, and by membrane-integral phosphatases CD45 and CD148. TCR-pMHC ligation leads to an increase in TCR phosphorylation.

Subsequently, fully (doubly) phosphorylated ITAMs constitute binding sites for the tandem SH2 domains (tSH2) of cytosolic zeta-chain associated protein kinase (ZAP70). ZAP70 is activated both allosterically upon TCR-binding, (as its tSH2 are forced to disengage from auto-inhibitory interaction at the PTK domain), and via trans-autophosphorylation [1]. Docked, activated ZAP70 phosphorylates transmembrane Linker of Activated T-cells (LAT). LAT nucleates a signalosome of SH2-containing components of diverse intracellular signaling cascades; downstream effects of which, include changes in gene expression, proliferation and cytokine secretion.

SH2-contaning Phosphatase-1 (Shp-1) is an inhibitory T-cell phosphatase, with ZAP70-mimetic domain structure N-SH2-SH2-PTP-C. The precise mechanism of inhibitory Shp-1 signaling is unknown. There exists empirical evidence for Shp1-mediated induction of TCR dephosphorylation which depends, at least in part, on SH2 domains [2]; Binding of pY-containing ligand by N-SH2 releases its auto-inhibitory, intra-molecular association with PTP, activating Shp-1 by allostery [3]. Motivated by structural data, we hypothesize a model in which (singly or doubly) phosphorylated ITAMs may constitute SHP-1 binding sites.

How ZAP70 and SHP-1 might compete for binding to, and exacting their opposing regulatory effects on, the TCR thus depends on their relative binding affinities and on the balance between singly and doubly phosphorylated ITAMs (I_1 and I_2 respectively).

II. Results / Hypotheses

We are interested in addressing the following questions:

Do inhibitory effectors bind activatory ITAMs?

We identify hypothetical parameter space for which I_1 dominates over I_2, and TCR is predominantly SHP-1 bound.

Why is there multiplicity and pairing of ITAMs?

The extent of ITAM multiplicity correlates with increasing potency, sensitivity and magnitude of response. ZAP70 engagement at paired ITAMs, coupled with its capacity for trans-autophosphorylation augments its activity in a manner which would be restricted on isolated ITAM equivalents [2].

Why do ITAM binding effectors, such as ZAP70, have tyrosines that regulate their activities?

Mathematical modelling indicates that without trans-auto-phosphorylation-based regulation, ZAP70 causes unwanted constitutive basal ITAM phosphorylation.

III. Discussion

The progress of downstream intracellular signaling events associated with T cell activation and ZAP70/SHP-1 regulation of T-cell sensitivity depend on the phosphor-state of the TCR.

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[1]Sir William Dunn School of Pathology, University of Oxford. E-mail: nicola.cokergordon@ox.ac.uk

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Restricted energy dissipation induces glass-like kinetics in high precision responses

Jayajit Das[1]

Short Abstract — **Cell signaling events usually occur in the absence of the detailed balance condition and continuously dissipate energy. Consequently, when energy supply is limited, specific chemical modification steps might not occur due to the lack of energy to support those reactions. How does the absence of such modification steps, that are intrinsically stochastic in nature, affect single cell signaling kinetics? I address this question in the context of a kinetic proofreading scheme used in a simple model of early time T cell signaling. I show using exact analytical calculations and numerical simulations that restricting energy dissipation leads to poorer discrimination in single cells for weak and low affinity ligands. Furthermore, restricting energy dissipation produced substantially larger intrinsic cell-to-cell variations of proteins with qualitatively different glass-like signaling kinetics in single cells marked by ergodicity breaking, dynamic facilitation, and, non-exponential waiting time distribution.**

Keywords — **Entropy Production, Kinetic Proofreading, Dynamic Facilitation, Energy Dissipation.**

I. Introduction

Living systems function in noisy environments and yet are capable of generating surprisingly precise responses. These responses are observed in scales ranging from the molecular to single cells to cell populations. A common feature of such high precision responses is involvement of non-equilibrium processes that requires constant supply of energy to execute the responses. How does the limitation in the available energy resources affect these responses? We address this question here in the context of a widely accepted kinetic proofreading mechanism describing ligand discrimination in single cells.

The concept of kinetic proofreading was proposed by Hopfield[1] and Ninio[2] in the 1970s to explain low error-rates in protein translation. This concept was applied to explain the remarkably sensitive antigen discrimination property of immune cells[3] which are able to distinguish between ligands, close enough to produce complexes of half-lives that differ only by few seconds. A key element in a kinetic proofreading scheme is the presence of a biochemical step that resets any activated state of the receptor to the original state. While this step increases sensitivity of the response it also requires a constant supply of energy that is dissipated away to sustain a non-vanishing probability current in the system. Usually intercellular sources or nutrients absorbed from the microenvironment provide sources for generating ATP, e.g., metabolism of glucose in T cells or tumor cells.

The dissipation of energy in systems functioning outside equilibrium can be quantified by the rate of entropy production in the system[4]. The relation between entropy production and sensitivity of responses has been recently investigated in populations of cells[5]. However, the discrimination is executed by individual cells where the involved biochemical processes is subject to stochastic fluctuations arising from the intrinsic random nature of biochemical reactions and cell-to-cell variations of protein abundances. Therefore, the amount of energy dissipated as the cells execute the discrimination program will vary from cell-to-cell and the results obtained by averaging over cell populations are not guaranteed to generalize at the level of single cells.

II. Results and Conclusion

I addressed the above issue by calculating energy dissipation in single cells and study the role of limiting dissipation by imposing boundary conditions in the Master Equation or by simulating the stochastic kinetics using a continuous time Monte Carlo simulation. I specifically investigated two cases: (1) a constant pool of energy is available, (2) energy is produced at a rate lower than the required energy. Limiting dissipation in the kinetic proofreading program changed the kinetics of the response qualitatively marked by slow kinetics, substantially large cell-to-cell variations, and, more importantly, by the presence of dynamic facilitation [6] and ergodicity breaking in single cell kinetics. Emergence of the last property points to a fundamental disconnect between the activation kinetics in single cells and the cell population averages. The results are likely to generalize in a large variety of systems working in non-equilibrium.

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[1]Battelle Center for Mathematical Medicine, The Research Institute at the Nationwide Children's Hospital and the Ohio State University, Columbus, OH. E-mail: das.70@osu.edu

Comprehensive Modeling and Validation of Glucose and Temperature Compensation of the *Neurospora* Circadian Clock

Andrey A. Dovzhenok[1], Mokryun Baek[2], Jennifer J. Loros[3,4], Jay C. Dunlap[4], Sookkyung Lim[1], and Christian I. Hong[2]

***Short Abstract* — Recently, a transcription repressor, CSP-1, was identified as a component of the circadian clock in *Neurospora crassa* functioning in a negative feedback loop on a circadian transcription factor WC-1. This feedback mechanism is suggested to maintain the circadian period in a wide range of glucose concentrations, which is referred to as glucose compensation. Here, we studied a mathematical model of the Neurospora circadian clock incorporating the above WC-1/CSP-1 feedback loop, and investigated molecular mechanisms of glucose and temperature compensation. Our model shows that glucose compensation is achieved by balancing the activation rates of *csp-1* and *wc-1*.**

***Keywords* — Neurospora, Circadian clock, Glucose compensation, Temperature compensation.**

Autonomous circadian oscillations arise from transcriptional-translational feedback loops of core clock components. The period of a circadian oscillator is relatively insensitive to changes in physiological temperature and nutrients (e.g., glucose), which is referred to as temperature and nutrient compensation, respectively. Recently, a transcription repressor, CSP-1, was identified as a component of the circadian system in *Neurospora crassa*. The transcription of *csp-1* is under the circadian regulation [1]. Intriguingly, CSP-1 represses the circadian transcription factor, WC-1, forming a negative feedback loop that can influence the core oscillator [2]. This feedback mechanism is suggested to maintain the circadian period in a wide range of glucose concentrations. In this work, we modified the existing mathematical model of the *Neurospora* circadian clock [3] incorporating the above WC-1/CSP-1 feedback loop, and investigated molecular mechanisms of glucose and temperature compensation.

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[1]Department of Mathematical Sciences, University of Cincinnati, Cincinnati, OH USA. E-mail: andrey.dovzhenok@uc.edu

[2]Department of Molecular and Cellular Physiology, University of Cincinnati College of Medicine, Cincinnati, OH USA. E-mail: christian.hong@uc.edu

[3]Department of Biochemistry, Geisel School of Medicine at Dartmouth, Hanover, NH USA

[4]Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH USA

Our model shows that glucose compensation exists within a narrow range of parameter space where the activation rates of *csp-1* and *wc-1* are balanced with each other, and that temperature compensation can be achieved by an intricate balance of synthesis and degradation of FRQ and WC-1. More importantly, we experimentally validated loss of glucose compensation in the *wc-1*^{ov} mutant, and maintenance of the abundance of nuclear FRQ as a function of temperature as predicted in the model.

Furthermore, our stochastic simulations demonstrate that the CSP-1-dependent negative feedback loop functions in glucose compensation, but does not enhance the overall robustness of oscillations against molecular noise. Our work highlights predictive modeling of circadian clock machinery and experimental validations employing *Neurospora* and brings a deeper understanding of molecular mechanisms of glucose and temperature compensation.

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Characterization of differences in IGF1 and insulin induced proteomic signaling cascades

Cemal Erdem[1], Alison M. Nagle[2], D. Lansing Taylor[1,3], Adrian V. Lee[2], and Timothy R. Lezon[1,3]

Short Abstract — **Understanding the roles of insulin-like growth factor 1 (IGF1) and insulin signaling in breast cancer is still a major challenge. To tackle this problem, an iterative experimentation-computation workflow is employed using a screen of time-series protein expression profiles. The computation step included utilization of different linear models, where directed networks of time translation are constructed and analyzed to find novel temporal differences between IGF1 and insulin stimulation conditions. Promising results have been obtained pointing out links between intercellular trafficking with Akt signaling, and lipid biogenesis with MAPK cascade. The results confirmed that these inferred interactions are acquired differentially downstream of IGF1 and insulin, in vitro.**

Keywords — **Insulin-like growth factor I (IGF1), insulin, signaling, time-translation, mathematical modeling, IGF1R, InsR**

I. Introduction

THE downstream effects of both insulin-like growth factor I (IGF1) and insulin are different in normal and disease states. Under normal conditions, IGF1 is a proliferation and development factor whereas insulin has a major role of glucose homeostasis [1]. Multiple investigations have shown evidence of the similar functions of the two hormones in cancer progression, escape from apoptosis, and proliferation. There are also studies showing the association of raised blood levels of IGF1 to increased cancer risk [2]. The IGF1 receptors (IGF1R) and insulin receptors (InsR) have high sequence and structural similarities. The two receptors are heterotetrameric receptor tyrosine kinases with disulfide linked two alpha-beta dimers. Each ligand can bind to the other receptor with a lower affinity than the original agonist. There are studies on how ligand binding affects subsequent auto-phosphorylation of the intracellular kinase domains the receptors [3, 4].

Hence, understanding system dynamics downstream of IGF1 and insulin, and finding possible pathway dysregulatory mechanisms are needed. Our approach selects a set of rationally ranked pathway protein candidates from which perturbation experiments are carried out to check the validity of predictions.

II. Methods & Results

The data used in this study included 108 protein expression profiles, either in total or phosphorylated form, at different time points of stimulation with IGF1 or insulin in 21 breast cancer cell lines.

A. Time translation models

The dataset is filtered and proteins with lowest variance values are excluded. Remaining 43 protein profiles are feature centered and normalized.

Linear time-translation (Eqn. 1) matrices (matrix $\boldsymbol{T}$) are constructed using three different methods; (1) covariance matrices, (2) inverse covariance matrices of maximum entropy, and (3) sparse regression matrices determined using lasso [5]. Each model is constructed using serum-free medium conditioned data as time zero (matrix $\boldsymbol{X}$) and a time point of stimulation as the next time point (matrix $\boldsymbol{Y}$).

$$\boldsymbol{Y} = \boldsymbol{T} \cdot \boldsymbol{X} \qquad \textbf{(Eqn. 1)}$$

The time-translation matrices obtained are different for each pair of time-translation and for each hormone stimulation condition.

B. Analyses of time-translation models

The different time-translation models for the same time point but with different hormone stimulation are compared using the highest magnitude interaction values and a list of ranked experimental candidates is generated. Top-ranked candidate experiments are currently under evaluation. Preliminary results confirmed differential effect of caveolin-1 knock-down on Akt phosphorylation in IGF1 and insulin conditions in MDA-MB-231 cells.

III. Conclusion

The computationally scalable reverse-engineering models of cellular networks in a temporal setting introduced here provided us a framework to elucidate experimental targets of pharmacological importance in a cost effective way

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[1]Department of Computational and Systems Biology, University of Pittsburgh, PA. E-mail: cemalerdem@pitt.edu

[2]Department of Pharmacology and Chemical Biology, University of Pittsburgh, PA.

[3]Drug Discovery Institute, University of Pittsburgh, PA.

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Dynamic Intercellular Communication Within Pluripotency Networks

Chad M. Glen[1], Todd C. McDevitt[2], Melissa L. Kemp[1]

***Short Abstract* — Gap junctions, formed from connexin hexamers, act as unique conduits for regulation cellular functions by mediating intercellular molecular transport. We observe connexin 43 (Cx43) to be largely distributed at the edges of embryonic stem cell colonies and more sparsely present at the colony interior. GAP-Fluorescence Recovery After Photobleaching (FRAP) was performed on cells with varying degrees of colony connectivity to characterize spatial features of inter-cellular communication. A preliminary computational agent-based model that incorporates gap junction communication as a function of differentiation produces spatial patterns being used to how direct intercellular molecular exchange may regulate differentiation and loss of pluripotency.**

***Keywords* — Gap junctions, connexins, embryonic stem cells, spatial differentiation.**

I. Introduction

Gap junctions are composed of two hexamers of connexins located in the plasma membrane of two neighboring cells, allowing diffusion of ions and small molecules below 1 kDa [1]. In cell colonies, the concurrent transfer through gap junctions creates an intercellular communication network for small molecules.

Multiple cellular functions are associated with gap junctions and connexins, such as modulation of cell signaling [2]. Specifically, inhibition of gap junctions results in abrogation of spatial patterns during differentiation, suggesting gap junctions provide a novel mechanism of regulating spatial differentiation within cell populations [3].

This work interrogates the intercellular network within ESC colonies and its effect on differentiation using convergent experimental and computational modeling techniques. Implementation of experimentally-determined bimodal transport rates associated with cell phenotypic state into an agent-based model of ESC colony growth and proliferation [4] allows us to examine propagation of spatial patterns within colonies.

II. Results

Mouse ESC cells were fixed, permeabilized, stained with Cx43 antibody, counterstained with Hoechst to identify mitotic cells, and analyzed by confocal microscopy. Cx43 was noticeably expressed by colony edge cells, and displayed distinct membrane localization in cells undergoing mitosis.

A. Intercellular Transport rates

GAP-FRAP is the measurement of fluorescence recovery from the diffusion of calcein, a low molecular weight molecule, into a previously bleached cell from neighboring cells. We observed a bimodal distribution of transport rates within ESC colonies with positional dependence. Cells undergoing mitosis yielded lower calcein intensity at recovery, however the kinetics matched that of non-dividing cells. This finding suggests that the two transport rates reflect changes in gap junction permeability.

B. Modeling Transport on Differentiation

We hypothesize that dynamic connexin expression as a function of colony position will result in different kinetics. Separate transport rates were assigned to differentiated and undifferentiated cells for a generic low molecular-weight molecule. Differentiation was predominantly a function of concentration thresholds of the molecule. Using these rules, spatial patterns of the molecular distribution across the colonies formed and preceded differentiation.

III. Conclusion

Interrogation of intercellular molecular mobility as a dynamic characteristic in ESC colonies via computational modeling provides hypothetical mechanisms of network communication which yield collective behavior of differentiation patterning. Our preliminary experimental and computational results suggest that differentiated cells have faster intercellular transport kinetics, thereby allowing regulatory molecules to propagate spatial differentiation within ESC colonies.

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[2]Gladstone Institute of Cardiovascular Disease Department of Bioengineering & Therapeutic Sciences, UCSF, San Francisco, CA

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Toward a Predictive Model of Spontaneous Clustering of VEGF Receptors

Emine Güven[1], Christopher Short[1], Meghan McCabe Pryor[2], Bridget S. Wilson[3], Jeremy S. Edwards[4] and Ádám M. Halász[1]

Short Abstract — **We analyze sets of transmission electron microscopy (TEM) micrographs of the distribution of VEGF receptors on cell membrane sheets. The images show these receptors tend to localize in small clusters. From a molecular perspective, the existence of these clusters has potentially far reaching implications given the role of VEGF signaling in a range of medical conditions.**

A simple hypothesis on the proximate mechanism of cluster formation is that the clusters form in specific domains in the cell membrane, the chemical and physical properties of these domains result in a locally increased density of receptors. We use this hypothesis to build and parameterize a mathematical model that should reproduce the distribution of cluster sizes across a moderately large sample of images.

I. Background

Signal transduction provides the logical inputs a cell needs in order to perform its role within the organism. The incoming information is processed and a complex bio-molecular network formulates the response. For the vascular endothelial growth factor (VEGF), the initial step is the binding of VEGF (ligand) to its membrane bound receptors. The subsequent activation of receptors concludes signal initiation.

Modern microscopic imaging and labeling techniques reveal certain receptor types tend to co-localize in clusters, ranging from a few to hundreds [1]; consequently, the distribution of membrane receptors on the cell surface is mostly heterogeneous [2]. Our data indicates that this is also the case for VEGF receptors. VEGF mediated signaling is involved in angiogenesis, important in normal development as well as in cancers [3].

Our goal is to go beyond characterization and attempt to provide a predictive model for clustering. We rely on a simple working hypothesis that has emerged from detailed analysis of static as well as dynamic imaging data [5]. We assume that the clusters form through transient residency in membrane domains, with the potential for rapid exchange.

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[1]Department of Mathematics, West Virginia University, Morgantown, WV. E-mail: {eisceviren,cshort3,halasz}@math.wvu.edu

[2]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD. E-mail: mmccabe9@jhu.edu

[3]Department of Pathology and Cancer Research and Treatment Center, University of New Mexico, Albuquerque, NM. E-mail bwilson@salud.unm.edu

[4]Department of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, NM. E-mail: jsedwards@salud.unm.edu

II. Methods

We study TEM images of PAE-KDR cells, porcine aortic endothelial cells that express VEGFR-2 (KDR) receptors. The receptors are labeled with gold nano-particles [4]. The distribution of the labeled receptors is not uniform. We identify receptor clusters through a hierarchic distance based algorithm with a globally optimized characteristic length, and then summarize the distribution of cluster sizes.

We used the hypothesis of attractive micro-domains to build a mathematical model, which provides the probability distribution of cluster sizes. The model parameters are related to the typical size and density of the domains and the relative affinity of the receptors for them. These parameters are not directly measurable. The size of a cluster in an image depends on the number of receptors present in the imaged area, as well as on the labeling efficiency; both may vary substantially. Our approach relies on comparing the model prediction for the cluster size distribution in each image with the one derived experimentally. A Metropolis-Hastings algorithm is used to minimize the overall square distance between the model and experimental distributions.

III. Results and Outlook

We have performed the cluster analysis for a moderately sized set of micrographs, and are working on implementing the global fitting algorithm. The global fit may validate or refute our simple hypothesis. In the first case, the resulting parameters will be useful in estimating the impact of this domain structure on signaling.

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Abrupt sister chromatid splitting in anaphase without obligatory positive feedback

Julia Kamenz[1,2,3], Tamara Mihaljev[4], Stefan Legewie[4], and Silke Hauf[1,2,3,5]

***Short Abstract* — Major transitions within the cell cycle are implemented with positive feedback loops in their regulatory pathways, which ensures that they occur quickly and are irreversible. Chromosome splitting in anaphase is abrupt and synchronous, and it seemed natural to assume that the regulation involves positive feedback. We now show in fission yeast that feedback happens, if at all, far upstream, but not at the level of the direct regulators. Hence, sister chromatid separation, being already irreversible by nature, may be one of the few major cell cycle transitions that can operate without positive feedback.**

***Keywords* — Cell cycle, mitosis, feedback regulation.**

I. Introduction

THE sudden splitting of sister chromatids at anaphase is visually one of the most remarkable transitions in the cell cycle. Anaphase is initiated when the protease separase cleaves cohesin, a protein complex that holds sister chromatids together. Prior to anaphase, separase is inhibited by securin (Nasmyth et al., 2000). Securin degradation proceeds over 4 - 20 min, depending on the organism, but separation of all chromosomes is typically a magnitude faster.

To explain the sudden onset of sister chromatid separation, a switch-like increase in separase activity has been suggested (Hellmuth et al., 2014; Holt et al., 2008; Shindo et al., 2012; Yaakov et al., 2012). Positive feedback is one of the regulatory mechanisms that can induce such switch-like changes and operates at many cell cycle transitions (Ferrell, 2013; Kapuy et al., 2009). A candidate positive feedback loop for sister chromatid separation has been identified in budding yeast (Holt et al., 2008), but it remains unclear whether it is physiologically important and functionally conserved across eukaryotes.

To address this question, we characterized the kinetics of both sister chromatid separation and the underlying securin degradation in fission yeast cells. Combining our quantitative results with computational models suggests that synchronous sister chromatid separation occurs without positive feedback regulation.

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[1]Department of Biological Sciences and [2]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, USA.

[3]Friedrich Miescher Laboratory of the Max Planck Society, Tuebingen, Germany.

[4]Institute of Molecular Biology (IMB), Mainz, Germany.

[5]silke@vt.edu

II. Findings

A. Perturbation experiments do not support the presence of feedback regulation

We find that synchronicity of sister chromatid separation strongly correlates with securin degradation kinetics. Our findings makes positive feedback downstream of separase release highly unlikely.

B. Mechanisms for abrupt anaphase without feedback regulation

Using computational modeling, we show that simple assumptions about securin-separase association or securin degradation are sufficient to explain rapid separase release. These mechanisms ensure a high synchronicity of sister chromatid separation, even without positive feedback.

C. Chromosomes show a tendency to separate in a certain order

Although there is no absolute order, there is a slight tendency of ordered separation. We show how this behavior can theoretically be explained.

III. Conclusion

Although positive feedback loops are ubiquitous in the cell cycle, such regulation may not be necessary for sister chromatid separation. Irreversibility is already ensured thermodynamically through loss of cohesion and the switch-like increase in separase activity can be accomplished by mechanisms other than positive feedback.

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Self-organization mechanism affects the time dependency of molecular diffusion

Takahiro Okuhara[1], Takeshi Kubojima[1], Keisuke Iba[1], Akito Tabira[1], Ryuichi Tanimoto[1], Mitsunori Ozeki[1], Takumi Hiraiwa[1], Yuichiro Nakai[1], Noriko Hiroi[1*] and Akira Funahashi[1]

***Short Abstract* — Intracellular heterogeneity exists to space, time, energy and force. These heterogeneity occurs by fluctuation of reactions which consists of small number of reaction species. At the same time, these elements are not independent each other, moreover affect each others. In this article, we focused the effect of organization mechanism of intracellular structures, which produce spatial heterogeneity and time dependency of molecular diffusion. By investigating spatial characteristics and the time dependent mean square displacement of mobile particles, we conclude that the history of the organization mechanisms of intracellular environment induces time-dependent behavior of mobile particles in the environment.**

***Keywords* — fractal dimension, spatial entropy, molecular crowding, anomalous diffusion**

I. Introduction

INTRACELLULAR environment is crowded with macromolecules [1]. The molecular crowding brings about 50 times higher viscosity to cytoplasm than water [2]. Such environment can result trapping the signaling molecules in cytoplasm. However, actual *in vivo* environment does not prevent molecular transferring from cell surface to nucleus. In order to realize such movement under the condition, heterogeneity of the intracellular environment is significant as once signaling molecules had been trapped, they can be released later. This type of diffusion manner is named continuous time random walk [3]. By considering the process to produce this type of molecular behaviour, the distribution pattern of the cause of viscosity is effective. That means the rules or mechanisms, which decide the distribution of macromolecular structures in a cell may be dominant to control the fate of molecular behaviors *in vivo*.

In this study, we investigated the organization mechanisms of structures of reaction space, which changes the final distribution pattern of structural obstacles. We classified the organization mechanisms into diffusion-limited or reaction-limited. We chose spatial parameters to investigate the characteristics of models and cell images and compared which organization mechanism is more plausible, and estimated the further affect of the difference on mobile particle in the reaction spaces.

II. Results

We constructed 3D volumes of intracellular environment based on transmission electron microscopy (TEM) images. We constructed additional 6 structural models of simply random model, diffusion limited aggregation model, cluster-cluster aggregation model (diffusion-limited models), modified DLA model, Eden model, and modified Eden model (reaction-limited models). We used these 3D volumes to compare the spatial characteristics to know which model could give better explanation of the self-organization mechanisms of intracellular structures.

A. Parameters to describe the spatial characteristics

We analysed 3D fractal dimension, spatial entropy, surface/volume ratio of free mobile space, the volume distribution of restricted volume, and the size of circling space. All these parameters indicated that reaction-limited models keep similar characteristics with TEM 3D image.

B. Comparison of Mean Square Displacement of a free mobile particle in the 3D spaces

We compared the anomalous characteristics of a mobile particle in the 6 simulation spaces. The anomalous diffusion constant of a particle in reaction-limited models showed the similar value with a particle in TEM 3D image.

C. Confirmation of the components of structures and the actual diffusion manner with experiments

We investigated the candidates of the cluster-like structures in 3D simulation spaces by immunocytochemistry. The protein-membrane complexes had the same range of their size with the clusters. Single particle tracking showed that the environment we prepared for the investigation also produced the same anomalous diffusion of a mobile particle.

III. Conclusion

Our results suggest that the heterogeneity to time of molecular diffusion depends on the organization mechanisms of environmental structures.

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[1]Department of Biosciences and Informatics, Keio University, Yokohama, Japan. E-mail: hiroi@bio.keio.ac.jp

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Queueing Entrainment – Downstream control of a synthetic oscillator

Philip Hochendoner[1], Nicholas C. Butzin[1], Curtis T. Ogle[1], and William Mather[1,2]

***Short Abstract* — Using microfluidic experiments, stochastic simulations, and analytical theory, we investigate how a synthetic oscillator in *E. coli* can be entrained via modulation of its protein degradation pathway. The interaction occurs primarily through "queueing" of components for degradation, where proteins compete for the oscillator's primary protease, ClpXP, which effectively acts as a queueing server with a finite bandwidth. We find that periodically varying the production rate of an otherwise independent protein targeted to ClpXP can lead to entrainment, which we understand analytically using a degrade-and-fire formalism.**

Keywords — **Entrainment, queueing theory, oscillators, synthetic biology, systems biology**

I. Introduction

Biological oscillators permeate our daily life, ranging from circadian rhythms, to cell cycles, to our very heartbeats. Control over these systems is often done through entrainment [1], but detangling the mechanism of entrainment tends to be difficult in natural oscillators due to their complex web of interactions.

A complementary strategy to understanding biological entrainment is the synthetic biology approach, where genetically encoded circuits are constructed using known parts with (mostly) known interactions. Previously, investigators successfully leveraged a synthetic oscillator in *E. coli* as a model for transcriptional regulation-based entrainment [2]. In the following, we seek to extend this investigation to explore a particular form of post-translational entrainment, where competition of components for proteolytic machinery leads to the coupling of environment to oscillator. This entrainment mechanism may arise in a number of natural oscillators, since many natural oscillators include analogous proteolytic pathways as one of their essential components.

II. A Synthetic Oscillator and ClpXP Queueing

Our model synthetic oscillator in *E. coli* functions based on two primary ingredients: delayed negative feedback and enzymatic degradation [3]. Focusing on the latter, the oscillator depends on the cell's natural degradation pathways to remove proteins from the system quickly. This degradation is due to the processive protease ClpXP targeting genetically encoded tags on oscillator proteins.

Recent work has revealed that the finite bandwidth of ClpXP naturally leads to a queueing interpretation of protein degradation [4,5], whereby the protease acts as a server for proteins. A consequence is that the protease exhibits classical queueing regimes, such as underloaded and overloaded regimes where competition for the protease is low and high, respectively [6]. These regimes can be experimentally realized using synthetic means [4].

III. Queueing Entrainment

We utilize queueing to couple two sets of tagged proteins: the oscillator proteins and a protein controlled by an externally controlled inducer. Competition for the protease is the primary source of the interaction between the two sets of proteins. This coupling allows us to entrain the oscillator with a wide array of external signals with variable strengths and periods. Entrainment is demonstrated experimentally using a microfluidic platform, which allows for tightly controlled and highly repeatable experiments. The theoretical basis for entrainment stems from the ability for queueing coupling to either dilate or contract the oscillatory period, depending on oscillatory phase. This conclusion is supported by both stochastic simulations and analytic arguments.

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[1]Department of Physics, Virginia Tech. plh5012@vt.edu, ncb@vt.edu, cogle@vt.edu, wmather@vt.edu.

[2]Department of Biological Sciences, Virginia Tech. wmather@vt.edu.

Role for *Clockwork Orange* Gene in Drosophila Circadian Clock

Jiayin Hong[1]

Previous study has suggested a two-loop model for the Drosophila circadian clock gene network which contains a negative feedback loop consists of PER-TIM dimer and CLK-CYC dimer and a regulation loop consists of VRI, PDP1 and CLK-CYC dimer. CLK-CYC dimer which contains a DNA-binding domain can recognize the E-box within the promoter region of *per, tim, vri* and *pdp1* gene. Once binding to the E-box, CLK-CYC will activate transcription of the genes. However, a novel core clock gene – *Clockwork Orange* (*CWO*) was identified in 2007 whose detailed function has not been clearly investigated. Here, I studied the *CWO* function using a mathematical model.

***Keywords* — Circadian clock, Drosophila, Clockwork Orange**

I. INTRODUCTION

CIRCADIAN clock is an internal oscillatory mechanism whose period is around 24 hours and can be regulated by zeitgeber. Circadian clock is widely conserved in fungi, plants and animals and is helpful for organism to sense and adapt the rhythm signal like light and temperature surrounded. Drosophila melanogaster is an ideal model organism to study circadian clock. Previous study has suggested a two-loop model for the Drosophila circadian clock gene network which contains a negative feedback loop consists of PER-TIM dimer and CLK-CYC dimer and a regulation loop consists of VRI, PDP1 and CLK-CYC dimer. Particularly, VRI repress the transcription of *clk* while PDP1 promote the expression of *clk* [1].CLK-CYC dimer which contains a DNA-binding domain can recognize the E-box within the promoter region of *per, tim, vri* and *pdp1* gene. Once binding to the E-box, CLK-CYC will activate transcription of the genes. PER and TIM will be phosphorylated by kinase DBT, CK2 and SGG in cytoplasm. Only by forming a dimer, can PER and TIM be stable or they will be degraded by further phosphorylation. When PER-TIM dimer accumulated to a degree in cytoplasm, they will translocate into nucleus and interact with CLK-CYC dimer to form a tetramer so that CLK-CYC dissociate with the E-box and repress transcription [2].

There were three labs that identified a novel core clock gene – *Clockwork Orange* (*CWO*) nearly in the same time in 2007 [3-5]. Applying microarray and Chip-Seq, they found that CWO will bind to the E-box competing with CLK-CYC dimer so that repress transcription. However, the mRNA level of *per*, *tim* and *vri* is lower in CWO deficient mutant than in wild type which seems inconsistent to common sense. It may be caused by the system effect. Here, I studied the *CWO* function using a mathematical model.

[1]Center for Quantitative Biology, Peking University. E-mail: hongjy@pku.edu.cn

II. RESULTS

The mathematical model consisted of 14 ordinary differential equations and was mainly based on assumptions listed below:

i. All biochemical reactions take place in the whole cellular environment so that the translocation time of protein and nucleic acid can be ignored.

ii. CLK-CYC binding to E-box activate transcription while CWO repress it.

iii. The concentration of CYC is high enough to form dimer with CLK for it is constituted expression in cells. So CLK-CYC is regarded as unity in this model whose concentration is represented by CLK.

iv. As there are kinases catalyzed the degradation of PER and TIM, the model ignored their spontaneous degradation.

Compared with experimental data before, the model can finely simulate the fluctuation of per, tim, clk mRNA level under light/dark condition [6].

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Mapping of deterministic versus stochastic network activity by feedback splitting

Chieh Hsu[1,2], Vincent Jaquet[1] , Farzaneh Maleki[1] , Mumun Gencoglu[1] and Attila Becskei[1]

***Short Abstract* — The functioning of cellular regulatory networks is jointly governed by deterministic nonlinear system dynamics and stochastic fluctuations. They can counteract or amplify each other, and their effects are often difficult to separate, particularly, in the context of feedback regulation. We developed an approach to separate these contributions at molecular level. The parameter space of deterministic bistability was mapped and the nonlinearity due to protein homo-dimerization that supports cellular memory was captured. Furthermore, we showed that with additional noise and deterministic transients characterized, the stochastic activity, the transition rates between the two states of the bimodal expression, can be well predicted.**

***Keywords* — Bistability, bimodality, cellular memory, positive feedback, stochastic transition, protein homodimerization, transcriptional regulation.**

NONLINEARITIES are essential to generate complex cellular behavior, such as bistability, pattern formation or oscillations. Modeling and control of nonlinear systems with the help of classical deterministic kinetics is valid when noise is absent. However, stochastic effects arise in cells due to the small number of molecules in single cells and due to the fluctuations in environmental stimuli and pathway components [1]. They can drive the system away from that predicted by classical kinetics [2].

When nonlinearities are incorporated into positive feedback loops the resulting autocatalytic circuit can maintain two stable activity states under identical conditions [3], a phenomenon termed bistability. Bistability can uphold alternative cellular differentiation states and store cellular memory of past stimuli. Noise induces transitions between the two states of the bistable system so that both states (phenotypes) are present in a cell population, termed bimodality, which is commonly considered to be a hallmark of bistability. However, noise can have deviant effects in the context of positive feedback loops. For instance, bimodality can arise in the absence of nonlinearities and even without feedback loops [4-5], which makes the distinction of deterministic and stochastic effects difficult. Since deterministic nonlinearities and stochastic fluctuations have different origins it is important to disentangle their effects to analyze and control network functioning.

Focusing on positive feedback circuits, we combined three concepts to devise a strategy to distinguish stochastic and deterministic effects. Firstly, opening of positive feedback loops reduces nonlinearity and consequently the deviant effects caused by noise, which permits a deterministic characterization. Secondly, a general mathematical theory states that opening of feedback loops yields input/output relations that map uniquely steady-state values of feedback expression states in a large class of systems [6]. Thirdly, the polymeric nature of biomolecules permits the creation of input/output pairs that are metrically equivalent, a strategy we termed feedback splitting. When these conditions are satisfied, deterministic mono- and bistability can be mapped.

We created well-defined synthetic positive feedback loops that incorporate protein dimerization and/or cooperative binding in yeast cells. With feedback splitting, we confirm that homodimerization can support robust cell memory. With the help of equivalence relations, the nonlinear responses obtained by feedback splitting delimit areas of true steady-state bistability from bimodality. Two distinct factors affecting stochastic transition between states were also identified: transient kinetics which predominately affect the transition in the parameter space outside of the bistable region and a fitted noise which determines the rates within. We showed that the kinetics of a feedback system over a two-dimensional parameter space can be well predicted with the model derived from feedback splitting.

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[1]Biozentrum, University of Basel, Basel, Switzerland

[2]School of Biosciences, University of Kent, Canterbury, Kent, United Kingdom. E-mail: C.Hsu@kent.ac.uk

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Dynamic control and quantification of bacterial population dynamics in droplets

Shuqiang Huang[1], Jaydeep K Srimani[1], Anna J Lee[1], Ying Zhang[3], Allison J Lopatkin[1], Kam W Leong[3] and Lingchong You*[1,2]

Quantification of bacterial population dynamics and gene expression is critical for developing a mechanistic understanding of bacterial physiology and for evaluating and optimizing performance of engineered gene circuits. Such quantification is typically carried out in bulk cultures that are not scalable and require large quantities of reagents. Droplet-based microfluidics can potentially overcome such limitations. But it also suffers from difficulty in dynamically manipulating the chemical environment or in maintaining droplet stability for long-term experiments. We developed a microfluidic system that integrates droplet injection and trapping to overcome these limitations.

***Keywords* — Quantitative biology, microfluidics, droplet injection and bacterial population dynamics.**

I. Introduction

Quantification of bacterial population dynamics and gene expression is critical for developing quantitative insights into gene regulation or bacterial physiology [1, 2]. Droplet-based microfluidics represents a promising alternative of bulk culture to overcome the limitations of large volume and high cost. However, it is also limited by manipulability of the chemical environment, and capability of long-term monitoring of population dynamics to acquire high temporal resolution.

We developed a microfluidic system that is integrated with an electrode-free injection technique for dynamic manipulation of droplets, and a trapping device to allow long-term, stable maintenance and monitoring of individual droplets. As a demonstration of our platform, we used this system to characterize both natural bacteria and engineered bacteria in response to diverse environmental cues, such as cell density and antibiotics.

II. RESULTS

A. Droplet injection and long-term data acquisition

In this work, we explored the electrode-free injection [3] to dynamically control the chemical environment of droplet. The custom device can used to monitor the population dynamics in individual droplets for at least 240 hours. To demonstrate the capability of this microfluidic system, we used it to quantify different population dynamics generated by either engineered or natural bacterial strains.

B. Programmed population control by an ePop circuit

When *ePop* cells were cultured in droplets, they generated population dynamics consistent with the circuit function. The population in each droplet started from a low density (1~5 cells per droplet), and reached its threshold density at approximately the 6th hour when the population crashed. The population then recovered at roughly the 20th hour.

C. Inoculum effect (IE) in response to antibiotics

IE refers to a population-dependent phenomenon in which bacteria at high initial densities are able to survive with intermediate antibiotic concentrations, while populations at low initial densities are eliminated. As our experiments in droplets, when streptomycin was 4μg/ml, the droplets with a high initial density survived, while those with a low initial density did not, which is the defining feature of IE.

D. Programmed altruistic death (PAD) of engineered bacteria in droplets

We then examined the dynamics of PAD of bacteria to test to capability of droplet injection technology. The survival and death of PAD cells are tunable by two chemicals, IPTG and 6-APA. When 6-APA was injected into the droplets containing PAD cells, the population without any induction of IPTG initiated growth for a short time window but stopped growing thereafter. In comparison, when the droplets were also injected with 1mM IPTG, the population in droplets grew to higher densities.

III. Conclusion

Our work demonstrated the feasibility of using liquid droplets to control and quantify dynamics of small bacterial populations, which has implications for the analysis of population dynamics of bacteria or other microbes in diverse contexts.

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[1]Department of Biomedical Engineering, Duke University
[2] Duke Center for Genomic and Computational Biology
[3] Department of Biomedical Engineering, Columbia University
Corresponding author: you@duke.edu

From Clinical Slides to Mathematical Prediction, a Twofold Approach.

Sergey Klimov[1], Remus Osan[2], and Yi Jiang[2]

***Short Abstract* — integrating actual pathological slides, of pre-invasive cancer, with a mathematical model will allow for a better understanding of the potential tumor biological properties which lead to malignancy. In our work we hope to use image analysis of DCIS to uncover areas of high proliferative cellular densities alongside other physical constraints and compare them alongside a cellular potts model via CompuCell3d.**

***Keywords* — DCIS, KI-67 quantification, Clinical parameterization**

I. Introduction

Ductal Carcinoma in Situ (DCIS) is a commonly computationally modeled cancer due to its potential to preclude invasive carcinoma [1]. In order to better understand possible cellular mechanisms which might explain this malignant transition we integrate a novel 2 part approach. For the first part we an automated method to quantify histological slides (IHC) of DCIS, stained with the proliferative marker KI-67, to find regions of high density proliferation and other spatial qualities. These images also allow us to identify basement membrane geometric structure, location of relevant blood vessels, and survival outcomes that are unique to the specific patient. For the second half we stimulate DCIS growth using a Cellular Potts model, via Compucell3d [2], in order to correlate potential similarities between the clinical slide and mechanistic explanations via the in-situ experiments. For our simulation we investigate if alterations in cell cycle length heterogeneity [3, 4] and cell/basement membrane compressibility/deformation [5], within patient specific glucose and oxygen microenvironments, can result in significant tumor structural alterations.

