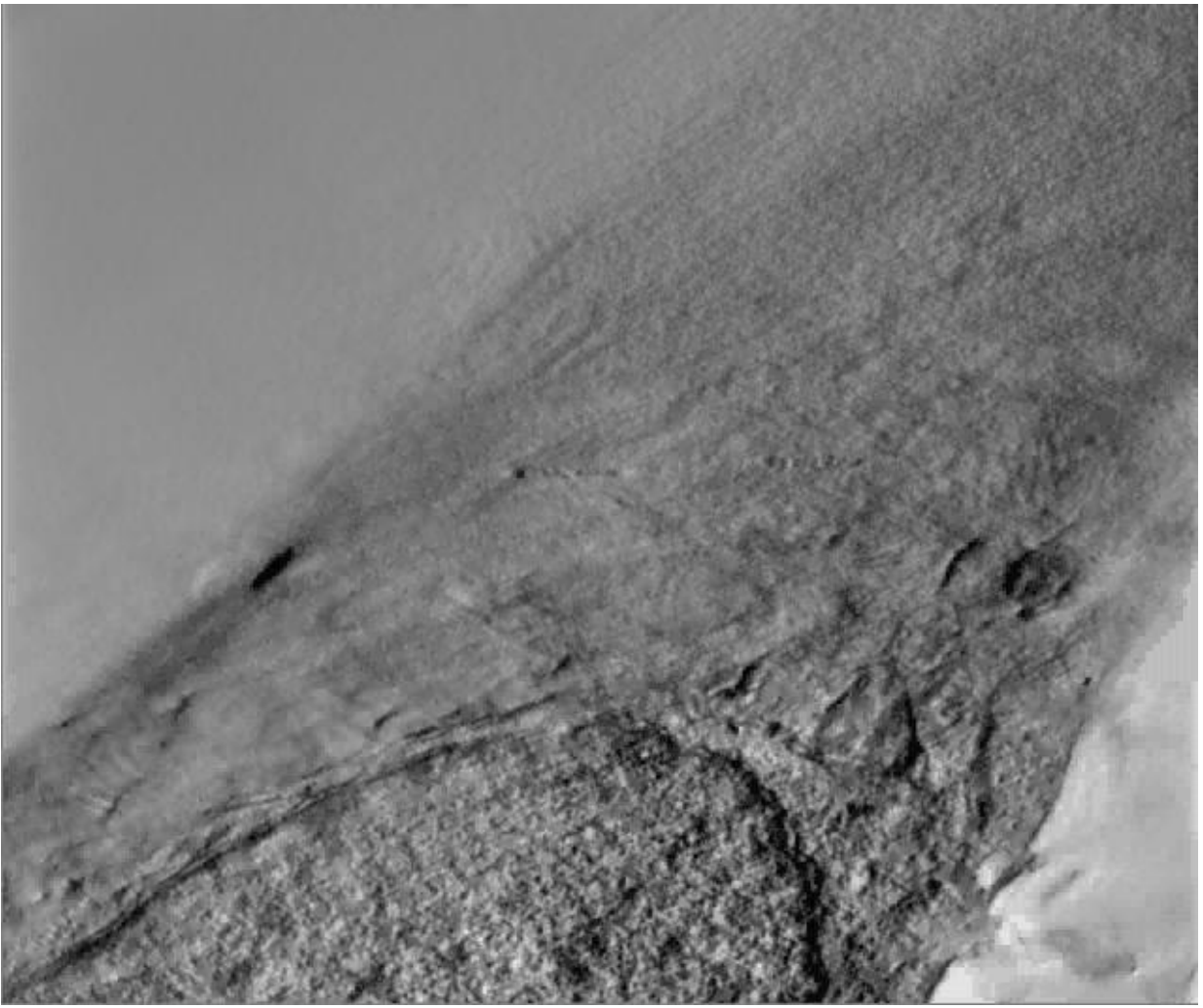
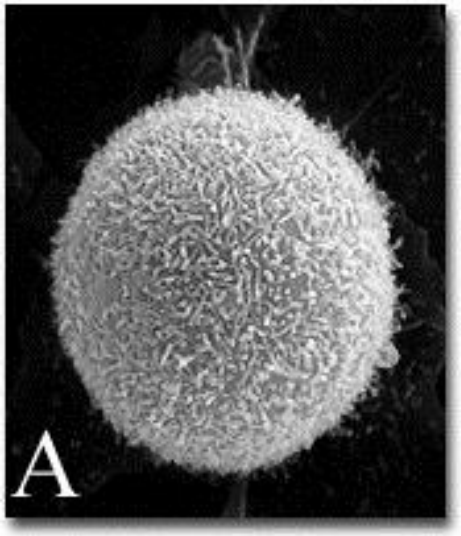
Bridget Wilson
Director, NM Spatiotemporal Modeling Center

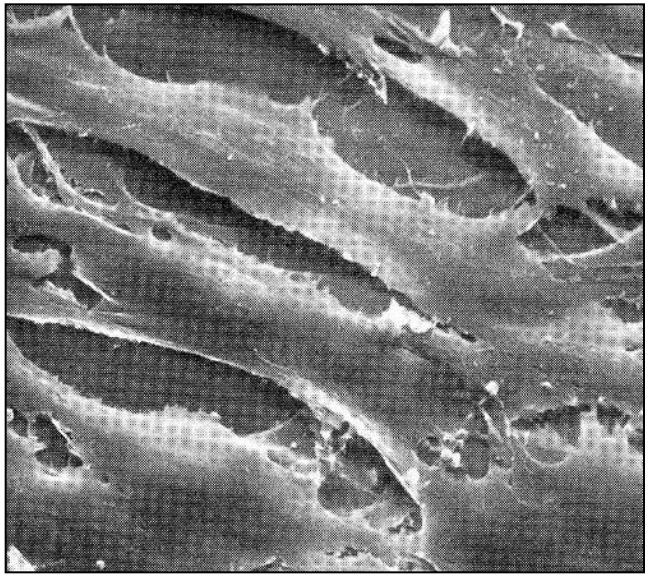
- **Fundamentals of Membranes**
- **Fundamentals of Signal Transduction**