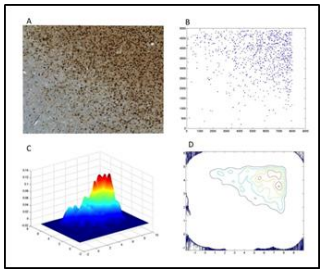
Although calibrations of DCIS models using patient specific IHC [6] and spatial analysis of tumor growth [7] have been done before our model is unique in that it combines membrane deformation, identification of spatial clinical patterns, and spatial alignment of blood vessels.

II. Results

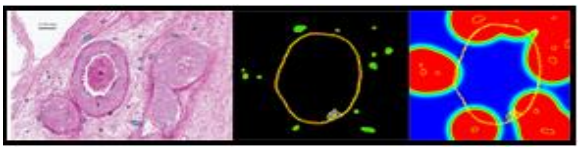
The work is currently in the process of integration, so results are reported alongside the results that we aim for.

A. KI-67 quantification

KI-67 cell pixels were properly stratified by setting a threshold of RGB values that was set at 2 standard deviations around the average of many KI-67 positive cells. The coordinates of these pixels were then ran through a kernel density estimator.



B. Model fit



Integrating CellDraw via CC3D we are able to outline the physical barriers that will be used within our model based on an actual biopsy slide (leftmost panel). The mock simulation of the cell field (middle) shows the basement membrane (red), blood vessels (green), and tumor cells (blue) while the oxygen field (rightmost) shows the potential diffusion limits of oxygen via the blood vessels. We aim to see what parameters can recreate the proliferative densities seen from part A, within the tumors physical constraints.

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[1]Georgia State University, Department of Biology, Atlanta, GA, United States

[2]Georgia State University, Department of Mathematics and Statistics, Atlanta, GA, United States

Robust Parameter Estimation for Biological Systems

Justin Krueger[1], Mihai Pop[2], and Matthias Chung[3]

***Short Abstract* — The use of modeling to interpret data and predict dynamics has significantly contributed to advances in the understanding of cellular regulatory systems, but quantitative methods must continue to adapt to handle the increasing complexity of this understanding. To this end, we have developed a method to address many of the issues associated with traditional parameter estimation techniques for differential equations models of complex dynamical systems. By defining a "nearby" problem, our method improves significantly on the efficiency and robustness of traditional approaches while providing accurate parameter estimates.**

***Keywords* — Parameter estimation, Differential equations, Optimization.**

I. Problem Statement

QUANTITATIVE experimentation and predictive modeling have become significant tools for enhancing biological research. Particularly in the study of cellular regulatory systems, the use of modeling has helped to analyze collected data and focus future research [3]. With experimental methods continuing to improve, the related quantitative techniques must evolve to remain relevant. This means, for example, developing methods for broader ranges of problems and bigger data sets.

In general, the problem statement for parameter estimation for differential equation models involves finding the model parameters that minimize some measurement of error between the solution of the initial value problem and the experimental data set, i.e.,

$$\min_{p} \left\| m(y) - d \right\|_2^2 \text{ subject to } y' = f(t, y, p),\ y(0) = y_0,$$

where d is the given data, t the time, and y the state solution of the initial value problem with parameters p. The function m projects the state solution onto the data d.

Traditional methods such as single shooting methods are used for solving parameter estimation problems for biological systems. These methods require a sequential scheme for solving the initial value problem and have been shown to be inefficient and to lack robustness [7].

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[1]Department of Mathematics, Virginia Tech. E-mail: kruegej2@vt.edu

[2]Department of Computer Science, University of Maryland, College Park. E-mail: mpop@umiacs.umd.edu

[3]Department of Mathematics, Virginia Tech. E-mail: mcchung@vt.edu

II. Robust Parameter Estimates

To improve on the efficiency and robustness of single shooting methods, we build on previous alternatives [2,4,5]. We replace the state solution y with a spline approximation s uniquely defined by spline parameters q and relax the hard constraints to achieve the new problem statement

$$\min_{p,q} \left\| m(s(q)) - d \right\|_2^2 + \lambda \left\| s'(q) - f(t, s(q), p) \right\|_2^2 + \alpha \left\| c(p, s(q)) \right\|_2^2.$$

This equation defines a "nearby" problem that can be solved using more robust numerical methods.

We test our method on *in silico* data generated by generalized Lotka-Volterra equations. We then test our method on data from an intestinal microbiota experiment, and we compare our results to a published parameterized model [1,6]. In the first case, we recover both the parameters and data, and in the second, our method provides improved data recovery relative to the published results.

III. Conclusion

Our approach of defining a "nearby" problem eliminates the computational inefficiency and robustness-limiting step of numerically solving an initial value problem at every optimization step. In doing so, we have not sacrificed parameter or data recovery. This suggests our method improves on the capabilities of traditional parameter estimation methods and is a valuable tool, particularly for the increasingly complex parameter estimation problems like those found in cellular regulatory systems.

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Serotonin-dependent Pulse-Width-Modulation control of food uptake

Kyung Suk Lee[1], Shachar Iwanir[2], Ronen B Kopito[1], David Biron[2,3], and Erel Levine[1]

Short Abstract — **In this study, we examined how food uptake is regulated in *C. elegans*. Combination of microfluidics and automated image analysis enabled longitudinal measurement of individual worms at high temporal resolution in precisely defined environment. We identified the bi-phasic nature of feeding regulation of the worms, where feeding behavior alternates between fast regular pumping and slow irregular pumping. The rate of food uptake is modulated in response to food availability by adjusting the duration of the two modes via the control mechanism as known as Pulse-Width Modulation, where the neurotransmitter serotonin plays essential role.**

Keywords — **Pharyngeal Pumping, Regulation of food uptake, Serotonin, Pulse-Width Modulation**

I. Background information

THE regulation of food uptake is a critical mechanism with major physiological impact [1-4]. To understand the mechanism of how food uptake is regulated, there has been interest in using feeding of *C. elegans* as a model system [5, 6]. The nematode feeds on bacteria, and it is facilitated by the action of the pharynx, a neuromuscular pump that draws bacteria suspended in liquid into the mouth from the surrounding environment, and transports them to the intestine after concentrating and grinding [7, 8]. Pharyngeal pumping is therefore a primary indicator of food intake and consequent growth.

Previous results suggest that pharyngeal pumping depends on feeding history and quality of food through a mechanism that involves the neurotransmitter serotonin (5-HT, 5-hydroxytryptamine) [9-11]. Serotonin increases feeding rate of *C. elegans* [12] and has been suggested as a putative food signal that controls feeding of the animal [13].

II. Summary of results

Conventional feeding assays are performed on dense bacterial lawns which do not allow fine control of food concentration. Thus, to probe how food uptake is regulated in response to food availability, we employed a custom microfluidic device [14] that enabled us to precisely control the concentration of available food and to monitor the dynamics of pharyngeal pumping at high resolution. At various food concentrations, we probed the time courses of pumping of individual worms, which revealed switching dynamics between two pumping modes. In one mode, worms pump regularly at maximum rate and, in the other mode, they pump sporadically. We show that the durations of the two modes are modulated in response to food concentrations, and the average feeding rate is determined by the fraction of time spent in the fast mode.

Using strains that lacks serotonin biosynthesis or serotonin receptors, the essential role of the neurotransmitter serotonin in the regulation of feeding in the nematode was demonstrated.

III. Conclusion

With our data, we show that feeding of *C. elegans* is controlled by a serotonin-dependent Pulse-Width-Modulation mechanism, with a duty cycle that depends on the availability of food.

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[1]Department of Physics and Center for Systems Biology, Harvard University, Cambridge, MA 02138. E-mail: klee04@fas.harvard.edu

[2]Department of Physics and the James Franck Institute, The University of Chicago, Chicago, IL 60637

[3]The Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL 60637

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Phenotypic models of T cell activation

Melissa Lever[1], Hong-Sheng Lim[1], Philip K. Maini[2], P. Anton van der Merwe[1] and Omer Dushek[1,2]

T cells are white blood cells that play an important role in immunity. While much progress has been made in discovering what molecules constitute the signaling network within the T cell, the functional relationship between these molecules remains unclear. We take the approach of generating a phenotypic model in order to understand this network, informed from a quantitative dataset generated by stimulating T cells expressing a high affinity therapeutic receptor with ligands that have a million-fold range in affinity. This modelling approach can explain the entire dataset, the majority of published data and help elucidate the signaling network.

Phenotypic models, T-cell signaling, systems biology, signal transduction, immunology

I. INTRODUCTION

T cells are important immune cells that initiate and regulate the adaptive immune response to infections and cancer. Much progress has been made in molecular immunology to identify the molecules that form the signaling network inside the T cell [1]. However, this network is complicated and it is unclear how these molecules functionally interact with each other. Numerous experimental studies have shown that it is the binding parameters between T cell receptors and their ligands that determine the functional response of the T cell [2-5]. Knowledge of the relationship between stimulation strength and response can offer insight into the structure of the signaling network. Despite extensive study, there is still no mathematical model that can explain this relationship consistently with the published data [6]. We have taken the approach of developing a phenotypic model inferred by a quantitative dataset in order to elucidate this signaling network.

II. RESULTS

We present a phenotypic model of T cell activation that has been inferred from a quantitative dataset. The dataset has been generated by stimulating T cells expressing a therapeutic high affinity T cell receptor with ligands that span a million-fold range in affinity. The phenotypic model consists of kinetic proofreading with limited signaling [6] coupled to an incoherent feed-forward motif [7]. The model is able to explain all key features of the dataset: ligand discrimination, an optimal ligand binding time and an inhibition in the response at high doses. It is also able to explain the majority of published data [2-5,8]. Furthermore, we can show how the model can inform where molecules lie in the signaling network. By comparing perturbations of the model with knock-down experiments, the role of a molecule within the signaling network can be found.

III. CONCLUSION

We have shown how the approach of generating a phenotypic model of T cell activation can yield a tractable model that can explain the experimental data and provide information on the structure of the underlying signaling network. It is an approach that can be applied to signaling networks more broadly.

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[1]1Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, Oxfordshire OX1 3RE, UK

[2]Wolfson Centre for Mathematical Biology, Mathematical Institute, University of Oxford, Oxford, Oxfordshire OX2 6GG, UK

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A Model for β-cell's death during ER stress

Xiang Liu[1]

The main causes and developing processes for the type II diabetes is still largely unknown. Yet obvious loss of β-cell in islet has been observed when diabetes happened. Both ER stress and apoptosis induced by it play important roles in β-cell loss. To answer how such apoptosis come into being, we have built a mathematical model to describe the molecule network from ER stress to cell death. Such model finds a way to illustrate the underline principle of single β-cell decision making facing with different inner environment.

***Keywords* — β-cell, Apoptosis, ER stress, Type II Diabetes.**

I. Introduction

The number of people living with, and dying from, diabetes across the world is shocking [1]. There are two major forms of diabetes, type I and type II. The latter type accounts for almost 90% of all cases of diabetes in adults worldwide [1]. As a chronic disease, the main causes and developing processes for the type II diabetes are of great complexity. Nevertheless obvious loss of β-cells in islets has been observed when diabetes happened [2]. It's has also been observed that at the early stage of diabetes, the number of β-cell in each islet suffers a large amount of decrease as well as the number of islets in pancreas [2]. Thus, deciphering how β-cells lost during the happening of diabetes is not only of great interest in science but also potentially important in clinic.

The β-cell in islets play a role in secreting insulin, a hormone regulates storage of glycogen in the liver and accelerates oxidation of sugar in cells. The β-cell is under a great 'pressure' when the blood glucose is high and it has to secret a great load of insulin. A part of such pressure comes from the ER stress. ER stress raise from the situation that a cell translates too much protein at a time. This unfolded protein has to be folded and processed accurately and efficiently in endoplasmic reticulum (ER) [3]. As a 'bottleneck', usually there will be considerable unfolded protein accumulating inside endoplasmic reticulum which makes a chain of reactions to release such bottleneck. This situation is the so called ER stress and the following reactions are unfolded protein response (UPR).

UPR has been extensively studied by many groups [3][4]. However, the role UPR play in the β-cell's death remains unknown. It is known that UPR can activate cell apoptosis [5]. But at what extend UPR will cause apoptosis almost surely is still a problem. The 'extend' here refers to the time, intensity and even the action pattern of ER stress. We aim to answer the problem above thoroughly.

II. Summary of Results

We have established an ODE model to simulate the process from the upstream ER-stress sensor to the whole downstream UPR system, including some proapoptosis gene such as CHOP. The input of this model is the amount of unfolded protein's mRNA. This model includes two main feedback to repress the unfolded protein accumulating in endoplasmic reticulum and one of its feedback, will simultaneously induced CHOP. We simulated the ODE model, compared it with the experimental data came from our collaborators. It shows that our model fits their data qualitatively. And we furthered our research on this model into analytical part. Especially on what kind of input situation will the final expression level of CHOP lead the cell to apoptosis. Some improvement has been made on this part of job, and we'll complete it in the future.

It has been found that ER stress induced apoptosis is not only type of cell death [6]. This means that the model has to update, including more relative pathway.

III. Conclusion

We have established a feasible ODE model to describe the whole process from different kind of unfolded protein input to the final CHOP expression level. And we have made some analytical job on this model in order to give an explanation of this process and answer the question of why β-cell choose to die.

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[1]Center for Quantitive Biology, Peking University, Beijing 100871, China. E-mail: lx199156@pku.edu.cn

Deep Learning of Cell Morphologies for Kinome Wide Screening

Mathias Engel[1,2], Xavier Robin[1], Jesper Ferkinghoff-Borg[1], James Longden[1], Rune Linding[1]

***Short Abstract* — Integrating individual and population based cell data, across mulitple experimental methods is crucial in gaining new biological understanding. We present a deep learning probabillity model that can handle the integration of different data types with predictive power. The model is deployed to understand resistance in a screen consisting of kinome-wide RNAi knockdowns throughout multiple cell lines and cancer therapies.**

***Keywords* — cell morphology, resistance, high content screening, kinome, deep learning architectures, RNAi.**

I. Introduction

Today the life science community has several large data producing methods at its disposal, such as next-generation sequencing, mass spectrometry and high content imaging. We can use these techniques to probe behavior and signalling of cells in a systematic and statistically robust manner. Furthermore we can now investigate these cell dynamics in vastly different length scales ranging from genes to full organisms. However although these techniques have led to extremely valuable insights they are often difficult to combine numerically in a predictive probabilistic model. Here we present a new method to handle data integration in combination with a large kinome wide screen aimed at understanding resistance.

II. Data Production

An Opera high content imaging system with cell:explorer robotics (PerkinElmer) was used to conduct multiple kinome wide RNAi knockdown screens. The full image data consists of 2.41 million images distributed on 17 cell lines with 11 anti-cancer therapies. The Opera microscope produces images each containing several hundreds of cells, while still maintaining sufficient resolution to extract cell-specific morphological information (textures, geometries, size). Cells were stained with Hoechst 33342 (nucleus) and Rhodamine phalloidin (F-actin).

Morphological features were extracted using Acapella image analysis software (PerkinElmer). This produced a feature vector containing thousands of entries per cell. This vector was stored in a database to facilitate easy access for the subsequent modeling work.

III. Purpose

It is already known that a connection exist between local signaling networks and morphological phenotypes (Bakal et. al., 2007)[1]. However, this study only aimed at discovering distinct subgroups of morphologies from the feature vectors.

In order to correlate and predict relations between morphologies and various prior information known from the experimental design (cell line, treatment, RNAi) in a bidirectional manner, a different model is needed. This model should be capable of combining uncertanties across different datatypes including both population and cell specfic information.

We will therefore exploit the properties of deep learning architectures (Hinton et. al., 2006)[2,3] to model the probability distribution of the morphological space across multiple conditions. All prior information as well as additional data (e.g. cell count, mRNA expression) can be directly included as input to the model through binary encoded vectors.

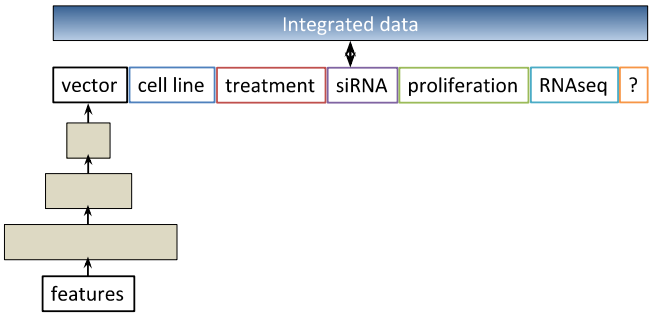

Figure 1. Schematic overview of the data integration in the deep architecture model.

IV. Conclusion

By exploiting deep learning architectures we will model the morphological feature space of multiple cell lines exposed to both kinome-wide RNAi knockdown and different treatment conditions. This will facilitate the systematic identification of the network rearrangements that occur after kinase knockdown, exposure to anti-cancer therapies and combinations thereof.

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[1]Linding Lab, Biotech Research & Innovation Center, UCPH, Denmark.
[2]Niels Bohr Institute, UCPH, Denmark.
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Rhythmic Degradation Explains and Unifies Circadian Transcriptome and Proteome Data

S. Lück[1 2], K. Thurley[1], P. F.Thaben[1], P. O. Westermark[1]

***Short Abstract* — In many mammalian tissues, 10% of all transcripts display a 24-hour rhythm in abundance. These abundane profiles are thought to be driven by the "circadian clock", a regulatory network of transcription factors.**

Recent studies have uncovered that these transcripts experience a widespread circadian post-transcriptional regulation. Using an ODE-model with time-dependent rates we have recently shown that the assumption of rhythmic half-lives can explain the mismatch of measured peaks of pre-mRNA and mRNA. The model predicts that peak phases of ca. 30% of oscillatory mRNA in mouse liver and fly brain are determined by rhythmic degradation. An expansion to a PDE allows us to include a measure for the molecule's age, and thus study oxidative protein damage or polyA-tail shortening.

***Keywords* — Circadian clock, rhythmic degradation, partial differential equation, transcriptome**

MANY behavioral, physiological, and biochemical activities show a circadian rhythm. This means they continue to oscillate under constant conditions with a period of about a day and are entrained to daily environmental cycles. On the cell level the circadian clock, a negative feedback loop in gene transcription and translation, influences several transcription factors [1]. Consequently, in many mammalian tissues 10% of all transcripts, and a possibly even higher percentage of all proteins, display a 24-hour rhythm [2,3].

Recent high throughput studies elucidate the circadian regulation on various levels of gene expression. Oscillating abundances can be found in nascent RNA, mature RNA and protein concentrations. The results have been enigmatic because transcript peak abundances do not always follow the peaks of gene-expression activity in time [4]. We posited that circadian degradation of mRNAs and proteins plays a pivotal role in setting their peak times. To establish guiding principles, we derived a theoretical framework that fully describes the amplitudes and phases of biomolecules with circadian half-lives [5]. We were able to explain the circadian transcriptome and proteome studies with the same unifying theory, including cases in which transcripts or proteins appeared before the onset of increased production rates. Furthermore, we estimate that 30% of the circadian transcripts in mouse liver and *Drosophila* heads are affected by rhythmic posttranscriptional regulation.

In a second approach we expand the view on a molecule's life and include a measure of a molecule's age. We address the question in which cases there is an advantage of rhythmic instead of constant degradation of long-lived proteins when they accumulate oxidative damage. Secondly, in a collaboration with Carla Green we use the same model to analyze sequencing data of poly(A) tails of mRNA in order to identify bottle necks in (rhythmic) mRNA degradation.

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Acknowledgements:

[1]Institute for Theoretical Biology, Charité – Universitätsmedizin, 10115 Berlin, Germany

[2] E-mail: sarah.lueck@hu-berlin.de

Minimal Regulatory Network of Extrinsic and Intrinsic Factors Recovers Patterns of CD4+ T Cell Differentiation and Plasticity

Mariana Martinez-Sanchez[1,2], Luis Mendoza[3], Carlos Villarreal[4,2], Elena R. Alvarez-Buylla[1,2]

***Short Abstract* — T CD4+ lymphocytes differentiate in different cell types in response to the cytokines present in their environment, this differentiation is not terminal, as this cells are plastic. In this work we construct a minimal regulatory network which recuperates the attractors corresponding to Th0, Th1, Th2, Th17, Tfh, iTreg, Th9 and various Foxp3-independent T CD4+ cells. Using this network we studied the effect of the cytokines in the environment and of directed perturbations in the differentiation and plasticity of this cells. Finally, we determined the key nodes of the network for the differentiation and plasticity of T CD4+ lymphocytes.**

***Keywords* — Regulatory boolean networks, differentiation, plasticity, T CD4+ lymphocytes**

I. Introduction

The immune system is a complex system of biological processes and structures that protects the organism against a variety of pathogens with specialized responses and is non-reactivity to itself maintaining the homeostasis of the organism. T CD4+ lymphocytes, also known as T helper (Th) cells, play an important role orchestrating the immune responses to various infectious agents. Naive T CD4+ lymphocytes (Th0) are activated when they recognize an antigen presented by an antigen presenting cell in a secondary lymphoid organ. Depending on the cytokine milieu and costimulatory signals in their environment CD4+ T lymphocytes differentiate into different cell types, expressing specific transcription factors, membrane molecules, and cytokines, which affect the behavior of the rest of the immune system[1,2]. This cell types include: Th1, Th2, Th17, Tfh,, iTreg, Th3, Tr1 and Th9. Once differentiated, T CD4+ lymphocytes can change their expression profile, making this cells plastic[3].

The differentiation and plasticity of CD4+ T lymphocytes depends on the complex molecular interactions between the molecular elements of the network. This networks have been studied with systemic and formal approaches mainly using dynamic and autonomous regulatory network models which recuperate the differentiation of this cells[4-6]. However, the effect of specific alterations in the elements of the molecular network and the cytokines in the environment in the differentiation and plasticity of this cells is still an open question

1 Departamento de Ecología Funcional, Instituto de Ecología, Universidad Nacional Autónoma de México, Coyoacán, México DF 04510, México
2 Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Coyoacán, México DF 04510, México
3 Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México DF, México
4 Departamento de Física Teórica, Instituto de Física, Universidad Nacional Autónoma de México, México DF, México

II. Results

We first explored models considering solely the interaction between master transcription factors as necessary and sufficient elements to explain immune system differentiation. Those models turned out to be false; they cannot explain the origin of all the expected T CD4+ cell types or their plasticity. After several refinements, we ended up with a minimal model including transcription factors, signalling pathways and, intrinsic and extrinsic cytokines as its components. This latter model was fully capable of explaining both the whole set of T CD4+ cell types (Th0, Th1, Th2, Th17, Tfh, Th9, iTreg and Foxp3-independent regulatory T CD4+ cells) and their plasticity.

The analysis of this minimal regulatory network also sheds light in the stability of the system's attractors -which correspond to different cell kinds- and the global plasticity of the differentiation process. We predict a cell fate map showing which perturbations of the components lead to transitions between subsets. This cell fate map changes in different polarizing environments, displaying how extrinsic signals alter the proportions and stability of the different T CD4+ subsets. Also, we analyzed the components of the minimal regulatory network in the global behavior of the system; in particular, the role of SOCS proteins , inhibitors of the signaling pathways, in the integration of molecular signals and plasticity is a novel discovery. The model is qualitatively congruent with the literature regarding how plasticity is affected by the micro-environment.

III. Conclusions

The quantity and diversity of the interactions involved in the differentiation of T CD4+ lymphocytes makes the behavior of this cells extremely complex, complicating the understanding and the clinic applications of the system, as the effects are not always direct. Studying the molecular network as a dynamic system lets us understand how the interactions between the components create the complex behaviors that let the immune system defend the organism against pathogens and maintain homeostasis and self-tolerance. T CD4+ lymphocytes are a complex, dynamic system, and modeling the system from this approach will give us insights into the richness of the system and its interactions.

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Ranking proposed yeast feedback networks through Approximate Bayesian Computation parameter estimation and model selection.

Patrick McCarter[1,3], Justin English[2], Henrik Dohlman[1,2,3], Tim Elston[1,2].

Stress-Activated Protein Kinase (SAPK) pathways utilize specialized Mitogen-Activated Protein Kinases (MAPK) to protect cells against environmental stressors. However dysregulated SAPK pathways are also found in several pathologies. In this work we apply a multi-disciplinary research strategy that combines mathematical modeling, model selection, biochemical and genetic experimentation in the *S. cerevisiae* (yeast) High-Osmolarity Glycerol (HOG) pathway to better understand how MAPKs coordinate responses to environmental stress.

I. Purpose

Mitogen-Activated Protein Kinases (MAPK) protect cells against ischemia[1], hyper-osmolarity, uv-irradiation[1], [2] and other stressors. However dysregulated MAPK pathways are also found in Alzheimer's Disease[5], Amyotrophic Lateral Sclerosis[3] and cancer[4]. In this work we detail a multi-disciplinary research effort that combines mathematical modeling, biochemical and genetic experimentation in the *S. cerevisiae* (yeast) High-Osmolarity Glycerol (HOG) pathway to better understand how MAPKs coordinate responses to environmental stress. The HOG pathway is a prototypical Stress-Activated Protein Kinase pathway that transmits osmostress to the Hog1 MAPK via two distinct signaling branches (Sho1, Sln1). Hog1, which is homologous to the mammalian p38 and JNK kinases, translocates to the nucleus upon activation, where it induces activation of stress response genes.

Using Phos-Tag polyacrylamide western blot analysis[5], we have shown that Hog1 activation is encoded via positive feedback and that deactivation is encoded via negative feedback[6]. We use mathematical modeling to identify the most likely feedback network that dynamically regulates Hog1 in response to sustained hyper-osmotic stress. We have thus far been successful in identifying models that agree with our preliminary experimental data. We now extend our modeling process to objectively rank the models using Approximate Bayesian Computation (ABC) techniques to perform model selection[7].

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Curriculum in Bioinformatics and Computational Biology[1], Department of Pharmacology[2], Department of Biochemistry and Biophysics[3], University of North Carolina School of Medicine, Chapel Hill, NC, USA

II. Conclusion

Many signaling mechanisms discovered in yeast are conserved in human cells. Understanding how MAPKs are regulated during stress and how MAPK dysregulation contributes to pathology is critical for human health.

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Actin-Membrane Interactions in Membrane Nanotubes

Sina Mirzaeifard[1] and Steven M. Abel[2]

***Short Abstract* — Membrane nanotubes are membrane structures that contain actin and connect cells over long distances. To gain insight into membrane nanotubes, we use theory and computer simulations to study continuum models of semiflexible polymers confined within elastic membrane tubes. Monte Carlo simulations allow characterization of typical configurations of the polymer and membrane as a function of parameters such as polymer persistence length and membrane bending rigidity. In the regime of low membrane bending rigidity, we find the presence of a polymer within the membrane suppresses membrane shape fluctuations, which is reflected in a decrease in the specific heat of the membrane.**

I. INTRODUCTION

MEMBRANE nanotubes are long and slender tubes formed from the plasma membrane, and can vary in size, structure, and formation processes. It is speculated that cells use membrane nanotubes as a means of intercellular communication over long distances, and it has been shown that viruses can propagate from one cell to another by means of membrane nanotubes [1-3].

A number of theoretical and computational studies have investigated tubular extensions from cells [3-5]. However, the relatively recent discovery of membrane nanotubes provides an interesting model system that may provide a greater understanding of membrane-cytoskeleton interactions. We study membrane nanotubes using a theoretical framework based on continuum models. On the length scales relevant to membrane nanotubes, many key attributes of actin filaments and cell membranes can be captured by regarding them as semiflexible polymers and thin elastic sheets, respectively. We employ analytical theory and Monte Carlo computer simulation methods to explore properties of polymer-membrane systems. We simulate discretized representations of both polymers and membranes in which particles are connected by edges to represent the objects. For the flexible membranes, we consider triangulated surfaces in which the vertex connectivity is not fixed as a way to confer fluidity to the membrane.

[1]Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville. E-mail: smirzaei@vols.utk.edu

[2]Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville. E-mail: abel@utk.edu

II. RESULTS

We begin by using analytical theory to calculate the energies of various polymer-membrane configurations. This provides a reference point for analyzing later results and suggests the favorability of certain polymer configurations. We then use Monte Carlo simulations to study the equilibrium properties of membranes in isolation. We consider effects of the membrane bending rigidity, and at small values, we observe a peak in the specific heat of the membrane, which is calculated based on energy fluctuations. Characterizing the membrane shapes sampled in simulations indicate that this peak is associated with a transition from a crumpled membrane to a locally flat membrane.

We next consider the effect of a semiflexible polymer, representing a bundle of actin, within an elastic tube. We find that fluctuating tubes allow enclosed polymers to equilibrate more quickly when compared with polymers confined within rigid tubes, but that the presence of a polymer has small effect on properties of the membrane at typical cell parameters. At low bending rigidity, we find that the peak in the specific heat of the membrane can be suppressed by the polymer in a manner that depends on the length and persistence length of the polymer.

III. CONCLUSION

Membrane nanotubes provide a novel system from the perspective of studying membrane-cytoskeleton interactions, and our results suggest an interesting interplay between polymer and membrane properties. Further efforts are needed to understand the role of additional factors (e.g., actin-associated proteins) and their behavior in biological contexts.

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How periodic signals can tell time

Michele Monti[1] and Pieter Rein ten Wolde[1]

***Abstract* — The coordination of biological activities in daily cycles provides an important advantage for the fitness of several organisms. The circadian clock in cyanobacteria *S. elongatus* drives daily genome-wide oscillations in mRNA expression levels, controls genome compaction and supercoiling, and modulates cell division. The clock contains a core oscillator consisting of the proteins KaiA, KaiB, and KaiC, which together generate circadian oscillations in the four phosphorylation states of KaiC. This clock controls the circadian expression of the transcriptome via phosphorylation of a single protein, RpaA. In this project we address how reliably temporal information can be transmitted from the clock to downstream genes. We have developed a mathematical model that describes how cells can infer the time from an ensemble of oscillatory genes. Using techniques of Statistical Mechanics and Information theory, we are able to compute how many time-states cells can uniquely distinguish. This enables us to analytically derive the error in measuring the perception of time.**

***Keywords* — Circadian Clocks, Mutual Information, cyanobacteria, noise**

PURPOSE

Circadian clock is one of the main ways to keep track of time for a wide range of biological organisms [1]. Generating rhythms in metabolic and behavioral processes helps cells distinguish between different moments in time [2] and this in turn optimizes the cells' biochemical functionality. Clocks are connected to the genome and drive oscillations in the whole transcriptome [3]. This connection is generally mediated by a single linking protein that oscillates along with the clock and triggers the expression of downstream genes. The purpose of this work was to study the dynamical features of this read out system that lead to an accurate perception of time in cells. To analyze this network we used information theory and statistical analysis [4]. We defined some of the relations occurring between oscillatory genes that maximize the information on time. Moreover, using analytical tools we were able to derive the error relative to the measure of time for each moment of the day [5].

CONCLUSION

In the study of the behaviour of genes that read out the clock we found the optimal region in the parameter space that optimizes the information and minimizes the error on time. It turned out that the key parameter is the phase shift between the oscillatory genes. The insertion of cross correlation among the noises [6] induces changes in the optimal phase shift. Therefore, having cross correlation could help cells infer better information on time. Since oscillations in both time and space are very common in biology, our analysis has a general validity and can be equally applied to all the systems that need to infer information from oscillatory signals.

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[1] FOM institute AMOLF monti@amolf.nl

Robust inference of expression heterogeneity from simultaneous single- and k-cell profiling

Manikandan Narayanan[1], Andrew J. Martins[1], and John S. Tsang[1,*]

Short Abstract — **Cell-to-cell gene-expression heterogeneity is a pervasive phenomenon, but its accurate quantification remains challenging as the level of many transcripts in single cells falls near/below the detection limit of even the most sensitive measurement technologies currently available. We present a combined experimental and computational strategy for inferring cellular heterogeneity parameters through Bayesian integration of simultaneously obtained expression profiles from single and random pools of k (e.g., 10) cells. Simulations/experiments show our strategy combines the direct interpretability of single-cell data with enhanced sensitivity of pooled-cell measurements to enable quantitative comparison of cell-to-cell variations across cellular states or conditions using modern multiplexed technologies.**

Keywords — **cell-to-cell heterogeneity, single-cell data analysis, k-cell data, Bayesian inference, stochastic gene expression, macrophage activation.**

I. Introduction

QUANTIFYING heterogeneity in gene expression across individual cells could help identify novel cell types in tissues and address fundamental questions such as how cellular fluctuations in gene expression propagate along the gene regulatory network. Despite rapid technological advances, accurate measurement of single-cell expression is a major challenge, particularly because many mRNAs are expressed at levels close to or below the detection limit of current profiling technologies [1]. Indeed, typical single-cell gene-expression profiles obtained by quantitative PCR (qPCR) or RNA-Seq contain a substantial number of zero or non-detected measurements, which are unlikely to be entirely attributable to cells expressing zero transcripts and instead may arise from technical factors such as missed capture/amplification of mRNA transcripts [1-2]. Measuring randomly sampled pools of a small number of cells (with the number of cells per pool denoted by k, such as k=10) offers more robust detection due to the increased amount of input mRNA and has been used to assess cell-to-cell heterogeneity within the sampled population, such as to infer whether expression distributions are bimodal [3]. However, information on single-cell variations using data from such k-cell pools is nonetheless indirect and the lack of measurements on individual cells would hinder applications such as novel cell type identification.

II. Results

Here we present a strategy for quantifying cellular heterogeneity that combines simultaneous expression profiling of single and k-cell samples from a cell population with a newly developed statistical model and computational method for Bayesian inference of heterogeneity parameters. Our method (called QVARKS) **q**uantifies the degree as well as the statistical uncertainty of expression **var**iation across cells by integrating **k**- and **s**ingle-cell data under explicit models of technical detection limits. Across diverse simulation scenarios representative of modern multiplexed technologies, we show that our approach allows robust inference of cellular heterogeneity parameters of difficult-to-detect transcripts even when technical noise or incomplete single-cell information hinder robust inference from either data alone.

When applied to single/10-cell expression data generated from human macrophages in resting vs. inflammatory conditions, we show our approach is able to effectively disentangle condition-specific biological cell-to-cell variation from detection limit induced technical noise. In addition, our analysis helped reveal several distinct modes of gene-specific responses upon cellular activation involving significant changes in the fraction of "on" cells, or in the average expression level in "on" cells, or both.

Thus QVARKS offers a promising way forward for statistically rigorous assessments of cellular heterogeneity, and can lead to compelling hypotheses on condition-dependent regulation of gene expression and cellular heterogeneity as demonstrated for an important immune cell type here.

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[1]Systems Genomics and Bioinformatics Unit, Laboratory of Systems Biology, NIAID, NIH.

[*]Correspondence E-mail: john.tsang@nih.gov

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Multi-Protease Queueing

Curtis T. Ogle[1], Philip Hochendoner[1], Nicholas C. Butzin[1], and William Mather[1,2]

***Short Abstract* — Cells depend on proteases to prevent the harmful accumulation of extraneous proteins and their unstable constituents, though degradation resources such as proteases are often limited. We employ queueing theory, a mathematical formalism describing systems of servers and customers, in characterizing the behavior of a multi-protease, multi-protein system, explicitly assuming limited degradation resources. We find that despite strong enzymatic preference of substrates, a correlation resonance phenomenon previously observed in single enzyme systems is also observed in some regimes. This may play a key role in scalability of synthetic systems where orthogonality of degradation pathways is often not plausible.**

Keywords — **synthetic biology, queueing theory, multi-protease, coupled degradation, correlation resonance**

I. Introduction

Queueing theory is a mathematical formalism first used to describe telecommunication networks where finite processing resources naturally lead to bottlenecks and waiting lines [1]. It typically employs both discrete and stochastic methods to effectively model the traffic of general server networks. This makes queueing theory a natural language for describing biochemical networks where resources are often limited, and where low copy number effects and natural noise play important roles in chemical processing.

Queueing theory has successfully characterized several biological systems in the past, in particular for systems involving enzyme kinetics [2,3]. In systems where enzymatic processing resources are limited, queueing theory predicts several regimes that systems may occupy. These regimes depict drastically different behaviors, each of which is relevant in cellular processing. For instance, it has been shown that the buildup of sigma factors consequent of enzymatic overload increases the expression of housekeeping genes, which help the cell cope with an unforgiving environment. This overload occurs as stress in the environment such as nutritional starvation causes misfolded or partially constructed proteins to accumulate. Thus the cell uses a bottleneck in degradation processing to trigger a stress response within the cell, which aids in coping with environmental stress. Such mechanisms would be essential in the construction of robust synthetic systems.

II. Multienzyme, multisubstrate kinetics

While many proteins, or protein constituents, exhibit strong enzymatic preference in the form of non-equal affinities for a host of enzymes found in cells, there are often multiple enzymes capable of degrading any one substrate. Queueing theory predicts that as resources such as enzymes become limited, substrates may more readily be degraded by other enzymes for which they have a relatively low affinity. Understanding how secondary degradation pathways affect a system experiencing a bottleneck in processing is likely essential in designing synthetic systems effectively. Many such systems would require components not to interact with one another to attain scalability. On the other hand, having a set of components that behaves in several fundamentally different ways based on some set of environmental controls could become a key design principle for efficient synthetic circuits.

III. Conclusion

It has been shown that the sharing of processing resources alone is sufficient to couple otherwise non-interacting subsystems operating within cells. Such coupling can drastically change the way a system evolves. For instance, in the context of a single enzyme that degrades two substrates, it has been shown that as the total rate of production of substrates approaches the degradation rate of the enzyme, coupling of substrate counts results in a phenomenon known as correlation resonance. Such coupling may also become an essential design principle for flexible, multi-functional circuits. As limitations on processing resources is a ubiquitous problem faced by organisms, a biological perspective rooted in queueing theory should have a wide range of applicability to different systems.

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[1]Department of Physics, Virginia Tech. cogle@vt.edu, plh5012@vt.edu, ncb@vt.edu, wmather@vt.edu.

[2]Department of Biological Sciences, Virginia Tech. wmather@vt.edu.

The TF binding mechanism role in the retroactivity impact

Libertad Pantoja-Hernández[1,3], Elena Álvarez-Buylla[1,3], Adriana Garay-Arroyo[1] and Juan Carlos Martínez-García[3,2]

***Short Abstract* — The effects of downstream loads in regulatory networks represent an issue for design in synthetic biology. Currently, retroactivity effects role in natural regulatory networks remains far from understood. A first step towards the understanding of such role is the dissection of biological systems where retroactivity is involved. In this work, we deal with different mechanisms that a transcription factor may use to bind to DNA and the impact that such downstream loads may have depending of the used mechanism.**

***Keywords* — retroactivity, transcription regulation, transcription factor.**

I. Introduction

Retroactivity is a signal that arises when connecting new elements to a biological system [1]. In a transcriptional regulation circuit, retroactivity is caused by the association of a transcription factor (TF) to its cognate binding sites in the genome.

The functional relevance of retroactivity still remains elusive, though multiple proposals regarding the potential functional roles of downstream loads have been posed as in [2] and [3], among others.

TFs bind their downstream targets by different mechanisms [4], [5]. These mechanisms are the steps needed for the transcription factor-binding site complex to drive downstream transcription. Here, we analyze four main binding mechanisms: simple monomer binding, dimerization prior to binding, two monomers' sequential binding, and sequential cooperative binding along with dimerization prior to binding. Our aim is to evaluate the impact of retroactivity in the system's behavior given that the transcription factor is regulating downstream sites with a specific mechanism.