Integrating Experimentation & Mathematical Modeling

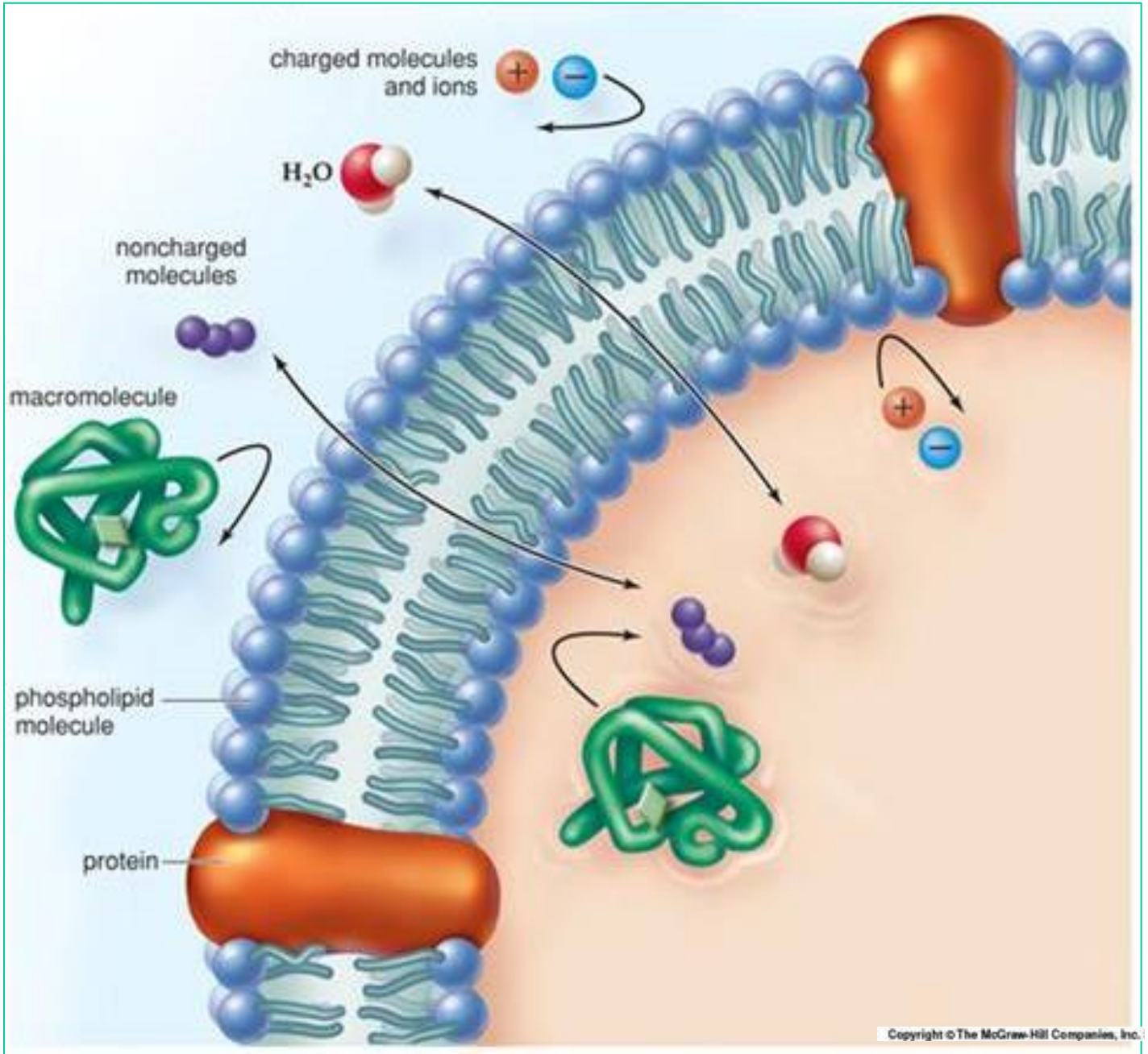
View of Cells by EM



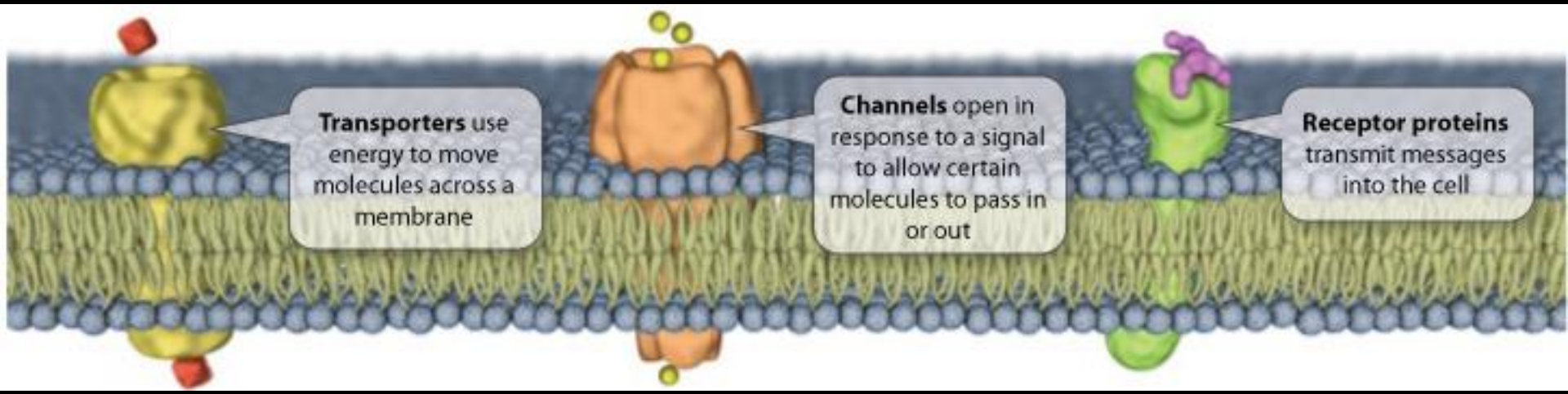




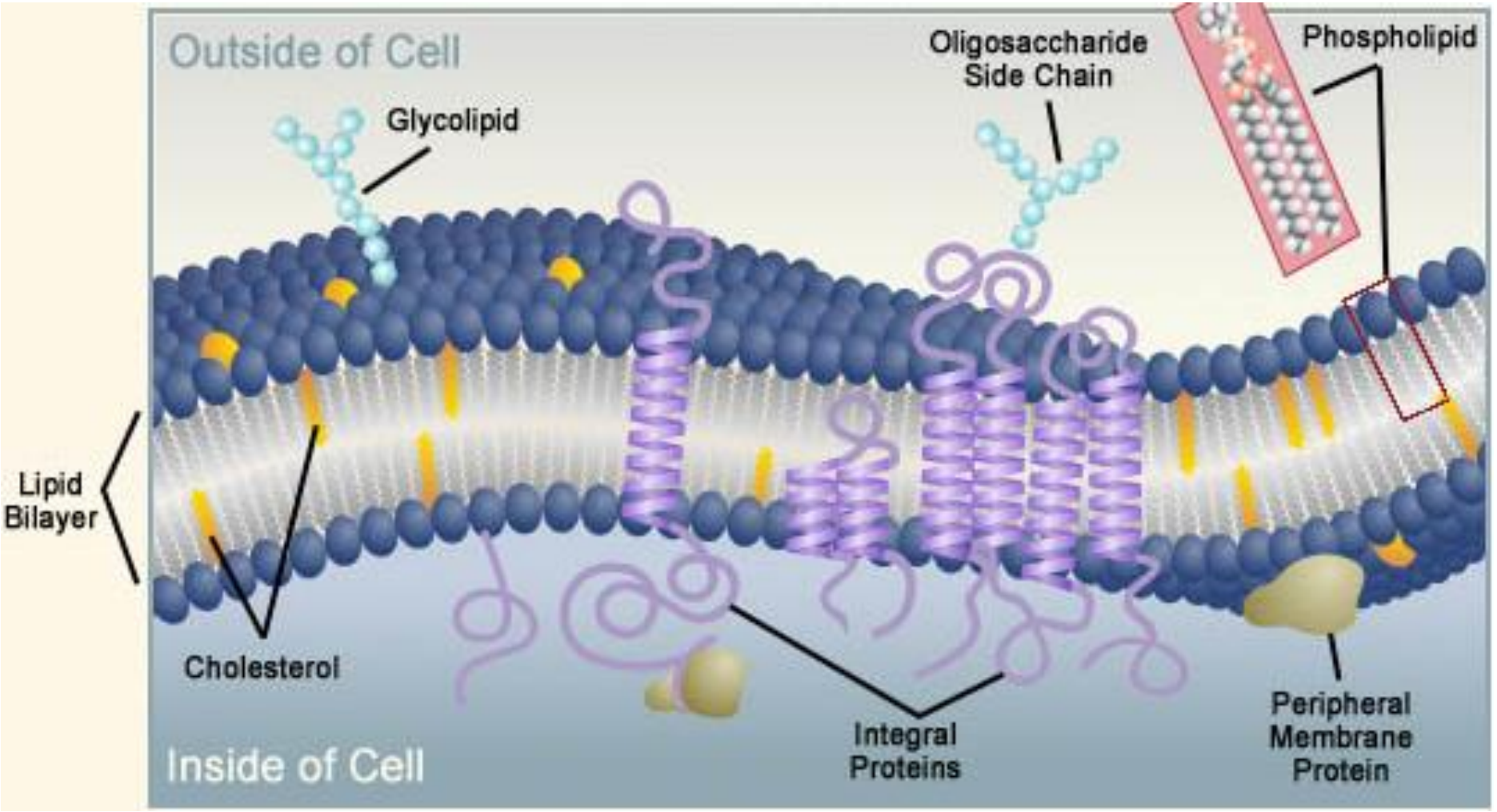
The Plasma Membrane is a Semi-Permeable Barrier



Because the cell membrane is such a highly selective barrier, integral membrane proteins are used to transmit signals and transport nutrients, ions, etc.

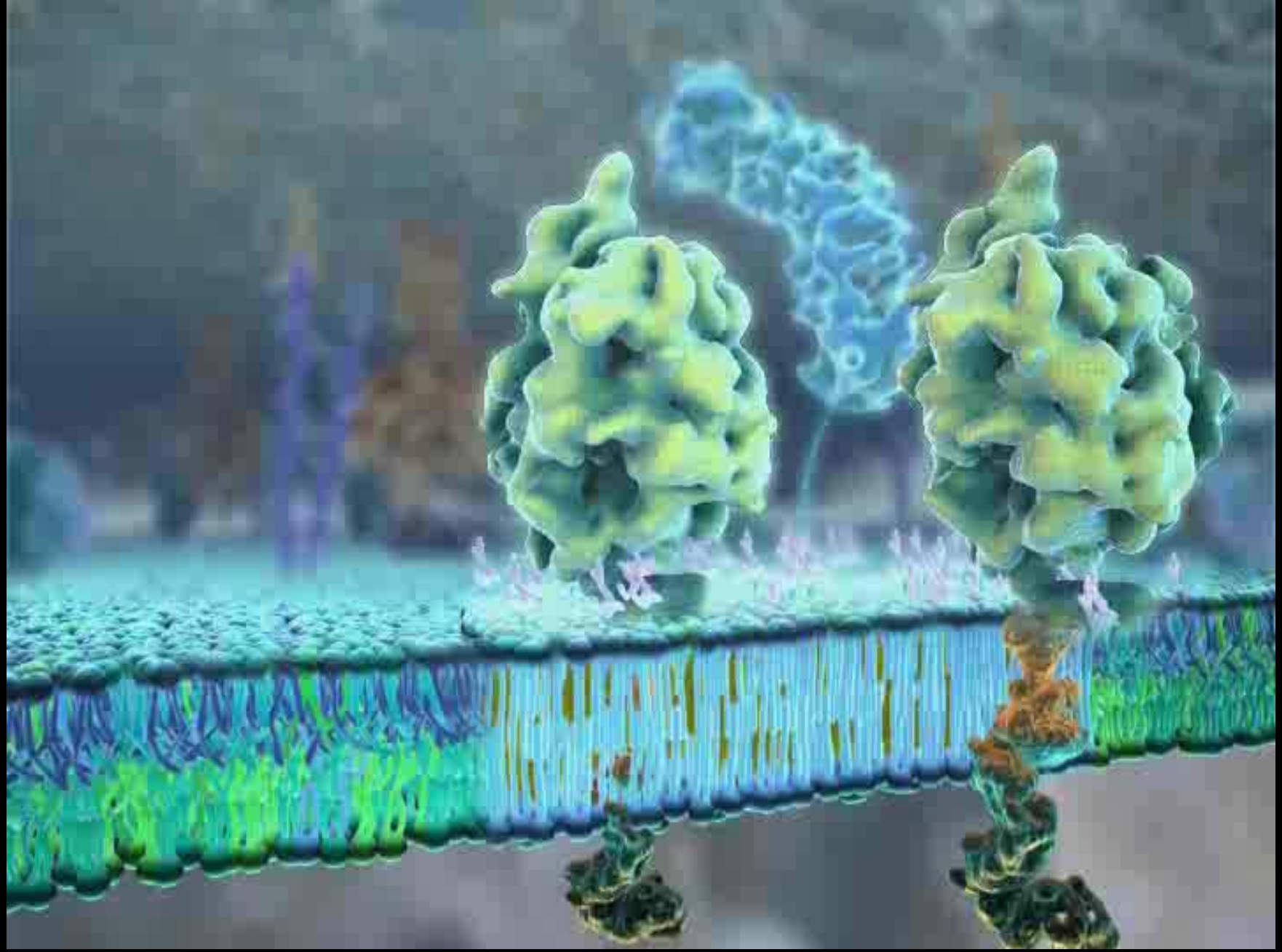


Textbook view of the plasma membrane



But important to remember that it is dynamic & renewable

www.biology.arizona.edu



The full length version of this award-winning animation from Harvard University is available on the Howard Hughes Medical Institute (HHMI) educational website**.**