II. Results

We analyze the change of three features due to interconnection: i) functional TF capable of binding downstream sites and ii) the total TF concentration, as this is easy to assess experimentally. We simulated our systems using ODE's and rule based models.

A. Deterministic models

The ODE's based modeling was curated using rule based models [6] and simulated using odeint in Python. Our models include whole tentative systems in two versions: original systems and retroactivity induced systems. Each one is simulated considering for the conditions of weak and strong association rate.

We assessed different degradation rates for each of the mechanism to analyze the extent at which they could be controlled.

B. Stochastic models

We simulated the stochastic versions of our systems using the Gillespie algorithm version included in BioNetGen to give further statistical support to the observed differences and gain insight in the noise role in this system.

III. Conclusion

Independently of the promoter strength, the sequential binding mechanism is conserved as the one with the most notorious difference between connected and disconnected cases, followed by sequential binding with dimerization. Regardless of the TF assessed (total or functional), the relation of connected vs. disconnected case seems to change in a way that is linearly dependent on the chosen degradation rate.

The prevalent change in variability upon interconnection, seems to be indicative of a side effect caused by downstream connections that makes the systems responses much more punctual in terms of the available TF. We find this interesting as it could have a role in fine tuning transcriptional responses.

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[1]Departamento de Genética Molecular de Plantas , Instituto de Ecología (Institute for Ecology), UNAM. E-mail: lpantoja@lcg.unam.mx

[2]Departamento de Control Automático, CINVESTAV, México. E-mail: juancarlos_martinez-garcia@conciliencia.org

[3]Centro de Ciencias de la Complejidad, UNAM, México.

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p53 pulses diversify and coordinate target gene expression

Joshua R. Porter[1], Brian E. Fisher[1], and Eric Batchelor[1]

***Short Abstract* — Cells employ complex systems to sense environmental conditions and execute appropriate responses. In response to DNA double-strand breaks, p53, a tumor-suppressing transcription factor, is expressed in a series of pulses. These pulses influence the fate of the cell, but their mechanism of action is unclear. We show that p53 pulses give rise to different target gene expression profiles, which can be predicted by the genes' mRNA decay rates, consistent with a mathematical model. Furthermore, we show that p53 coordinates expression of certain target genes and that this coordination changes as the DNA damage response progresses.**

***Keywords* — p53, pulsing, dynamics, signal transduction, DNA damage response, gene expression**

I. Introduction

As methods for observing and quantifying intracellular signaling improve, we can increasingly appreciate that cells transmit information in the dynamics (changes in time) of molecules involved in signaling [1]. In particular, several recently discovered signaling pathways contain components that pulse in time [2,3]. Pulsing is believed to serve different purposes in different contexts, including coordinating gene expression, keeping track of time, generating diverse patterns of gene expression or phosphorylation, and improving signal-to-noise ratios in information transmission.

The tumor suppressor protein p53 pulses as part of its response to DNA double-strand breaks [4]. Since p53 is a transcription factor, regulating over 100 genes [5], this pulsing potentially impacts numerous downstream processes, including apoptosis, cell cycle control, DNA repair, and metabolism. p53 pulsing is ultimately linked to cell fate, as suppressing pulsing pharmacologically while keeping p53 at a constant high level leads to changes in cell fate patterns [6]. The direct mechanistic consequences of p53 pulsing, however, are unknown. Here we investigated two hypotheses about what p53 pulsing accomplishes mechanistically.

II. P53 Pulsing Diversifies Target Gene Dynamics

We had previously proposed that p53 pulsing could enable a wider range of target gene expression dynamics than would be possible if p53 were raised to a constant high level [4]. To test this, we treated MCF-7 breast carcinoma cells with a drug to induce DNA double-strand breaks, then measured expression of 93 p53 target genes over a 10-h period. We found that target gene expression profiles clustered into distinct groups, including those which pulsed with p53 and those which simply rose in response to p53. Moreover, we found that a gene's mRNA decay rate was a significant predictor of whether its expression would pulse or rise, consistent with a simple mathematical model of target gene activation by a transcription factor. These findings suggest that each target gene acts as a low-pass filter for p53 pulses, tuned by its mRNA decay rate.

III. P53 Pulsing Coordinates Target Gene Expression

We also investigated whether p53 coordinates expression of its target genes, similar to the pulsing transcription factor Crz1 in yeast [7]. We performed single-cell transcriptional profiling on cells treated to induce DNA double-strand breaks, then looked for correlations in the expression of pairs of genes. This revealed two coordinated subsets of p53 target genes. One subset was p53-independent and largely composed of DNA repair genes; these genes maintained correlation throughout the DNA damage response. The other subset was p53-dependent and largely composed of genes with pulsing dynamics. The "pulsing" subset peaked in correlation after the third p53 pulse, at which time it became negatively correlated with the "repair" subset.

IV. Conclusion

Our results suggest that p53 pulsing generates diversity in target gene dynamics, enabling a complex response to DNA damage, and coordinates expression of a subset of its target genes in a time-varying manner, likely driving the DNA damage response through different stages.

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[1]Systems Biology Section, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD USA. Emails: joshua.porter@nih.gov, brian.fisher@nih.gov, batchelore@mail.nih.gov.

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Receptor binding, membrane deformations, and signal propagation at the cell membrane

Robert H. Pullen[1] and Steven M. Abel[2]

***Short Abstract* — Cell signaling plays a key role in many cellular processes such as immune cell activation by antigen. We investigate two processes important to lymphocyte activation: membrane-mediated interactions between receptor-ligand pairs at cell-cell junctions and subsequent signal propagation by a network with positive feedback. Using spatially resolved, deterministic simulations, we study membrane-mediated interactions between receptor-ligand pairs and characterize the influence of spatial clustering and diffusion on the spread of a chemical signal through space and time in a positive feedback network.**

I. Introduction

CELL communication is vital for biological systems and is often highly dependent upon the interaction between a transmembrane surface receptor and its ligand. Lymphocytes such as T cells and B cells can be stimulated by cell-cell contact, with the ligand being presented on the surface of the other cell. This is an example of juxtacrine signaling, and receptor-ligand binding can couple with membrane mechanics to deform the membrane and locally exclude other surface molecules that are longer than the length of the receptor-ligand complex [1]. This can lead to effective membrane-mediated interactions between receptor-ligand pairs, thus contributing to their spatial organization on the membrane. In many cases, signaling networks downstream of the receptors contain feedback motifs that confer useful dynamical and steady state properties. The spatial organization of surface receptors can significantly influence the dynamics of signaling [2, 3]. Positive feedback networks are interesting because they can support bistability and fast propagation of a signal through space [4,5].

II. Results

We begin by considering a model of receptor-ligand binding at cell-cell interfaces. Drift-diffusion partial differential equations provide a useful framework for describing the concentration profiles of long surface molecules (LSMs) present in the intercellular junction given that a receptor-ligand bond has formed [1]. We have explored the characteristics of LSMs and membrane energetics given that one or more receptor-ligand bonds have formed. The coupling of the drift-diffusion partial differential equations and the Euler-Lagrange equation describing membrane shape and energetics has given insight into the time-dependent evolution of molecular concentrations in the system.

Given that membrane mechanics can promote the spatial clustering of receptor-ligand complexes, we consider the effects of clustering and diffusion on switching to an active state in a bistable positive feedback network. We consider a simple two-component network and seek to understand how diffusion influences various properties of the reaction network. Coupled reaction-diffusion partial differential equations describe the spatiotemporal evolution of molecular concentrations. We obtain numerical solutions for the spread of an "active" chemical signal through space and time, given an initial localized pulse of the active species. The biological motivation includes clustering of receptors at the cell membrane and stochastic fluctuations that could trigger signaling. We find that a slower diffusion coefficient results in a more pronounced interface between active and inactive regions, and that the fastest signal propagation occurs at intermediate diffusion coefficients. It is interesting to note that the diffusion coefficient corresponding to the minimum time for the system to be in the active state over the entire domain is dependent on the ability of the active species to accumulate in a localized region.

III. Conclusion

A general understanding of communication at cell-cell interfaces remains a challenging problem. Methods that couple receptor binding with membrane mechanics and spatial organization have the potential to inform studies of signal transduction networks to give greater insight into receptor-mediated juxtacrine signaling.

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[1]Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville. E-mail: rpullen2@vols.utk.edu

[2]Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville. E-mail: abel@utk.edu

Correlation of Gene Expression Noise During Cell Fate Transition

Shanshan Qin[1], Chao Tang[1,2]

Isogenic cells often exhibit different phenotypes under different circumstances, but the dynamic process of cell fate transition remains elusive. Here, by studying the correlation of gene expression noise of a ten-nodes gene regulatory network under different circumstances, we show that cell fate transition point corresponds to the bifurcation point of the gene regulatory network, where strong correlations of fluctuations among certain genes, which is defined as a core network, can be observed. Our preliminary results verify that correlation of fluctuations between genes might be used as an indicator of cell fate transition.

***Keywords* — Cell fate transition, Noise, Bifurcation, Correlation**

I. Introduction

Cell fate transition is widespread among unicellular and multicellular organisms[1-3]. Unicellular organism often change phenotype such as proliferation, quiescence or sporulation, in responding to sudden change of environment, such as starvation, UV-exposure. Cell differentiation is important for the development and maintaining hemostasis of multicellular organisms. Diseases, such as cancer, have long been regarded as an catastrophic transition after a long time of accumulation of mutations[4]. All these cell fates transition are governed by the underlying complex genetic regulatory networks. The ultimate goal in the research field of cell fate transition is to effectively control cell fates. Previous studies mostly focus on identifying the so called key regulators of this process. Yet little is known about the complex dynamic process. Recently, the dynamic systems view of cell fates has gained increasing attention, which regards cell fates as high dimensional attractors[5,6]. Cell fate transition in respond to external environment or clues is regarded as the qualitative change of the epigenetic landscape produced by the underlying gene regulatory network. Can we predict when cell fate transition happens? Is there any early signature that can be get from experimental measurements? Is there core network that responsible for the transition? How can we control cell fate transition effectively? To answer these questions we studied a ten-nodes random gene regulatory network under various circumstances.

[1] Center for Quantitative Biology, Peking University, Beijing, China. E-mail: qinss.pku@gmail.com

[2] Peking-Tsinghua Center for Life Sciences, Peking University Beijing, China. E-mail: tangc@pku.edu.cn

II. Results

Using ordinary differential equations, stochastic simulation, as well as analytical calculation, we show that when the gene regulatory network is tuned at bifurcation point, strong correlation of gene expression noise among certain genes can be observed.

We first study the behavior of toggle switch and binary cell fate decision motif when tuned at critical point. Using Langevin equation and Linear Noise Approximation method, we find both correlation of gene expression noise and variation of gene expression increasing rapidly when control parameter approaches the bifurcation point.

When the random ten-genes regulatory network is subjected to saddle-node bifurcation or pitchfork bifurcation, strong correlation of fluctuation among some genes can be observed. To mimic the real situation of cell fate transition, we gradually change the control parameter from one regime(monostable) to another(metastable). Under this circumstance, time series analysis of gene expression profiles shows that only when the control parameter changes slowly enough can strong correlation of fluctuations be observed. This result give some constraint that only when cell fate transition process is slow enough can early indicator of transition observed experimentally.

III. Conclusion

Gene regulatory network shows strong correlation of fluctuations when it is tuned at bifurcation point. Only when cell fate transition is slow enough can we find early indicator of transition from experimental measurements.

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Propagation of signals from the transcription factor MarA to downstream genes

Nicholas A. Rossi[1], Mary J. Dunlop[2]

***Short Abstract* — Bacteria live in rapidly changing, uncertain environments. As such, they need to take advantage of complex control systems to properly anticipate and respond to sudden, potentially dire changes. Here, we examine how signals in the transcription factor MarA are processed by downstream targets, altering the dynamics and distributions of gene expression. These data have implications for understanding bacterial stress response.**

***Keywords* — Signal Processing, Bacteria, Stress-Response, Optogenetics**

I. Purpose

THE purpose of this study is to understand how dynamic expression of MarA impacts the downstream genes it regulates. We selected this transcription factor as it has been shown to upregulate over 40 downstream genes and play a critical role in the general stress-response phenotype [1]. Previous computational results suggest that it may have interesting pulsatile expression dynamics as well [2]. The importance of MarA from a biological point of view, combined with potentially interesting endogenous dynamics make MarA an ideal target for our work. Our goal is to understand how a given MarA signal is interpreted and processed by a number of downstream promoters, providing insight into how general stress response is coordinated, or alternatively how each gene response is specialized through differential processing of a common input signal. In order to analyze the signal processing characteristics of each promoter, we are engineering systems to control the dynamic levels of MarA and simultaneously measure the output activity of downstream targets. First, we are developing constructs that place MarA under the optogenetic control of the CcaS/CcaR light controllable plasmids [2], as well as the IPTG inducible lacUV5 promoter. The combination of these two approaches gives us precise control over the levels of MarA in terms of both single-cell dynamics and population-wide distributions. To measure the output, we are constructing a number of transcriptional fusion reporter plasmids for downstream genes [3], which will allow us to measure the modulation of downstream promoter activity as a function of MarA concentration. By measuring the fluorescence with single-cell, time-lapse microscopy and flow-cytometry, we are able to generate both dynamics and distributions of downstream promoter activity levels. These data will allow us to develop possible models for how promoter activity shapes the phenotype distribution of the population in response to a signal from MarA.

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[1]College of Engineering and Mathematical Sciences, University of Vermont nrossi1@uvm.edu

[2] College of Engineering and Mathematical Sciences, University of Vermont mjdunlop@uvm.edu

II. Results

Our preliminary data show correlation between MarA levels and downstream targets given a step input in MarA. By collecting data at the single-cell level we are measuring how MarA alters the shape of downstream protein distributions across populations and the dynamic concentrations within individual cells. Preliminary dynamic data suggest that the downstream gene *micF* pulses and that levels of MarA modulate the frequency and amplitude of these pulses. Initial flow-cytometry data show that the shape of population distributions in two of the transcriptional reporters responds differently to varying levels of MarA, indicating the potential for differential interpretation of MarA signals at the population level

III. Conclusion

The above data represents the first step in developing an understanding of how MarA can dynamically facilitate a multifaceted stress response. Having a distinct input/output relationship for several downstream genes will raise interesting questions about how unified the role of MarA is. Can a single MarA signal produce a multitude of promoter activities – or are all of the downstream promoters coordinated? Furthermore, do endogenous MarA dynamics have advantages over those generated using the synthetic optogenetic system? Additional experiments studying the greater *marRAB* operon will allow us to contrast naturally occurring MarA levels to those present in our synthetic systems.

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Simulating Emergent Behavior in Host-Microbiome Systems using Robots and Synthetic Biology

Keith C. Heyde[1], Patrick W. Gallagher[2], and Warren C. Ruder[3]

***Short Abstract* — The microbiome's underlying dynamics play an important role in regulating the behavior and health of its host. In order to explore the details of these interactions, we created an *in silico* model of a living microbiome, engineered with synthetic biology, that interfaces with a biomimetic, robotic host. By analytically modeling and computationally simulating engineered gene networks in these commensal communities, we reproduced complex, emergent behaviors in a physical robotic within an arena. Our system is a novel tool for exploring inter-kingdom ecological relationships while potentially impacting fields ranging from ecology to medicine.**

***Keywords* — Synthetic Biology, Computational Biology, Microbiome, Inter-kingdom, Robotics, Biomimicry**

I. Background

COMMENSAL microbes, and in particular the microbiome, have been shown to play a critical role in regulation the behavior of their hosts, with influence ranging from reproductive affinity[1] to anxiety and motility[2]. Although targeted microbiome engineering remains challenging, synthetic biology and biomimetic robotics provide us with two invaluable tools for understanding host-microbiome interactions. Using these two tools, we designed a robotic host with a synthetically engineered simulated microbiome in order to create a model system for studying host-microbiome interactions [3].

II. Results

Our system architecture relied upon information exchange between an engineered cell population and the onboard robotic microcontroller. We conceptualized this system as having three different modules. (1) Inducer chemicals enter the cell population and activate gene circuits encoding for fluorescent proteins. (2) Changes in cell coloration are converted into a digital signal via a microscope. (3) The digital signal is processed by the robot microcontroller and converted into robot subroutines. Here, we computationally simulated modules 1 and 2 in MATLAB® and built and programmed a physical robot for module 3.

We designed the cells to contain plasmids with canonical gene circuits from synthetic biology [4]. The cells were modeled and simulated using a system of differential equations based off of first principles.

Upon interfacing our physical robot with the simulated cell population, we found that simple engineered gene networks caused nuanced emergent robot behavior. These behaviors included preferential resource selection as well as predation behavior similar to those found in vertebrates [3,5]. It should be noted that at no point was the robot's firmware altered, and all variations in robot behavior were a direct result of changes in the cell's morphology.

III. Conclusion

By engineering and testing a robot that could interface with a simulated cell, we designed a novel tool for understanding host-microbiome interactions in nature. Our simulated cell population provides a predictive tool for effectively engineering living cell lines for selective robotic behaviors.

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[1]Department of Biomedical Engineering and Mechanics, Virginia Polytechnic Institute and State University, Blacksburg VA USA. E-mail: kch2118@vt.edu

[2]Department of Biological Systems Engineering, Virginia Polytechnic Institute and State University, Blacksburg VA USA. E-mail: patgall@vt.edu

[3] Department of Biological Systems Engineering, Virginia Polytechnic Institute and State University, Blacksburg VA USA. E-mail: wruder@vt.edu

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Visualizing Regulation in Rule-based Models

John A.P. Sekar[1], José-Juan Tapia[2], and James R. Faeder[3]

***Short Abstract* — Rule-based models are kinetic models where biochemical structures are modeled explicitly as graphs and kinetic mechanisms are modeled explicitly as reaction rules. The regulatory network of the system, envisioned as a bipartite diagram of sites and processes, is implicit in the overlaps between reaction rules. Visualizing individual mechanisms and identifying pathways and feedback loops would facilitate communicating about the model with other experts. In this work, we have developed an algorithm to automatically infer and organize the network structure of a BioNetGen rule based model. We have provided automated tools for visualizing individual rules as well as the inferred network. These tools are freely available with the latest distribution of BioNetGen software at http://bionetgen.org.**

***Keywords* — rule-based modeling, visualization, BioNetGen, reaction rules, regulatory network**

I. Background

RULE-based frameworks such as BioNetGen [1], Kappa [2] and Simmune [3] use a graph syntax to represent biochemical structures and kinetic mechanisms. Visualizing these models as a regulatory network is necessary to improve communication and usability. The directed bipartite graph showing relations between sites and processes is a classical abstraction used for visualizing regulatory networks. Naïvely automating a bipartite graph for rule-based models encounters combinatorial complexity in overlaps between reaction rules and lack of appeal to expert intuition. Prior to this work, regulatory interactions between reaction rules were inferred manually (Extended Contact Map [4]), automated as a unipartite graph (Rulebender [5]), automated for a subset of overlaps (Simmune Network Viewer [5]), part of the model specification (Rxncon [6]), or inferred by simulation [7]. Here we provide automated inference of the regulatory network by static analysis of a BioNetGen rule-based model and user-guided organization and coarse-graining of the inferred network. The tools described here generate visualizations in Graph Modeling Language (GML) format, which is compatible with dedicated graph layout tools such as yEd (yworks.com/yed) and Cytoscape [8].

II. Results

A. Rule Visualization

BioNetGen structures, called *patterns*, are visualized as site graphs: graphs with nested nodes and edges representing binding interactions. A kinetic mechanism, modeled as a *reaction rule*, is composed of reactant and product patterns. We provide two rule visualizations: (i) *syntactical*, where reactant and product patterns are embedded in a bipartite graph of the rule, and (ii) *compact*, where the action of the rule is shown as a set of graph operations on structures.

B. Regulatory Network Inference

The pattern is coarse-grained from an explicit graph to a set of discrete 'sites' or *atomic patterns*. A reaction rule is then summarized using bipartite relations to atomic patterns: consumption (reactant), production (product) or requirement (context). These are visualized on the bipartite *regulatory graph*. Regulatory graphs of individual rules are aggregated into a regulatory network of the model.

C. Regulatory Network Organization

We have provided flexibility to optimize the visual complexity of the regulatory network. Background sites and constitutive processes that obscure the regulatory structure can be tagged and removed. Rules modeling conditional variants of the same process are automatically identified and grouped together. This grouping can be seeded with equivalence classes for sites, which can be imposed as expert input. Collapsing groups to single nodes leads to compact and coarse-grained network diagrams. Using these tools, we were able to generate regulatory diagrams for large sets of reaction rules, such as the FcεRI interaction library [9] with 162 rules, and the ErbB receptor family pathway model [10] with 544 rules.

III. Conclusion

Rule visualization allows side-by-side display and comparison of kinetic mechanisms. The regulatory graph enables identification of pathways and feedback loops in the system. The user is also able to flexibly organize the network to appeal to expert intuition. Systematic coarse-graining enables compact visualization of large networks.

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[1,2,3] Department of Computational and Systems Biology, University of Pittsburgh. E-mail: {jas237[1], jjtapia[2], faeder[3]}@pitt.edu.

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Directional Accuracy in a Model of Gradient Signaling during Yeast Mating

Rati Sharma[1] and Elijah Roberts[2]

I INTRODUCTION

The mating response of the yeast *Saccharomyces cerevisiae* is widely used as a model system for studying chemotropism. Haploid yeast sense nearby cells of the opposite mating type by detecting a pheromone gradient and then polarize and grow a mating projection in the direction of the gradient in an attempt to mate with a partner [1]. Experiments monitoring individual yeast cells in an artificial gradient show that they polarize with a broad distribution of directions centered on the gradient [2], but the probability distribution of the direction is not theoretically understood. Here, we present a hybrid model that uses both deterministic and probabilistic features to study the response of the circuit architecture to a gradient stimulus. In particular, we use a simplified model of the reactions that lead to the formation of the pheromone-receptor complex and activation of the mitogen-activated protein kinase (MAPK) cascade [3]. We simulate the model using a fully probabilistic method, the reaction-diffusion master equation (RDME), with novel gradient boundary conditions accounting for a point pheromone emitter a short distance away, the gradient for which has reached a steady state. We analyze the response to the gradient for different shapes of the simulation volume.

II METHODS

A point pheromone emitter a short distance away from the matα cell creates a gradient. This gradient is modeled deterministically via partial differential equations (PDE) using the diffusion equation with constant flux conditions at the source and constant gradient conditions at the boundaries. The concentrations of the pheromone at the desired distance from the source are then selected and fed into the simulation volume which contains all the other species, namely the kinases, phosphatases and the receptors. The kinases themselves can exist in two states, unphosphorylated or phosphorylated and in the region of parameter space where both are stable states, the system is considered bistable, while the space where only one stable state exists, the system is considered monostable [4]. We model the reactions between these species in the bistable as well as the monostable conditions and carry out RDME based stochastic simulations of these reactions.

III RESULTS

RDME simulations were carried out through *lattice microbes* [6] on the set of reactions after the inclusion of a pheromone gradient across the diagonal in a 3D cubic simulation volume and in a cell shape simulation volume. In a 3D cubic volume, the reactions occur randomly across the entire volume and hence are more distributed. This leads to decreased clustering of the kinases and therefore higher switching times between the two stable states. On the other hand, in the cell shape simulation volume, all the reactions occur on the membrane and hence there is more localized distribution of the species which leads to enhanced clustering and lower switching times between the two states. We also compared the direction of the pheromone gradient to that of the phosphorylated kinases in the theta-phi plane and notice that it is only at lower diffusion coefficients that the phosphorylated kinases follow the direction of the pheromone.

IV CONCLUSIONS

The rates of reactions, the switching times between the two states and clustering of molecules depend a lot on the simulation volume. The signaling cascade is more efficient in a smaller and more compact simulation volume and this is the strategy that most cells use to survive and grow. In addition, diffusion coefficients also play a very important role in the enhancement of the signal in the direction of the pheromone gradient and therefore it is important to understand diffusion related dynamics within the cell.

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[1] Dept. of Biophysics, Johns Hopkins University, Baltimore, Maryland, USA. E-mail: rsharm28@jhu.edu

[2] Dept. of Biophysics, Johns Hopkins University, Baltimore, Maryland, USA. E-mail: eroberts@jhu.edu

VEGF Binding with High Affinity Domains

Christopher Short[1], Emine Güven[1], Jeremy S. Edwards[2], and Ádám M. Halász[1]

***Short Abstract* — TEM imaging indicates that VEGF receptors tend to co-locate in clusters ranging from a few to a hundred molecules. Dimerization of receptors (usually ligand dependent) is necessary for signaling, but there is no known mechanism for larger bound aggregates.**

We hypothesize pre-existing high affinity regions on the cell membrane that preferentially exchange receptors with the remaining part of cellular membrane. This would increase the observed dimerization rates by increasing the concentration of receptors in the high affinity regions. We explore the implications of this mechanism through a compartmentalized version of a kinetic model of VEGF signal initiation [1].

***Keywords* — Chemical Reaction Networks, Receptors, Ligands, VEGF, Clustering**

I. Background

We discuss a Chemical Reaction Network (CRN) model of the binding of Vascular Endothelial Growth Factor (VEGF) to its receptors. VEGF has important roles in the spread of cancer; it facilitates tumor growth by summoning blood vessels to continue to feed the tumor once it has surpassed the size at which diffusion alone can provide oxygen and necessary nutrients [2].

High resolution imaging studies have revealed that the cell membrane is far from homogeneous; it has a varied landscape with elements of the cytoskeleton, accumulations of membrane proteins, and inhomogeneities in the lipid composition of the membrane. This landscape is reflected in the mobility and localization of membrane receptors and of other molecules involved in early signaling.

Certain families of membrane bound receptors, such as receptor tyrosine kinases (RTK), require dimerization in order to activate. Ligand-induced dimerization is a central feature of the respective signaling machinery, whose precise kinetics ensures the proper behavior of cells. Molecular changes that result in increased or decreased dimerization rates may have far reaching consequences. The mobility of membrane-bound receptors can potentially have a similar impact on signaling, by facilitating or hindering receptor-receptor collisions and impacting dimer formation.

II. CRN Model of VEGF Signal Initiation

Our starting point is a mathematical model [1] developed by Mac Gabhann and Popel (MGP), based on experiments quantifying the binding of VEGF to cells and the overall cellular response. VEGF receptors are monovalent (they bind to only one ligand) whereas the VEGF ligand is bivalent, binding to two receptors. The MGP model allows for a direct receptor-receptor bond, resulting in ligand independent dimer species; for a single receptor type, the resulting CRN has 7 species and 14 reactions [1]. This baseline model represents a spatially homogeneous, "well-mixed" system where the receptors are distributed evenly and can move unobstructed over the entire membrane.

III. Model with High Affinity Domains

Based on TEM imaging of VEGF receptors, as well as a host of indications of clustering behavior in other receptor systems [3], we developed the following hypothesis. The membrane contains small attractive regions that concentrate the receptors in their random movement. The observed clusters reflect the size and distribution of these "high affinity domains".

We explore this hypothesis with a version of the MGP model where copies of the VEGF system are placed in multiple domains. Transfer reactions added to the system allow movement between the high affinity regions and the rest of the membrane. Computer simulations of this model indicate increased receptor concentrations in the attractive regions result in increased signaling as compared to the baseline MGP model. Analytical calculations provide an independent check of the simulations, as well as more direct insight into the behavior of the system. We are investigating the possibly multiple steady states, which are not forbidden by basic CRN theory.

IV. Outlook

Beyond this, we are interested in the effect high affinity regions have on the speed at which cellular response occurs, which must be done through simulation. We plan to use our framework of combining spatial and chemical networks for stochastic simulations of early VEGF signaling on the scale of the entire cell.

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[1]Department of Mathematics, West Virginia University, Morgantown, WV, E-mail: {cshort3,eisceviren,halasz}@math.wvu.edu

[2]Deptartment of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, NM. E-mail: jsedwards@salud.und.edu

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Phenotypic heterogeneity of nutrient-starved *E. coli* cells

Emrah Şimşek[1] and Minsu Kim[1]

***Short Abstract* — Bacteria in nature spend most of their life in a nutrient-deprived state. It is well known that when transferred to a nutrient agar plate, a large fraction of these nutrient-starved bacterial cells fails to form colonies. Currently, little is known about the physiological and metabolic states of these non-rejuvenating cells. Here, we characterized physiology, metabolism and gene expression of nutrient-starved *E. coli* cells with single-cell resolution. Our study reveals surprising phenotypic heterogeneity in nutrient-starved cells.**

***Keywords* — Quantitative single cell microbiology, bacterial physiology, starvation, growth**

I. Background

In nature, bacteria spend most of their lifetime in nutrient-depleted environments. When transferred to a nutrient agar plate, only a fraction of them forms colonies. A similar phenomenon was observed in laboratory experiments; when bacterial cells are deprived of nutrients, the number of cells that form colonies (when transferred to a nutrient agar plate) decreases by half within a couple of days (for reviews [1, 2]). Currently, little is known about physiological and metabolic states of these cells that fail to form colonies.

Here, employing time-lapse fluorescence microscopy and fluorescence markers, we characterized physiological and metabolic states of the non-rejuvenating cells.

II. Results

A. Correlating membrane integrity and nutrient-uptake ability to rejuvenation.

Cells that were previously growing exponentially were subjected to nutrient deprivation. At different times, we took an aliquot, treated the cells using propidium iodide (PI) and 2-NBDG (fluorescent glucose analog) and transferred them to a nutrient agar plate. PI and 2-NBDG report membrane integrity and nutrient-uptake ability, respectively. Then, using time-lapse fluorescence microscopy, we monitored the rejuvenation of cells with single-cell resolution. Cells that have intact membrane (unstained by PI) and nutrient uptake ability instantly rejuvenated. As more time elapses in starvation, the number of instantly-rejuvenating cells decreases; the decrease quantitatively agrees with decrease in the number of colony forming units (CFU).

[1]Department of Physics, Emory University. E-mail: esimsek@emory.edu, minsu.kim@emory.edu

B. Phenotypic heterogeneity of non-rejuvenating cells.

Next, we characterized non-rejuvenating cells. We see that they consisted of (i) membrane disrupted cells (stained by PI), (ii) ghost cells (judged by poor phase contrast image), and, importantly, (iii) healthy looking (based on phase contrast image) cells with intact membrane and nutrient uptake ability. The fraction of the category (iii) cells was observed to be ~0% of the total population at the onset of the starvation, increased to ~5% within ~2 days, and remained about the same level afterwards (~for a week). When we monitor the category (iii) cells for an extended period of time, we see that they rejuvenate at much later times. Hence, they are dormant cells.

Further characterization reveals their lack of ability to produce proteins and, also, their lack of resilience during starvation.

III. Conclusion

Our finding reveals phenotypic heterogeneity of nutrient-deprived cells. Importantly, the high percentage of dormant subpopulation is of particular interest to microbial ecology and medicine.

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Analysis of Ras as a tumor suppressor

Chelsey Poling[1] and Edward C. Stites[2]

***Short Abstract* — Oncogenic Ras mutations are common drivers of human cancer. One poorly understood aspect of Ras biology is the apparent tumor suppressor activity that wild-type Ras sometimes appears to exhibit. This problem was investigated with a computational model of Ras signaling. Modeling demonstrates that tumor suppressor activity is actually not needed to explain data commonly interpreted to support the tumor suppressor argument. Modeling also finds that small changes in oncogene expression after the loss of the wild-type allele would have an effect that could be interpreted as the wild-type allele having acted as a tumor suppressor.**

***Keywords* — oncogene, tumor suppressor, Ras, cell signaling**

I. Introduction

MUTATIONS to the Ras GTPases are among the most common cancer promoting mutations [1]. It is now understood that oncogenic Ras mutations lead to constitutive proliferative signals [2]. Still unexplained is the tumor suppressor behavior that the Ras proto-oncogene sometimes appears to demonstrate [3-7].

A mathematical model of Ras signaling that accounts for the multiple biochemical mechanisms that regulate Ras activity has previously been developed and applied to the study of cancer promoting Ras mutations [8,9] and Ras pathway mutations [10]. The model allows one to find the behaviors that logically follow from what is already known and quantified about Ras biology. Here, the model is applied to the problem of whether or not wild-type (WT) Ras has "tumor suppressor" properties.

II. Results

Arguments that Ras has tumor suppressor activity commonly refer to the frequent loss of heterozygosity (LOH) in Ras genes when a Ras oncogenic mutant is present. Common mechanisms for LOH not only result in a loss of the WT allele, but also in the duplication of the mutant allele [3,5]. Simulations find that doubling mutant expression results in a large increase in Ras signaling. This modeling result is consistent with experimental data that examines the consequences of Ras mutant dosage [6]. This suggests that tumor suppressor activity is not necessary to explain the LOH data.

Arguments that Ras has tumor suppressor activity also refer to the inhibition of mutant Ras signals by dominant negative (DN) Ras mutants [5]. However, model simulations find DN Ras results in less oncogenic Ras signaling. This follows from the inhibition of Ras GEFs by DN Ras. This suggests that tumor suppressor activity is not needed to explain DN Ras data.

A compelling argument for tumor suppressor activity comes from mouse studies [4]. In these studies, *Kras* mutations were chemically induced. Mice with only one wild-type allele ($Kras^{+/-}$) developed more tumors than mice with two wild-type alleles ($Kras^{+/+}$). Our simulations suggest that *Kras* mutants would generate a higher level of Ras signal in the +/- mice if the +/- mice express more than 50% as much KRas protein as the +/+ mice. The exact amount of expression varies based on the concentrations of Ras network proteins, but ranges from as low as 51% to as much as 65% of the amount of KRas expressed in the $Kras^{+/+}$ mice. Experiments quantifying protein expression in these studies have typically been used to demonstrate less KRas in the +/- mouse, not to precisely quantify how much less KRas is in the +/- mouse.

III. Conclusion

Our analysis finds much of the data used to argue that WT Ras has tumor suppressor activity is actually consistent with the well-established activity of mutant Ras. Our simulations also suggest that a low level of increased expression from a single Ras allele could explain the increased tumor burdens in $Kras^{+/-}$ mice compared to $Kras^{+/+}$ mice. Quantitative measurements of Ras protein expression that are capable of detecting small changes in expression could distinguish our hypothesis from the tumor suppressor hypothesis. Overall, this study demonstrates how quantitative modeling can contribute to the study of unresolved problems in cancer biology.

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[1]Translational Genomics Research Institute, Scottsdale, AZ.
[2]Department of Pathology and Immunology, Washington University School of Medicine, Saint Louis, MO. E-mail: estites@path.wustl.edu

Frequency of multiply infecting bacteriophage in natural environments exposed by spatial models

Bradford P. Taylor[1], Catherine Penington[2], and Joshua S. Weitz[3,1]

***Short Abstract* — It is generally assumed that multiple infections of microbes by viruses is rare. This assumption stems, in part, from the use of mean field models of virus-microbe population dynamics. Here, we investigate an explicitly spatial model of interactions and assess the frequency of multiple infections given biophysically relevant rates of interaction and movement. We find that multiple infection are significantly more common than in mean field models, and we investigate how such infections can alter ecological dynamics and virus-microbe coexistence.**

***Keywords* — Viruses, Bacteria, Multiple infections, Spatial Models, Multiscale Models**

I. Purpose

Viruses are able to directly interact when coinfecting a host cell. This direction interaction benefits the viruses by allowing recombination leading to quicker adoption of positive mutations in the population [1]. However, coinfection allows direct competition leading to within-host niche specialization by viruses and the emergence of cheaters for shared viral products due to the modular structure of viruses [2]. Investigations of these phenomenon are in vitro settings mediated by high multiplicity of infection (MOI) inoculants. The relevance of coinfection in vivo settings is poorly understood. We address this gap in understanding via simulation. The goal of this project is to quantify the rate and magnitude of multiple infections within a stochastic individual based spatial model (IBSM).

Previously, IBSM have resolved apparent paradoxes in viral ecology such as the tragedy of the commons [3]. In addition, ecological IBSM feature increased ranges of coexistence [3]. These results stem from the spatial correlations between hosts and viruses and the existence of density dependent effects. Meanwhile, experimental effects of coinfection include delayed lysis and altered burst size [4,5]. Hence, coinfection is a phenomenon that affects dynamics across multiple scales. Our approach demonstrates what condition coinfection is amplified or tempered due to multi scaled feedback.

[1]Department of Physics, Georgia Institute of Technology, Atlanta, GA, USA. E-mail: bradfordptaylor@gmail.com

[2]Department of Mathematics, Queensland University of Technology, Queensland, Australia. E-mail: catherine.penington@qut.edu.au

[3]Department of Biology, Georgia Institute of Technology, Atlanta, GA, USA. E-mail: jsweitz@gatech.edu

II. Results

By considering the dynamics of viruses and three classes of hosts (susceptible, infected, and coinfected) in an individual based stochastic spatial model. We consider parameter space corresponding to viral-host dynamics involving in autotrophs marine environments such as prochloroccocus. We demonstrate coinfection occurs frequently across parameter space. In addition we quantify the distribution of MOI across the host population. We show that coinfection occurs frequently and that high intracellular MOI can be achieved even when population level virus to bacteria ratio is low. Spatial correlation between host and virus populations account for this increased level of coinfection as compared to an analogous mean field model. We implicitly model intracellular effects due to coinfection by including parameter dependence on individual MOI. We characterize how altered lysis times and burst sizes affect the rates of coinfection within and coexistence of the virus host populations.

III. Conclusions

Coinfection and its effect on viral-host dynamics phenomenon is inherently multiscaled affecting intracellular decision-making to population level evolution. By demonstrating increased frequency of coinfection in a spatial environment as compared to a mean field model, we argue that modelers and experimentalists alike should consider multiple infections a more common phenomenon.

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Ultrasensitivity and Bistability arising from miRNA-mRNA Reciprocal Interaction

Xiao-Jun Tian[1], Hang Zhang[2], Jingyu Zhang[1], and Jianhua Xing[1, 3]

***Short Abstract* —It is found that miRNA and mRNA reciprocally regulate each other. However, the functionality of this mutual regulatory relationship is not fully understood. Here, through mathematical modeling, we found that depending on the recycle ratio of miRNA, the reciprocal regulation between mRNA and miRNA shows subsensitive activation, ultrasensitive and subsensitive inhibition. Bistability is generated when the ultrasensitivity from the miRNA-mRNA reciprocal regulation is equipped with a positive feedback loop. Furthermore, the degree of ultrasensitivity is amplified when a stronger competitor (ceRNA) is involved. Interestingly, bistability can also be generated from mRNA-miRNA reciprocal interactions when considering more than one binding sites.**

Keywords — Reciprocal, miRNA, mRNA, ultrasensitivity, bistability, recycle ratio.

I. INTRODUCTION

In the gene regulatory networks, miRNAs serve as important post-transcriptional regulators of gene expression to control a large variety of essential cellular processes, such as EMT [1]. During the last decade, there are accumulated studies on the basic molecular mechanisms of miRNA biogenesis, function and degradation.

Through base-pairing interactions, miRNA inhibits its target mRNA by two modes, translational repression and mRNA degradation. Furthermore, under some circumstance, miRNAs can stimulate mRNA translation. Quantitative measurements show that miRNA regulation establishes a threshold level of target mRNA [2]. However, the functionality of the gene expression threshold regulation by miRNAs remains to be established.

Furthermore, recent results also provide evidence that mRNA targets can reciprocally control the stability and function of miRNAs. Kinetic analysis already provided support that miRNA could be recycled following regulating mRNA [3]. However, the endogenous functions of mRNA-directed miRNAs degradation remain elusive. Interesting, it is also found that target interaction could stabilize miRNA by preventing its release from Ago and subsequent destabilization. Furthermore, each miRNA may target tens or hundreds of mRNA molecules, enabling cross-talk between competing endogenous RNAs (ceRNAs) targeted by the same miRNA. This appreciated reciprocal regulation between miRNAs and their targets adds a significant level of complexity to the miRNA-mRNA relationships. Thus, how the ceRNAs cross-talk and the miRNA-mRNA reciprocal regulation tune the miRNA-mediated regulation need to be further elucidated.

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[1]Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, 15260, USA

[2]Genetics, Bioinformatics, and Computational Biology Program, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24060, USA

[3]Email: xing1@pitt.edu

II. MODEL AND RESULTS

To explore the features of the regulations between mRNA and miRNA, we built a mathematical model by considering the formation of mRNA-miRNA complex via base-pairing with complementary sequences, degradation of the complex and the recycle ratio of miRNA during the degradation.

First, regulation of mRNA by miRNA generates ultrasensitivity in a recycle ratio dependent manner. The larger of the recycle ratio, the less sensitive inhibition of mRNA by miRNA and vice versa. That is, ultrasensitivity is generated by sacrificing efficiency.

Second, regulation of miRNA by mRNA also generates ultrasensitive inhibition under small recycle ratio, subsensitive inhibition under large recycle ratio, or protection under near complete recycle.

Taken together, the regulation between miRNA and mRNA are reciprocal and shows different level of sensitivity, either ultrasensitive or subsensitive.

Third, ultrasensitivity from the miRNA-mRNA mutual regulation can also contribute to the generation of bistability. ceRNA with stronger binding affinity further enhances the ultrasensitivity of miRNA regulation on mRNA.

Fourth, several kinds of response curves exist when considering two binding sites, including inhibitory subsensitivity, inhibitory ultrasensitivity, protective subsensitivity, duality, and especially bistability. The bistability generated from mRNA-miRNA reciprocal interaction in the absence of any imposed feedback regulation are never reported theoretically or experimentally.

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Bistability and Hysteresis in the Pheromone Response Pathway

Lior Vered[1], Beverly Errede[2], and Timothy Elston[3]

***Short Abstract* — Cell polarity is the asymmetric organization of cellular structures. Many mathematical models of polarity rely on bistablility, or the existence of two stable steady states. Bistable regulation of polarity has yet to be tested experimentally.**

Hysteresis is a hallmark of bistability. We tested for the existence of hysteresis in polarity establishment during the pheromone response of the *Saccharomyces cerevisiae*. Interestingly, mother cells display hysteresis, whereas in daughter cells do not. The results for daughter cells show that bistability is not a necessary condition for polarity. The hysteresis observed in mother cells opens the possibility for bistability in certain cellular contexts.

***Keywords* — Polarity, Bistability, Hysteresis, Pheromone Response, Saccharomyces cerevisiae.**

I. Introduction

Cell polarity is the asymmetric organization of cellular structures and morphology. Polarity establishment is critical for differentiation, morphogenesis and migration in all eukaryotes [1]. Many mathematical models of polarity rely on bistablility, or the existence of two stable steady states [2-6]. In the context of polarity establishment in response to an external stimulus, bistability means that cells can exist in either a polarized or unpolarized state for a range of stimulus strengths. Which steady state is observed depends on past conditions. Bistable regulation of polarity has yet to be proven or refuted experimentally.

II. Methods and Results

One of the hallmarks of a bistable process is hysteresis [7]. In the context of stimulus induced polarity, hysteresis means that the stimulus strength needed to establish polarity is greater than that needed to maintain polarity once it is established. We tested for the existence of hysteresis in polarity establishment during the pheromone response of the budding yeast Saccharomyces cerevisiae. Using a custom microfluidic system, we determined the minimum pheromone concentration required to establish polarity and compared it with the maximum concentration at which polarity is lost. Interestingly, we found that mother cells display hysteresis, whereas in daughter cells do not. Mother cells require a higher pheromone concentration to establish polarity than to lose polarity, while daughter establish and lose polarity at the same pheromone concentration.

III. Conclusion

The results for daughter cells show that bistability is not a necessary condition for polarity establishment. The hysteresis observed in mother cells opens the possibility for bistability in certain cellular contexts.

[1]Department of Chemistry, University of North Carolina, Chapel Hill, NC, USA. E-mail: vered@live.unc.edu

[2]Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC, USA. E-mail: errede@email.unc.edu

[3]Department of Pharmacology, University of North Carolina, Chapel Hill, NC, USA. E-mail: telston@med.unc.edu

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Stochastic Simulation of Bio-molecular Networks in Dynamic Environments

Margaritis Voliotis[1], Philipp Thomas[2], Ramon Grima[2], and Clive G. Bowsher[3]

***Short Abstract* — Simulation algorithms have become indispensable tools in modern quantitative biology, providing deep insight into many biochemical systems including gene regulatory networks. However, current stochastic simulation approaches handle the effects of fluctuating extracellular signals and upstream processes poorly, either failing to give qualitatively reliable predictions or being very inefficient computationally. Here, we introduce the Extrande method, a novel approach for simulation of bio-molecular networks embedded in the dynamic environment of the cell and its surroundings. The method is accurate and computationally efficient, and hence fills an important gap in the field of stochastic simulation. We employ it to study a bacterial decision-making network and demonstrate that robustness to fluctuations from upstream signaling places strong constraints on the design of networks determining cell fate.**

***Keywords* — Stochastic simulation, biochemical networks, fluctuating environment, time-varying propensities.**

DYNAMIC simulation is an essential and widespread approach for studying models of bio-molecular networks in cell biology [1]. Often such models need to take into account biochemical stochasticity [2] as well as the effects of interactions with other fluctuating processes in the cell and/or with signals arising extracellularly [3].

The stochastic simulation algorithm (SSA) [4] allows the random timing of reactions in the network model to be taken into account (often known as intrinsic noise). However, the SSA assumes constant propensities between reactions and cannot be used when other processes interacting with the network cause its propensities to fluctuate between reaction occurrences. Here, we introduce a new approach relaxing this assumption, which we call *Extrande*. The method allows stochastic simulation of a bio-molecular network of interest embedded in the dynamic, fluctuating environment of the cell and its surroundings.

There are two existing approaches to stochastic simulation of reaction networks subject to dynamic, fluctuating inputs. The first class of algorithms [5-7] simply implements the SSA, under the approximation that the input remains constant between the occurrences of any two reactions. We term these collectively the naive method. The second class of algorithms [8-10] involves step-wise numerical integration of reaction propensities until a target value for the integral is reached. We term these collectively the integral method. The naive method can yield qualitatively misleading predictions (even when dynamic inputs change relatively slowly) while the integral method can impose large and impractical computational burdens due to numerical integration of propensities.

We demonstrate the clear advantages of *Extrande* in terms of speed and accuracy using two illustrative case studies. In the first case study, we study how various biological sources, including effects related to circadian oscillations, chromatin remodeling, the cell cycle, and pulsatile transcription factors, affect variation in gene expression levels across cells and over time. In the second case study, we use *Extrande* to study how fluctuations in the protein componentry of signal transduction networks affect downstream networks determining cell fate. We find that robustness to fluctuations from upstream signaling places strong constraints on the design of networks determining cell fate.

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[1]School of Mathematics, University of Bristol, UK E-mail: m.voliotis@bristol.ac.uk

[2]School of Biological Sciences, University of Edinburgh, U.K

[3]School of Mathematics, University of Bristol, UK

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Using NF-κB modules and DNA elements to engineer combinatorial and dynamic gene regulation

Qiuyue Wang[1,3], Jun Jiang[2], Huaiqiu Zhu[1,3], Ping Wei[1,2,4]*

In response to large variety of stimuli, mammalian NF-κB transcriptional factors (TFs) promote diversified series of genes expression. The unique DNA-binding properties of NF-κB dimers is a key signal integration step. To investigate these properties, we build a library of NF-κB binding DNA elements (κB sites) in a synthetic promoter and use flow cytometer to evaluate the transcriptional activity in yeast. We further characterize these dimer-specific promoters and then rebuild genetic circuits, such as logic gates etc. These study provides us new insight of how NF-κB dimerization act as regulatory function and become a powerful toolbox for synthetic biology practice.

I. Introduction

LIVING cells regulate gene expression by well-designed gene circuits. After the process of upstream signal transduction pathway, the delicate interaction between DNA and TFs play an important role[1].

Several modular tools, such as zinc finger [2], TALE (transcription activator-like effector) [3], and CRISPR [4] system have been used to regulate gene expression in synthetic biology. Their excellent orthogonality and few off-target rate make them a broad application prospect.

In this paper, we use NF-κB natural and refabricated protein to control programed promoters. First, our TFs derived from nature, which will be true reflection of real gene regulation mechanism. Second, NF-κB family proteins can form homo- and hetero- dimers, which exhibit specific DNA binding properties. Third, we can program extended logics into transcriptional regulation through controlling NF-κBs' dimerization process.

II. Results

A. Rapid Identification of NF-kB dimer specific gene activation DNA elements in yeast

Based on database [5] and results from high throughput technology [6], we build a library of 260 different NF-κB binding sites and insert them in front of minimal CYC1 promoter. Then we transform all the plasmids into 11 kinds of yeast with different combination of NF-κB proteins.

[1]Center for Quantitative Biology, and [2]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, [3]College of Engineering, [4]School of Life Sciences, Peking University, Beijing 10086, China.

*Corresponding to: pwei@pku.edu.cn

The sequence specificity of NF-κB dimers is obvious, which helps us to rediscover the specificity of NF-κB dimers. We also have got powerful toolbox and orthogonal pairs for synthetic genetic circuits. Well-conserved sequence motifs are shown for those dimers.

B. Programmable gene regulation with rational designed synthetic promoters and refabricated NF-kB proteins.

Combining NF-κB proteins with 18 other co-factors, such as med6 and hda3, the properties of natural NF-κB proteins are widened. A quicker repressor is born, which will dynamically change the regulation of downstream genes.

Systematically engineering NF-κB binding position makes promoters, such as ADH1 and CYC1, have different basal expression and fold of activation and repression, which provide us an abundant source of material.

C. Synthetic devises and logic gates coded by NF-kB protein dimerization

Through changing different kinetic κB sites, we build different self-activation and inhibition curves, which will fit well with computation models. Taking use of the dimerization process of NF-κB protein, we have rebuilt almost all the logic gates with a single promoter, which is simpler and more modularized.

III. Conclusion

The ability to manipulate gene regulation is the most fundamental business in genetic engineering. Here, we rigorously measure the specificity of eukaryotic TF, NF-κB. We find that properties of TFs and promoters can be fine-tuned. Beyond these, with the dimerization process of NF-κB proteins, we simply rebuild useful synthetic devises.

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The limitations of model-based experimental design in sloppy systems

Andrew White[1], Malachi Tolman[1], Mark K. Transtrum[1]

Complex models in systems biology can include a large number of unknown parameters. Many such models are "sloppy", i.e., exhibit an extreme insensitivity to coordinated changes in many parameter combinations. Because of this extreme insensitivity experimental design methods have been developed to optimally select those experiments that allow accurate estimates of all the parameters. These methods typically assume that the model is a complete representation of the system. In practice, however, this is assumption is almost never true--models always involve simplifying approximations. We explore the effects of these approximations on model-based experimental design methods. We conduct several numerical experiments in which data is generated from a complex model (acting as a surrogate for the actual system) but experiments are selected based on an approximate model. We find that although the simple model is able to fit data generated by the complex model for many potential experiments, it is unable to fit data for those experiments selected as "optimal" as determined by experimental design methods. This is because the "optimal" experiments are those most likely to make microscopic details more important, including those omitted from the model.

MODELS of complex biological systems can involve a large number of unknown parameters. Considerable attention has been given to the problem of parameter inference in systems biology. Many models are "sloppy," i.e., exhibit an extreme insensitivity to coordinated changes in the parameters. Because of the near-universal appearance of sloppiness among systems biology models it was suggested that sloppiness was an inherent feature of such models and that accurate parameter inference would be practically impossible [1]. Subsequently, it was shown that model-based experimental design could be used to identify a collection of experiments that would enable accurate parameter estimates. The idea was that although the model of each experiment would be sloppy individually, complementary experiments could be identified that would allow the accurate estimates of all the parameters [2].

There has been considerable interest in experimental design techniques for parameter inference in systems biology [2, 3, 4, 5]. However, nearly all of these methods assume that the model is a complete representation of the system. In practice, however, this assumption is almost never true. Models always employ simplifying. Indeed, it would be hard to imagine a "complete" model of systems biology. Any model will have rates and binding affinities that will be altered by the surrounding complex stew of proteins, ions, lipids, and cellular substructures. Furthermore, in such systems there is no clear distinction between which parameters are important and which are not.

We consider the model of EGFR signaling due to Brown et al. [6] for which optimal experiments were later identified by Apgar et al., [2]. We generate data for the Apgar et al. experiments using a model similar to that of Brown et al. but with Michaelis-Menten reaction replaced by the more accurate mass-action reactions. We find that although both models can fit the data for the experiments in Brown et al. [6], the Michaelis-Menten model is unable to fit data generated by the mass action model for the experiments proposed by Apgar et al.

We argue that this result will be generic for systems in which there is no clear separation between important and unimportant system features. We describe such systems as "sloppy," a natural extension of "sloppy" models. Optimal experiments are those that highlight features of the system that were unimportant for other experiments. This includes those components of the system that were omitted from the model. When these experiments are carried out, the model will typically be unable to fit the resulting data. Our results suggest that more careful uncertainty quantification is necessary when modeling and selecting experiments for such systems.

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[1]Department of Physics & Astronomy, Brigham Young University, Provo Utah. E-mail: mktranstrum@byu.edu

Mathematical Modelling Reveals Missing Mechanism in AKT Activation

Martin KL. Wong[1,2*], Dougall Norris[2], James R. Krycer[2], James G. Burchfield[2], Westa Domanova[1,2], Zdenka Kuncic[1,2] and David E. James[2,3,4].

***Short Abstract* — AKT is a central regulator of growth and is important in the transmission of the insulin signal to a range of biological processes such as protein synthesis and glucose uptake in fat and muscle cells. AKT does not phosphorylate its substrates uniformly as they exhibit different temporal profiles in terms of shapes and speeds. Additionally, the phosphorylation kinetics of AKT itself, which is commonly used as a marker for its activity, does not match the phosphorylation kinetics of its substrates. Mathematical modelling revealed that variations in substrate phosphorylation speeds are in sufficient to explain the mismatch. This suggests that there is some missing mechanism of AKT activation that requires further investigation.**

***Keywords* — Insulin Signalling, AKT, Systems Biology, Kinetic Modelling.**

I. AKT AS A KEY HUB OF SIGNALLING

AKT is a key component of insulin signaling that potentiates many downstream processes such as glucose uptake, protein synthesis, lipid synthesis as well as inhibiting glycogen metabolism [1]. After insulin triggered translocation of AKT to the plasma membrane, it is phosphorylated at its T308 residue by PDK1 and S473 residue by mTORC2 [2], [3]. These result in activation of AKT, enabling it to phosphorylate its target substrates. In this work, we explore the relationship between AKT phosphorylation and AKT activation and hypothesise that phosphorylation order and kinetics are the determining factors of activity.

II. RESULTS

Insulin signaling was explored in the 3T3-L1 cell model. Initial results suggested that T308 and S473 kinetics are very similar. At maximal doses of insulin, the AKT phosphorylation and AKT substrate phosphorylation, such as AS160 at the T642 residue and GSK3β at the S9 residue, have matching temporal patterns. However, at a submaximal dose, they are very different. This is because at 1 nM insulin stimulation, AKT phosphorylation features overshoot behavior, peaking at 2 minutes then reducing. Its substrates, however, peak at 5 minutes then sustain their response.

Through simulation of described system using our newly developed dQSSA model, we found that AKT activity cannot be directly related to T308 or S473 phosphorylation [4]. It showed that substrate phosphorylation was not slow enough for them to be insensitive to the overshoot in AKT activation.

Since AKT phosphorylation is not directly linked to substrate phosphorylation, we are now determining if AKT phosphorylation relates directly to AKT activity itself using an *in vitro* kinase assay.

Given that singly phosphorylated AKT possesses some kinase activity, we will test to see whether this contributes to the disconnect between AKT and substrate phosphorylation. We will separate singly phosphorylated AKT from doubly phosphorylation AKT by finding the perturbation in S473 and T308 phosphorylation time profiles from PDK1 and Sin1 (an mTORC2 component) knockout experiments, respectively, then analyzing them using mathematical modelling. These can then be used to determine the link between AKT activity and the pools of AKT phosphorylation.

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[1]Institute of Medical Physics, School of Physics, Unviersity of Sydney, Sydney, NSW, Australia.
[2]Charles Perkins Centre, University of Sydney, NSW, Australia.
[3]School of Molecular Bioscience, University of Sydney, NSW Australia.
[4]Sydney Medical School, University of Sydney, NSW, Australia.
* Corresponding to: martin.wong@sydney.edu.au

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Quantitative Analysis on Mitochondrial Apoptosis Pathway

Yin Peipei[1]

Short Abstract — **Apoptosis is a biological process that eliminates the damaged or useless cells in order to maintain inner balance in organisms. It's responsible for many vital processes such as development, morphogenesis, homeostasis and deletion of dangerous cells. Escaping apoptosis can cause a lot of diseases such as cancer. As a vital process, apoptosis is regulated by a complex network. It can be divided into 2 pathways: extrinsic and intrinsic apoptosis network, which will give instructions to drug design for related diseases in future.**

Keywords — **Apoptosis, Caspase 3, Caspase 9, XIAP**

I. Intruoduction

THE apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of the cell populations in tissues upon physiological and pathological conditions.[1]Apoptotic processes are of widespread biological significance, being involved in development, differentiation, proliferation, regulation and function of the immune system and in the removal of defect and therefore harmful cells. As a vital process, apoptosis is regulated by a complex network. [2][3][4] `In the vision of systems biology, the most important factor is the dynamics of Caspase3 activity in the mitochondria apoptotic pathway. The dynamics of Caspase3 mainly depends on the concentration of Caspase9, proteasome and XIAP. The questions I take most interest in are the network consists of these four nodes, in other words, I concerned about the dynamics of Caspase3 activity and apoptosis percentage which are caused by these three feedback loops. Based on experiments have been taken, I would like to take three measures to address my questions, the small molecule inhibitors, knocking down the expression of Caspase9 and XIAP, and knocking out those genes in the means of CRISPR.

In order to monitor the dynamics of Caspase3, Goldstein et.al designed FRET reporter[5] which its principle is measuring the extent of fluorescence resonance energy transfer within a recombinant substrate containing cyan fluorescent protein (CFP) linked by a short peptide possessing the Caspase3 cleavage sequence, DEVD, to yellow fluorescent protein (YFP). When Caspase3 was not activated, we can see the yellow fluorescent (YFP) if it was given the excitation spectrum of CFP. While we can see the CFP when Caspase3 was activated because DEVD was cleaved by Caspase3. Rehm et.al monitored the dynamics of Casp3 using FRET report.[6] They found that Casp3 is always activated quickly and absolutely no matter what was the apoptotic inducing signal. We concerned about the dynamics of Caspase3 activity and apoptosis percentage which are caused by these three feedback loops.

II. Results

I used siRNAs to knock down the expression of Casp9 and XIAP. By the results of quantitative western blot, I successfully knocked down the expression of Caspase9 and XIAP with approximately 60% and 40% efficiency each. Then we will monitor the dynamics of Casp3 by the imaging analysis.

III. Conclusion

I successfully knocked down the expression of Caspase9 and XIAP with approximately 60% and 40% efficiency each.

In the following two years, I plan to knock out Caspase9 and induce exogenous Caspase9 dimerization in HeLa cells. By this way, I can work out the influence of Caspase9 dimerization on the three-node network consists of Caspase3, XIAP and Caspase9 that play a vital role in the mitochondria apoptotic pathway

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[1]Center for Life Sciences, Peking University, No.5 Yiheyuan Road Haidian District, Beijing, P.R.China. E-mail: peipeiyin@pku.edu.cn

Construction of Potential Landscape Uncovers Robust Dynamical Structure in Prostate Carcinogenesis

Ruoshi Yuan[1], Ping Ao[1], Leroy Hood[2], and Xiaomei Zhu[3]

***Short Abstract* —We explored endogenous molecular-cellular network hypothesis for prostate cancer by constructing relevant endogenous interaction network model and analyzing its dynamical properties. Molecular regulations involved in cell proliferation, apoptosis, differentiation and metabolism are included in a hierarchical mathematical modeling scheme. This dynamical network organizes into multiple robust functional states, including physiological and pathological ones. Some states have characteristics of cancer: elevated metabolic and immune activities, high concentration of growth factors and different proliferating, apoptotic and adhesion behaviors. The molecular profiling of calculated cancer state agrees with existing experiments. We developed a novel numerical method of constructing potential landscape for large scale interacting biological network. Robust topological structures such as invariant surface in the phase space are revealed by the potential landscape obtained.**

***Keywords* - Prostate Cancer, Endogenous Molecular-cellular Network, Dynamical System, Potential Landscape, Attractors.**

I. Background

MANY ideas have been put forward for carcinogenesis. At the two ends of the spectrum are accumulation of mutations in somatic cells and endogenous molecular -cellular network hypotheses [1-5]. The former focuses on a few accidental hits due to environmental insult. The later blames the whole biological structure formed by evolution: The molecular-cellular interactions which enable organisms to cope with different living conditions also lay down "traps", robust pathological states, in the endogenous molecular-cellular interaction network. While events leading to the pathological states vary, these disease causing states are well defined and therefore, having common features among individuals, according to the hypothesis.

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[1]Key Laboratory of Systems Biomedicine, Ministry of Education, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, 200240, China.

E-mail: rsyuan.acm06@gmail.com; aoping@sjtu.edu.cn

[2]Institute for Systems Biology, 401 Terry Ave. N., Seattle, WA 98109-5234, USA. E-mail: lhood@systemsbiology.org

[3]GeneMath, 5525 27th Ave. N.E., Seattle, WA 98105, USA. E-mail: xiaomeizhu@yahoo.com

II. Method

We explored endogenous molecular-cellular network hypothesis for prostate cancer by constructing relevant endogenous interaction network model and analyzing its dynamical properties. We developed a new numerical method of calculating potential landscape for large-scale endogenous network.

III. Results

This dynamical network organizes into multiple robust functional states, including physiological and pathological ones. Some states have characteristics of cancer: elevated metabolic and immune activities, high concentration of growth factors and different proliferating, apoptotic and adhesion behaviors. The potential landscape constructed even suggested the existence of more complex topological structure beyond stable states, such as invariant surface in the phase space.

IV. Conclusion

We developed a hierarchical mathematical model of endogenous molecular-cellular interactions for prostate cancer. By utilizing a new numerical method of calculating potential landscape, we effectively and intuitively demonstrate the dynamical behavior of the endogenous network, and even find out more robust topological structure hidden inside the complex biological interactions.

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Crosstalk among TGF-β, Hedgehog and Wnt signaling pathway during EMT

Jingyu Zhang[1+], Xiao-jun Tian[1+], Hang Zhang[2] and Jianhua Xing[1*]

***Short Abstract* — Epithelial to mesenchymal transition (EMT) is a key step in cancer metastasis. It involves cooperation of signaling pathways, such as Transformation growth factor-β (TGF-β), Wnt and Hedgehog (HH) pathways. These signaling pathways cooperate together and converge to Snail upregulation to turn on the core switches of EMT. The functional roles of multi-signaling pathway crosstalks in EMT remain to be explored. In this project, we use integrated computational modeling and quantitative experimental studies to investigate TGF-β induced signaling crosstalk to promote Snail expression and EMT.**

***Keywords* — TGF-β, Hedgehog, Wnt, EMT, signal trasduction.**

I. Purpose

THE process of cells transformed from health to cancer cells and the promotion of cancer cell metastasis involves multi-steps, such as evading growth suppressors, avoiding immune destruction, enabling replicative immortability, genome instability and mutation, activating invasion and metastasis, and so on [1]. EMT, which transforms the regular-shaped epithelial cells with tight cell-to-cell attachment to spindle-like mesenchymal cells with loose or no cell-to-cell attachment, plays a key role in cancer metastasis. Previous studies showed that EMT can be induced in most of the mammalian cell lines by exogenesis signals, such as TGF-β, epithelial growth factor (EGF), HH, *etc.*, and is regulated by a delicate signaling network [2].

TGF-β is a major inducer of EMT. The core process of the transformation involves two transcription factors, Zeb and Snail, and two families of microRNAs, miR-34 and miR-200. These four components form two coupled double-negative feedback loops that enable EMT process following two steps, first transition to partial EMT then to full EMT [3, 4]. The canonical TGF-β signal transduction pathway involves TGF-β receptor (TGFBR) to SMAD family and finally to Snail [5, 6]. However, many bypasses also exist in response to TGF-β signaling, resulting crosstalk to other signaling pathways. For instance, SMAD3 and SMAD4 that are promoted by TGF-β also induce Gli1/2. As two are major transcription factors in HH signaling pathway, Gli1/2 also regulate Snail expression. Meanwhile, TGF-β also activates β-catenin, which is associated to the Wnt pathway and binds to the promoter region of Gli to upregulate its expression [7]. The question then lay on the reason behind the fact that a single signal input promotes actually more than one signaling pathways and how they operate together to regulate Snail1 and EMT.

II. Experiment procedure

We constructed a mathematical model based on experimental results that were collected from previous studies. After systematical analysis of the model, we found that the the three pathways are coordinately regulate Snail1 and EMT. Now we are confirming our predictions qualitatively by traditional biochemistry experiments (flow cytometry, qPCR and western plot) and combined with cut-edge technology such as CRISPR for quantitatively verification.

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[1]Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA, 15260, USA
[2]Genetics, Bioinformatics, and Computational Biology Program, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24060, USA
E-mail: xing1@pitt.edu

Examining Genetic Background and Synaptic Morphology with Heterozygotes

Charles L Zhao[1], Patrick McGrath[2], Kang Shen[3], and Hang Lu[4]

***Short Abstract* — Most genetic studies in *Caenorhabditis elegans* are done in the N2 background, but it is unclear how this laboratory background affects these genetic studies. To examine these subtle effects, particularly on synapse development, we examine subtle synaptic mutants, using high-throughput microfluidics and computer vision to obtain the requisite sample size. With heterozygotes between these and wildtype strains, we examine different backgrounds rapidly without extensive breeding, identifying an unknown interaction between a true wildtype and the genes *jkk-1* and *unc-104*. This provides a methodology for studying multigenic and background interactions, particular their interaction with genetic studies in *C. elegans*.**

***Keywords* — Microfluidics, *C. elegans*, Quantitative Phenotyping, Computer Vision, Epistasis, Synapses, Synaptic Trafficking, Synaptic Morphology, High-throughput**

I. Background

THE model organism *Caenorhabditis elegans* is prized for ease of handling and genetic manipulation. This encourages use for genetic studies, an endeavor that has yielded ground-breaking results. However, the vast majority of genetic studies in *C. elegans* have been done on the strain N2, in which decades of cultivation has resulted in behavioral, physiological, and genetic divergence from wild populations [1]. It is probable that this genetic background modifies the results obtained in genetic studies, but the exact significance of these effects is unknown.

Evaluation of this has been bottlenecked by difficulties in experimental procedure, as well as the subtlety of background effects. Large-scale phenotypic effects drown out genetic background effects, while subtler effects require much larger sampler sizes, fluorescent markers introduced into every background under study, and detailed observation, rendering examination of more than a handful of genetic backgrounds impracticable.

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[1]Walter H Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA E-mail: czhao34@gatech.edu

[2]School of Biology, Georgia Institute of Technology, Atlanta, GA E-mail: patrick.mcgrath@biology.gatech.edu

[3]Department of Biology, Stanford University, Stanford, CA E-mail: kangshen@stanford.edu

[4]Department of Chemical Engineering, Georgia Institute of Technology, Atlanta, GA E-mail: hang.lu@gatech.edu

II. Methodology and Results

To address these difficulties, we introduce a methodology tying together several innovations made by this lab. These include the use of **microfluidics for high-throughput imaging**, **computer vision** for rapid and accurate quantitative phenotyping, and the use of **heterozygotes for comparison of genetic backgrounds** without burdensome, repeated outcrossing. The former two reflect innovations demonstrated previously by this lab [2], while the latter is a novel approach to a challenging experimental problem.

We choose to use a combination of the two recently introduced **dominant** synaptic mutants *jkk-1 (km2)* and *unc-104 (wy673)*, along with the synaptic marker *Pmig-13:snb-1::yfp*, all in the N2 background. These were chosen because of the relative subtlety of the phenotypes involved, as well as the relatively unexplored nature of synaptic morphology.

By crossing these strains with wildtype strains of *C. elegans*, producing heterozygous F1 progeny, we show that **background effects on a subtle feature like synaptic morphology can be discerned**, using the N2-cross as a control. In particular, we show that the genetic background of the Hawaiian strain CB4856 exerts an effect on synaptic morphology similar to the *km2* and *wy673* alleles, without reinforcing these mutations when present. Since CB4856 does not carry mutations known to affect synaptic morphology, the CB4856 wildtype background exerts a novel effect on these phenotypes. The choice of genetic background used for a given study can thus have a strong effect, and it is important to understand the interactions of genetic background with phenotype.

We thus demonstrate that our method is **capable of detecting subtle genetic background effects**, and also that **these effects are an important confound to genetic discovery**.

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A. Aptekmann[1], A.Nadra[1]

Archean core promoter, information content and its relation with optimal growth temperature.

We studied the relation between optimal growth temperature (OGT) and information content (IC), in the core promoter region of all the archeal genomes published to date, by calculating the information content of the motiff that represents the TATA binding site (TBS).
We have tested several different approaches to predict transcription start sites (TSS) in a given genome we then used motiff prediction software in the flanking regions to the TSS, we constructed a database, compiling already available information from published sources, that contains characteristic growth conditions for each strain.
Our work hipotesis is that protein-dna interfase in thermophiles should be different from that of mesophiles, in particular we propose and test a positive correlation between information content of binding sites and OGT in archeas.
We show that the information content increases with increasing optimal growth temperature, and this effect cannot be explained solely by an increased CG composition.
Selective pressure towards binding sites with higher binding affinity to the protein could be the reason for this correlation.
The established Rseq = Rfreq from molecular information theory doesnt take into account the effect of temperature as a selective pressure acting to skew the posible binding sites, and creating another cause for an increment in Rseq that doesnt apply to Rfreq.
Since entropy effects increase with temperature, Shannon entropy effects might as well.

***Keywords* — Information Content, Thermophiles, TATA binding protein, Basal Promoter.**

I. Purpose

Life is limited by physical and chemical extremes that define the "habitable space" within which it operates.
Aside from its requirement for liquid water, no definite limits have been established for life under any extreme[1].
We know about some aspects of the adaptations to extreme environments, in particular there has been a lot of research regarding the adaptations to temperature in order to maintain protein stability[2] and some possible mechanisms for stabilizing genomic DNA in archeas have also been proposed [3], but trough what means the fine regulation that is normally exerted trough protein-DNA interactions is maintained, still is relatively unknown and might give insights into the determinants that mediate the inter-molecular recognition process and how the extreme environments favor certain responses. Even tough our interest is in the general process of protein-DNA interaction we choose to limit our study to the realm of the archeas since they comprehend the majority of the hyper thermophiles.
One possible approach to understanding the adaptation process is looking for trends in the sequence changes of the organisms, this line of thinking lead to many proposed ideas, as for example the relation between GC content and the OGT[4].
We propose a novel relation between the information content of a protein binding site and the temperature at witch the organism lives.
We choosed to work with archeas due to the wide range of temperatures they endure.
Protein-DNA interactions are central to cell activity regulation including transcription initiation, one of the more studied cases available for archeas , being the TATA box binding protein (TBP).
TBP is involved in promoter recognition, the first step of transcription initiation. TBP is universally conserved and essential in archaea and eukaryotes. In archaea, TBPs have to be stable and to function in species that cover an extremely wide range of optimal growth temperatures (OGTs), from below 0ºC to more than 100ºC.
Thus, the archaeal TBP family is ideally suited to study the evolutionary adaptation of proteins to an extremely wide range of temperatures [5].
Organisms that do thrive in extreme environments might have in some way been affected by the selective pressure imposed by this conditions, for the particular case of the TBP we expect TBP and TATA box to co evolve responding to a number of factors, adaptation to temperature, pressure, salinity, and other extreme biophysical conditions.

II. Conclusion

Our preliminary results confirm our hypothesis, there is an apparent correlation between information content and optimal growth temperature.

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[†]This work was funded by CONICET.
[1]Department of Biological Chemistry, University of Buenos Aires , Argentina. E-mail: arielaptekmann@qb.fcen.uba.ar

Comparison of Domain Nucleation Mechanisms in a Minimal Model of Shoot Apical Meristem

Dorjsuren Battogtokh[1,2] and John J. Tyson[2]

Short Abstract — Existing mathematical models of the shoot apical meristem (SAM) explain nucleation and confinement of a stem cell domain by a Turing mechanism, assuming that the diffusion coefficients of the activator (WUSCHEL) and inhibitor (CLAVATA) are significantly different. As there is no evidence for this assumption of differential diffusivity, we recently proposed a new mechanism based on a "bistable switch" model of the SAM. Here we study the bistable-switch model in detail, demonstrating that it can be understood as localized switches of WUSHEL activity in individual cells driven by a non-uniform field of a hypothetical hormone. By comparing domain formation on a cell-network driven by Turing and bistable-switch models, we show that better domain control is possible with the new mechanism.

***Keywords* —minimal model of SAM, reaction-diffusion systems, Turing instability, bistability, fast diffusive field.**

I. Purpose

The stem cells residing in the shoot apical meristem (SAM) give rise to above ground tissues [1]. Hence, maintenance of stem cell niches is of central importance to plant growth [2,3]. Negative feedback between the proteins WUSCHEL (WUS - a homeodomain transcription factor) and CLAVATA (CLV - a receptor kinase) is at the core of the signaling pathway controlling the central domain – the reservoir of stem cells [1]. Recently, theorists have proposed reaction-diffusion models of the SAM [4-7] that explain nucleation and confinement of the central domain as a Turing instability.

The most well-known mechanism of pattern formation in dissipative systems is associated with a Turing instability [8]. In this case, a spatially uniform steady state, which is globally stable with respect to uniform perturbations, becomes unstable with respect to non-uniform perturbations, provided that the diffusion range of an inhibitor significantly exceeds the diffusion range of an activator [9]. Under these conditions, a periodic pattern emerges in a monostable system at a certain critical wavenumber [10]. For a mathematical model of SAM, a Turing mechanism requires that the diffusion coefficient of CLV (inhibitor) significantly exceeds that of WUS (activator). At present, the diffusive properties of CLV and WUS are not well established; therefore, there is no clear experimental evidence on whether the Turing condition of differential diffusivity is applicable within the WUS and CLV expression zones of the SAM.

Existing models of the molecular biology of SAM regulation have positive and negative feedback loops that can generate not only Turing patterns but also alternative stable steady states (bistability) in a certain range of parameter values [2,6,7]. Recently bistable reaction-diffusion models have been studied to simulate experimental data on cytokinin controlled domain confinement in SAM [3]. In our previous work [11], a mechanism different from Turing instability was proposed for pattern formation in a minimal, bistable model of SAM. In the present work, we study in detail the mechanism of domain nucleation reported in Ref. [11].

II. Conclusion

Here we illustrate how a spatially non-uniform field of a peptide hormone synthesized by WUS can drive domain nucleation in a SAM model exhibiting bistability. We compare central-domain formation by Turing and bistable-switch mechanisms on a polygonal cell-network and show that, in the latter case, domain nucleation at a target location is possible without the additional assumptions required by the former.

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[1]The Institute of Physics and Technology, Mongolian Academy of Sciences, Ulaanbaatar 51, Mongolia. E-mail: dbattogt@vt.edu
[2]Department of Biological Sciences, Virginia Polytechnic and State University, Blacksburg, Virginia 24061, USA. E-mail: tyson@vt.edu

Ensemble Learning for Correlated Substitution Analysis

Kevin S. Brown[1], Christopher A. Brown[2]

***Short Abstract* — There are a variety of methods that attempt to infer networks of positional correlations in proteins from multiple sequence data. However, method accuracy is inconsistent from sequence alignment to sequence alignment, depends strongly on sequence preprocessing and method parameters, and the predicted networks from different methods show little overlap. We use ensemble learning to combine the results of multiple scoring methods. When tested on a large set of alignments, the ensemble method outperforms the individual scoring methods in the ensemble.**

I. Introduction

EMBEDDED in an evolving protein is a complex network of amino acid correlations. The constraints induced by this network of correlated fluctuations drive residue substitutions at single sites. Correlations can be strong even between pairs of residues widely separated in the folded structure because of allostery [1], charged interactions [2], or other forms of energetic coupling [3].

In order to infer this correlation network from multiple sequence data, many methods under the names *correlated substitution analysis* have been developed. The methods for scoring pairs of residues for high correlation include chi-squared tests [4], explicit likelihood [5], variants of mutual information [6,7], and maximum entropy models [8].

Unfortunately, method accuracy – as assessed by comparison to protein contact maps – is inconsistent from sequence alignment to sequence alignment and can be strongly dependent on other preprocessing steps and scoring parameters. In addition, predicted networks from different methods often show relatively poor overlap [9].

II. Results

In some machine learning problems, combining several models yields better results than can be achieved by any individual model [10]. We use an ensemble approach to blend the results of multiple correlated substitution scoring methods.

We first convert the set of scores each method assigns into a set of ranks. The ranks are then aggregated, using a metric similar to those employed for web meta-search engines [11]. We combined nine different scoring methods on a large (~ 3500) set of high-quality protein alignments from the Pfam database [12]. The methods used include both newer, more sophisticated methods [7,8,9] and older, simpler ones [4,6]. The ensemble approach shows a marked improvement in scoring accuracy when compared to the individual ensemble members.

III. Conclusion

A large amount of effort in the field of correlated substitution analysis is directed towards developing ever more complicated, and hopefully more accurate, scoring methods. Our mixture-of-experts results suggest that even older, relatively simple methods can still yield impressive predictions when properly blended. Given that many scoring methods are derived from others, in the future it might be desirable to blend the models with a more sophisticated scheme [13].

[1]Biomedical Engineering, University of Connecticut, Storrs, CT. E-mail: kevin.s.brown@uconn.edu

[2]Palomidez, LLC. E-mail: chris.al.brown@gmail.com

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Coupled Reaction Theory

William R. Cannon[1]

***Short Abstract* — A statistical mechanical formulation of the law of mass action has been developed, coupled reaction theory, that is on equal theoretical footing with kinetic rate laws and describes chemical kinetics exactly. The formulation is based on a fluctuation theorem for coupled reactions and uses chemical potentials instead of rate constants. Furthermore, using this approach, it can be demonstrated that for many systems, the highest rate of flux at steady state corresponds to the optimal thermodynamic conditions as well. We discuss the significance of coupled reaction theory for applications in systems biology.**

***Keywords* — kinetics, thermodynamics, law of mass action, transition state theory, stochastic.**

I. Motivation

ONE hundred and fifty years ago Peter Waage and Cato Maximillian Gulberg published their first article describing the law of mass action, that the rate of a chemical reaction is proportional to the concentration of the reacting species [1].

Forty years after Waage and Gulberg's initial publication, efforts were made to merge the law of mass action with Gibbs ensemble formulation of statistical thermodynamics, which culminated in 1935 with the Absolute Rate Theory of Eyring [2], and Polanyi and Evans [3]. While transition state theory, as it is now known, has been enormously successful as a framework for understanding chemical reactions, it has not had success in being a convenient formulation of statistical mechanics that can be used for large-scale modeling of coupled reactions [4].