Know the “Parts of a Membrane Protein”

Extracellular Domain (often glycosylated)

Transmembrane Domain

Cytoplasmic Tail (may be phosphorylated ubiquitinated, etc)

Large Variety in Membrane Protein Topology

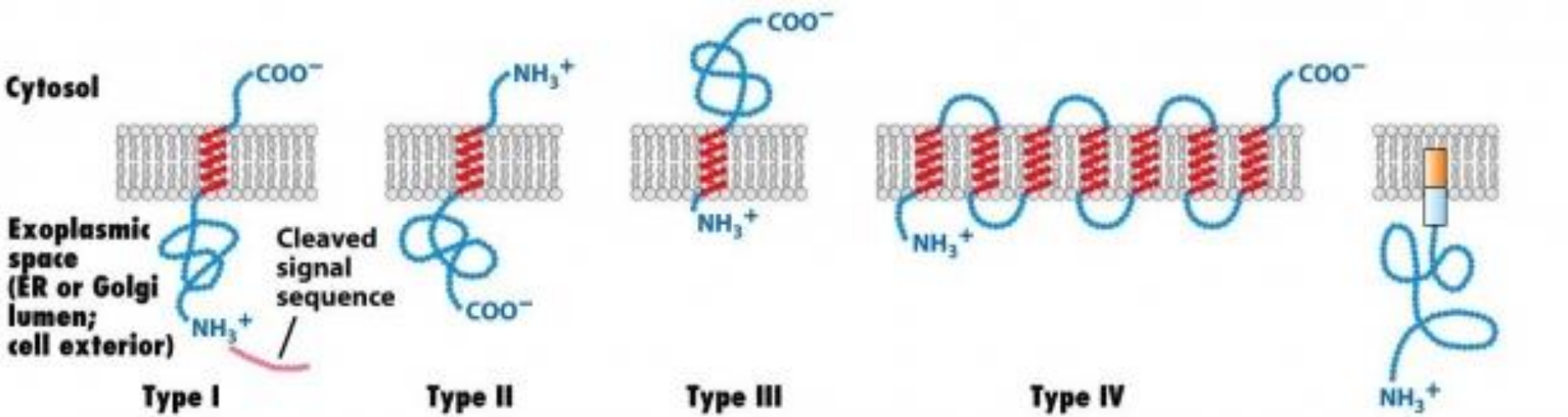
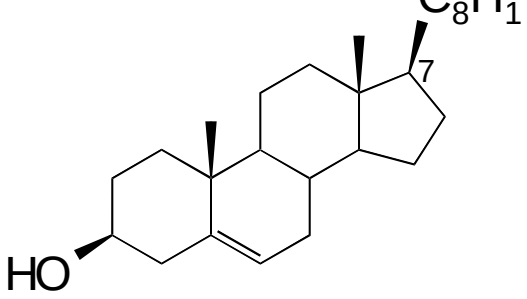