II. A Fluctuation Theorem for Coupled Reactions

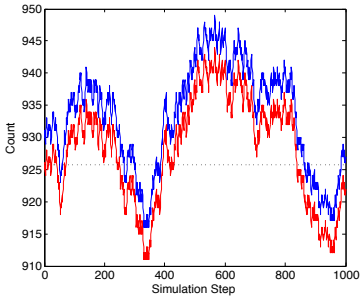
Fluctuation theorems are used to describe stochastic models of dynamics [5]. In stochastic models of reactive systems (Markov models) the usual differential equation relating rate to concentrations is replaced by the change in probability of a state as a function of time. Fluctuation theorems relate conjugate processes from the same original state via the dissipation,

$$\frac{\mathcal{P}r(J)\pi_1(t \mid J)}{\mathcal{P}r(J)\pi_{-1}(t \mid J)} = e^{\Omega_1(J)}$$

where $\Omega_1(J)$ is a dissipation function for reaction 1 from state J. This is a significant aspect of fluctuation theorems – they relate the ratio of the probabilities of conjugate processes to a value that is potentially measurable or calculable. If one could determine $\Omega_1(J)$ then the relative reaction probabilities could be determined and the system could be modeled without the use of the rate constants first described by Waage and Gulberg over 150 years ago.

Using a fluctuation theory, we have been able to model the dynamics of coupled chemical reactions with the same precision as kinetic rate laws using differential equations or stochastic kinetic models.

The dynamical trajectory of a reaction intermediate *B* from a stochastic simulation using coupled reaction theory is shown below along with a trajectory from a stochastic kinetic simulation and the steady state solution to the deterministic ordinary differential equation for comparison. When using the same set of random numbers, the trajectories are exactly the same, which indicates that the reaction probabilities are also exactly the same. In this case the ratio of the forward rate constants for the two coupled reactions was 10^{-4}. However, we have also been able to demonstrate that the exact results are obtained regardless of the difference in rate constants between sequential reactions. That is, coupled reaction theory represents one solution to multiscale modeling challenges.



A trajectory from coupled reaction theory (blue, offset by +5) compared to stochastic kinetic simulation (red).

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[1]Computaional Biology and Bioinformatics Group, Pacific Northwest National Laboratory, Richland WA 99352. E-mail: william.cannon@pnnl.gov

Non-equilibrium relaxation in the vicinity of the extinction critical point in a stochastic lattice Lotka-Volterra model

Sheng Chen[1], Uwe C. Täuber[1]

***Short Abstract* — Spatially extended stochastic models for predator-prey competition and coexistence display complex, correlated spatio-temporal structures and are governed by large fluctuations. We specifically study a stochastic lattice Lotka-Volterra model. Generically, the system tends to relax into a quasi-stationary state, independent of the initial conditions. We investigate the non-equilibrium relaxation in the vicinity of the critical point. We obtain a power law dependence between the relaxation time and predation rate (critical slowing down), and measure the critical aging scaling exponents.**

***Keywords* — non-equilibrium relaxation, stochastic lattice Lotka-Volterra model, critical point, critical exponent.**

I. BACKGROUND

THE Lotka-Volterra model [1,2] describes a two-species predator-prey coexistence / competition system with 'predators' A and 'prey' B. Predators spontaneously die with rate μ. They also may consume prey and reproduce with rate λ > 0. Prey may reproduce with rate σ.

$$A \xrightarrow{\mu} 0$$
$$AB \xrightarrow{\lambda} AA$$
$$B0 \xrightarrow{\sigma} BB$$

We study a stochastic lattice Lotka-Volterra model [3,4] by means of Monte Carlo simulations performed on a 2D square lattice with periodic boundary conditions. Site restrictions are applied to the system. Each lattice site can either be empty, occupied by a 'predator' or by a 'prey'.

If we fix μ and σ, the system relaxes into one of two possible states which are governed by predation rate λ. There is one stable state in which both species survive. The other state is an absorbing state with only prey remaining. A critical predator extinction threshold exists between these two states.

II. SUMMARY OF THE RESULTS

We set the initial condition as a random configuration with both species densities 0.3 on a 1024*1024 lattice. The system relaxes into a quasi-stationary state with reaction rates σ = 1, μ = 0.025 and $\lambda_1 = 0.25$. Then we change the predation rate from λ_1 to λ_2, followed by an ensuing relaxation to another state.

A. Relaxation between two quasi-stationary states

We obtain non-equilibrium relaxation between two quasi-stationary states when λ_2 is in the stable region. The density relaxes into a new state exponentially. The ensuing relaxation times are measured via the peak width of the population density Fourier transforms. The damping rate is equal to the inverse of the relaxation time.

Away from the critical point, we find that the initial configuration influences the oscillations for the duration of one relaxation time.

B. Quench to the critical point

In the vicinity of the critical point λ_c, we obtain a power law dependence of the relaxation time on $(\lambda-\lambda_c)/\lambda_c$ (critical slowing down). The associated dynamical critical exponent is measured to be $z\upsilon \approx 1.9$ for a 512*512 system.

We employ different system sizes to carry out finite-size scaling [5] in order to accurately measure the aging scaling exponents.

III. CONCLUSION

In the lattice Lotka-Volterra Model, there is a species coexistence state where both predators and prey can stably survive. We observe relaxation between two quasi-stable states when changing the predation rate. As expected, we find that the initial state generically only influences the oscillations for the duration of about one relaxation time, implying that the system quickly loses any memory of the initial configuration.We have measured the critical aging scaling exponents following a quench of the system to the predator extinction threshold.

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[1]Department of Physics, Virginia Tech, Blacksburg, VA, USA. E-mail: csheng@vt.edu

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Global effects from errors at single kinetochores

Bin He[1], Emanuele Roscioli[1], Alex Winemiller[1], Gul Civelekoglu-Scholey[2], and Daniela Cimini[1]

***Short Abstract* — Correct chromosome segregation during mitosis relies on the mitotic apparatus, a complex macromolecular machine that specifically assembles during this cell cycle stage. We previously established a quantitative model to describe metaphase chromosome dynamics via kinetochore-microtubule (KT-MT) interactions mediated by viscoelastic linkages. Here, we used this quantitative framework in combination with experimental approaches to characterize the metaphase and anaphase KT/chromosome dynamics in cells with merotelic KT mis-attachments. We found that mis-attachment can affect the dynamics of KTs other than the mis-attached one. Moreover, we investigated the role of Kif2a in correction of KT mis-attachments in PtK1 cells.**

***Keywords* — Kinetochore, microtubule, merotelic, Kif2a**

I. Background

We previously developed a quantitative framework integrating forces that control the attachment to MTs, positioning, and dynamics of amphitelically (correctly) attached KT pairs during metaphase [1]. The model describes and predicts many aspects of spindle dynamics. Here, we utilized this model to address important biological questions. First, we investigated the chromosome dynamics (both in metaphase and anaphase) associated with erroneous KT-MT attachment, specifically merotelic attachment (a single KT bound to both spindle poles instead of just one). Merotelic KT mis-attachment is a major cause of aneuploidy in mammalian cells [2] and a major cause of chromosomal instability in cancer cells [3]. These mis-attachments arise frequently in healthy mitotic cells [4], but most of them are corrected before anaphase onset. This correction process depends upon microtubule dynamics fine-tuned by many mechanical and molecular signaling mechanisms, some of which have been extensively dissected and others that are not well understood. MT poleward flux is one such mechanism because, although there is evidence for a role of MT poleward flux in correction of KT mis-attachments [5], the exact mechanism is not clear.

II. Results

We combined mathematical modeling and quantitative live-cell microscopy to study the effect of KT mis-attachment on chromosome dynamics and the contribution of MT poleward flux to correction of KT mis-attachments.

[1]Dept. of Biological Sciences and Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, 24061 – USA E-mail: cimini@vt.edu

[2]Dept. of Molecular and Cellular Biology, University of California, Davis, CA 95616 – USA E-mail: egcivelekoglu scholey@ucdavis.edu

A. *Merotelic KT attachment and metaphase dynamics*

Our mathematical model reproduced the observed shift of merotelic KTs closer to the cell equator and their lack of oscillation [6] and predicted the sister of a merotelic KT displays reduced oscillation. Quantification of KT oscillation in live cells confirmed that the sister of a merotelic KT displayed shorter oscillation period and amplitude compared to KTs of normally attached chromosomes.

B. *MT poleward flux and correction of mis-attachments*

Model simulations predicted that reduced MT poleward flux resulted in larger numbers of MTs bound to KTs, suggesting reduced correction of KT mis-attachments. Experimental reduction of MT poleward flux resulted in a larger fraction of KT-bound MTs and increased rates of cells progressing through mitosis with merotelically attached KTs, confirming the model predictions.

C. *Merotelic KT attachment and anaphase dynamics*

We finally found that reduction of MT poleward flux did not affect overall rate of chromosome movement in anaphase. But strikingly, in anaphase cells with merotelic KTs that lagged behind at the spindle equator, not only the merotelic KT did not move poleward, but the poleward movement of all other, normally attached KTs was slower compared to anaphase cells without merotelic KTs.

III. Future directions

Computer simulations will be used to dissect the mechanism responsible for the merotelic KT-dependent effect on anaphase chromosome dynamics and formulate predictions that we can test experimentally.

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Accumulation versus propagation: coding dynamics in motion discrimination

Bryan C. Daniels[1], Jessica C. Flack[1,2], and David C. Krakauer[1,2]

***Short Abstract* — We propose two temporal phases of collective computation in a visual motion direction discrimination task by analyzing recordings from 169 neural channels in the prefrontal cortex of macaque monkeys. Phase I is an "intensive" or incompressible phase in which uncertainty is substantially reduced by pooling information from many cells. Phase II is an "extensive" or compressible phase in which numerous single cells contain all the information present at the population level in Phase I. Intriguingly, the most informative cells in Phase I are least coupled to each other on short timescales, as measured by size distributions of synchronous events. We suggest that this dynamic of independent accumulation followed by cooperative propagation is a generic feature of robust collective computing systems related to consensus formation.**

***Keywords* — Neural coding, population coding, collective behavior, criticality**

I. Introduction

THE nervous system is the paradigm of a distributed information processing system, with information present at multiple levels that span single cells, correlated modules, the hemispheres, and the whole brain. How these scales interact, how activity across scales becomes coordinated, and how adaptively significant information is encoded are among the primary concerns of cognitive neuroscience.

Here, we explore these issues with data from an experiment developed by Newsome and collaborators [1,2] that tracks neuronal processing in a visual discrimination task. Macaque monkeys are trained to discriminate directions of motion in a stochastic random dot display. In each trial, the stimulus is presented, and after a delay a "go" cue prompts the subject to indicate their decision about the dots' direction of motion using an eye movement. A multi-electrode array simultaneously measures times of action potentials in 169 neural units in prefrontal cortex. Neurons in this area are known to carry high-level signals specific to salient visual targets and eye movements [2].

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[1]Center for Complexity and Collective Computation, Wisconsin Institute for Discovery, University of Wisconsin, Madison. E-mail: bdaniels@discovery.wisc.edu

[2]Santa Fe Institute, Santa Fe, New Mexico.

II. Population to Individual Coding

During the visual stimulus presentation (Phase I), a subset of cells becomes increasingly informative about the eventual decision. Just before the go cue, Linear Discriminant Analysis can use their rates of firing to predict the monkey's decision with ~85% accuracy. The information, however, is not stored in any individual unit—maximal performance requires integrating over the activity of >20 cells. After the go cue, when the decision is translated into an eye movement (Phase II), many more cells become predictive, and can predict the output with near perfect accuracy. Conversely, the number of units needed to attain maximal performance drops to 1–2. In this way, the decision process includes a switch from *accumulation* in a population code to *propagation* of information to individual rates. Implementing this switch has implications for the units' collective dynamical properties.

III. Criticality and Information Localization

Multiple other neuronal systems have behavior suggestive of tuning toward a specific point between complete independence and complete correlation, a phase transition that implies maximal sensitivity to perturbations (e.g. [3-5]). For the units in our study that carry negligible information about the decision, we also find evidence of this near-critical state in their distribution of sizes of synchronous events.

However, the cells whose firing rates contain the most information about the eventual decision are decidedly not critical, firing largely independently of one another, with an event size distribution similar to an independent null. This more generally suggests a robust method for collective decision making: localized accumulation of evidence by independent individuals followed by a consensus process that propagates a single decision to the global scale.

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Analysis of inexact Krylov subspace methods for approximating the matrix exponential

Khanh N. Dinh[1], Roger B.Sidje[2]

Abstract* — Krylov subspace methods have proved quite effective at approximating the action of a large sparse matrix exponential on a vector. Their numerical robustness and matrix-free nature have enabled them to make inroads into a variety of applications of great importance. A case in point is solving the chemical master equation (CME) that models a system of biochemical reactions. This is a challenging problem that gives rise to an extremely large matrix due to the curse of dimensionality. Inexact Krylov subspace methods combined with truncation techniques have helped solve some CME models that were considered computationally out of reach as recently as a few years ago. However, as models grow, truncating them means using an even smaller fraction of their whole extent, thereby introducing more inexactness. But experimental evidence suggests an apparent success and the aim of this study is to give theoretical insights into the reasons why. Essentially, we show that the truncation can be put in the framework of inexact Krylov methods that relax matrix-vector products and compute them expediently by trading accuracy for speed. This allows us to analyze both the residual (or defect) and the error of the resulting approximations to the matrix exponential from the viewpoint of inexact Krylov methods. Numerical experiments demonstrating the theory are reported.

[1]Department of Mathematics, University of Alabama. E-mail: kdinh@crimson.ua.edu

[2]Department of Mathematics, University of Alabama. E-mail: roger.b.sidje@ua.edu

Regulation of intestinal crypt homoeostasis: A balance between Wnt mediated expansion and proliferation inhibition

Huijing Du[1], Qing Nie[1] and William R. Holmes[1,2]

***Short Abstract* — A hybrid stochastic model is used to investigate how exogenous niche signaling (Wnt and BMP) and auto-regulation promote homeostasis. This model uses sub-cellular element method to account for three-dimensional structure of the crypt, external regulation by Wnt and BMP, internal regulation by Notch, as well as regulation by internally generated diffusible signals. Results provide an alternative view of crypt homeostasis where the niche is in a constant state of expansion and the spatial structure of the crypt arises as a balance between this expansion and the action of various sources of negative regulation that hold it in check.**

I. Introduction

THE epithelium of the intestinal crypt is an incredibly dynamic tissue, constantly replenishing itself every 4-5 days, which is fueled by approximately 15 CBCs [1], dividing roughly once per day even in healthy tissue [2]. Numerous investigations have shown the canonical Wnt / b-catenin pathway to be critical in maintaining homeostasis [3]. There are two sources of Wnt signaling in the crypt [3]. The mesenchyme that surrounds it produces graded expression of Wnts; Paneth cells also produce Wnt3a. Genetic deletion of this "local", Paneth cell derived Wnt source does not impair stem cell populations in the *in vivo* crypt [4], suggesting the global Wnt gradient is sufficient for homeostasis. However, i*n vitro* studies of "mini-guts" grown from CBCs have shown that Paneth derived Wnt3a alone is also sufficient to maintain crypt structure in the absence of the other exogenous Wnt sources [5]. Additionally, Eph / ephrin signaling interactions generate repulsive forces that drive Paneth cells to migrate down the crypt wall while all other cells passively migrate upward from the base, driven by proliferative pressure [6]. Bone morphogenic proteins (BMPs) are also known to influence crypt homeostasis by suppressing proliferation of stem cells [7].

How do these signaling components contribute to maintaining the spatial structure of the crypt and how do they interact? Extensive computational modeling has been employed to address this and related questions.

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[1]Center for Complex Biological Systems and Department of Mathematics, University of California Irvine, Irvine, CA 92697. E-mail: huijingd@uci.edu, qnie@math.uci.edu

[2]Department of Mathematics and Statistics, University of Melbourne, Melbourne Australia 3010. E-mail: holmesw@unimelb.edu.au

II. Results

Paneth derived Wnt promotes uncontrolled expansion of the stem cell niche. Simulations results show that stem and Paneth cells together create a mutually sustaining feedback that drives expansion of both populations. Thus, Paneth cell derived Wnt signaling alone cannot both fully sustain the niche and promote homeostasis at the same time.

Regulation of proliferation by BMP constrains niche expansion. Simulation results suggest that there is a balance between expansion and repression that is required to maintain homeostasis. Wnt, which influences differentiation, promotes niche expansion while BMP, which influences proliferation, constrains that expansion.

Eph/Ephrin mediated Paneth cell motion is required to constrain niche expansion. These results suggests that rather than being required to maintain the niche, Paneth cell migration is instead required to maintain proper structure in the upper walls of the crypt, and in particular to constrain niche expansion. Also the rate of cellular proliferation and the drag between cells and the crypt wall induced by adhesion are also observed to investigate the role of cell motions.

III. Conclusion

We find that there are redundant signals created by both the epithelium itself and surrounding tissues that act in parallel to maintain epithelial structure. However, this redundancy introduces the possibility of explosive stem cell population growth. Additional results suggest that other signals along with choreographed motion of cells are responsible for repressing this expansion. Taken together, our results provide a novel hypothesis for how robust but fast renewal of the crypt is achieved: as a balance between expansion, which drives fast renewal and repression, which holds that expansion in check to maintain the crypt's structure.

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Explore design principles of signaling networks with *in silico* evolution of rule-based models

Song Feng[1], Julien F Ollivier[2], Peter S Swain[3] and Orkun S Soyer[1,4]

***Short Abstract* — Systems and synthetic biologists aim to decipher the structure and dynamics of cellular networks underpinning specific responses, then to alter existing networks or engineer de novo ones. Both tasks could benefit from study of which structural and dynamic features can emerge from evolutionary processes, through which intermediary steps these arise, and whether they constitute key "design principles". Here, we present a design approach that focuses on *discovering* a range of possible signalling circuits with a given response dynamics. This approach combines *in silico* evolution and rule-based modelling of signalling proteins and their interactions. In particular, we evolve ultrasensitive and bistable signalling circuits that display both known and hereto unknown design features.**

***Keywords* — Design Principles, Signalling Networks, Evolution *in silico*, Rule-based Models, Computational Design**

I. Introduction

SIGNALING networks allow organisms to sense and process environmental information and thereby implement phenotypic behaviors that enable survival. It is of fundamental interest to understand the structure and dynamics of these cellular networks. In particular, systems biologists hope to be able to define common structural and dynamical features of networks that can be seen as "design principles" that are re-used in diverse systems, while synthetic biologists aim to utilize such design principles for reliable and modular engineering of biology [1]. One approach for understanding the evolutionary processes that lead to existing network elements, and for exploring the space of possible solutions, is to re-create the evolutionary dynamics of cellular networks *in silico*. Here, we present a design approach based on a novel combination of *in silico* evolution with a specific rule-based modeling of signaling proteins called Allosteric Network Compiler (ANC) [2]. The use of rule-based models allows us to define biochemical features of signaling proteins in detail, while overcoming the combinatorial explosion in model structure that arises from evolving protein interactions [3]. At its core, the rule sets in the ANC framework allows us to define any number of signaling proteins, each with a number of domains, and their interactions, i.e. a complete signaling circuit. Combining the ANC with an *in silico* evolutionary algorithm, we are able to evolve such signaling circuit models according to a user-defined fitness function [4].

[1]School of Life Sciences, University of Warwick, United Kingdom
[2]Centre for Nonlinear Dynamics, Department of Physiology, McGill University, Montreal, Canada
[3]SynthSys, The University of Edinburgh, United Kingdom
[4]Warwick Center for Integrative Synthetic Biology, University of Warwick, United Kingdom E- mail: O.Soyer@warwick.ac.uk

II. Results

We applied this approach to explore signaling circuit design exhibiting switch-like (i.e. ultrasensitive) and bistable response dynamics. These types of response dynamics are particularly important in information processing and decision-making in cells [5,6].

For signaling circuits with ultrasensitive response dynamics emerged from evolutionary simulations, approximately half of them utilize the zero-order sensitivity to get ultrasensitivity [6]. More interestingly, some evolved circuits displayed bistability, while, from previously reported works, the only suggested cases for bistability in phosphorylation based signaling networks were multi-site phosphorylation and positive feedback loops where phosphorylated proteins acted upon their own, upstream kinases [5,6,7]. The evolved bistable circuits we find displayed neither of these features. To better understand the role of allosteric regulation, we analyzed the simplest found circuit with bistability and further reduced its complexity by removing reactions from it. This led to a minimal design for bistability, in which we had a protein with a single phosphorylation site that is phosphorylated by an allosteric kinase.

III. Conclusion

This analysis demonstrates the power of an *in silico* evolution approach in designing signaling networks as well as the potentials for discovering design principles of ultrasensitive and decision making in cells.

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Distinct mechanical roles for bacterial biopolymers in sensing and strength

Chris Rodesney[1], BJ Cooley[1], Kristin Kovach[1], Megan Davis-Fields[1], and Vernita D. Gordon[1]

Short Abstract — **Biofilm bacteria are embedded in extracellular polymers (EPS). Multiple types of EPS can be produced by a single bacterial strain - the reasons for this redundancy are not well-understood. Our work suggests that different polymers may confer distinct mechanical benefits. Biofilms initiate when bacteria attach to a surface, sense the surface, and change their gene expression. The EPS PEL enhances surface sensing by increasing mechanical coupling of single bacteria to the surface. For the mature biofilm, the EPS PSL stiffens and strengthens biofilms. For bacteria in chronic infections, EPS expression evolves to combine mechanical fitness with complementary, chemical fitness benefits.**

***Keywords* — *P. aeruginosa*, biofilm, mechanosensing, signaling, cyclic-di-GMP, extracellular polysaccharide (EPS), motility, shear stress, rheology, evolution.**

I. Biofilm initiation, mechanics, and evolution

Pseudomonas aeruginosa is an opportunistic human pathogen that forms chronic infections in the form of biofilms, a phenotypic state associated with increased antibiotic resistance and evasion of the immune defense. In biofilms, sessile microbes are embedded in a matrix consisting largely of self-produced extracellular polysaccharides (EPS). PAO1 is a lab strain that, *in vitro*, produces two types of EPS, PEL and PSL.

In vitro biofilm formation initiates when bacteria encounter, and attach to, a surface. Cyclic-di-GMP, a second messenger whose intracellular levels increase upon adhesion of *P. aeruginosa* to a surface, regulates the expression of many genes for biofilm initiation. What cues notify bacteria that they are attached to a surface to increase cyclic-di-GMP production are unknown. This is a gap in our understanding of a fundamental microbiological process.

The biofilm matrix can protect bacteria chemically and mechanically. *P. aeruginosa* infections in the cystic fibrosis (CF) lung often last for decades, ample time for the infecting strain(s) to evolve. Production of a third EPS material, alginate, is well-known to tend to increase over time in CF infections and to be associated with poorer outcomes for patients. Alginate chemically protects biofilms, but also makes them softer, which seems to be a mechanical disadvantage. It was recently found that bacteria in chronic CF infections also evolve to increase PSL production [1].

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[1]Center for Nonlinear Dynamics, The University of Texas at Austin, Austin TX 78731. E-mail: gordon@chaos.utexas.edu

II. Mechanosensing of surfaces

We use a green fluorescent protein (GFP) reporter for intracellular cyclic-di-GMP levels [2]. With increased flow rate of liquid media, and thus increased shear stress, the intracellular cyclic-di-GMP levels increase in a dose-response fashion. Moreover, at low shear stress we find that PEL enhances the cyclic-di-GMP signaling response– populations of wild-type (WT) and Δ*pel* have indistinguishable GFP intensity distributions when in liquid suspension and at high shear rates, but at low shear the WT are brighter than the Δ*pel*. Motility measurements suggest that PEL may increase frictional interactions between the surface and the bacteria. To date, the role of PEL in PAO1 biofilms has seemed relatively minor and redundant with PSL. We infer that a major role of PEL is to enhance surface sensing by increasing the mechanical coupling.[3]

III. PSL stiffens and strengthens biofilms

We use oscillatory bulk rheology to determine the unique contributions of EPS materials to the mechanics of biofilms grown from isogenic PAO1 variants and from sets of chronological clinical isolates from four CF patients over decades of infection [1]. We find that PSL stiffens biofilms and PEL and alginate make biofilms more ductile. Comparing, biofilm mechanics to estimated forces exerted by phagocytosing neutrophils [4], we infer that increased PSL could confer a mechanical fitness benefit. [5]

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Mechanism Inference from Single Cells (MISC)

Rachel A. Haggerty[1], Jeanette Baran-Gale[1] and Jeremy E. Purvis[1]

***Short Abstract* — Mechanism Inference from Single Cells (MISC) is a method we have developed to extract the structure and strength of kinetic interactions among two or more signaling molecules. The algorithm considers all possible network structures of a certain size that could relate the measurements and then tests each network structure against the single-cell data. Well performing network structures are used to find a consensus model that suggests potential underlying signaling mechanisms.**

***Keywords* — Signal transduction, single-cell, mechanism inference, networks, machine learning.**

I. Background

Network motifs in cellular signaling pathways are indicative of the underlying function of the signaling network [1]. Sometimes the interactions between two signaling molecules can be easily inferred, for example, if one directly activates or represses another. However, there are many instances where multiple molecules are involved in a signaling response, but the exact mechanism in which they interact with each other is not clear. To address this problem, we developed a computational method called MISC (Mechanism Inference from Single Cells) that uses paired time-series measurements from individual cells to predict the underlying network structure with no prior knowledge about the network architecture.

II. Detailed Algorithm

The input for MISC is two sets of time series traces from individual cells, such as those generated from fluorescent biosensors used in live-cell imaging experiments. The output is a ranked list of signaling mechanisms from a complete list of network topologies of a certain size that describe the kinetic relationships among the biosensors.

The steps of the algorithm are as follows: (1) All possible network structures of a given size that relate an input signal and output signal are enumerated. This is to say that every possible network with a set number of nodes or less with a path from the input signal to the output signal is generated. If it is unknown which signal is upstream, the algorithm may be run both ways. The ability to include additional nodes beyond those observed allows for the possibility of other unknown factors to influence the network. (2) Ordinary differential equations are automatically generated. MISC allows the user to specify the functional form of the equations for positive and negative links, so the method can be applied to a large range of systems. (3) Signals are simulated using the single-cell data from the input biosensor. These are fed through all networks and evaluated for how well each reproduces the output biosensor. (4) All possible networks are given a score based on how well it fits the output biosensor signal and ranked. (5) A consensus network is calculated to best describe the network.

III. Method Validation

A. Synthetic Data

To validate that our method works correctly, we generated a network with three nodes involved, but only associated biosensors with two nodes. Using this network, we generated ordinary differential equations to describe the system and used them to simulate single-cell data for the input and output biosensor by varying the initial conditions and adding noise.

B. Results

We tested the synthetic biosensor data on MISC to determine whether we could recover the original network we used to create the synthetic data. We ran the algorithm with the possible number of nodes set to three or less. Links that were present in the original network were enriched in the top ranked models. In addition, the consensus model, in this case created by clustering the top 10% of models and finding the centroid of the cluster with the lowest error, was very similar to the original network. Additionally, we then generated synthetic signals for if we had a biosensor on the third node, and the behavior of this signal was very similar for the original network and the consensus model network.

IV. Conclusion

MISC is an algorithm that allows us to discover the mechanism by which one factor is influenced by another via single-cell data. There is a large range of potential applications for which this method could also be applied. In general, it searches for plausible interaction networks between any two (or more) signals. Because it ranks all possible networks, it also suggests which sets of signaling motifs may have equivalent functions. Moreover, the ability of MISC to account for unobserved signaling molecules in the network allows for the discovery of novel factors and interactions.

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[1]Department of Genetics, Bioinformatics & Computational Biology Graduate Program, University of North Carolina at Chapel Hill. Correspondence E-mail: haggerty@unc.edu

Control of inflammatory gene expression at the step of transcription elongation

Yingli Shang[1], Teng He[2], Li Yu[1], Maddalena Coppo[3], Inez Rogatsky[3], Chao Tang[2] and Xiaoyu Hu[1]

Recruitment of RNA polymerase II (PolII) to target gene promoters to initiate RNA synthesis has long been considered the key step for gene regulation. However, recent genome-wide studies have revealed transcription of many genes are regulated post transcription initiation. Therefore, regulation of PolII transcription elongation, which is controlled by positive elongation factor complexes b (P-TEFb) and negative elongation factors (NELF), may be an important rate-limiting step in gene expression[1]. While many genes are known to be regulated at the PolII initiation step, little is known about how gene expression is modulated at the PolII elongation step.

Introduction

Pol II transcription elongation is regulated by positive elongation factor complexes b (P-TEFb) and negative elongation factors (NELF). Pol II pausing occurs shortly after transcription initiation and involves the association of pausing factors DSIF and NELF.(d)Pause release is triggered by the recruitment of the P-TEFb kinase. P-TEFb kinase phosphorylates the DSIF/NELF complex and CTD. Then the paused Pol II escapes into productive elongation.[2]

Results

We have found that LPS-induced expression of *Cxcl1*, a gene encoding a chemokine crucial for neutrophil recruitment, is regulated at the elongation step by transcription repressor hairy and enhancer of split 1 (Hes1) in mouse bone marrow-derived macrophages (BMDMs). Mechanically, Hes1 suppressed recruitment of the P-TEFb complex and subsequently attenuated e occupancy of serine 2-phosphorylated PolII at the *Cxcl1* gene locus. To directly evaluate PolII binding throughout the entire gene locus we analyzed genome-wide Pol II occupancy by chromatin immunoprecipitation followed by deep sequencing (ChIP-seq)in wildtype (WT) and Hes1-deficient BMDMs. Consistent with ChIP-PCR data, ChIP-seq data showed that Hes1 deficiency did not affect PolII binding near the transcription start site of the *Cxcl1* gene. Instead, PolII binding patterns at the *Cxcl1* gene body region significantly differed between WT and Hes1-deficient macrophages, validating our hypothesis that Hes1 indeed regulated *Cxcl1* gene transcription via targeting post-initiation steps by inhibiting transcription elongation.

By bioinformatic analysis of the PolII ChIP-seq data set, we wish to identify additional *Cxcl1*-like genes whose expression is regulated at thetranscription elongation step. In addition, we will further assess the role of transcription elongation in macrophages by genetically targeting P-TEFb and NELF complexes. We hope our results will elucidate mechanism and functional significance of regulation of inflammatory gene transcription at the elongation step, which may provide a rapid and efficient way for fine-tuning gene expression in response to environmental stimuli.

Summary and Future Plan

In this Study, Hes1 downregulated Cxcl1 gene transcription via targeting post-initiation steps by inhibiting transcription elongation. And we will further assess the role of transcription elongation in macrophages using RNAi to knockdown P-TEFb subunit (Cdk9) and NELFe or using NELFb knockout mice.

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[1] Institute for Immunology, Tsinghua University, Beijing

[2] Center for Life Sciences, Peking University, Beijing

[3] Weill Cornell Medical College, New York NY

Markers of Astrocyte Reactivity in *in vitro* Models of Blast-Induced Neurotrauma

Nora Hlavac[1], Samuel Miller[2], and Pamela J. VandeVord[3,4]

Short Abstract — **Astrocyte reactivity is a response often to mechanical stimulation that is defined by biochemical changes and increased proliferation. There is a growing need to understand this reactive response in relation to insult associated with common mechanisms of traumatic brain injuries. This study aimed to characterize the response of astrocytes after exposure to overpressure using both two- and three-dimensional models. Structural and other related biomarkers were quantitatively assessed at acute time points after exposure. Results indicate that cytoskeletal structure of the cells was not compromised and some reactive markers indicate different time periods of activation of the two- and three-dimensional models.**

Keywords — **astrocyte, reactivity, neurotrauma, cytoskeleton**

I. INTRODUCTION

Blast-induced neurotrauma is a growing concern in military personnel, with more than 73% of casualties in recent military endeavors involving explosives [1]. The prevalence and long-term impacts of these injuries dictate a need to better understand cellular responses to injury in this context in order to be able to design targeted therapeutics. Astrocytes play a critical role in the central nervous system's response to injury [2]. Moreover, they have a reactive response, termed astrogliosis, as a result of exposure to mechanical stimuli. Astrogliosis is characterized by increased proliferation as well as up-regulation of activation markers including glial fibrillary acidic protein (GFAP) [2-4]. The role of astrocyte activation in both neuroprotection and degeneration has been explored [2, 5-6], however, the response is still not completely understood. In this study, cells were exposed to overpressure profiles characteristic of blast exposure. This study aimed to compare two- and three-dimensional models of astrocyte reactivity to quantitatively assess the effect of both exposure and environment on gene expression for several structural proteins as well as a proliferation marker. Each target was chosen as a potential biomarker for activation in response to overpressure.

II. EXPERIMENTAL APPROACH

C6 astroglioma cells (ATCC, CCL-107) were used in two- and three-dimensional *in vitro* models to characterize acute astrocyte response and reactivity to an overpressure of 18-20 psi. A custom chamber was used to measure and record overpressure in real time. Quantitative assessment was conducted using reverse transcription, real-time polymerase chain reaction (RT-PCR). RNA was extracted from samples at 48 and 72 hours post exposure and was used to synthesize cDNA for RT-PCR. Fold changes for each target (Table 1) were calculated by using a delta-delta ($\Delta\Delta$) C_t method and by normalizing to a sham group.

Table 1. Biomarkers of interest for astrocyte reactivity.

Target	Classification/Function
Glial fibrillary acidic protein	intermediate filament, mechanical strength
β-actin	cytoskeletal protein, shape, integrity
Vinculin	cytoskeletal protein, anchors actin
Piezo2	transmembrane protein, cation channel, mechanosensor
Ezrin	peripheral membrane protein, adhesion, communication
Mitogen-activated protein kinase kinase 1	enzyme, stimulates MAP kinases pathways (proliferation)

III. RESULTS

In both models there were significant differences in fold change of GFAP expression from the sham groups ($p<0.05$), with opposite responses at 48 hours. For the two-dimensional model, GFAP expression was elevated to a fold change of 1.66, whereas it was decreased to 0.51 for the three-dimensional model. Both models showed a return to normal levels by 72 hours. This suggests different time periods of activation relative to environment. There were no significant differences from sham for other structural components at either time point, however, several targets had trending increases from the 48 to the 72-hour time point. While analysis suggests no significant structural damage to the cells, it does show potential for differential activation markers between the two- and three-dimensional *in vitro* models.

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[1]Department of Biomedical Engineering and Mechanics, Virginia Tech, Blacksburg, VA, E-mail: nhlavac@vt.edu
[2]Department of Biomedical Engineering and Mechanics, Virginia Tech, Blacksburg, VA, E-mail: msam@vt.edu
[3]Center for Injury Biomechanics, Virginia Tech, Blacksburg, VA, Email: pvord@vt.edu
[4]Salem Veteran Affairs Medical Center, Salem, VA

Optimizing protein expression levels as a function of network topology minimizes nonfunctional complex formation

David O. Holland[1], Margaret E. Johnson[2]
[1]Johns Hopkins School of Medicine, Department of Biomedical Engineering, [2]Johns Hopkins University, Department of Biophysics

Cells are crowded by macromolecules, posing challenges for proteins to locate functional partners and avoid misinteractions. Overexpressed proteins may saturate partners, leaving leftovers for nonspecific binding. To avoid this, protein expression levels may be balanced according to the structure of their binding networks. We simulated several such networks under varying protein concentrations while allowing for nonspecific interactions. It was found that relative concentrations could be optimized to minimize misinteractions, and that network motifs determined how sensitive the networks were to non-optimal concentration levels. We conclude that there is evolutionary pressure on both protein abundance and network topology.

***Keywords* — Cellular crowding, dosage balance hypothesis, misbinding, protein-protein interaction network, network motif**

I. Introduction

To perform multiple functions reliably, cells have evolved a vast network of protein-protein interactions (PPI). Human cells alone contain about 20,000 genes encoding for at least 30,000 unique protein types[1]. One challenge that cells face is ensuring that their proteins bind to functional partners reliably as they diffuse through the cell. The cell interior is crowded: 5-40% of cell volume is occupied by macromolecules[2], posing challenges for proteins to both locate functional partners and avoid misbinding. Misbinding – nonspecific interactions that pose no benefit to the cell – can be hazardous to cell function, depleting resources and leading to pathogenic aggregations[3,4]. Highly abundant proteins are at particular risk for misbinding since they may saturate functional partners, leaving leftovers for nonfunctional binding. These "supersaturated" proteins have been linked to neurodegenerative diseases[5]. To avoid leftover proteins, cells may have evolved stoichiometrically balanced gene expression levels, a theory known as the "dosage balance hypothesis" (DBH)[6]. Indeed, copy number variations of genes have been linked to increased susceptibility to a number of diseases, including cancer and multiple sclerosis[7,8]. While the DBH has been explored for single protein complexes, one unexplored question is whether protein expression levels are balanced according to their overall binding networks.

II. Results

To study the effects of relative protein abundance on nonspecific complex formation, we first simulated five simple network motifs under varying protein concentrations using the Gillespie algorithm. While the motifs formed roughly the same proportion of nonspecific complexes under optimal conditions, they varied in sensitivity to initial concentrations (ICs), with the hub being the most sensitive and triangle being the least, and high sensitivity correlating with motifs that allow more ways to form nonspecific complexes. We then simulated 500 large networks of 90-200 nodes with varying topological properties under equal, random, and optimized ICs. Binding affinities for all specific and nonspecific interactions were determined using a coarse-grained protein sequence model. The proportion of protein in nonspecific complexes was recorded as a function of degree distribution, network density, average binding strength, local topology, and ICs. It was found that optimizing the local topology via introducing more hubs and less chains and flags; similar to real networks; decreased the number of nonspecific complexes under optimal ICs, but also increased sensitivity to ICs. Degree distribution, surprisingly, had little influence once local topology was optimized. A lower average binding strength resulted in a lower proportion of nonspecific complexes, in agreement with the hypothesis that abundant proteins are less sticky to avoid misinteractions[9].

III. Conclusion

We conclude that there is evolutionary pressure to both favor certain network motifs and to balance protein abundance to avoid misinteractions. Future work will add noncompetitive binding to the model and perform the analysis on real protein networks to compare with experimental expression level data.

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Semi-adaptive response and noise attenuation in BMP signaling

Tian Hong[1,2], Ernest S. Fung[2,3], Lei Zhang[4], Grace Huynh[3], Edwin S. Monuki[2,3], Qing Nie[1,2]

Short Abstract - **Temporal dynamics of morphogen-driven signaling events are critical for proper embryonic development. During development, cells translate extracellular bone morphogenetic protein (BMP) gradients, often subject to noise, into graded intracellular tail-phosphorylated SMAD (TP-SMAD) levels. Using modeling and experimental approaches, we found that BMPs induce TP-SMAD responses in neural precursor cells (NPCs) in a concentration-dependent manner, which are semi-adaptive within a specific intermediate range of BMP concentration. These semi-adaptive TP-SMAD responses involve an intrinsically-slow deactivation of BMP receptors, which attenuates noise by prolonging SMAD deactivation time after BMP withdrawal, but increases response time. Interestingly, negative feedback on BMP receptors is also required for semi-adaptation, which benefits both noise attenuation and response time, and therefore balances the tradeoff seen with slow BMP receptor deactivation. These results highlight the rich dynamics of SMAD regulation in response to graded BMP concentration, and elucidate general design principles for balancing noise attenuation and activation speed in signaling systems.**

Keywords — **Activation time / adaptive response / noise attenuation / signaling speed / deactivation**

I. Background

Bone morphogenetic proteins (BMPs) play critical roles in embryogenesis and tissue patterning. As morphogens, BMPs regulate patterning by forming concentration gradients within developing tissues [1] and specify multiple cell fates in a concentration-dependent manner. Central to BMP-induced intracellular signaling is phosphorylation of SMAD transcription factors [2]. Tail-phosphorylated SMADs (TP-SMADs) are imported into the nucleus to regulate transcription.