Figure 13-10
Molecular Cell Biology, Sixth Edition

Major Lipid Species

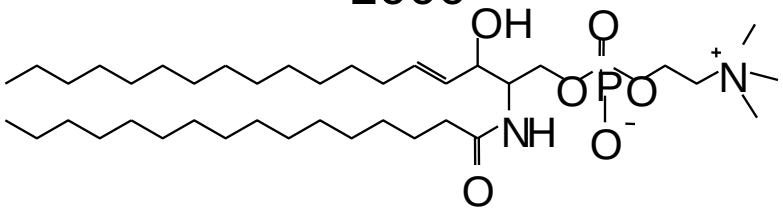
Major Single Species

Cholesterol C_8H_1

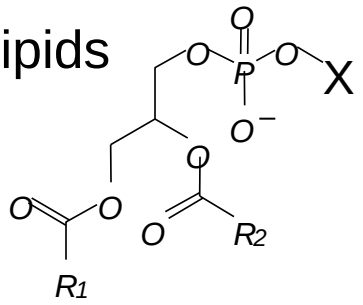


Abundant Molecular Species

Sphingolipids
1000+

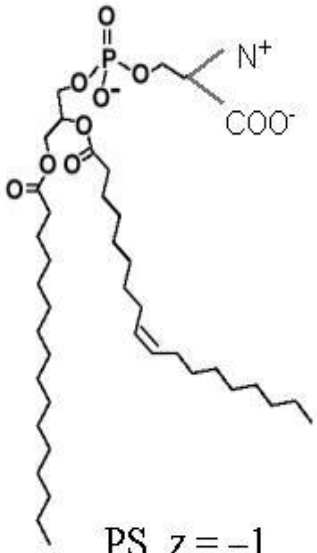


Phospholipids
1000+



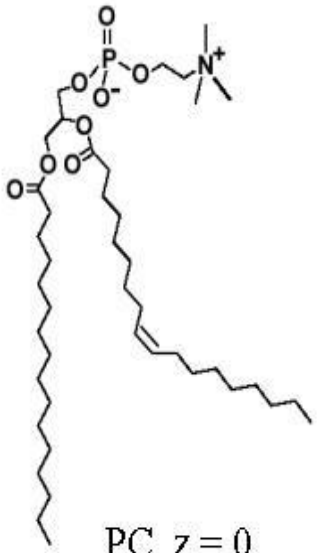
Phosphatidylinositol
4,5-bisphosphate

Phosphatidylserine

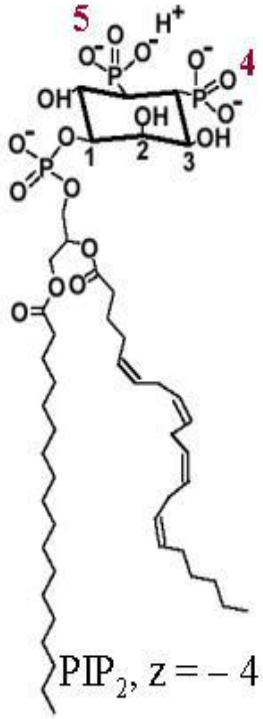


PS, z = –1

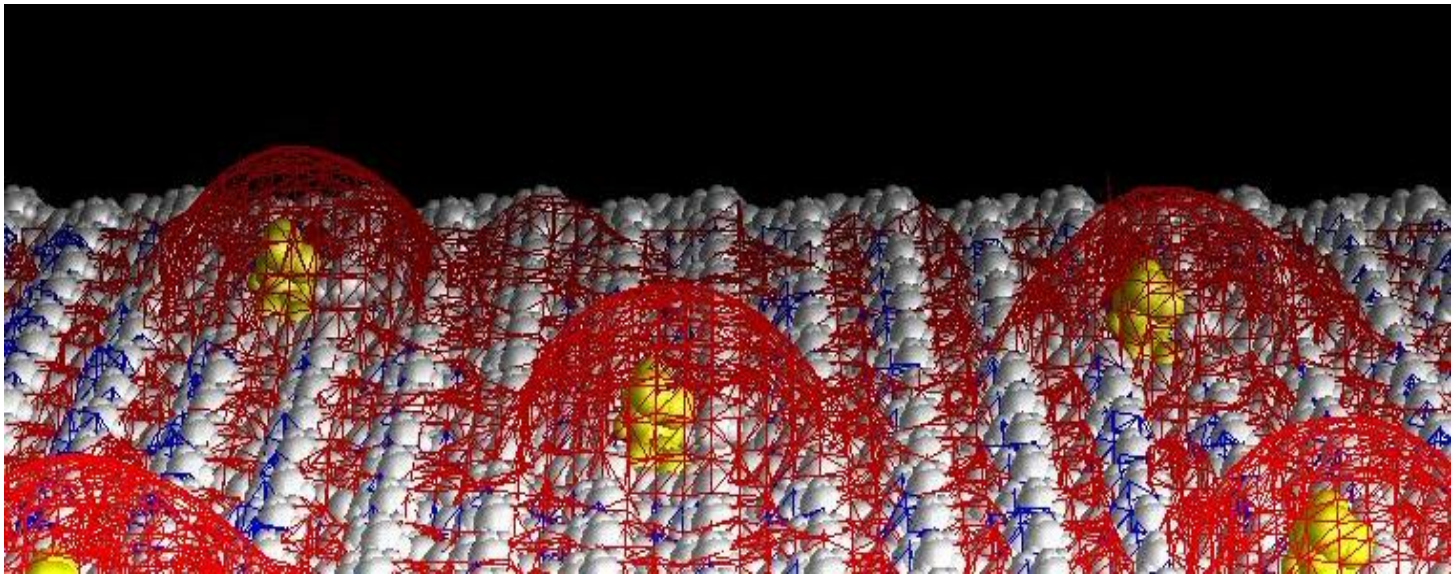
Phosphatidylcholine



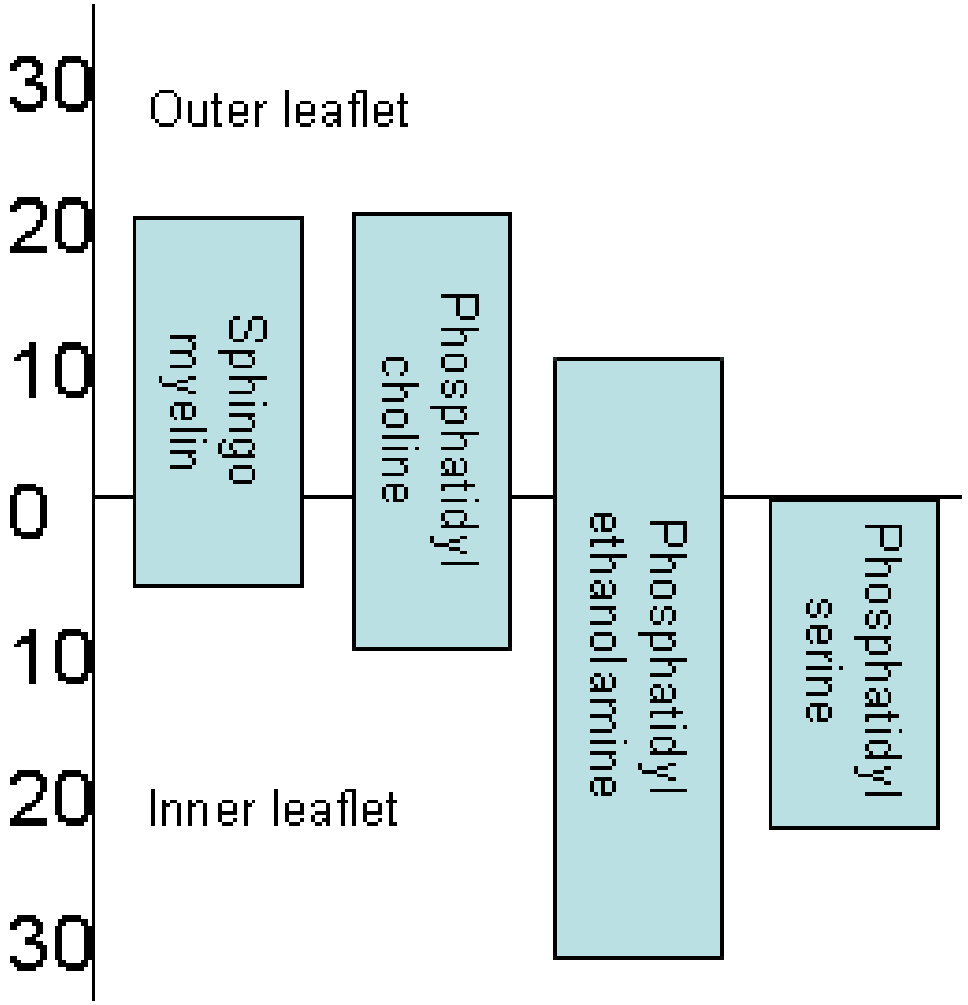
PC, z = 0



PIP_2, z = – 4



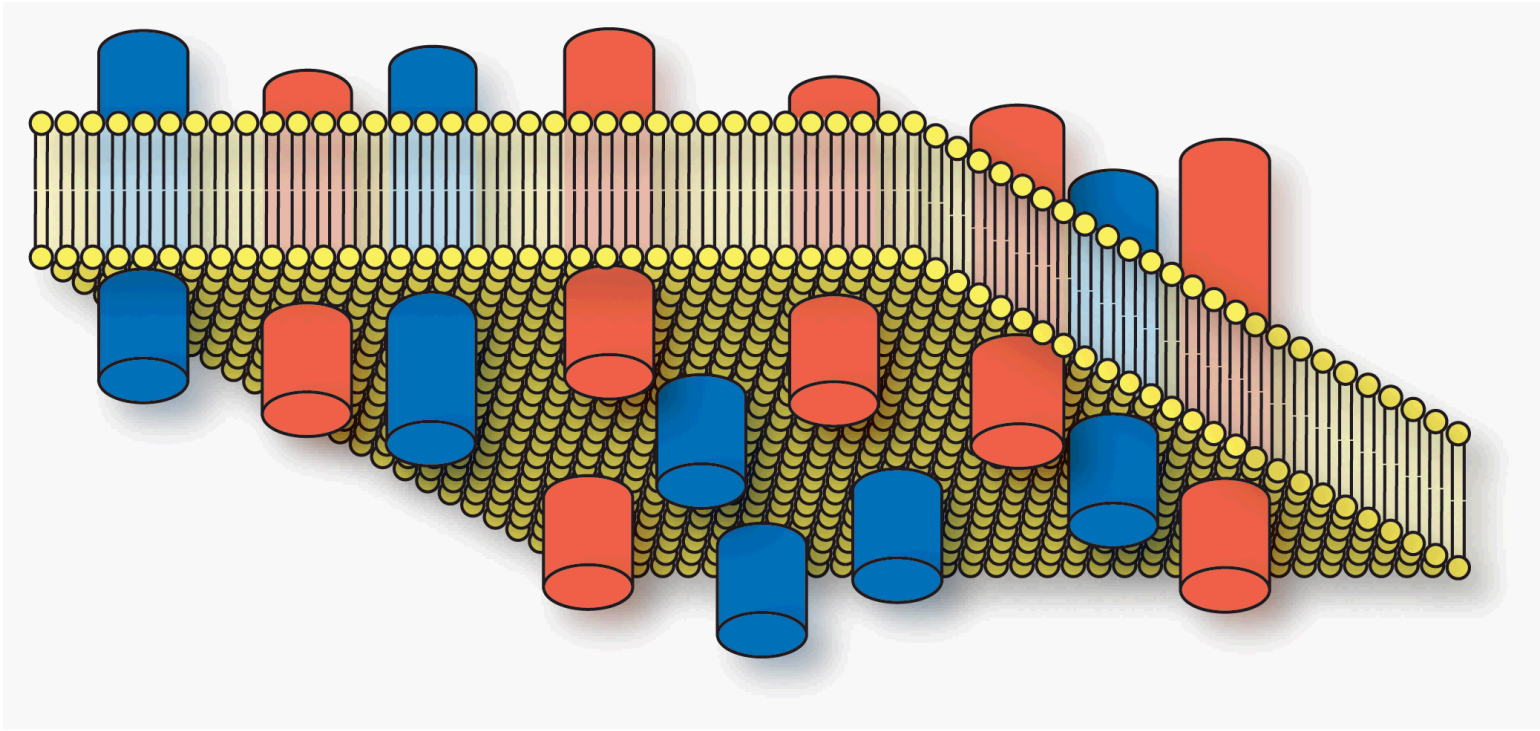
MEMBRANE LIPIDS ARE ASYMETRICALLY DISTRIBUTED



www.steve.gb.com/.../lipids_and_membranes.html

the Fluid Mosaic Model could be viewed this way

RANDOM!