In neural precursor cells (NPCs) of the developing cerebral cortex, steady-state TP-SMAD levels form a dorsoventral gradient *in vivo* [3] and approximate extracellular BMP concentrations *in vitro* [4], suggesting that TP-SMAD is a direct and proportional readout of extracellular BMP concentration. In addition to steady-state responses, pulse-like responses to morphogens can be critical for tissue development [5]. While BMPs can generate this type of response at the level of SMAD1 [6], the temporal dynamics of SMAD1 activation to graded BMP signals is poorly understood.

[1]Department of Mathematics, [2]Center for Complex Biological Systems, [3]Department of Pathology and Laboratory Medicine, University of California, Irvine, CA, USA E-mail: hong.tian@uci.edu

[4]Beijing International Center for Mathematical Research, Peking University, Beijing, China

II. Results

We combined experimental and modeling approaches to investigate the dynamics of SMAD1 activation in NPCs responding to graded BMP signals. We showed that an intermediate range of BMP concentration triggers semi-adaptive SMAD1 responses, which differ from the non-adaptive responses stimulated by higher or lower BMP levels and accelerate cell responses.

Using sensitivity analysis, we found that BMP receptor deactivation rate has pronounced effect in attenuating fluctuations of BMP signals. In particular, slow receptor deactivation rate benefits noise attenuation, but exhibits the tradeoff of increasing response time. Interestingly, BMP receptor inhibition through negative feedback, which is required for the semi-adaptation, exhibits no such tradeoff.

III. Conclusion

Our experimental observations and computational analysis demonstrate unique dynamic features of cellular responses to graded BMP signals. Our findings suggest a general cell-intrinsic control mechanism for creating fast adaptive responses with attenuated noise within a morphogen gradient. Combination of slow morphogen receptor deactivation rate with negative feedback can optimize both activation speed and noise attenuation property.

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Spatial Quantification of Morphological Changes in Retinal Pigment Epithelium

Haitao Huang[1], Micah Chrenek[2], John Nickerson[2], Hans Grossniklauss[2], Yi Jiang[1]

***Short Abstract* — Morphological changes in retinal pigment epithelium (RPE) is often associated with the disease progression of age-related macula degeneration (AMD). We applied different statistical methods to quantify the morphology of RPE in both mouse and human eyes as well as in simulated AMD eyes. Distinct pattern of second-order spatial properties reveal the RPE pattern signatures of AMD eyes. Classification of genotypes and ages by RPE cell morphometric measures suggests there is little difference in prediction rates in angular locations, but significant differences in radial locations.**

***Keywords* — retinal pigment epithelium, age-related macula degeneration, quantification, morphological changes.**

I. Introduction

RETINAL pigment epithelium (RPE) is a monolayer of cells key to the wellbeing of photoreceptor cells in eyes. Previous study has quantified the RPE morphological changes in AMD [1, 2]. In this study we further quantify such changes in spatial locations, which will help to understand whether AMD progresses as well as how RPE morphologies differ in individuals with different genotypes and ages during and after AMD. Developing a set of quantitative tools for this purpose will also have practical applications in the early diagnosis of AMD.

II. Methods

RPE images were obtained from mouse and human (donor) eye flatmounts, with RPE cell borders stained by anti-ZO-1. We developed a reproducible segmentation procedure to identify cells with ImageJ and represented 2D cell distribution by a spatial point process of cell centroids. We then studied the second-order properties to investigate the changing patterns of clustering in both experimental and simulated RPE [2]. Cell morphometric features (24 in total) including cell shape, area, etc. were extracted using CellProfiler [4]. K-nearest neighbor (KNN) algorithm, combined with leave-one-out cross validation, were applied to classify the genotype and age in C57BL/6J (wild type) and RD10 mice and to calculate the prediction accuracy in various spatial regions.

[1]Department of Mathematics and Statistics, Georgia State University.
[2]Department of Ophthomology, Emory University.

III. Results

Spatial analysis of simulated human RPE images showed that the oscillations in the variance stabilized K-function and pair correlation function (PCF) for normal RPEs gradually disappear as hexagonal cells stretch and distort. The AMD-like RPE patterns show an increased clustering between distance 100 and 200 (in pixels), indicative of the disordered RPE pattern in AMD. The same analysis on experimental human RPE images shows the same change from normal eyes to diseased eyes.

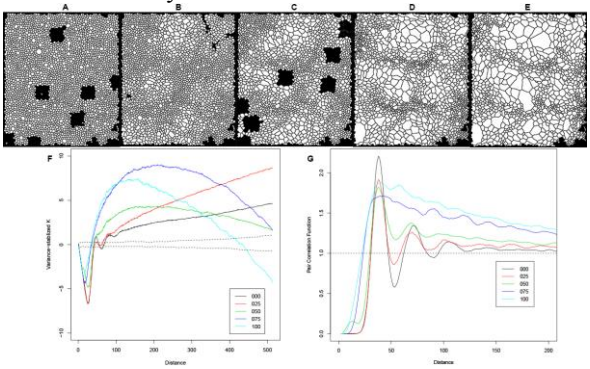

Figure 1. Simulated RPE tissue undergoing repeated clustered damage (black area: regions of cell apoptosis) and recovery from a normal RPE pattern.(A-E). F: Variance stabilized K function; G: Pair correction function.

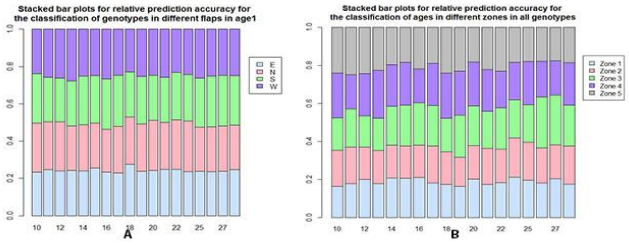

Figure 2. Stacked bar plots of relative prediction rates

Previous research hypothesized a certain difference exists in different spatial regions of RPE sheets. The KNN classification showed little difference in RPE pattern in our defined 4 flaps, yet an increasing accuracy rate with zone numbers [1]. Morphometric variables such as eccentricity consistently perform well in a classification (prediction rates over 90%). We also find the linear weighted combination of the morphometric variables as morphometric signatures that best distinguish the disease progression of AMD.

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Characterizing gene expression kinetics in nutrient-starved *E.coli* cells

Sukanya Iyer[1], Bo Ryoung Park[1], and Minsu Kim[1]

***Short Abstract* — Bacteria are frequently exposed to starvation in their natural environments. Expression of relevant genes during early starvation is essential for their long-term starvation survival or resumption of growth when other nutrients become available. However, it is not clear how starvation limits cells' ability to make gene products and how cells overcome such limitation. Here, using a synthetic biology approach and FISH (fluorescence in situ hybridization), we quantitatively characterized the process of gene expression in carbon starved cells and nitrogen starved cells. Our findings reveal that the different types of starvation limit gene expression differently and cells employ a distinct strategy to overcome the limitation.**

***Keywords* — Starvation response, fluorescence in situ hybridization, transcription and translation coupling, ppGpp**

I. Introduction

MOST bacteria experience nutrient poor conditions for most of their lifetime. Starvation imposes significant limitation on various processes essential for growth and survival in bacteria. Expression of relevant genes under starvation conditions, especially during early starvation, is critical for long-term starvation survival [1] or for rapid switching to other nutrient sources if such nutrient sources are available, e.g., during diauxic shift. However, starvation affects transcriptional and translational machineries, as well as availability of substrates needed to make gene products. As such, cells' ability to express these genes is expected to be significantly limited. Currently, however, it is not clear how starvation limits gene expression and how cells overcome such limitation. In this work, we precisely controlled transcriptional activation and characterized the kinetics of gene expression in carbon-starved cells and nitrogen-starved cells.

II. Approach

To precisely control transcriptional activation, we designed a synthetic construct in which the synthetic promoter drives the expression of a *lacZ* reporter gene. For precise measurement of the low lacZ mRNA levels produced, Fluorescence in situ hybridization, (FISH) was used. Concomitant changes in lacZ protein expression were quantified using a standard β-galactosidase assay.

III. Results and Discussion

We observe that carbon starvation and nitrogen starvation have different effects on the kinetics of gene expression. Nitrogen starvation imposes strong limitation on translation, reducing the protein synthesis rate (per mRNA) as well as the speed of translation by ribosomes (i.e., polypeptide elongation speed). If unchecked, such a reduction would expose naked regions of mRNA, which is known to result in premature termination of transcription and have detrimental effects on gene expression [2]. We find that, cells avoid such effects by slowing down the speed of transcription to match the speed of translation through the stringent response alarmone, ppGpp. By contrast, carbon starvation imposes strong limitation on transcription, reducing mRNA synthesis rate as well as the speed of transcription. In this case, ppGpp is not needed to match the speed of transcription and translation. Our findings show that different types of starvation limit gene expression differently and distinct strategies are employed to overcome such limitation.

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[1]Department of Physics, Emory University, Atlanta ,USA E-mail: siyer9@emory.edu

From START to FINISH: Computational Analysis of Cell Cycle Control in Budding Yeast

Pavel Kraikivski[1], Katherine C. Chen[1], Teeraphan Laomettachit[2], T.M. Murali[3] and John J. Tyson[1]

Short Abstract — **In the cell division cycle of budding yeast, START refers to a set of tightly linked events that prepare a cell for budding and DNA replication, and FINISH denotes the interrelated events by which the cell exits from mitosis and divides into mother and daughter cells. Based on recent progress made by molecular biologists in characterizing the genes and proteins that control START and FINISH, we crafted a new mathematical model of cell cycle progression in yeast.**

Keywords — **Cell Cycle, Budding Yeast.**

The cell division cycle is the ordered sequence of events by which a cell replicates its genome and segregates the replicated chromosomes to two daughter cells during mitosis. In budding yeast, *Saccharomyces cerevisiae*, START refers to a set of tightly linked events that prepare the cell for budding and a new round of DNA replication and FINISH denotes the interrelated events by which the cell exits from mitosis and divides into mother and daughter cells. Based on the noteworthy progress made by molecular cell biologists in characterizing the genes and proteins that control cell cycle progression in budding yeast, we have built a comprehensive mathematical model of the molecular mechanisms underlying START and FINISH. For this mathematical model, we use a new modeling framework in which all reactions are classified into three basic types: protein synthesis and degradation ($\rightarrow C \rightarrow$), phosphorylation and de-phosphorylation ($C \leftrightarrow CP$), and binding to activator or inhibitor partners ($C+A \leftrightarrow C:A$).

Results: The model successfully explains the observed phenotypes of 263 mutant yeast strains and can be used to predict the phenotypes of novel combinations of mutant alleles. The credibility of these predictions has been assessed by distinguishing between fragile predictions (which are sensitive to values of adjustable parameters) and robust predictions (which depend on the regulatory network itself rather than specific parameter values).

Conclusions: Our comprehensive model of the molecular events controlling cell cycle progression in budding yeast has both explanatory and predictive power. Future experimental tests of fragile predictions will be useful to constrain adjustable parameters of the model, and future tests of robust predictions will either confirm the underlying molecular mechanism or provide new insights into how the cell division cycle is regulated.

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[1]Department of Biological Sciences, Virginia Polytechnic Institute and State University, E-mail: pavelkr@vt.edu

[2]BioPark Technology King Mongkut's University of Technology Thonburi.

[3]Department of Computer Science Virginia Polytechnic Institute and State University

Automation of Model Design and Analysis for Big Mechanisms

Anuva Kulkarni[1], Cheryl Telmer[2], and Natasa Miskov-Zivanov[1]

Short Abstract — **In this paper, we describe a framework for automated development of executable models using information extracted from literature. The framework also includes model analysis and correction methods. The final objective is to have a representation for models of complicated mechanisms that allow for easy model exchange and improvement, facilitating the discovery of interventions (e.g., treatments or drugs in case of cell mechanisms).**

Keywords — **automation, modeling, inference, interaction graphs, simulation, model checking, cell signaling pathways**

I. Purpose

Understanding complicated mechanisms usually requires collecting information from various sources such as published literature and integrating it all within a model. A large number of models in existing literature that were developed over the years for a particular biological system, for example, are rendered useless when they cannot be updated, validated or corroborated with each other. By designing a unified model knowledge database that can be exchanged, tested and improved, we can advance and accelerate knowledge exchange. Our aim is to automate reading and model building procedures, followed by model checking and improvement. With the assistance of experts in natural language processing, causal inference and cancer immunology, we hope to achieve the goal of developing a system that can learn, execute and manage models for large, complicated mechanisms, enabling informative simulations.

II. Proposed Framework

Here we briefly discuss our framework for automation. Information extracted from literature using natural language processing algorithms is entered into a standardized format that can be further processed by causal inference algorithms. This yields an interaction graph with nodes and edges for the connections in the model. Models from databases such as [1], [2] and Biological Expression Language (BEL) can also be translated to generate the interaction graph.

This is followed by inference of an executable model that involves automated inference of element update functions by combining qualitative interaction graphs and any other available quantitative information. We perform simulations of such models using deterministic and stochastic approaches [3], [4]. The simulation results are further used for sensitivity and controllability analysis of the modeled system. Probabilistic and statistical model checking is performed using tools described in [5]. Perturbation analysis considers the effects of altered causal relationships in steady-state and transient behavior [6], [7]. We are currently developing automated hypotheses extraction that follows model analysis. This step will lead to refinements to the model, guide new literature search and help design new wet lab experiments to validate generated hypotheses.

III. Conclusion

The focus of our project is development of a completely automated model design and analysis procedure. We are applying this approach on models of cell signaling and metabolism networks, as well as to cell-cell communication scenarios in cancer.

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[1]Electrical and Computer Engineering, Carnegie Mellon University. E-mail: {anuvak, nmiskov}@andrew.cmu.edu

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Applications of Precision, Real-time Cell Biomass Measurements in Cell Physiology and Drug Development

K.A. Leslie and J. Reed [§]

Short Abstract — **Live Cell Interferometry (LCI) is a new technology for biomass profiling of single living cells or cell clumps (3D structures) with picogram sensitivity. It has shown highly repeatable (<1% coefficient of variation) quantification of cellular biomass, mass accumulation or loss rates, and mass distributions for medium-sized populations of cells (10^3) on a single cell basis, and has broad potential application in studies of normal cell physiology, cancer, and additional diseases showing aberrant growth and deregulation of cell biomass control. Our poster will discuss our group's recent applications of LCI technology for conducting single cell drug response assays, studying biomass dynamics in stem cell differentiation and for detecting subtle drug-induced aberrations in mass partitioning during cell division.**

Keywords — **Biomass Profiling, Single-Cell, Drug Development, Stem Cell Differentiation, Cell Division**

I. Background Information

LIVE Cell Interferometry (LCI) is a new technology for biomass profiling of single living cells or cell clumps (3D structures) with picogram sensitivity. LCI quantifies the shift in phase imparted to light propagating through a transparent cell body, which is proportional to biomass. It has shown highly repeatable (<1% coefficient of variation) quantification of cellular biomass, mass accumulation or loss rates, and mass distributions for medium-sized populations of cells (10^3) on a single cell basis, and has broad potential application in studies of normal cell physiology, cancer, and additional diseases showing aberrant growth and deregulation of cell biomass control [1, 2].

II. Results

Our group has successfully utilized LCI to accurately quantify the sensitivity of single cell and colony-forming human breast cancer cell lines to the HER2-directed monoclonal antibody, trastuzumab (Herceptin). Relative sensitivities were determined tens-to-hundreds of times faster than possible with traditional proliferation assays [1].

Currently, we are using LCI to investigate the sensitivity of established human melanoma cell lines to the B-Raf enzyme inhibitor vemurafenib (Zelboraf). Additionally, our LCI has demonstrated accuracy in measuring smaller human cell types in a recent collaboration investigating mast cells and their degranulation process.

Finally, our group is in the initial phases of using LCI to study the recovery of immune system cells following stem cell transplantation in patients with blood and bone marrow cancers in order to predict the onset of graft-versus-host disease.

III. Conclusion

Live Cell Interferometry enables real-time quantification of single-cell and cell cluster mass with picogram sensitivity. It can be used to accurately predict drug sensitivity in cell samples hours faster than gold-standard clinical growth assays. Our LCI's increased speed of analysis and quantification of therapeutic responses for aggregated cell clumps, sheets, and spheres provides exciting new opportunities for agent selection, prognosis in solid tumor therapy, applications in the study of immune cell function, and delving further into basic cell physiology.

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[§] VCU Department of Physics

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Transient DNA looping bridged on low-affinity sequences substantially promotes gene transcription

Yaolai Wang[1], Feng Liu[1,2] and Wei Wang[1]

***Short Abstract* — Although abundant structural data are available on the transcription apparatus (TA), little is known about how it operates kinetically. By analyzing the organization architectures of proteins binding to the regulatory DNA sequences, we first propose a model of how the TA operates on the *glnAp2* promoter. We then characterize the transcription behaviors in response to various concentrations of NtrC (transcription factor). Specifically, an enhancer and a low-affinity binding site can be transiently bridged by an NtrC oligomer at its low and intermediate levels, contributing to transcription initiation. This work also clarifies different roles for two enhancers in gene transcription.**

***Keywords* — transcription initiation, DNA looping, kinetics**

I. Background

Using a newly developed fluorescence technology, Friedman and Gelles recently dissected the major steps of transcription initiation at an activator-dependent bacterial promoter [1]. The study revealed the formation of two sequential closed complexes and an open complex, as well as the release of σ^{54}. Nevertheless, how the DNA-bound activators and the promoter-bound holoenzyme σ^{54}RNAP are positioned properly remains unclear. Here, we address this issue in terms of activity of the *glnAp2* promoter in *Escherichia coli*.

The *glnAp2* gene expresses the nitrogen assimilation enzyme glutamine synthetase under the regulation of NtrC (nitrogen regulatory protein C). Intracellular free NtrC molecules exist as dimers. Upon activation in nitrogen-starved cells, NtrC dimers are phosphorylated and can nucleate formation of hexamers. $NtrC_P$ binds to two strong sites (enhancers) at -140 (site I) and -108 (site II) and to three weaker sites at -89, -66 and -45 (sites III-V) relative to the transcription start site [2], while σ^{54}RNAP binds the -24~-12 region. It is generally held that enhancer-bound NtrC hexamers can contact and catalyze the holoenzyme, which then opens the DNA double strands and initiates mRNA synthesis. Whether and how those low-affinity sites also play a role is largely unknown.

Although the transcription apparatus (TA) on the *glnAp2* promoter involves only NtrC, σ^{54}RNAP and promoter DNA, it exhibits rather complex behaviors [2], some of which cannot be accounted for by traditional models. To elucidate how the TA operates, it is essential to associate the binding kinetics of NtrC with transcription initiation.

II. Results

By structurally and kinetically analyzing the organization architectures of NtrC and σ^{54}RNAP on the *glnAp2* gene, here we present a model of how the TA dynamically operates [3]. We propose that enhancer II and low-affinity site V can be transiently bridged by an enhancer II-bound NtrC tetramer/hexamer at low and intermediate activator concentrations. During the short lifetime of this conformation, another hexamer bound to enhancer I is just around the -24~-12 region; a newly recruited holoenzyme can be immediately activated to initiate mRNA synthesis, thus elevating transcriptional levels. At high concentrations of NtrC, the three low-affinity sites are occupied, rendering the DNA more rigid and hindering DNA bending to repress transcription. Stochastic simulation results further reproduce the experimental observations quantitatively. Experimentally testable predictions are also made.

III. Conclusion

A dynamic mechanism for transcription initiation on the *glnAp2* gene is provided, and weak molecular interactions can play a critical role in transcriptional regulation.

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[1] School of Physics, Nanjing University, Nanjing 210093, China.
[2] E-mail: fliu@nju.edu.cn.

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Adhesive forces play key roll in pattern formation and stability in chemotaxing cells

Chenlu Wang[1,2], Joshua Parker[2,3], and Wolfgang Losert[1,2,3 *]

***Short Abstract* — The slime mold cell *Dictyostelium discoideum* is well studied, serving as both a model for amoeboid motion as well as for g-protein mediated chemotaxis up gradients of cyclic adenosine monophosphate (cAMP). Since cells also secrete cAMP, they end up in heat-to-tail "streams" that help the colony collapse into an aggregate to facilitate sporulation. We investigated the role of cell-cell and cell-surface adhesion on these heat-to-tail patterns. Using cell shape analysis, we found evidence of actin wave transmission across cell neighbors, a phenomena that disappears upon the loss of surface adhesion. Additionally, by tuning external buffer concentrations to diminish cell-cell adhesion, we found that large scale streaming structures were progressively absent from self-aggregation experiments with low adhesion, suggesting a role for mechanical interactions in stabilizing migratory patterns. Using numerical simulations, we demonstrate that adhesion preserves cell-cell contacts over time, which stabilize otherwise transient streams into cohesive structures.**

Keywords* — *Dictyostelium discoideum, chemotaxis, streaming, adhesion, actin, simulations

I. Introduction

THE slime mold cell *Dictyostelium discoideum* is well studied, providing a model organism for the study of amoeboid motion as well as for g-protein mediated chemotaxis up gradients of cyclic adenosine monophosphate (cAMP) [1,2]. When in a nutrition poor environment, cells begin to secrete cAMP and migrate, which leads to heat-to-tail "streams" that help the colony collapse into an aggregate to facilitate sporulation. The migration itself is due to preferential polymerization of actin, which has been seen to create protrusion waves that travel down the cell [2].

II. Do mechanical cues stabilize streams?

We focused our attention on investigating possible adhesive cues to stream formation and stability, both due to cell-cell and cell-substrate contact. Ion concentration is known to be able to vary cell-cell adhesion and cell substrate adhesion, because, by screening out the negative charges on cells and some substrates, e,g, glass surfaces, ions reduce the electrostatic repulsion between them [3]. We studied migration of cells in medium with different ion concentrations and found that diluted medium greatly inhibits the formation of cell-cell contact but retains cell-surface contact and cell motility [4]. Cells exhibit significant steaming defect and aggregate through very short streams when cell-cell contact is partially inhibited. With even lower ion concentration, cells remain active motion but do not form multicellular streams or cell-cell contact. Thus, these experiments elucidate that inhibiting cell-cell/substrate contact results in significant collective streaming defect: cells do not align in a head-to-tail fashion without proper adhesions.

To focus only on cell-cell adhesion, we developed a numerical model of chemotactic migration. Treating the center-of-mass motion of each cell as being driven by the relative local cAMP concentration and the resultant motion as a sum over protrusions [2], we compared the stability of cell-cell contacts in ensembles of cells migrating in an external gradient [5]. In particular, we measured the fraction of broken contacts over a period of time, F, which is normalized by the same measure on experiments with secretion inhibited. We demonstrated that this measure properly distinguishes "boundaries" in the pseudo-phase diagram (with respect to density and external concentration) representing regions of steady state individual motion, streaming, and aggregation. By then including cell-cell adhesion, we found that the region of individual motion shrinks in favor of streaming, representing an earlier onset of stream stability.

III. Conclusion

We've shown that cell-cell and cell-surface adhesion play a role in stabilizing the local neighborhood allowing for the transmission of actin protrusive waves across cell-cell boundaries as well as preventing loss of neighbor contacts. This in turn allows for easier stream formation and stabilizes those streams that form, leading to faster formed aggregates containing a larger portion of the cell population.

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[1]Dept. of Physics, U. of Maryland, College Park, MD, USA.
[2]Dept. of Biophysics, U. of Maryland College Park, MD, USA
[3]Institute for Research in Electronics and Applied Physics, U. of Maryland, College Park, MD USA
* E-mail: wlosert@umd.edu

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Gabi: Network inference from antibody-based proteomics data

Alexander L. R. Lubbock[1], Michael W. Irvin[1], David J. Harrison[2], Carlos F. Lopez[1], Ian M. Overton[3]

***Short Abstract* — Gabi is a novel algorithm for inference of small-scale networks from human tumor tissue samples scored for protein expression using quantitative antibody-based technologies. These signed, directed networks provide insight into pleiotropy, complexity and context-specificity. Inferred networks successfully recover the information flow between proteins on synthetic data generated by the PySB simulation framework. Directionality predictions have high precision (79%) if input network connectivity is accurate. The Gabi algorithm was applied to study multiple carcinomas (renal, breast, ovarian), providing novel insights into the relationships between epithelial–mesenchymal transition (EMT) players and fundamental processes dysregulated in cancers e.g. apoptosis and proliferation.**

***Keywords* — network biology, systems medicine**

I. Introduction

THE biological network has become a key concept within systems medicine. Network analysis shows particular promise for cancer biology, where the underlying causes are complex and heterogeneous. Dysregulation of networks/pathways is key to oncogenesis and clonal selection within tumors [1]. Indeed, changes in network structural properties can be predictive of patient outcome [2].

Functional proteomics platforms, including tissue microarrays (TMAs) and reverse-phase protein arrays (RPPAs) are particularly relevant for understanding cancer signaling activity, wherein protein abundance and post-translational modifications are key determinants. The antibody-based TMA and RPPA platforms both enable study of *ex vivo* tissue from carcinomas of interest, providing insight into the context specific and pleiotropic activity of proteins.

Here we present Gabi: a bespoke method to infer biological networks from functional proteomics data typically containing 6–100 protein markers. Key improvements over existing methods include detection of a broad array of coexpression patterns by combining Spearman correlation and symmetric uncertainty (normalized mutual information), relevance thresholding using an automated parametric approach, and directionality inference based on conditional independence detection, including graph theoretic evidence weighting based on the maximum clique algorithm.

[1]Department of Cancer Biology, Vanderbilt University, Nashville, TN
[2]School of Medicine, University of St Andrews, UK
[3]MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, UK

II. Results

A. Incorporation of functional scaffolds

During Gabi calibration on data from the DREAM4 network inference competition [3], it was noted that directionality precision improved from 56% to 79% if the correct network connectivity was supplied. Thus, we added the ability to include high confidence prior knowledge edges [4] into the procedure.

B. Benchmarking

A tool was developed for extracting gold standard information flow networks from models in the PySB framework [5]. The Apoptosis Necrosis Reaction Model (ANRMv2.0) was used. Gabi achieved similar directionality precision to methods pcalg [6] and ggm [7] but with twice and four times the directionality recall respectively.

C. Carcinoma networks

Gabi networks were generated from renal, breast and ovarian cancer datasets from Western General Hospital, Edinburgh, UK and The Cancer Genome Atlas (TCGA) project [8]. Existing knowledge is recapitulated e.g. clusters of epithelial adhesion markers and mesenchymal/invasion markers. Novel insights include connections between tumor weight and key proteins in the TCGA breast cancer network, and elucidation of ER-β2's role in high grade serous ovarian cancer.

III. Conclusion

Gabi is a network inference algorithm for small-scale proteomics data providing key improvements in connectivity and directionality inference. The algorithm was calibrated and benchmarked using separate synthetic modeling approaches. Performance on synthetic data from ANRM exceeded rival methods overall. Known cancer biology and novel insights are observed in Gabi networks generated on cancer proteomics datasets.

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Regulation of P53 Oscillations by MircoRNA-mediated Positive Feedback Loops

Richard Moore[1,2*], Hsu Kiang Ooi[1*], Taek Kang[1,2], Leonidas Bleris[1,2,3] and Lan Ma[1]

Short Abstract — **The tumor suppressor p53 oscillates in response to DNA double-strand breaks. We model a class of ubiquitous post-transcriptional regulators, termed microRNAs, which form positive feedback loops with the p53 regulatory network. Simulations reproduce the oscillation of p53 under DNA damage stimulus. Importantly, model analysis show that specific microRNA abrogation leads to loss of the wild-type phenotype. For evaluation, we perform microRNA-perturbation experiments in MCF7 breast cancer cells. Quantitative microscopy analysis confirms that the p53 oscillatory performance is compromised under specific microRNA perturbation. Our results provide evidence of the impact of microRNA-mediated positive feedback loops on the stress-induced p53 oscillations.**

I. Introduction

THE behavior of the tumor suppressor protein p53 could be significantly dynamical in response to stress signals [1]. Experiments demonstrate that DNA double-strand breaks trigger oscillations of p53 and its core antagonist MDM2 [2]. Previous studies have shown that the p53-MDM2 negative feedback loop is essential for the stress-induced p53 oscillations. However, the role of positive feedback loops in p53 oscillations remains largely elusive.

MicroRNAs are small noncoding RNAs serving as post-transcriptional regulators. Intriguingly, recent studies have revealed extensive crosstalk between the p53 network and microRNAs [3]. In this work, we investigate the role of microRNA-mediated positive feedback loops that interface with the p53 regulatory pathways.

We develop a mathematical model of a p53-MDM2-microRNA network that involves three different microRNAs forming positive feedback loops. We perform simulations and robustness analysis of p53 oscillations under abrogation of microRNA-mediated feedback loops. Specifically, bifurcation analysis is used to probe the system behavior under parametric variability in relationship to cellular noise. To experimentally evaluate our predictions, we introduce microRNA inhibitors in the MCF7 breast cancer cells, and perform time-lapse microscopy to track the p53 dynamics under drug-induced DNA double-stranded breaks. Our experimental results reveal that the three microRNA-mediated positive feedback loops confer different level of control to the stress-induced p53 oscillations.

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[*]Joint first author

[1]Department of Bioengineering, The University of Texas at Dallas, Richardson, TX. E-mail: lan.ma@utdallas.edu, bleris@utdallas.edu

[2]Center for Systems Biology Biology, [3]Electrical Engineering Department, The University of Texas at Dallas, Richardson, TX

II. Results

A. MicroRNAs mediate positive feedback loops with p53

The miR-192 family, miR-34 family and miR-29 family are transactivated by p53. In turn, miR-192 inhibits MDM2. In addition, miR-34 inhibits SIRT1 and YY1, while miR-29 inhibits CDC42 and Wip1, which are direct or indirect negative regulators of p53. As a result, these microRNAs all form positive feedback loops with p53.

B. Modeling and analysis of p53-MDM2-miRNA network

A mass-action model of the p53-MDM2-miRNA network is developed based on our previous work [4], incorporating the three different microRNAs that form positive feedback loops. We simulate DNA damage-induced p53 oscillations under inhibition of each of the microRNAs. Furthermore, we perform bifurcation analysis to account for the significant cellular variability due to extrinsic noise in parameters. The results suggest that only the repression of miR-192 could effectively abrogate the p53 oscillations in single cells.

C. Experimental evaluation of microRNA abrogation on p53 oscillations

We experimentally track the p53 response in single MCF7 cells under wild-type and microRNA repressed conditions. By quantifying the percentage of oscillatory cells in a population, we confirm that cells transfected with the inhibitor of miR-192 show markedly decreased portion of p53-oscillating cells compared to the wild-type phenotype.

III. Conclusion

Using theoretical modeling in combination with single-cell experiments, we provide the evidence that microRNA-mediated positive feedback loops can control the robust manifestation of stress-induced p53 oscillations.

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Mechanistic Insights into Early Endoderm Differentiation of Human Embryonic Stem Cells using Systems Analysis of Signaling Interactions

Shibin Mathew[1], Sankaramanivel Sundararaj[1], Hikaru Mamiya[2], Kelly Donovan[1], Biswas Bodhisatva[1] and Ipsita Banerjee[1,2,3]

Human embryonic stem cells (hESCs) are an attractive raw material for regenerative medicine due to their potential to deliver a variety of clinically important mature lineages. Several experimental studies have identified the signaling players that govern endoderm lineage specification of hESCs, however the precise mechanisms by which these molecules work together to orchestrate the dynamics of this process are not clearly known. Using a combination of mathematical modeling and model informed experiments, we evaluated the systems level interactions in the TGF-β/SMAD pathway and the role of crosstalks with the self-renewal pathway (PI3K/AKT) in controlling signal propagation and variability during differentiation.

Keywords **— SMAD-AKT crosstalks, Endoderm differentiation, Parametric ensembles, Global sensitivity analysis, Dynamic Bayesian Networks.**

I. Background

THE process of endoderm differentiation in hESCs is initiated by elevating the levels of signaling molecules called SMADs by adding Activin A to the growth medium. However, this alone is not sufficient, the context of the survival pathway PI3K/AKT is extremely important in determining the efficiency of differentiation. Particularly, the signaling activity of this pathway has to be inhibited to get high endoderm differentiation. But this comes at a cost of high cell death. In this work, we evaluated the nature of signaling interactions that govern the balance of signaling interactions during the entire differentiation process.

II. Materials and Methods

A. Experimental setup and analysis

H1 hESCs were maintained on matrigel-coated plates in mTeSR1 and endoderm differentiation was performed using 100 ng/ml Activin A ± modulation of PI3K/AKT pathway using Wortmannin. The phosphorylation dynamics was measured using MagPix multiplex technology. The initial selection of key molecules was based on the study by Singh *et al.* [1]. Nucleo-cytoplasmic shuttling rates were measured using Fluorescence Recovery After Photobleaching (FRAP).

B. Quantitative analysis

We first employed Dynamic Bayesian Network Analysis (DBN) to identify possible interactions from the signaling time series [2,3]. Detailed mechanistic Ordinary Differential Equation (ODE) model for the TGF-β/SMAD2,3 pathway with crosstalk interactions of PI3K/AKT was then developed for a systems level analysis. The model was calibrated using Affine Parallel Tempering based MCMC to identify parametric ensembles and sensitive reactions were identified by meta-model based Global Sensitivity Analysis (GSA) [4].

III. Results and Discussion

Application of DBN on the experimental signaling dynamics showed that the molecules p-SMAD2, SMAD4 and p-SMAD3 are influenced by p-AKT in the early phases of the signaling dynamics. This crosstalk is removed under PI3K inhibition, in spite of recovery of p-AKT levels back to the basal levels. Further, the receptor RII levels influenced the downstream molecules during the entire phase of the signaling dynamics. hESCs further showed divergence in the dynamics of regulatory SMADs. When evaluating this divergence using a mechanistic model, the parametric ensembles of p-SMAD2 and p-SMAD3 showed that there are differences in negative regulation by the negative feedback molecule SMAD7. Further, time to peak response of SMAD2 and SMAD3 was sensitive to the receptor levels and negative feedback. This affects the variability in the availability of SMAD molecules in the nucleus and the nuclear shuttling rates, ultimately controlling the variability seen during differentiation.

IV. Conclusion

Our analysis showed that modulation of crosstalk interactions in combination with inherent variability of specific signaling nodes affects the differentiation response of hESCs. This approach provides a new avenue for rational design and optimization of differentiation media of hESCs.

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[1]Department of Chemical and Petroleum Engineering, University of Pittsburgh, PA, USA, E-mail: shm82@pitt.edu, sankaramanivel@gmail.com, ked77@pitt.edu, bodhi.biswas@pitt.edu

[2]Department of Bioengineering, University of Pittsburgh, PA, USA, E-mail: him25@pitt.edu

[3]McGowan Institute for Regenerative Medicine, University of Pittsburgh, PA, USA E-mail: ipb1@pitt.edu

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Computational Model of Cortical Actomyosin

Callie J Miller[1,2], Demetrius Harris[3], Robert Weaver[1], G. Bard Ermentrout[4], Lance A Davidson[1], and Tim Elston[2]

***Short Abstract* — Cortical actomyosin contractions have been implicated in a broad range of morphogenetic tissue movements. Actomyosin consists of two cytoskeletal proteins, filamentous actin (f-actin) and non-muscle myosin II. We consider the biomechanics of actomyosin, how force within the cell is produced, and how these forces remodel the actin cytoskeleton. We have constructed a 2D agent-based model representing a patch of cell cortex. We compare experimental actomyosin to our simulated 2D network in order to gain insight into the biophysical origin of pulsatile contractions, how intra-filament forces modulate f-actin array morphologies, and how these arrays drive cell shape and tissue morphogenesis.**

***Keywords* — actomyosin, Monte Carlo, agent-based modeling, cell mechanics, cytoskeleton function & dynamics**

I. Purpose

DYNAMIC actomyosin networks play a critical role in morphogenesis by providing forces to move cells and establishing tissue mechanics. For example, contractile actomyosin networks drive cell shape change, resulting in bending of epithelial sheets during *Drosophila* gastrulation [1], and are responsible for the viscoelasticity and force production in *Xenopus* embryonic tissues [2,3]. It is surprising that we know little about the biophysical connection between active, dynamic pulses in the actomyosin network, which occurs on the molecular level, and the mechanical processes of cell rearrangement and bulk movements on the tissue level.

Actomyosin dynamics have been studied *in vivo* utilizing fluorescently tagged f-actin and actomyosin targeting drug perturbation studies [4,5]. *In vitro* models use reconstituted gels [6] and micropatterned arrays [7] to understand the mechanical properties of cortical actomyosin and characterize biophysical properties. *In silico* models investigate the biophysical principles and processes leading to emergent behaviors of actomyosin arrays [8,9]. In the work presented here, we have built on our previous rotational model [10] to address the gaps of understanding dynamic actomyosin networks from the molecular to the cellular level. We have developed a two-dimensional model that incorporates dynamic aspects of *in vivo* actomyosin interactions, captures the observed behaviors of *in vitro* model system actomyosin, and lays the simplified groundwork for future efforts.

[1]Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA Email: lad43@pitt.edu

[2]Department of Pharmacology, University of North Carolina, Chapel Hill, NC Email: millecaj@email.unc.edu, timothy_elston@med.unc.edu

[3]Department of Bioengineering, Penn State University, College Park, PA

[4]Department of Mathematics, University of Pittsburgh, Pittsburgh, PA

II. Results

For physiologically relevant parameters, we observe the emergent morphology of actomyosin as a f-actin aster with a punctuated concentration of myosin II at the center. The aster morphology arises from an isotropic contraction within the actomyosin network, but is a stable morphology. In order to investigate ways of making the aster contraction dynamic, we performed a parametric analysis on the actomyosin biophysical parameters to identify key candidates in the regulation of aster emergence and disappearance.

To investigate *in silico* results, we simulated cases where f-actin plus ends were tethered into a bar geometry, and where myosin II were tethered to a specific location within the domain. As a further way of determining the specificity of various actomyosin morphologies as they relate to stress fiber generation, for example, we introduced a population of non-motile motors to serve as actin cross-linking proteins. The crosslinkers were able to interrupt the aster morphology from forming, and instead resulted in a ring of actomyosin.

III. Future Directions

The model has multiple applications, some of which will be discussed in this poster. For example, understanding the role of signaling in actomyosin biophysical properties, and predicting organization of actomyosin based on externally applied forces.

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Diffusion- and Geometry-Influenced Stochastic Switching in a Reaction Network with Positive Feedback

Paul Mlynarczyk[1] and Steven M. Abel[1]

***Short Abstract* — In signal transduction, cells propagate information in response to various stimuli by means of biochemical reaction networks. Positive feedback between two molecular species in a network can lead to bistability, and spontaneous fluctuations in numbers of molecules can lead to stochastic switching between the two states. In this work, we use stochastic simulations [1] to investigate the role of spatial confinement and diffusion on stochastic switching in a simple reaction network with positive feedback. Characteristics of the bistability and stochastic switching depend on system shape, reaction volume, and diffusion coefficients.**

***Keywords* — Bistability, positive feedback, stochastic switching, signal transduction**

I. Purpose

STOCHASTICITY and spatial organization can each play important roles in the emergent behavior of biochemical reaction networks with positive feedback. Fluctuation-induced transitions between stable steady states may occur in the bistable regime by a phenomenon known as stochastic switching. Unlike well-mixed systems, in a spatially resolved system, such transitions may occur by spatially inhomogeneous pathways. For example, fluctuations in the local concentration can nucleate a cluster of active molecules that then spreads in space [2]. Spatial clustering often leads to fast nucleation of active molecules and thus a rapid transition to the active steady state [3]. Biologically, we are interested in the difference between stochastic switching in the cytoplasm and stochastic switching in confined regions such as the membrane.

A simple and well-studied two-component reaction network that exhibits bistability by means of positive feedback can be described by the following reactions:

$$A \leftrightarrow X$$

$$A + 2X \leftrightarrow 3X$$

Here, we say that X is the molecular species that prompts system activation and that a system with high X molecule concentration is in the active state. The rate constants allow for bistability under reasonable reaction conditions.