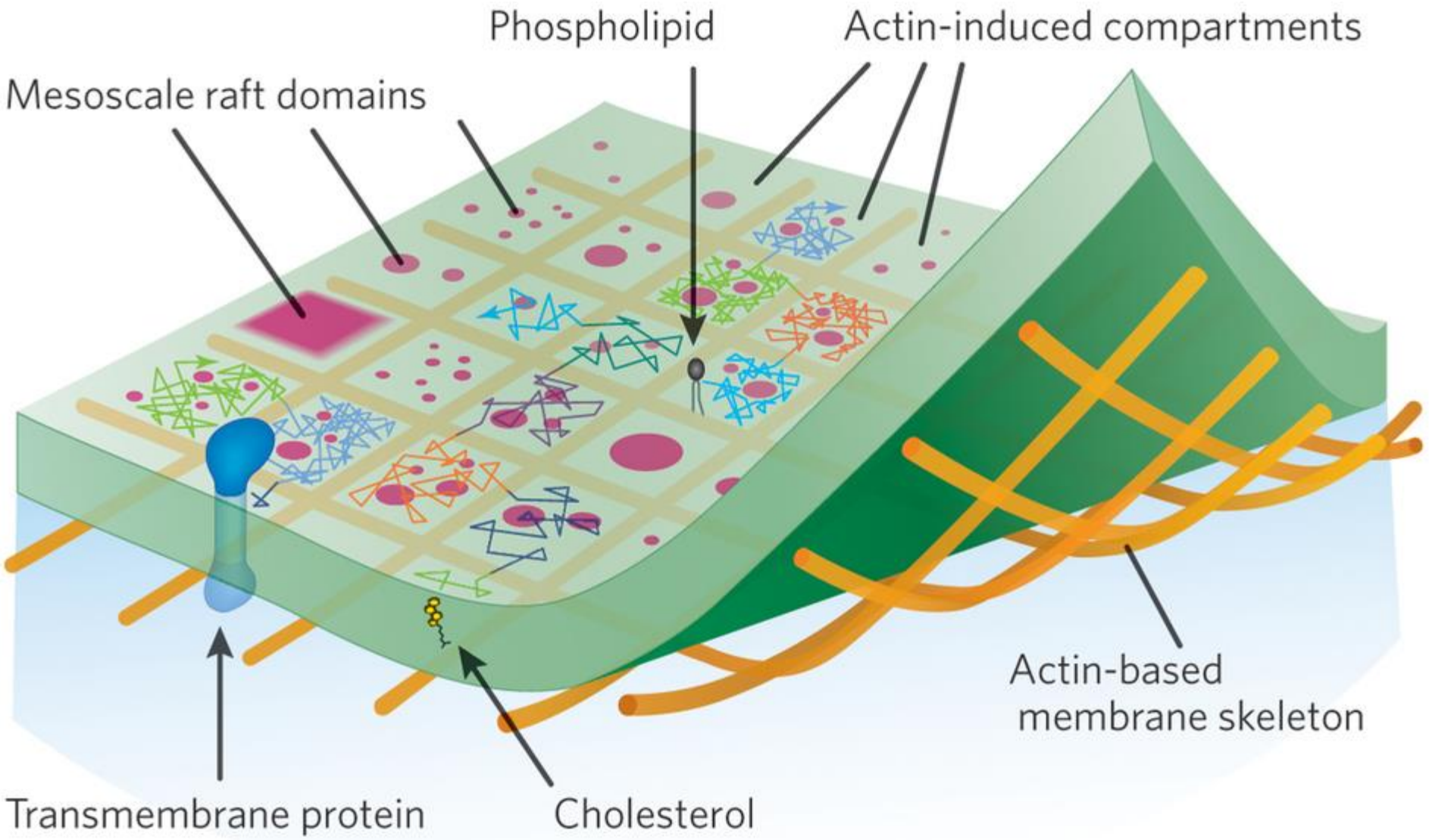


transmembrane proteins

Membrane lipids

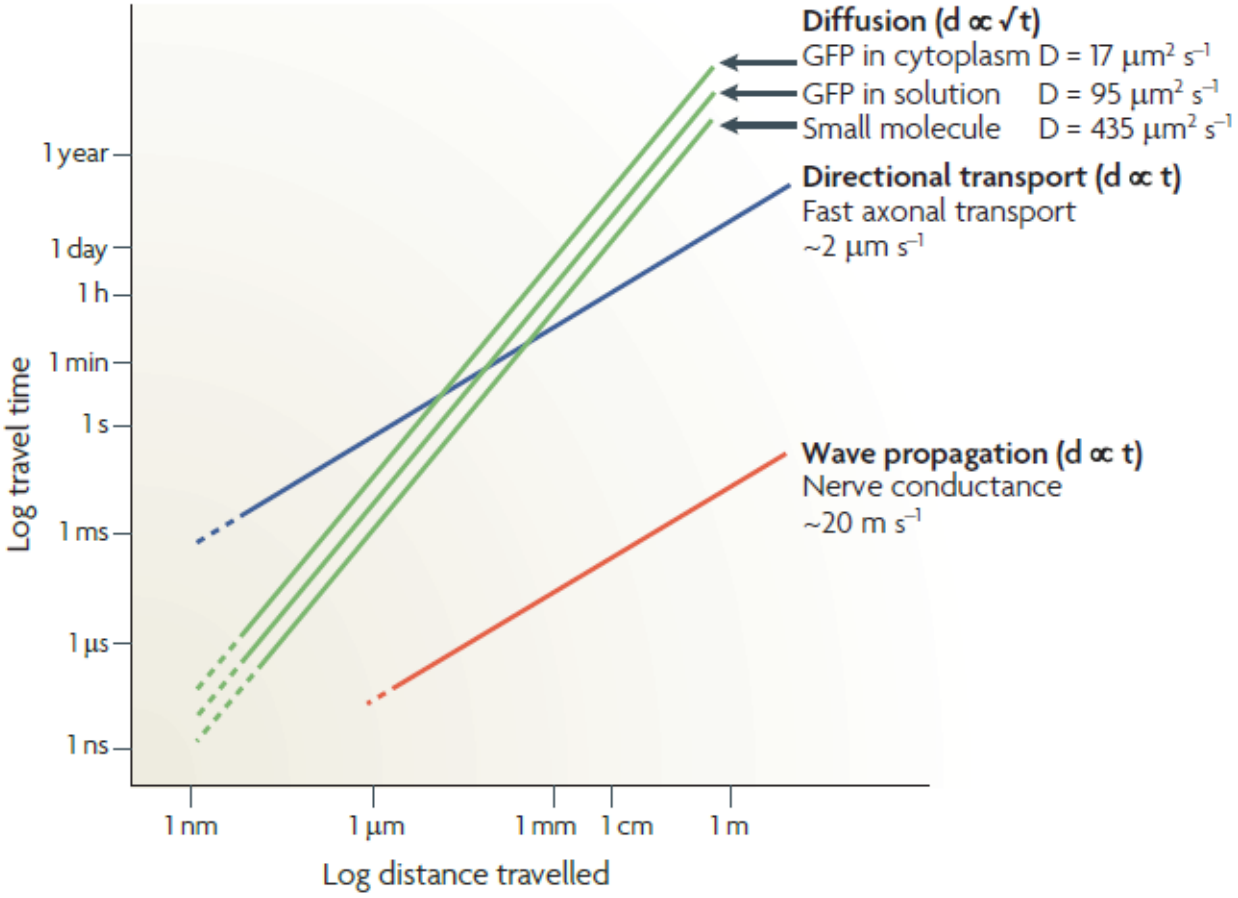
Björn F. Lillemei

More like this?



Kusumi et al, Nature Chemical Biol. 2014

Diffusion-Limited Reactions? By comparison, diffusion in membranes is slower than in buffer or even in the "crowded" cytosol.