II. Results

We utilize the Gillespie algorithm to generate many spatially resolved stochastic trajectories under various conditions [1]. In the well-mixed case, the initial concentration of each species influences the distribution of steady states sampled within a fixed time window. In the spatially extended case, the molecular diffusion coefficients as well as the system size and shape also influence the distribution of stable steady states sampled within a fixed time window.

In the well-mixed case, we observe unidirectional stochastic switching from the active to inactive state in cases in which the system is in the bistable regime near the bifurcation point. To gain insight into stochastic switching from the inactive to active state in the spatially resolved system, we perturb the system with a pulse of X molecules in a localized region. The probability that the system is in the active state at a given time depends on the diffusion coefficient, and it increases when the initial X distribution is spatially clustered rather than homogeneously distributed. In three dimensions, the system shape plays a key role in the emergent dynamics. Systems of equal volume that have small aspect ratios are more likely to switch to the active state. Faster diffusion also results in faster activation and a larger steady state value of X molecule population.

III. Conclusion

Faster diffusion and larger concentrations promote the active steady state in the positive feedback network considered in this work. In a spatially resolved system, stochastic switching from the inactive to the active state often occurs as a result of nucleation of clusters of X molecules. Such clusters can form naturally by means of localized fluctuations or artificially by implementation of a pulse of desired size and concentration.

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[1]Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN 37916, USA

Reverse Engineering Signaling Cascade from High Throughput Data

Sayak Mukherjee[1], William Stewart[1], and, Jayajit Das[1]

***Short Abstract* — The high throughput experiments opened for the first time a possibility of inferring the cell signaling networks from data. Presence of extrinsic noise in a population as well as tonic signaling however offers a challenge for inference. Using a combination of Maximum Entropy based inference scheme and simulated annealing we have developed a method that is capable of constructing an effective linear description of the underlying biochemical network. We have validated our method for synthetic data acquired from linear and non-linear reaction cascades and used it to infer signaling networks for primary NK cells from multi parameter CyTOF data.**

***Keywords* —CyTOF data, Maximum Entropy, simulated annealing, data driven modeling, NK cell signaling**

I. Purpose

The advent of high throughput measurements ushered in a new era in systems biology. The simultaneous monitoring of expression levels of different proteins at different time points for each single cell showed us that the time evolution of the proteins are highly co-regulated and opened the door for inferring the underlying network of interactions.

There are two major sources of noise [1] in a high throughput data. First, the presence of large amount of cell-to-cell fluctuations in the protein copy numbers as well as fluctuations in the kinetic binding and unbinding rates (extrinsic noise). Second, the fluctuations those arise due to the inherent probabilistic nature of biochemical reactions (intrinsic noise). Even in the limit of large copy numbers of proteins (weak intrinsic noise), the presence of large extrinsic noise can pose a formidable challenge in inferring networks. It can dominate over the variations in time evolution of proteins coming exclusively from the internal network architecture and can potentially render the inference scheme ineffective. In addition, there can be a substantial amount activation present in the unstimulated cells due tonic or basal signaling which makes it difficult to separate out the changes in activation that truly occurred due to a signaling response to external stimuli.

The purpose of this work is to develop a method that can identify and isolate the extrinsic noise and contribution from the tonic signaling from variations arising solely due to a signaling response and help us reverse engineer the directed causal architecture of the signaling network from the single cell time series data.

II. Method

We seek for an effective linear description of the real biochemical reaction cascade. To this end we sample for a linear module characterized by a corresponding **M** matrix given by $\partial_t C = \mathbf{M}C$, where $\boldsymbol{C}$ is the concentration vector, using simulated annealing [2] that minimizes the Euclidean distance between the moments generated by the sample and once calculated from the *in-silico* networks/experiments. The simulated annealing yields an effective linear description that is endowed with causal information. A Maximum Entropy based method is used to choose between different initial network topologies that are used in simulated annealing.

III. Conclusion

The method is very general; it probes the network architecture directly, it is insensitive towards extrinsic noise fluctuation, and separates out the contribution from basal signaling. We have validated the method against *in-silico* linear and non-linear reaction cascades. We have used this method for data acquired in CyTOF experiments performed at Lewis Lanier's lab (UCSF) with Jurkat T cells and primary NK cells. The primary NK cells are stimulated with cognate ligands for NKG2D activating receptors and we use the combined computational and experimental method to search for hitherto unknown novel interactions in NK cell signaling.

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[1]BCMM, Research Institute at The Nationwide Children's Hospital. E-mail: mukherjee.39@osu.edu

Light, Imaging, Vision: An interdisciplinary undergraduate course

Philip C Nelson[1]

***Short Abstract* — I'll describe an undergraduate course, for students in several science and engineering majors, that takes students from the rudiments of probability theory to the quantum character of light, including modern experimental methods like fluorescence imaging and Förster resonance energy transfer. After a digression into color vision, the course closes with the remarkable signaling cascade in our photoreceptors, and a glimpse of further processing beyond the first synapse. Course materials are available upon request.**

THE vertebrate eye is fantastically versatile instrument. Using eyes as a common thread helps motivate undergraduates to learn a lot of physics, both fundamental and applied to scientific imaging and neuroscience. I'll describe an undergraduate course, for students in several science and engineering majors who have taken one year of introductory physics and math. The course takes students from the rudiments of probability theory to the quantum character of light, including modern experimental methods like fluorescence imaging and Förster resonance energy transfer. After a digression into color vision, we then see how the Feynman principle explains the apparently wavelike phenomena associated to light, including applications like diffraction, subdiffraction imaging, total internal reflection and TIRF microscopy. Then we see how scientists documented the single-quantum sensitivity of the eye seven decades earlier than "ought" to have been possible, and finally close with the remarkable signaling cascade that delivers such outstanding performance. Course materials are available upon request. They are free-standing, independent of another recent book by the author [1]. Two separate resources are available that help students to acquire needed computer programming skills [2,3].

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[1]Physics and Astronomy, University of Pennsylvania. E-mail: nelson@physics.upenn.edu

Automated tropical algebra reduction and hybridization of biological models

Oscar O. Ortrega[1,2], Shawn Garbett[1], and Carlos F. Lopez[3,*]

***Short Abstract* — We present TroPyc, a python module integrated into the PySB modeling framework that performs automated reduction and hybridization of biological models. It allows identification of key driver interactions of these complex networks and the most important parameters at different time-scales. Additionally, we validated this module with three different apoptosis models (EARM) and report the key species and parameters of these models. Importantly, this novel approach could find new targets for cancer drugs**

***Keywords* — Systems biology, model reduction, tropical algebra, network analysis.**

I. Purpose

The extrinsic apoptosis reaction model (EARM), is a family of novel and previously published models of extrinsic apoptosis, focusing on variant hypotheses for how the Bcl-2 protein family regulates mitochondrial outer membrane permeabilization (MOMP) [1]. These models possess distinct spatial and temporal dynamic behaviors as well as multiple molecular interactions molecules and interaction sites. Due to this complexity, large models are difficult to interpret yet they are necessary to understand the complexity of systems-level behaviors that lead to cell decision processes. In this work we develop an automated formalism employing algebraic geometry paradigms [2] to identify the important parameters as well as the time-dependent execution where multiple parameters commit a cell to apoptosis. We present the TroPyc Python module that interacts with our PySB modeling framework to perform tropical algebra transformations. TroPyc can hybridize and reduce large biochemical models like EARM into simpler models – also known as– dominant subsystems, that employ a quasi-steady state and quasi-equilibrium approximation to reduce the parameter space and therefore facilitate analysis. As shown in Figure 1, the tropicalization approach enables the extraction of signaling activity in a time-dependent manner, whereas this information is obscured in a typical chemical species dynamic plot.

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[1]Department of Cancer Biology, Vanderbilt University, 2220 Pierce Ave, Nashville TN.

[2]E-mail: oscar.ortega@vanderbilt.edu

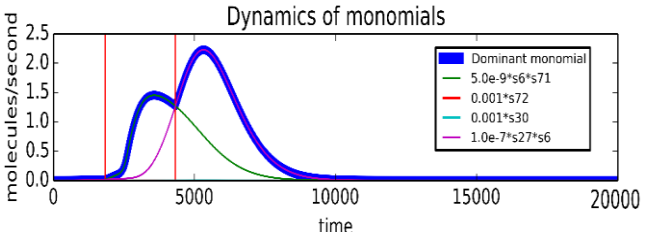

Figure 1. Caspase 3 tropicalization. The red lines identifies the time points where there are sharp transitions between dominant subsystems.

II. Approach

Based on a theoretical analysis by Radulescu *et al* [3], we developed an automated tool to reduce network complexity within the open source PySB framework using tropical algebra. The method consists of a simplification step followed by a tropical algebra calculation step which yields the key driver interactions of a complex network, and the steady state modes that the network can occupy. In addition, state-change drivers can also be identified when comparing multiple network modes. We present validation of the automated algebra tool using three different EARM models: Embedded, direct and indirect [4]. For each model, we identified the driver species in the different time-scales and their related parameter of association or dissociation. Then we validated the relevance of this parameters by changing the parameter value at different time points. We found that when we changed the parameters in time points outside their dominant time-scale, there were no drastic changes in the dynamics of the model. On the other side, when the parameter were changed in time points within their dominance, small changes of the parameter value generated extreme changes in the dynamic behaviors. The results of this analysis could lead to discoveries of new targets for cancer drugs.

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Mitochondrial Energetic Homeostasis and Parallel Activation

Yanjun Li, Vipul Periwal

***Short Abstract* —** Both skeletal muscle and cardiac muscle have the special ability to maintain energetic homeostasis in response to drastic elevations in energy expenditure. To meet this tremendous demand of ATP, mitochondrial oxidative phosphorylation has to increase in coordination. A metabolic network requires coordinated changes in fluxes, leading to the `in parallel activation' hypothesis. We propose a simple hypothesis that Ca^{2+} modulates mitochondrial energetic metabolism. We show that a mathematical model incorporating this hypothesis matches experimental data.

***Keywords* — mitochondria, oxidative phosphorylation, calcium**

I. Purpose

MITOCHONDRIAL oxidative phosphorylation supplies the vast majority of energy in metazoans. In humans, the change in ADP and Pi concentrations between exercising and resting muscle is small even though the ATP demand increases by almost an order of magnitude[1]. The canonical feedback regulation of energetic homeostasis by ADP and Pi is supported by some studies, but may not induce enough ATP production due to the minor changes in concentrations of metabolites. The `in parallel activation' hypothesis due to Korzeniewski[2] suggests using activation factors for metabolic enzymes to achieve the required coordinated changes in fluxes via simultaneous activation. The exact mechanisms for such an activation remain unclear. Metabolic enzymes are associated with regulation at different levels, e.g. phosphorylation, but perhaps calcium is the most important and ubiquitous factor impacting metabolism. Ca^{2+} not only is needed for muscle contraction, but also directly regulates various key enzymes in mitochondria. Recently, Glancy et al. evaluated the effect of Ca^{2+} on mitochondrial respiration in situ[3]. Their study provided new evidence that inter-mitochondrial Ca^{2+} alone can stimulate the entire energetic pathway simultaneously with similar magnitudes. Therefore, mechanisms may exist to coordinately induce the activities of those enzymes in vivo, as seen, for example, in [4-6].

II. Methods

This hypothesis was incorporated into a published mathematical model of mitochondrial metabolism[7]. We assumed that the metabolic enzymes have two forms: a basal form or an active form in the absence or presence of Ca^{2+}. Ca^{2+} can activate reactions by inducing the conversion from the basal form to active form. This new model was validated to fit the steady-state Ca^{2+} -dependent responses of muscle mitochondrial respiration in State 4 or State 3. Model simulations were compared with experimental results from the creatine kinase clamp protocol.

III. Results

Model simulations match experimental data[3]. The metabolic responses in State 4 and State 3 are remarkably different. In the absence of Ca^{2+}, oxygen consumption (JO_2) in State 3 was almost three times that in State 4. With added Ca^{2+}, oxygen consumption in State 4 was almost constant. In State 3, it increased and reached a plateau. In the absence of Ca^{2+}, the fraction of NADH in total NAD+NADH (%NADH) was ~100% higher in State 4 than in State 3. With the addition of Ca^{2+}, %NADH in both states increased with similar patterns. The membrane potential in State 4 was much higher than in State 3 in the absence of Ca^{2+}. Compared with its effect on JO_2 and %NADH, Ca^{2+} had negligible effect on membrane potential. JO_2 is almost linearly related to ΔG_{ATPe}, and the slope in the presence of Ca^{2+} is greater than in its absence. With the increase of ADP, the increase of JO_2 in the presence of Ca^{2+} was much larger than its increase in the absence of Ca^{2+}. The Eadie-Hofstee plots of JO_2 and ADP exhibit a near linear relationship. JO_2 is almost linearly related with membrane potential and the slope in the presence of Ca^{2+} is larger than in its absence.

IV. Conclusions

In parallel activation modulated by calcium concentrations is a viable mechanism for the observed ability of muscle mitochondria to maintain almost unchanged concentrations of metabolites under large changes in energy demand.

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Laboratory of Biological Modeling, National Institute of Diabetes Digestive and Kidney Disorders, NIH. E-mail: yanjun.li@nih.gov, vipulp@mail.nih.gov

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Redesigning the response of T cell signaling networks using *in silico* evolution

Aaron M. Prescott[1] and Steven M. Abel[2]

***Short Abstract* — The T cell receptor (TCR) signal transduction pathway allows T cells to activate upon binding molecular signatures of pathogens. To gain insight into the T cell signaling network, we utilize *in silico* evolutionary algorithms to produce a variety of signaling profiles as a function of TCR-antigen binding strength. Repeated, independent *in silico* trials produce a variety of potential solutions for a given target signaling profile, and analysis of the data provides insight into relations between various reaction rates that produce the profile.**

I. Introduction

CELLS have evolved an array of signal transduction pathways that allow them to detect and respond to their environments. T lymphocytes use the T cell receptor (TCR) signal transduction network to identify molecular signatures of pathogens. The pathway exhibits a sharp activation threshold as a function of the binding strength of the TCR with peptide-MHC (pMHC) ligands presented on other cells. Following pMHC binding, the cytoplasmic region of the TCR complex is phosphorylated by a kinase associated with coreceptors that also bind to the pMHC complex [1]. It has been shown that coreceptor-mediated recruitment of the kinase has a dramatic effect on the rate of TCR phosphorylation [2]. TCR phosphorylation is an important early signaling event that results in the recruitment of other proteins that promote downstream signaling.

The TCR network topology is the result of natural evolution. Novel signal transduction network topologies have also been produced through the use of *in silico* evolutionary algorithms (EA) [3-5]. EAs are a class of heuristic optimization techniques that utilize a selective pressure in order to discover a system that produces a desired output. In this work, we consider a fixed-topology network and allow kinetic rates to vary. The parameters are screened by evaluating them with a fitness function that measures the deviation of the actual output from the target output. Parameter sets with improved performance are selected for and then mutated and/or recombined with other desirable parameter sets to produce new networks. This process is repeated until a desired level of fitness is reached [6].

[1]Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville. Email: apresco1@vols.utk.edu

[2]Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville. Email: abel@utk.edu

II. Model and Methods

We consider a deterministic, well-mixed model of the TCR signaling network [2] and utilize an *in silico* evolutionary algorithm to search for sets of kinetic parameters that give a variety of altered output responses as a function of the off rate between pMHC and TCR. With fixed network topology, we allow variation in all reaction rates in the model. The evolutionary algorithm can produce sets of reaction rates that shift the activation threshold typically observed for the TCR network to both higher and lower values of the TCR-pMHC off rate. Interestingly, we find that the TCR signaling network topology can achieve even more dramatic output profiles, such as an inversion of the activation pattern in which weak TCR-pMHC binding achieves activation and strong binding does not.

By running multiple independent instances of the evolutionary algorithm for each desired output, we find many different sets of reaction rates consistent with the desired output. Distinct patterns of solutions become apparent when analyzing the resulting data sets using techniques such as k-means cluster analysis.

III. Conclusion

In silico evolutionary algorithms can be applied to existing biochemical network topologies to produce novel outputs not seen in nature. We demonstrate that through moderate adjustments of kinetic parameters, the TCR signal transduction network has the potential to produce a wide array of input-output relations. Such studies can help to shed insight into T cell signaling and may provide a means for designing artificial networks with desired signaling properties.

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A network model of cellular aging and its applications.

Hong Qin[1]

Why would a genotypically homogeneous population of cells live to different ages? We propose a mathematical model of cellular aging based on gene interaction network. This model network is made of only non-aging components, and interactions among genes are inherently stochastic. Death of a cell occurs in the model when an essential gene loses all of its interactions. The key characteristic of aging, the exponential increase of mortality rate over time, can arise from this model network with non-aging components. Hence, cellular aging is an emergent property of this model network. The model predicts that the rate of aging, defined by the Gompertz coefficient, is proportional to the number of active interactions per gene and that stochastic heterogeneity is an important factor in shaping the dynamics of the aging process. Hence, the Gompertz parameter is a proxy of network robustness. Preliminary studies on how aging is influenced by power-law configuration, synthetic lethal interaction, and allelic interactions can be modeled. A general framework to study network aging as a quantitative trait has also been found, and the results has implication on missing heritability. Preprint for the basic model is available at http://arxiv.org/abs/1305.5784.

***Keywords* — Cellular aging, gene networks.**

I. Background of our model

Aging is a fundamental question in biology, yet its mechanism remains elusive. Aging can be quantified by the normalized decline of viability (s) over time (t),

$$m = -\frac{1}{s}\frac{ds}{dt} = f(t), \qquad \text{Eq. 1}$$

where, m is called the mortality rate, and $f(t)$ is a function of time. It can be shown that change of mortality rate over time follows the Weibull model for homogenous systems like machiner and Gompertz model for heterogenous systems like organisms, using a model with serial connected-blocks with redundant components [1].

Cellular aging is the basis of physiological aging. The unicellular eukaryotic organism, budding yeast *Saccharomyces cerevisiae*, is a model organism for cellular aging. Replicative lifespan of the budding yeast has been shown to follow the Gompertz model of aging [2].

To provide a unifying theoretic framework on cellular aging, we proposed a mathematical model for cellular aging based on gene networks.

In our probabilistic gene network model for cellular aging, there are essential genes and non-essential genes (Figure 1). Genes are nodes, and gene interactions are edges. We assume the efficacy of each gene interaction is non-aging and that it declines with a constant mortality rate λ. Each gene interaction is active within cells with a probability of p. Each essential gene interacts with n number of non-essential genes. It can be shown that the mortality rate of the entire network, i.e. a cell, grows exponentially with time (age),

II. Applications of our model

One important application of our model is to evaluate a hypothesis that the conserved mechanism of lifespan extension is through improving reliability of gene interactions. With the availability of replication lifespan measures of thousands of yeast single gene deletion mutants, we can fit our network model with these mutants, and compared the fitted network parameters.

Another application is to use mixture distributions to model gene interactions with limiting effect on yeast lifespan.

Our model also provides a mechanistic explanation for aging as a quantitative trait. By comparing our network model with linear models in quantitative genetics, we may answer the question of missing heritability.

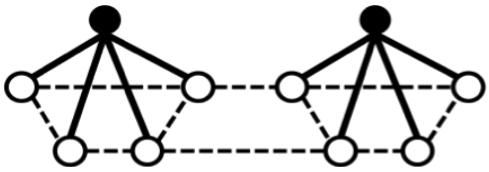

Figure 1. Network reliability model for cellular aging.

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[1] Department of Biology, Spelman College, Atlanta, Georgia 30314, U.S.A. E-mail: hqin@spelman.edu

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Modeling the spatiotemporal dynamics of Cdc42 activity at dendritic spines accounting for membrane geometry

Samuel A. Ramirez[1], Sridhar Raghavachari[2] and Daniel J. Lew[3]

***Short Abstract* —Long lasting remodeling of dendritic spines induced by synaptic activity has been associated with learning and memory. Upon synaptic stimulation, the activity of the Rho GTPase Cdc42 localizes to the stimulated dendritic spine in a sustained manner. Long lasting activity localization occurs even though Cdc42 can rapidly diffuse in and out of the spine. Here we describe the spatiotemporal dynamics of Cdc42 activation at dendritic spines as the numerical solution of Reaction-Diffusion equations on spine-like geometries. We propose that positive feedback of activation together with the geometry of the spine can account for long lasting localization of Cdc42 activity.**

***Keywords* — Cdc42, dendritic spine, signaling localization, Reaction-Diffusion equations.**

I. Introduction

SYNAPTIC activity induces sustained structural remodeling of dendritic spines, the input site of most synapses, in a process associated with learning and memory. This long lasting remodeling is specific to the stimulated spine; neighbor spines not stimulated remain unchanged. The molecular basis of the specificity of sustained spine remodeling has not been fully elucidated, however, upon spine stimulation, the activity of Cdc42, a GTPase known to regulate dendritic spine structure, localizes at the membrane of the stimulated spine in a long lasting manner [1]. Interestingly, sustained localization of active Cdc42 occurs even though Cdc42 itself can rapidly diffuse in and out of the spine.

Cdc42 also localizes in yeast, where it forms a cluster on the membrane that directs budding and mating. In this system a positive feedback of activation that recruits inactive Cdc42 from the cytosol has been shown to be necessary for clustering [2]. Furthermore, it is believed that depletion of a cytosolic substrate of the activation reaction [3, 4], is required to prevent the positive feedback from completely covering the membrane with Cdc42.

Depletion of a cytosolic substrate is not likely to occur in dendritic spines as they are connected to the much larger dendrite. Taking this into consideration we propose a model for sustained localization of Cdc42 activity at dendritic spines that does not require the substrate depletion condition.

[1]Program in Computational Biology and Bioinformatics, Duke University, Durham, NC. E-mail: samuel.ramirez@duke.edu

[2]National Science Foundation. E-mail: sraghava@nsf.gov

[3]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC. E-mail: daniel.lew@duke.edu

II. Results

A. We describe the spatiotemporal dynamics of Cdc42 activation at the dendritic-spine membrane as the numerical solution of Reaction-Diffusion equations on spine-like geometries. We use a model for positive feedback of Cdc42 activation that describes the spread of Cdc42 activity as a traveling wave front [4]. In this scenario, membrane geometry results in sustained localization of active Cdc42 at dendritic spines without requiring depletion of cytosolic substrates such as inactive Cdc42.

B. Our simulations suggest that the width of the spine neck is a critical geometrical parameter that controls sustained localization of activity. Thin spine necks promote activity confinement, whereas thick spine necks result in activity spread. The simulations also show that higher diffusion coefficients of Cdc42 on the membrane promote confinement of activity, which seems counterintuitive.

C. The results of our simulations are in qualitative agreement with theoretical predictions of the effect of surface geometry on wave front dynamics. However, we observe quantitative differences between simulations and theory. In particular, the predicted critical values of parameters controlling localization of Cdc42 activity differ between simulations and theory. We investigate the cause of such discrepancy.

III. CONCLUSION

We propose that positive feedback of activation coupled with the unusual spine geometry can explain the sustained localization of active Cdc42 at dendritic spines upon synaptic activity.

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A Synthetic Biology Approach to Ribosome Collisions and Stalling

Julia A. Roth[1], Nicholas C. Butzin[2], Curtis T. Ogle[2], Royce K. P. Zia[2], William Mather[2,3]

***Short Abstract* — Translation is well understood, but few predictions at the whole cell level have been experimentally tested regarding ribosome collisions and stalling. In this work, we modify a TASEP model to account for the influence of rare codons on collective ribosomal dynamics. We test this model using a synthetic biology approach, where we are able to obtain quantitative data concerning the influence of ribosome collisions and stalling on translational output.**

***Keywords* — Ribosome Collisions, Ribosome Stalling, TASEP, Systems and Synthetic Biology, Monte-Carlo**

I. ABSTRACT

THE fundamentals of translation are well established, but the cellular response to ribosomal stalling and collisions is poorly understood. Previous detailed models for translation, one of which is Totally Asymmetric Simple Exclusion Process (TASEP), predict that ribosomes naturally and frequently interact with each other [1-6]. However, TASEP and similar models typically do not consider the fact that each ribosome's footprint spans many codons, that each ribosome moves along mRNA with a non-uniform rate, or that ribosomes can prematurely dissociate from mRNA.

To this end, we construct a detailed stochastic model for translation that extends earlier efforts [6]. This new model allows for ribosome collisions when their respective footprints overlap. These collisions can then accelerate ribosome rescue, whereby ribosomes insert a fast degradation tag on partially completed peptides and then detach from mRNA. The model also allows for ribosome rescue in the case of stalling, where a ribosome simply pauses at a rare codon. Using this model, we explore the influence of ribosome collisions and stalling on translational output. In the near future, this model will also include the influence of collisions and stalling on mRNA degradation.

We then experimentally test our model predictions using a synthetic biology approach. This involves batch results (96-well microplate experiments) and single cell results (microfluidics and microscopy) for a synthetic circuit in *E. coli*. We designed our synthetic circuit so that two adjacent coding regions for fluorescent proteins are under control of a common inducible promoter. In between the coding region for these proteins is a region we call the "gate," a potential bottleneck (or stalling site) of elongation. The gate consists of codons that are normally rare in *E. coli*. We then synthetically control the cellular level of tRNA specific to these gate codons, and thus, we can vary these codons from rare to abundant.

Measurement of the two fluorescent proteins provides insight into the influence of ribosome collisions and stalling on translational output. By controlling the tRNA concentration corresponding to our gate, we can continuously transition the gate from an open to a closed state. If the gate is open, then there is a plentiful amount of the tRNA for the gate codons, and translation will continue at a fast pace for all fluorescent proteins. If the gate is closed or infrequently open due to minimal tRNA concentration, then translation of the downstream protein is either stalled or aborted.

The advantage of using our synthetic approach is it allows for quantitative data for (1) rareness of codons related to tRNA levels, and (2) an *in vivo* analysis of ribosomal collisions and stalling. By combining experimental findings with TASEP simulation, it allows us to create a more accurate model of ribosomal interactions than previously possible.

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[1]Department of Biological Systems Engineering, Virginia Tech. jroth242@vt.edu.

[2]Department of Physics, Virginia Tech. ncb@vt.edu, cogle@vt.edu, rkpzia@vt.edu, wmather@vt.edu.

[3]Department of Biological Sciences, Virginia Tech. wmather@vt.edu.

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Optimal feeding regulation in noisy environmental conditions

Monika Scholz*‡ , Aaron R. Dinner* , and David Biron*†

***Short abstract*—Any organism needs to respond to a spatially and temporally dynamic environment and modulation of behavior assists in favorably exploiting the environment. In the case of simple eating behavior, performing eating motions and obtaining nutrients represent a cost and a benefit, respectively, and optimal exploitation is defined by the maximum of a cost-benefit curve. Models of regulatory strategies can potentially be tested using detailed experimental data and may assist in conceptualizing the data in terms of an optimality principle. Within this framework, the implications of limitations, such as noisy sensory cues or imperfect memory, can be assessed. Here, we introduce novel measurements and analysis tools of eating behavior in the nematode *C. elegans*. We use the observes statistics of eating to compare between models based on different optimality criteria and assess the constraints that could make particular strategies favorable.**

***Keywords*—feeding behavior, optimal regulation, decision-theory, information maximization**

I. Background

THE nematode *C. elegans* is a simple model system with feasible genetics and easily quantifiable behaviors that facilitate high-throughput assays. *C. elegans* feeding depends on the action of a muscular pump, the pharynx. During pharyngeal pumping the nematode sucks in bacterial food and surrounding liquid, expels the liquid, and traps the food [1]. The rate of pumping is thus the primary indicator of food intake. he traditional pumping assay scores a mean rate by counting the number of muscle contractions over short intervals, typically 30-60 sec in duration [3].

To measure the statistics of pumping in detail, we use a microfluidic device that enables continuous measurements of pumping and control of the feeding conditions [2]. We can thus alter food concentrations precisely and assay animals continuously for prolonged periods. In addition, we developed a set of machine vision tools which enable us to automatically obtain times series of pumping events from the raw images. These developments allow us to characterize pumping in a controlled environment and obtain unprecedented quantitative information about the dynamics of this process.

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* James Franck Institute and Institute for Biophysical Dynamics, University of Chicago, Chicago, IL 60637

‡ Graduate Program in the Biophysical Sciences

†Department of Physics, University of Chicago, Chicago, IL 60637, USA

II. Results

We continuously measure feeding rates in the presence of various concentrations of bacterial food for hour-long periods (the optical density (OD) of the bacterial food ranges between 0.5 and 5) and obtain a time-series of pumping events for each animal. Under these conditions, the distribution of intervals between pumps is approximately bimodal and that brief periods of regular pumping are interspersed with periods in which pumping appears irregular. Moreover, the statistics of pumping depend on the availability of food. Thus, a single mean rate fails to capture the richness of pumping dynamics.

In our assays, the animal can ingest relatively small numbers of (discrete) bacteria through each single pump, such that shot noise can affect individual samples of the environment. We hypothesize that there exists an optimal pumping strategy given a fixed cost per pumping event, a fixed benefit per bacterium, and the possible limitations discussed above. We show that the simplistic approach of memory-based thresholding (i.e., asking at each step if the expected gain would be positive) does not describe the experimental data. In contrast, an information-maximization strategy [4] intrinsically reproduces experimentally observed features such as switches between regular and irregular pumping.

III. Conclusion

Novel tools allow the investigation of eating behavior in more detail than was previously possible. These data can be conceptualized in terms of optimal strategies for sensory-based decision processes given various limitations.

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Role of Stem Cell Niche Structure in Cancer

Leili Shahriyari[1] and Natalia L. Komarova[2]

***Short Abstract* — Using recent modern imaging techniques, scientists have found evidence of collaboration between different types of stem cells (SCs), and proposed a bi-compartmental organization of the stem cell niche. Here we create a class of stochastic models to simulate the dynamics of cells at the stem cell niche. We examine this model in the context of 2-hit mutant generation, which is a rate-limiting step in the development of many cancers. We discover that a cooperative pattern in the stem niche with two groups leads to a significantly smaller rate of double-hit mutant production compared with a homogeneous and one-compartmental SC niche. Furthermore, the optimal architecture (which minimizes the rate of 2-hit mutant production) requires a large proliferation rate of stem cells which are close to the transit amplifying (TA) cells along with a small, but non-zero, proliferation rate of the central stem cells. This result is remarkably similar to the niche structure described recently by several authors, where one of the two stem cell compartments was found more actively engaged in tissue homeostasis and turnover, while the other was characterized by higher levels of quiescence (but contributed strongly to injury recovery).**

***Keywords* — Stem cell niche, Mutation, Cancer, Tumor suppressor genes, Stochastic process, Moran process.**

I. Purpose

Anatomical and molecular heterogeneity has been reported to be a common feature between mammalian SC niches across different tissues [1, 2, 3]. In [4], it was suggested that SCs in many tissues are characterized by a bi-compartmental organization. One SC group engages more readily into new growth, while the other one contributes more to the long-term turnover and regeneration of the tissue. Such patterns have been identified in several adult SC niches, such as hair follicles, blood, intestine, and brain [4]. So SC populations exhibits a certain degree of complexity that cannot be captured by simple, one-compartment models. More details of the bi-compartmental niche structure were recently uncovered by [5]. Researchers were able to follow the fate of individual intestinal stem cells and their progeny over time *in vivo*. In particular, two distinct groups of SCs have been identified: the "border cells" located in the upper part of the niche at the interface with TAs, and "central cells" located at the crypt base. The proliferative potential of the two groups was unequal and correlated with the cells' location (central or border). Further, it was reported that the central SCs could divide and migrate downstream replacing SCs in the border part. A similar dependence of self-renewal potential on proximity to the niche border was reported in the hair follicle, in an *in vivo* live-imaging study [6].

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[1]Mathematical Biosciences Institute, Ohio State University, Columbus, OH, USA. E-mail: shahriyari.1@mbi.osu.edu

[2]Department of Mathematics, University of California Irvine, Irvine, CA, USA. E-mail: komarova@uci.edu

II. Stochastic Model of the Stem Cell Niche

Here, we incorporate the bi-compartmental structure in our modeling approach, to see how this complexity might affect the evolutionary forces that shape the cells' division patterns. We focus on the stem cell niche and create several general models of the architecture, which include the model of [7] as a special case. Following evidences of collaboration between cells in the niche and their neighboring cells [5, 6], we divide SCs in the niche into two groups. One stem cell group S1 (the border cells) regulates the number of TAs and SCs, and the group S2 (the central cells), is only responsible for controlling the total number of SCs. We also include a possibility of migration of cells from one group to the other. In particular, we investigate which architecture type lead to the maximum delay in 2-hit mutant production. We obtain the optimal niche structure and the division patterns for each group, which minimize the rate of 2-hit mutant production.

III. Conclusion

We found that a certain pattern of cooperative stem cells in the bi-compartmental niche, along with symmetrically dividing SCs, leads to a significantly lower rate of two-hit mutant generation compared with the architecture that involves only one group of stem cells. In the optimal niche architecture, most divisions happen in the S1 group, with an occasional symmetric division in the S2 compartment followed by a S2 to S1 SC migration.

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How to Achive Perfect Adaptation

Wenjia Shi[1], Wenzhe Ma[2], Liyang Xiong[3] and Chao Tang[4]

***Short Abstract* —Understanding design principles of certain biological function is an amazing task. Adaptation is our best candidate to study. First, we analyzed and enumerated topologies for gene regulatory process with three regulatory rules to find those adaptive ones. Second, we evolutionally searched adaptive topologies with more than three nodes for enzymatic reactions. We found two kinds of three-node topologies are adaptive and they partly differ from that of enzymatic ones; 15 four-node topologies are adaptive, and a coherent feed-forward loop coupled with a negative feedback loop emerged as a totally new one. Our study may provide a design table for adaptive circuits for different network size and biologoical processes.**

***Keywords* — Design principle, Adaptation, Transcriptional regulation, Enzymatic reaction, Three node, Four node.**

I. Introduction

In the quantitative era, with the huge accumulative experimental data. We need a simplified way to try to approach the underlying mechanism while every single perturbation may cause an inconvenient phenotype change of the complex biological networks. On one hand, a very exciting indication from previous studies really lightens the way [1]: *There should be a limited group of network motifs to execute certain functions*. On the other hand, small network is no doubt to achieve related function while its sufficiency to present complex biological system remains unclear due to the ignorance of many details and feature of the systems. So if we extend the small network to even one more node larger, will something new emerge? We chose adaptation as the best candidate function to study the network design principles because of its universality and clear mathematical definition.

II. Topologies for Adaptative Processes

First of all, despite the fact that enzymatic topologies for adaptation have been investigated systematically, the topologies for gene regulatory networks that are capable of adaptation are still unknown, due to the complexity of transcriptional regulations. For simplicity, we model transcription as a Hill function with three kinds of logics: AND logic(multiplied all the activation and repression terms); AND&Additive rule(taking he average effects of all the activation terms and multiplying the product of all repression terms); Competitive repression(the repressors merely decrease the effect of activators instead of blocking gene expression, and gene expression becomes weaker in the absence of repressors). From linear analysis and simulation, we conclude that: 1) neither one- nor two-node (separate input and output nodes) topologies adapted perfectly; 2) three-node topologies that were capable of perfect adaptation fell into two classes, NFBLs(negative feedback loops) and IFFLs(incoherent feed-forward loops). However, some new skeletons emerged compared with enzymatic reactions, due to the consideration of complex regulatory rules and the degradation of gene products. Most important, all the NFBLs must have an auto-activation on buffer node B working in its linear region with Hill coefficient equals to 1. For the IFFLs, an inversed proportional mechanism breaks the necessity of proportional mechanism for enzymatic topologies and two new types of IFFLs are also feasible for perfect adaptation in gene regulatory networks.

Next, we evolutionally[2] searched enzymatic topologies with more than three nodes that can adapt. We found a mechanism that when the output node is catalyzed by enzymes which are proportional to each other in their steady states, the output node will be independent of input signal and then achieve perfect adaptation. We then explored the simplest proportional network motifs, and used the motifs as blocks to build networks with more nodes in which such motifs are coupled. Primarily, we started to build four-node networks which can be analytically handled. We found 15 four-node adaptive networks, and a totally new one emerged: A coherent feed-forward loop does not achieve adaptation by itself, but it is capable with the help of a negative feedback loop. This new one works as an “anti-spring” -firstly transmitting signal synchronously with input, while then giving an anti-response and make the coherent feed-forward loop capable of adaptation with the help of a negative feedback loop.

III. Conclusion

In this study, we systematically approached the design principles of adaptive networks involving in gene regulations and enzymatic reactions with different network size. Through this kind of study, we can help synthetic biologist build the functional tools they want and get closer to a dream that we may make a biological machine as electronic industry developed. Meanwhile, there still are questions afterward our study: How does God design a complex system with multifunctional modules? Follow these studies, we may get a clear clue about how nature designs.

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[1,3,4]Center for Quantitative Biology, Peking University, Beijing, China.

Email: wenjia.shi@gmail.com. xiongliyang219@gmail.com.

tangc@pku.edu.cn.

[2] Department of Systems Biology, Harvard Medical School, Boston, Massachusetts, United States of America. Email:wenzhe1980@gmail.com.

Classifying and quantifying parameter nonlinearity in biological models

Alex Shumway[1], Mark K. Transtrum[1]

Models of biological systems typically include many parameters. Furthermore, the model behavior often responds to changes in these parameters in highly nonlinear ways. This nonlinear response is responsible for many of the unique emergent behaviors of biological systems. We discuss how model nonlinearity can be both quantified and classified. We consider the tensor of model second derivatives, i.e., the vector of Hessian matrices for each model prediction or alternatively the Jacobian of the Jacobian matrix. We use a higher-order singular value decomposition to identify the principal parameter combinations exhibiting the greatest nonlinearity (generalizations of singular vectors) and quantify this nonlinearity using generalizations of singular values. We further classify types of nonlinearity by decomposing the second derivative tensor into geometrically motivated components, including extrinsic, intrinsic, and parameter-effects nonlinearity. We discuss applications to model interpretation as well as for numerical methods.

MODELS of biological systems, such as those describing dynamics of protein signaling, gene regulation, and other cellular activity typically include a large number parameters. In many cases these parameters are unknown and must be estimated from data. The response of the model behavior to changes in these parameters is often highly nonlinear. The nonlinearity in the model parameters leads to challenges for numerical methods, such as data fitting [1]. Furthermore, parameter nonlinearity makes it difficult to interpret the model. In particular, the nonlinearity makes it challenging to identify the particular parameter or parameter combination that controls a feature of the model behavior. Consequently, highly nonlinear models often exhibit nontrivial, emergent behavior that is obscured by this nonlinear parameter response [2].

Understanding the role of parameter nonlinearity in models is important for a host of modeling activities including numerical algorithms (such as data fitting or Bayesian posterior sampling), model interpretation, model construction, and experimental design. We present a theoretical and computational framework for understanding the effect of nonlinear parameters in complex biological models that uses techniques from differential geometry, information theory, and linear algebra.

The response of a model to *small* changes in parameters can be studied using a local linearization of the model: characterized by either a Jacobian matrix (derivatives of model predictions with respect to each parameter) or through the closely related Fisher Information Matrix (FIM). Considerable effort has been devoted to understanding how these objects characterize the model, including "sloppy" model research [3] with applications to experimental design and numerical methods.

We extend these methods by considering the tensor of second derivatives, i.e., the Jacobian of the Jacobian matrix. We use the higher-order singular value decomposition [4] of this tensor to quantify the nonlinearity and identify the principal parameter directions corresponding to this nonlinearity. We further construct other measure of nonlinearity motivated by the information geometric interpretation of the model, including intrinsic curvature, i.e., Riemann and Ricci tensors, extrinsic curvature, and parameter-effects curvature, i.e., the connection coefficients [5].

We find that for many systems biology models, most of the parameter nonlinearity is dominated by a few parameter combinations. This result is analogous to "sloppy" models in which the Jacobian matrix has an exponential distribution of singular values. The nonlinearity quantifies how principal parameter combinations "rotate" into one another and lead to compensatory effects. Using this, we identify groups of parameters that act as effective control knobs for model behavior. We compare these groups of parameters with the functional relationships identified by the manifold boundary approximation method [6] and discuss how these insights can guide the interpretation of the model and improve numerical methods for data fitting and posterior sampling.

[1]Department of Physics & Astronomy, Brigham Young University, Provo Utah. E-mail: mktranstrum@byu.edu

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Microfluidic Study of a Stochastic Genetic Circuit Carefully Modulated by Environmental Inputs

Minjun Son[1], Delaram Ghoreishi[1], Sang-Joon Ahn[2], Robert A. Burne[2], and Stephen J. Hagen[1]

***Abstract* — Genetic competence in the bacterial pathogen *Streptococcus mutans* is regulated by many environmental inputs, including two quorum-sensing molecules and pH. We are using microfluidics to control the environmental inputs and examine the output of the competence regulatory circuit at the single cell level. Our studies reveal that the composition and pH of the growth medium are key inputs; different combinations of these inputs can tune an internal feedback circuit to create a stochastic response that ranges from no response to bimodal/unimodal responses. Our findings suggest how cells incorporate environmental inputs to determine their behavior.**

***Keywords* — Stochastic genetic circuit, quorum sensing, bimodal, unimodal, positive feedback, microfluidics, *Streptococcus mutans*, competence, environmental inputs, pH.**

I. Introduction

GENETIC competence is a transient physiological state during which a bacterial cell can internalize exogenous DNA from its environment. In the oral pathogen *Streptococcus mutans*, competence is important not only because it contributes to genetic diversity, but also because its regulation is closely intertwined with virulence-related behaviors [1, 2]. Early stages of *S. mutans* competence are governed in part through two secreted quorum-sensing signal molecules: CSP and XIP [1-3]. Interestingly, the activity of these signal molecules depends on environmental cues, including pH and medium composition, through mechanisms that are not well understood [4-6].

Using planktonic or biofilm cultures it is difficult to unravel the separate regulatory effects of signal molecules and environmental conditions, because *S. mutans* continuously modifies its environment during growth, through acid production, generation of other signal molecules, etc. To precisely define environment and inputs to the competence system, we employed a two-layer microfluidic mixer device. Activation of competence genes was then studied at the single cell level using fluorescent protein reporters. We found that the composition and pH of the growth medium modulate activity of the ComR/ComS positive feedback system that controls *S. mutans* competence. This leads to diverse patterns of expression of key competence genes.

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[1]Department of Physics, University of Florida, Gainesville, FL 32611, USA. E-mail: sjhagen@ufl.edu

[2]Deparment of Oral Biology, University of Florida, Gainesville, FL 32611, USA.

II. Results

A. Bimodal response to CSP vs. Unimodal response to XIP

The two quorum signals CSP and XIP stimulate the competence genes in a very different manner: bimodally vs. unimodally, respectively. The data suggest that the bimodal activation by CSP is due to auto-activation of the ComR/ComS feedback loop.

B. Composition of medium determines which quorum sensing molecules are active

CSP stimulates competence in peptide-rich medium, but not in peptide-free medium. On the other hand, XIP stimulates competence in peptide-free medium, but not in peptide-rich medium. This effect of medium can be understood in terms of control of feedback strength.

C. pH determines onset/offset of the competence phase

Regardless of the growth stage of the cells, both CSP and XIP elicit a response only if the pH of medium is nearly neutral. Experiments and simulation suggest that extreme pH acts to hinder the positive feedback circuit.

III. Conclusion

Although *S. mutans* competence regulation is sensitive to many environmental inputs, its complex behavior can be understood in terms of simple tuning of parameters in the ComR/ComS positive feedback circuit. Our findings propose how *S. mutans* manages competence regulation and other virulence behaviors as it copes with environmental stresses in the human mouth.

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Mechanistic analysis of reaction network models

José-Juan Tapia[1], John A.P. Sekar[2] and James R. Faeder[3]

***Abstract*— Chemical reaction networks have been used for a long time to model biochemical systems. Rule-based modeling is a newer graph-based approach that allows explicit consideration of molecular structures and reaction configurations. We have previously developed a tool called Atomizer that infers molecular structures and interactions from reaction network models to generate rule-based representations. Here, we have atomized reaction network models in the BioModels database and performed a statistical analysis of their composition in terms of the five basic graph operations that can occur in reactions.**

I. Background

RULE-BASED MODELING is an alternative approach to building kinetic models, that explicitly encodes information about structures and reaction mechanisms [1]. In the rule-based framework, molecules and complexes are explicitly represented as graphs. Reactions, modeled as *reaction rules*, are explicit graph rewritings using five basic operations: add and delete molecules, add and remove bonds and change internal states [2]. However, in the reaction network framework, each reaction naïvely models the creation and deletion of structureless chemical species. Any structure present in the species has to be manually encoded, either using annotations or using an *ad hoc* labeling convention (e.g. `A_B` to represent a complex of `A` and `B`). The Atomizer algorithm [4] attempts to learn these conventions and extract this hidden structure from each RNM. Using clues from reaction stoichiometry and common naming conventions, the algorithm relearns the explicit mechanistic interactions that were implicitly encoded in the reactions.

BioModels is a repository of user-submitted reaction network models (RNMs) focusing on cellular biochemistry and other biological processes [3]. It includes 540 models curated by the BioModels group and 650 uncurated models. Here, we use Atomizer to analyze the composition of RNMs in both the curated and uncurated sets of the BioModels database and compare this to a control set of rule-based models.

II. Results

The balance of composition between bond and state changes versus creation and deletion is indicative of the degree to which mechanistic information can be extracted from a particular reaction network. The fractional occurrence of a particular graph operation within a model can be compared across a whole suite of models. Figure 1 shows a histogram of the fractional occurrence for each graph operation in three sets of models: atomized curated BioModels, atomized non-curated BioModels, and a control set of BioNetGen rule-based models. The control set is predominantly composed of bond and state changes, reflecting the explicit encoding involved in the rule-based framework. The non-curated set is predominantly composed of molecule creation and deletion. These operations do not provide information about the underlying structure of the species involved and their extensive use in a model limits the amount of structural information that can be recovered. The curated set lies between the two, showing that manual curation can, to some extent, be used to resolve molecular interactions.

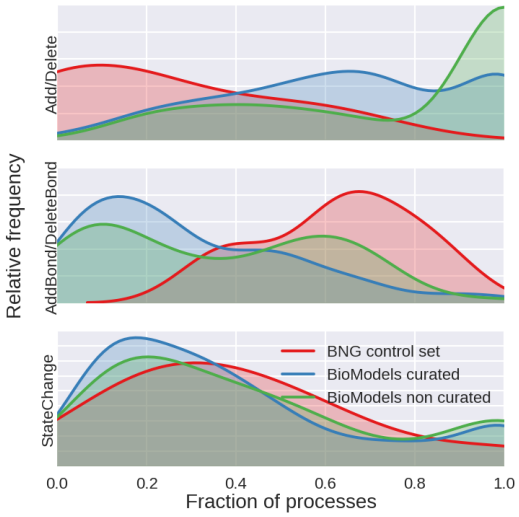

Fig. 1. Distribution in the fractional occurrence of three basic sets of graph operations over the curated (blue) and non-curated (green) sections of the BioModels database and a control set of rule-based models (red).

III. Conclusion

The results of our analysis suggest that the network approach does not limit the use of explicit mechanisms, but makes it harder to recapitulate or infer them after construction. This is because the network abstraction is not optimal for structured species, requiring manual encoding for each species and reaction. On the other hand, the rule-based framework was designed to be a more appropriate abstraction for hierarchically structured biochemical entities such as proteins and signaling complexes.

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[1,2,3]Department of Computational and Systems Biology, University of Pittsburgh E-mail: {jjtapia[1],jas237[2],faeder[3]}@pitt.edu

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Improved Model Fitting for Complex Self-Assembly Reaction Networks

Lu Xie[1,3], Gregory R. Smith[2], Marcus Thomas[2], and Russell Schwartz[2, 3]

Abstract — **Self-assembly is a crucial component of nearly every cellular function, yet quantitative modeling of self-assembly remains primitive due to the experimental challenges of monitoring assembly reactions and the computational challenges of accurately and efficiently simulating them. Coarse-grained rule-based models have made accurate and efficient sampling of reaction trajectories possible but learning parameters for real systems remains challenging given the limits of experimental assays of assembly progress. We describe advances in model inference, specifically exploring the potential of improved fitting algorithms and improved data sources to more accurately and efficiently learn correct assembly models from bulk experimental measures of assembly progress. Exploration of simulated virus capsid assembly data suggests that better algorithms and better data sources can each independently lead to more accurate and precise model fits, although the advantage of better algorithms diminishes with richer data. Application of the methods to real viral data provides novel insights into pathway selection in unprecedented detail as well as a platform for exploring the effects of changes to better mimic the cellular assembly environment versus the *in vitro* conditions under which kinetic data is gathered.**

Keywords **— Self-Assembly, Virus Capsid, Stochastic Simulation, Rule-based Models Data Fitting, Optimization.**

I. Motivation

VIRUS capsid assembly has long been a model system for general macromolecular assembly due to its high complexity and relative experimental tractability. Nonetheless, detailed quantitative understanding of subunit-level assembly pathways has remained elusive. There are no experimental methods to observe fine-scale assembly dynamics directly, only to monitor bulk assembly *in vitro*. Simulation methods provide a window into the unobservable fine details of assembly, but are hindered by the huge potential pathway space of even small complexes. We have previously developed methods to simulate realistic scales and parameters ranges of capsid assembly through the use of coarse-grained, rule-based models [1] and their combination with fast stochastic sampling algorithms [2]. We later addressed the lack of direct data through simulation-based model fitting to static light scattering (SLS) measurements of bulk assembly *in vitro* [3,4]. This approach made it possible for the first time to model subunit-level pathway space for real capsids and to explore how pathway usage might vary in more realistic models of the intracellular environment [5]. Nonetheless, limited data sources and uncertain fit quality call into question the reliability of the model inferences.

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[1]Joint Carnegie Mellon – University of Pittsburgh Ph.D Program in Computational Biology and [2]Department of Biological Sciences and [3]Computational Biology Department, Carnegie Mellon University.

II. Modeling and Methods

We develop local rule models for viral capsids and simulate them via stochastic sampling as in our prior work. We use these to explore three experimental assays in current use: SLS, time-resolved non-covalent mass spectrometry (NCMS), and dynamic light scattering (DLS). We use simulated capsid models with artificially chosen rate parameters to simulate idealized data from each source. We then fit model parameters to simulated data using either our prior gradient-based algorithms or variants on derivative-free optimization (DFO) to minimize root mean square deviation between the model and data. We evaluate quality of fit by accuracy of inferred parameters and reaction trajectories. We also apply the methods to real SLS data to assess fit quality and explore pathway space in real viruses.

III. Discussion

Our results indicate that learning accurate models of complex assembly reaction networks is feasible via simulation-based data fitting. Richer data for monitoring bulk assembly can yield substantial improvements in fit quality over past work, although the best algorithms can learn generally accurate models even from older SLS data. Our results suggest that further work on experimental methods and algorithms is needed. Nonetheless, they show that these approaches already have enormous and largely unappreciated potential for exploring a critical but still poorly handled aspect of cellular reaction networks.

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Simulating Yeast Polarization in the Cloud

Michael Trogdon[1], Brian Drawert[2], Michael Lawson[3], Salman Toor[4], Ben Bales[1], Debjani Banerjee[2], Andreas Hellander[4], Tau-Mu Yi[5] and Linda Petzold[1,2]

***Short Abstract* — We have developed a discrete spatial stochastic model of cellular polarization during mating of *Saccharomyces cerevisiae*. Specifically we investigate the ability of yeast cells to sense a spatial gradient of mating pheromone and respond by forming a projection in the direction of the mating partner. Our results demonstrate that higher levels of stochastic noise result in increased robustness, giving support to a cellular model where noise and spatial heterogeneity combine to achieve robust biological function. This also highlights the importance of spatial stochastic modeling to reproduce experimental observations.**

***Keywords* — Spatial stochastic modeling, yeast polarization, cloud-computing, yeast mating.**

I. Background

The importance of randomness (or stochasticity) in biological systems is well documented [1-2]. Within a cell, proteins critical to cell signaling pathways often exist in low copy numbers. Under these conditions, methods such as the stochastic simulation algorithm (SSA) [3] are a more accurate mathematical representation of the system compared to deterministic models, which rely on ordinary differential equations. Another important consideration is the spatial nature of many biological systems, which is not captured in a "well-mixed" description. Here we look specifically at the spatial polarization of proteins during the mating of yeast.

The yeast *Saccharomyces cervisiae* exists in both a haploid and a diploid form. The haploid cells (of type **a** or **α**) can sense a spatial gradient of mating pheromone and respond by forming a projection in the direction of the mating partner. In [4] a combination of computational modeling and biological experiments showed that, in comparison to a deterministic model, a discrete spatial stochastic model can more robustly reproduce two key features of polarization observed in wild-type cells: a highly polarized phenotype via spatial stochastic amplification and the ability of the polarisome to track a moving pheromone input. This work demonstrated that stochasticity plays a critical role in yeast polarization and provided the foundation for more detailed mechanistic models.

II. Current Modeling Efforts

Although the model presented in [4] did provide novel insight into key features of the polarization system, there is still much to be understood. We are currently working to extend this model by adding additional pathways known to be involved in the polarization cascade. In particular, our current mechanistic model integrates three components of the polarization process: the heterotrimeric G-protein cycle activated by pheromone bound receptors, the focusing of a Cdc42 polarization cap, and the formation of the tight localization of proteins on the membrane known as the polarisome. Open questions to be addressed by this model, in combination with biological experiments, include the role of stochastically diffusing extracellular ligand, the effect of stochasticity on the yeast cell's ability to form a localized Cdc42 cap, and what feedback mechanisms exist between the components of the polarization process.

III. Conclusion

We have developed a discrete spatial stochastic model of cellular polarization during yeast mating. Past work has demonstrated the necessity of spatial stochastic modeling to reproduce experimentally observed features of the polarization process. Our current model includes three components of the polarization process: the G-protein cycle, the focusing of Cdc42, and the formation of the polarisome. Due to the complexity of modeling these systems and the need for massive computational resources to simulate them, we have developed StochSS, an integrated development environment (IDE) for discrete stochastic simulations of biochemical networks with cloud-computing capabilities that easily allows for large-scale computational experiments. StochSS provides access to state-of-the-art algorithms, and supports a wide range of hardware from desktop workstations to high-performance clusters in the cloud.

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[1]Department of Mechanical Engineering, University of California – Santa Barbara.

[2]Department of Computer Science, University of California – Santa Barbara.

[3]Department of Cell, Molecular, Computational, and Systems Biology, Uppsala University.

[4]Department of Information Technology, Uppsala University.

[5]Department of Molecular, Cellular, and Developmental Biology, University of California – Santa Barbara.

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A PROJECTION METHOD FOR SOLVING THE CHEMICAL MASTER EQUATION

ROGER B. SIDJE AND HUY D. VO

Abstract. The mathematical framework of the chemical master equation (CME) uses a Markov chain to model the biochemical reactions that are taking place within a biological cell. Computing the probability distribution of this Markov chain allows us to track the composition of molecules inside the cell over time, with important practical applications in a number of areas such as molecular biology or medicine. However the CME is typically difficult to solve, since the state space involved can be very large or even countably infinite. This study investigates a numerical method based on the stochastic simulation algorithm (SSA) to address this challenge. Supported by NSF Grant 1320849

Extended Abstract

Models of cellular processes promise great benefits in important fields such as molecular biology or medicine. Within a cell , some key regulatory molecules exist only in small numbers, in which case it becomes appropriate to formulate the models in a discrete and stochastic setting. The mathematical framework that underpins this is a continuous-time, discrete-state, Markov process, and computing its probability distribution amounts to solving the chemical master equation (CME).

While promising many insights, the CME is difficult to solve, especially for large models. Consequently, researchers often resort to simulating trajectories, using most notably Gillespie's stochastic simulation algorithm (SSA) [2] or its improved variants, e.g., [1]. A direct approach to the CME was the catalyst of the finite state projection (FSP) algorithm of Munsky and Khammash [3] that truncated the state space to a more tractable size. The efficiency of the method depends on how well one selects the truncated state space.

We prototype a SSA-driven reduction that builds on the principle that the CME aims at computing a probability vector $\boldsymbol{p} = (p_1, \ldots, p_n)^T \in [0,1]^n$, with components that sum to one, $\sum_{i=1}^{n} p_i = 1$. For very large problems, $n \gg 1$, the probability sum condition implies that some of the components must necessarily be zero or negligible, Dropping those negligible components allows us to reduce the size dramatically. We tested the SSA-driven projection which is a central element, and early results are quite promising.

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The University of Alabama, Department of Mathematics, P.O. Box 870350, Tuscaloosa, AL 35487, USA. roger.b.sidje@ua.edu, hvo@crimson.ua.edu. Supported in part by NSF grant DMS-1320849.

Dynamic environments reveal specialized roles in the HOG network

A. Granados[1, 2], M. Crane[1], L. Montaño[1], M. Voliotis[3], R. Tanaka[2] and Peter Swain[1].

***Short Abstract* — The ability of biological systems to grow and survive depends upon the detection of specific environmental signals and the organization of adequate physiological responses. At the cellular level, information from the environment is processed using molecular networks that orchestrate an appropriate response, which often includes changes in gene expression. The high osmolarity glycerol (HOG) pathway in yeast serves as a prototype signaling system for eukaryotes. Its main function is to sense changes in osmotic pressure and coordinate a wide range of molecular processes that lead to the adaptation of cell volume. The signaling properties of the HOG pathway have been widely studied both experimentally and theoretically. An integrative framework, however, that connects the dynamic properties of the pathway with its Y-shaped structure and its ability to process information is still missing. Using novel microfluidic experiments and mathematical modeling, we are determining how the two branches of the HOG network combine to allow both a response at short times to enable survival and at longer times to enable adaptation in dynamic environments.**

***Keywords* — cell signaling, HOG pathway, microfluidics, mathematical modeling.**

I. Introduction

The High Osmolarity Glycerol (HOG) pathway in yeast (*Saccharomyces cerevisiae*) serves as a prototype system for MAPK signaling in eukaryotes. Its main function is to respond to hyper-osmotic shocks by driving a wide range of molecular processes that lead to the adaptation of cellular volume. The pathway consists of two input branches (SLN1 and SHO1) that activate Pbs2 (MAPKK). Subsequent activation of Hog1 (MAPK) by Pbs2 enables regulation of glycerol levels in various ways; for example, activating regulatory enzymes in glycolysis but also up-regulating the expression of enzymes required for glycerol production (Gpd1) and glycerol import (Stl1). Although the signaling properties of the HOG pathway have been widely studied both experimentally and theoretically [1-4] an integrative framework that connects the dynamic properties of the HOG pathway to its ability to process information is still missing.

II. Results

Using microfluidic experiments and mathematical modeling, we are determining how the two branches of the HOG network combine to allow cellular response both at short times—to enable survival to severe shocks—but also at longer times—to enable better adaptation to dynamic environments.

Using a novel design of the ALCATRAS microfluidic device [5] we measure single-cell signaling responses and fitness dynamics under various dynamic environments. We find that ste11 mutant cells (only SLN1 branch active) do better under sudden, step-like hyper-osmotic shocks, while, skk1 mutant cells (only SHO1 branch active) adapt better in slowly varying environments. Therefore, our results indicate that the SLN1 branch is essential when response speed is important, while the SHO1 branch allows precise cellular adaptation to gradually fluctuating environments.

[1]Center for Sythetic and Systems Biology, University of Edinburgh, UK
[2]Department of Bioegineering, Imperial College London, UK
[3]School of Mathematics, University of Bristol, UK

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Mathematical modeling of insulin secretion from a network of coupled islet β-cells via glucose-induced changes in membrane potential, intracellular calcium, and insulin granule dynamics

I. Johanna Stamper[1,2], Xujing Wang[3]
[1]Department of Physics, University of Alabama at Birmingham, Birmingham, Alabama, 35294
[2]The Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, Alabama, 35294
[3]Bioinformatics and Systems Biology Core, Systems Biology Center, National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892, USA

Tel: 301-451-2862

In this study we present a new mathematical model of the biphasic insulin secretion and β-cell mass. It is built on two of our previous models: an inter-cellular Hodgkin-Huxley (H-H) type model of a hexagonally closed packed (HCP) network of electrically connected pancreatic islet β-cells (Plos One, 2007), and an intra-cellular model of glucose-induced insulin secretion based on insulin granule dynamics (J. Theo. Bio 2013). In order to couple these two models we assume that the rate at which the primed and release-ready insulin granules fuse at the cell membrane increases with the intracellular calcium concentration, one of the variables in the HH model. Moreover, by assuming that the fraction of free K_{ATP} -channels decreases with increasing glucose concentration, we are able to take into account the effect of glucose dose on membrane potential and, indirectly via the effect on the potential, on intracellular calcium. Numerical analysis of our model shows that a single step increase in glucose concentration typically yields the characteristic biphasic insulin release often seen experimentally. Our model's biphasic response can be either oscillatory or non-oscillatory in nature depending on the glucose-concentration; at high concentrations the oscillations tend to vanish due to a constantly elevated membrane potential of the β-cells. Furthermore, with increasing glucose dose, the area under the insulin curve increases, as does the plateau fraction (the time that the β-cells spend in their active firing phase). To our knowledge our model is the first to explicitly connect insulin secretion from intracellular insulin granules to glucose-stimulated intercellular electrical activity within a network of coupled β-cells.

Improved Enzyme Kinetics Model for Simulating Complex Biochemical Networks

Martin KL. Wong[1,2,4*], James R. Krycer[2,4], James G. Burchfield[2,4], David E James[2,3,4] and Zdenka Kuncic[1,2]

***Short Abstract* — Quasi steady-state enzyme kinetic models are commonly used in systems modelling. Current models require the reactant stationary assumption (also known as the low enzyme concentration assumption), which may not always be valid *in vivo*. We have developed the differential quasi-steady state approximation (dQSSA) kinetic model, which eliminates the reactant stationary assumption while only requiring two kinetic parameters to model irreversible enzyme action. We validated the dQSSA *in silico* and found that it is consistent with mass action kinetics and correctly replicated *in vitro* kinetics of the enzyme LDH. This presents an accurate method of modelling complex biological systems.**

***Keywords* — Systems Biology, Enzyme Kinetics, ODE Modelling, Biochemical Networks, Quasi-steady state assumption.**

I. Enzyme Kinetic Models

ENZYME kinetic models are integral parts of chemical kinetic models as many biochemical networks are enzyme mediated [1]. However, modelling of large networks leads to a high dimensionality model of many unknown kinetic parameters, which increases the amount of data required to tune unambiguously [2]. As such, the Michaelis Menten model is commonly as it simplifies the mass action model by reducing the required parameter number by one per reaction [3]. However, this model requires two assumptions: the quasi-steady state assumption and the reactant stationary assumption. These may not always be satisfied in complex biochemical networks under *in vivo* conditions.

The total quasi steady state approximation (tQSSA) model proposed by Tzafriri overcomes the reactant stationary assumption by using total concentrations as the state variable [4]. Which this approach is viable for the simplest biochemical networks, it becomes mathematically intractable to apply when an enzyme targets more than two substrates [5]. To address these issues, we set out develop an improved model that improves accuracy by overcoming the reactant stationary assumption [5].

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[1]School of Physics, Unviersity of Sydney, Sydney, NSW, Australia.
[2]Charles Perkins Centre, University of Sydney, NSW, Australia.
[3]School of Molecular Bioscience, University of Sydney, NSW Australia.
[4]Diabetes and Metabolism Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia.
* Corresponding to: martin.wong@sydney.edu.au

II. Results

The new dQSSA enzyme kinetic model was derived by explicitly modelling the complex concentration as functions of its constituent substrate and enzymes. The evolution of the complex concentration was then determined using a linearized form of the differential equation to avoid the use of simultaneous equations.

We found the dQSSA to replicate the mass action model when on a hypothetical complex network that includes negative feedbacks and substrates competitively targeted by multiple enzymes. Furthermore, we found the dQSSA and Michaelis Menten model to differ when predicting the kinetics of pyruvate to lactate reaction by LDH when NAD^+ is present. It was found that the dQSSA made the correct prediction, thus showing that the dQSSA is a model that is accurate enough to be able to replicate kinetic behaviors in *in vitro* scenarios.

III. Conclusion

The dQSSA is an enzyme kinetic model that can model complex biochemical networks with improved accuracy of *in vitro* scenarios by overcoming the reactant stationary assumption.

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Controlling the Heterogeneous Quiescent State by an Rb-E2F Bistable Switch

Sarah Jungeun Kwon[1], Xia Wang[1], and Guang Yao[1,2#]

Short Abstract — **Cellular quiescence is a reversible non-proliferative state that is critical to tissue homeostasis. Deregulation of the quiescent state can lead to a wide range of diseases. Control mechanisms of cellular quiescence are until now poorly understood. By combining modeling and single-cell measurements, we show that quiescent depth is determined by the activation threshold of an Rb-E2F bistable switch. We identified factors within the Rb-E2F pathway that modulate quiescent depth with different efficacy. We also show that Notch pathway and circadian rhythm pathway crosstalk with the Rb-E2F bistable switch and modulate the heterogeneous quiescent depth.**

Keywords — **Quiescence, proliferation, heterogeneity, cellular state, Rb, E2F, bistable switch, activation threshold, Notch pathway, circadian rhythm.**

I. INTRODUCTION

QUIESCENCE is a non-proliferative state associated with many cell types (e.g., fibroblasts, lymphocytes, and stem cells) in the body. Distinct from other non-proliferative states that are irreversibly arrested such as senescence and terminal differentiation, the quiescent state is reversible. Reactivation of quiescent cells to enter the cell cycle under appropriate signals is fundamental to tissue repair and regeneration. Quiescence is often described as a "G0 phase" outside of the active cell cycle, but it is in fact not a single uniform state. Studies in the '70s and '80s have shown that when lymphocytes and fibroblasts were kept quiescent for a prolonged duration, they moved progressively "deeper" into quiescence and underwent a longer pre-replicative phase when reentering the cell cycle [1, 2].

Cells at an abnormally deeper or shallower quiescent state become hypo- or hyper-proliferative, respectively, which can lead to a wide range of diseases. It is therefore important to understand what controls the heterogeneous quiescent state and depth. Recently, Coller et al. compared the transcriptional profiles of human fibroblast cells that remained quiescent for different durations; they found that cells remaining quiescent for longer periods (at deeper quiescence) exhibited larger expression changes of a transcriptional "quiescence program" than cells remaining quiescent for shorter periods (at shallower quiescence) [3]. The transcriptional quiescence program suggests a likely regulatory mechanism of quiescent depth. However, it remains to be determined what activities in the transcriptional program were causal, instead of correlative.

[1]Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721, USA.
[2]Arizona Cancer Center, University of Arizona, Tucson, AZ 85719.
[#]Correspondence to: guangyao@arizona.edu

II. SUMMARY OF RESULTS AND CONCLUSION

Here we first show that as fibroblast cells go deeper into quiescent state (with prolonged serum starvation), they require stronger serum stimulation to reenter the cell cycle. On the other hand, these deep quiescent cells can still be reactivated to proliferate with sufficient serum stimulation, demonstrating that deep quiescence is distinct from senescence or cell death.

We then show that the depth of cellular quiescence can be defined by the activation threshold of an Rb-E2F bistable switch. Previously, we showed that the Rb-E2F pathway functions as a bistable switch, converting graded and transient growth signals into a binary (ON or OFF) and long-lasting E2F activity, which controls the quiescence-to-proliferation transition [4]. Here by combining modeling and single-cell measurements, we show that the degree of difficulty (i.e., threshold) to activate the Rb-E2F bistable switch accounts for the degree of difficulty to exist quiescence (i.e., quiescent depth). We identified different cellular factors within the Rb-E2F pathway with different efficacy to modulate the Rb-E2F activation threshold using computer simulations. Such model predications were further validated in experiments. We found that deep quiescent cells feature a higher Rb-E2F activation threshold and a delayed commitment to quiescence exit and cell cycle entry.

We further show that the Rb-E2F activation threshold can be modulated by the Notch pathway and circadian rhythm pathway. Such pathways crosstalk with the Rb-E2F pathway, affect the bistable region of the Rb-E2F switch, and modulate the heterogeneity of quiescence exit in response to growth signals.

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Novel Single-Molecule Resolution Method for Spatio-Temporal Simulations of Protein Binding and Recruitment on the Membrane

Osman N. Yogurtcu, Margaret E. Johnson. Department of Biophysics, Johns Hopkins University, Baltimore, MD, USA.

In the early stages of clathrin-mediated endocytosis (CME), a variety of distinct proteins can bind to the membrane and engage in further interactions with proteins on the membrane and in solution. Understanding the dynamics of this process requires correctly accounting for the behavior of protein interactions while restricted to the 2D membrane surface, as it is fundamentally distinct from binding in solution due to changes in the dynamics of the proteins. Here we introduce the 2D Free-Propagator Reweighting (2D-FPR) method that accurately models the spatial and temporal dynamics of proteins as they are recruited to the membrane surface and as they interact with one another while bound to the membrane. In this method the position of each diffusing protein is tracked, and reactions between binding partners can occur upon collisions. Reaction probabilities are determined by the solution to the 2D Smoluchowski diffusion equation with reactive boundary conditions, allowing us to take large time steps. Molecule positions are propagated by free diffusion, but by using a trajectory reweighting approach we can recover the exact association rates for all reactive pairs. This approach is uniquely able to capture the changes in protein binding dynamics that can occur upon membrane binding because it accounts for both the diffusional motion of proteins and their binding reactions. These important details are absent from models that lack spatial resolution. We present our simulation results on modeling adaptor protein interaction dynamics, and discuss the effects of varying local protein concentration on both recruitment to the membrane and complex formation in the confined 2D geometry.

Integrative Proteomic and MicroRNA Approaches Reveal a Novel Post-Transcriptional Motif Regulating Human Definitive Endoderm Differentiation

Yue Teng[1], Hang Zhang[2], Xiao-Jun Tian[3], and Jianhua Xing[3,4]

***Short Abstract* — Systematic investigation of the developmental stage from human embryonic stem cells (hESCs) to definitive endoderm (DE) may shed light on the underlying mechanisms of human liver development. Here, using two-dimensional difference gel electrophoresis in conjunction with mass spectrometry, we identified two significantly inversely altered splicing-related gene products during the differentiation process, heteronuclear ribonucleoprotein A1 (hnRNP A1) and KH-type splicing regulatory protein (KHSRP). Combined bioinformatics and microRNA-Array data analysis suggests hnRNPA1 and KHSRP antagonizing each other through miR-375 and miR-135a respectively. Further mathematical modeling analysis demonstrated that this motif could generate switch-like responses to the differentiation signal, which can serve as a noise filter to control hESCs self-renewal and differentiation. Simulations predicted that elevated hnRNP A1 or miRNA-375 expression lead to rapid and efficient differentiation of hESCs into DE was further experimentally validated. Taken together, we revealed a potential mechanism which functions in post-transcriptional level to regulate stem cell differentiation.**

***Keywords* — Stem cell differentiation, miRNA, post-transcriptional regulation.**

I. INTRODUCTION

Human embryonic stem cells (hESCs) can self-renew and differentiate into any cell type found in the three embryonic germ layers, making them an attractive source of cells for use in regenerative medicine. The ability to efficiently generate definitive endoderm (DE), the precursor cell type to the liver, pancreas, lungs, thyroid, and intestines, is of great clinical importance. However, differentiation of hESC towards DE is a complicated process and the underlying mechanism remain elusive.

Directing embryonic stem cell differentiation towards definitive endoderm has been achieved by manipulating the Nodal and Wnt signaling pathways. Activin A, which activates the Nodal pathway, directs DE formation from mesendoderm precursors in mouse and human ESCs. In human ESCs, synergistic activation of Nodal and Wnt–β-catenin signaling promotes more efficient DE generation.

While most previous studies in this area have focused on identifying gene expression and signaling pathways, we chose to investigate the key proteins associated with the differentiation process. Here, we report results of a comparative proteomic analysis on DE derived from hESCs in feeder layer-free conditions, using two-dimensional difference gel electrophoresis (2D-DIGE) and mass spectrometry (MS), followed by bioinformatics and mathematical modeling analysis on the function of identified regulatory motif. The identified post-transcriptional level motif may shed light on the underlying mechanism of hESC fate decision.

II. CONCLUSION

Combined experimental and modelling tools, we identify a possible post-transcriptional motif regulating DE differentiation. The newly identified motif could generate switch behavior corresponding to the differentiation. Perturbing the system with hnRNPA1 and miR375 could promote differentiation. The post-transcriptional level switch may serve as a noise filter for ESC differentiation regulation which may prevent inadvertent differentiation by random exogenous signal fluctuations.

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[1]Stem Cell and Regenerative Medicine Laboratory, Beijing Institute of Transfusion Medicine, 27 Taiping Road, Beijing 100850, P.R. China
[2]Genetics, Bioinformatics, and Computational Biology Program, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24060, USA
[3]Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, 15260, USA
[4]Email: xing1@pitt.edu

The F-ATP synthase: what advantages might the rotary mechanism confer?

Zining Zhang[1,2] and Daniel M. Zuckerman[2]

***Short Abstract* — F-ATP synthase is an enzyme complex that synthesizes ATP using energy from the passage of protons down their electrochemical gradient across the membrane. People are familiar with its rotary mechanism and the complex structure. However, no literature has addressed what the advantages the rotary mechanism may have over the more common "transporter" mechanism. Two simplified model was made using BioNetGen to compare effectiveness of rotary and the alternating-access mechanisms. We found that (i) the peak operating conditions for both mechanisms, (ii) the superior performance of the rotary mechanism in two regimes, and (iii) the requirement for locally enhanced proton concentration near the FOF1 complex.**

***Keywords* — F-ATP synthase, rotary mechanism, quantitative modeling, elastic coupling, BioNetGen**

I. Introduction

F-ATP synthase, or F-ATPase, is found in bacterial plasma membranes, in mitochondrial inner membranes and in chloroplast thylakoid membranes. It uses a proton gradient to drive ATP synthesis by allowing the passive flux of protons across the membrane down their electrochemical gradient [1]. In 1997, a Japanese group first observed how this protein works. It uses a rotary mechanism to carry out its cellular function of manufacturing ATP. After that FOF1 is well known for its intricate rotary mechanism and the complex dual-ring structure [2-3]. F type is the only type that could both works as an ATP synthase and a proton pump that utilize the energy from ATP to maintain the proper pH difference across the membrane.

Many investigations have studied the rotation details, and advanced techniques allow people to observe how this machine works in a higher resolution [4-6]. But these experimental studies of necessity take the mechanism and structure for granted. However, the literature does not appear to have addressed to what significant extent the basic advantage of a rotary mechanism over the more common alternating-access mechanism coupled to ATP hydrolysis/synthesis. In principle, an ATP-coupled alternating-access transporter should be reversible under the same conditions that reverse the FOF1.

Here we use BioNetGen, a rule based modeling method to make two simple quantitative models to compare the effectiveness of rotary and alternating-access mechanisms for ATP synthesis [7]. Our approach allows the models to be identically matched in a thermodynamic sense, with only mechanistic differences.

II. Results and Conclusions

A 180 mV potential across the membrane was set in all the system. The parameters were referenced from published literatures [8-10]. Although the simplicity of the models makes the results highly speculative, some findings appear to be robust:

(i) The existence of peak operating conditions for both mechanisms.

(ii) The superior performance of the rotary mechanism in two regimes.

(iii) The requirement for locally enhanced proton concentrations near the FOF1 complex compared to bulk values implied by pH measurements.

These simple models are the first steps toward a longer-term goal of building a comprehensive modeling framework for both F and V-type ATPases with an arbitrary number of c subunits and with elastic coupling between FO and F1 sub-complexes.

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[1]School of Medicine, Tsinghua University. E-mail: ziz13@pitt.edu

[2]Department of Computational & System biology, University of Pittsburgh. E-mail: ddmmzz@pitt.edu

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Mechanistic dissection of Drosophila apoptotic switch

Riccardo Ziraldo[1] and Lan Ma[1]

***Short Abstract* — The molecular switch mechanism of apoptosis in *Drosophila* is studied for the first time by mathematical modeling. Enumeration of the elementary reactions in the model demonstrates that the molecular interactions among the signaling components are considerably different from their mammalian counterparts, despite the conserved apoptosis pathway. The model is calibrated by an experimental input-output relationship and the simulated trajectories exhibit all-or-none behavior. Bifurcation diagrams confirm that the model of *Drosophila* apoptotic switch possesses bistability. The bistable activation of the effector caspase to the apoptosome scaffolding protein is reversible, rather than irreversible as in mammals. Further analysis shows that the key to the systems property of reversibility lies in the double-negative feedback from the effector caspase to the initiator caspase.**

***Keywords* — Drosophila, apoptosis, molecular switch, mathematical model, bistability, double-negative regulation**

I. Introduction

APOPTOSIS is an evolutionarily-conserved process of autonomous cell death [1]. In contrast to the intense theoretical modeling work on mammalian apoptosis pathways, the apoptotic signaling mechanisms in the fruit fly, *Drosophila*, have not been investigated theoretically, to our best knowledge. Previous theoretical studies from the viewpoint of systems theory suggest that the mechanistic property of bistability can achieve the switch-like behavior of apoptosis. Consensus exists that the models of apoptosis networks are necessarily bistable, with one discrete stable steady state (inactive effector caspase) corresponding to cell survival, and the other (active effector caspase) to cell death [2, 3]. We ask, for the conserved function of apoptosis, whether the cellular regulatory system in *Drosophila* behaves the same as that in mammals or not.

II. Results

A. Modeling of the Drosophila apoptosis pathway

We first identify the essential signaling components from literature, which all have mammalian homologs. However, although the schematic regulations along the *Drosophila* pathway resemble their mammalian counterparts, the underlying molecular reactions have substantial degree of distinction. The most-upstream signaling proteins are upregulated by extrinsic stimuli, making the model an *intrinsic-extrinsic* hybrid type [4]. Moreover, the regulations of the initiator caspase DRONC is complicated, due to the combinatorial cleavage of two functional protein domains. A notable distinction in the network organization is the double-negative feedback from the effector caspase to the initiator caspase, while mammals have a direct positive feedback [5]. The calibrated model presents all-or-none time trajectories.

B. Reversible bistability and feedback topologies

Bifurcation diagrams of the steady state of the effector caspase versus varying single parameters or pairs of parameters show that the model of *Drosophila* apoptosis pathway is bistable in an extended region surrounding the nominal parameter set.

The bifurcation diagram of the response of effector caspase versus DARK input, the homolog of mammalian APAF1, shows that this essential bistable response is reversible. Further analysis of the *Drosophila* models containing different combinations of topologies, either with only the double-negative feedback, or with only the direct positive feedback, or with both feedback loops, demonstrate that the distinct double-negative feedback is the mechanism responsible for the reversible bistability in *Drosophila*.

III. Conclusion

The model of the *Drosophila* apoptosis pathway presents robust bistability. However, in contrast to the irreversible bistability of the caspase response to the APAF1 induction in mammals, the caspase activation elicited by DARK is reversibly bistable, which arises from the absence of the direct feedback activation of the initiator caspase by the effector caspase. Due to the essential role played by irreversibility in the robust apoptosis decision in mammals, our finding highlights an important mechanistic distinction between the apoptotic switch in flies and that in mammals. The results indicate plausible systems-level evolution of a conserved cellular function.

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[1]Department of Bioengineering, Erik Jonsson School of Engineering and Computer Science, The University of Texas at Dallas, 800 W Campbell Rd, ECSS 3.908, Richardson, TX 75080, E-mail: lan.ma@utdallas.edu