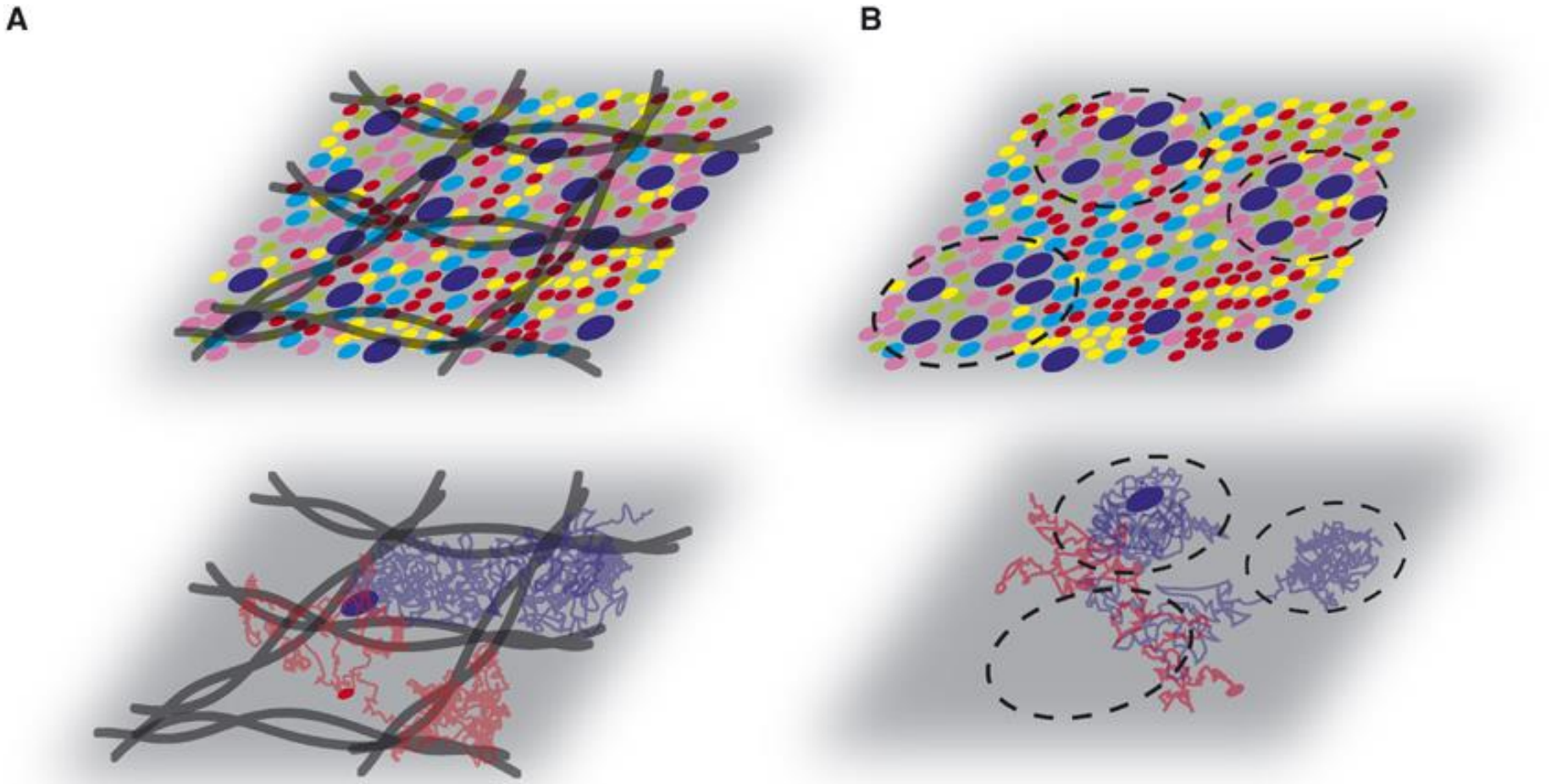
Leif Dehmelt and Philippe I. H. Bastiaens
NATURE REVIEWS | MOLECULAR CELL BIOLOGY
JUNE 2010 | VOLUME 11

Membranes:

*Lipid	3.39 μm²/s
***GMI-FITC	0 .1μm²/s
***GPI-GFP	0.621μm²/s
* Ras	1.2 μm²/s
**FcεRI	0.074 μm²/s
**ErbB3	0.038 μm²/s

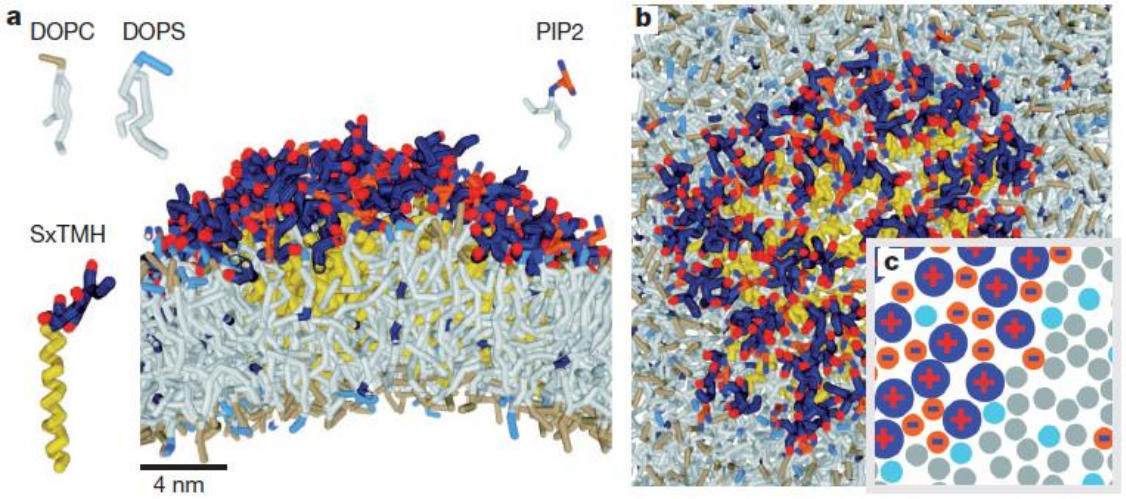
*Lin et al, PNAS 2014
**Andrews et al NCB 2008, Steinkamp MCB 2014
***

Some cartoon models to describe Restricted Diffusion at smaller scales



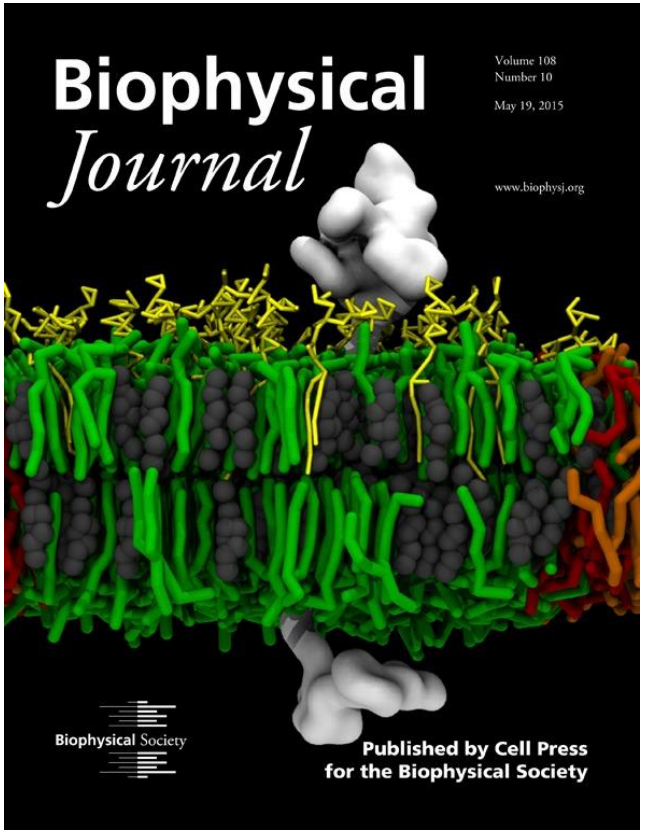
EMBO Journal (2006) 25, 3446–3457
Marguet et al.

Protein Interactions with Lipid Head Groups May Drive Microdomain Formation and Signaling

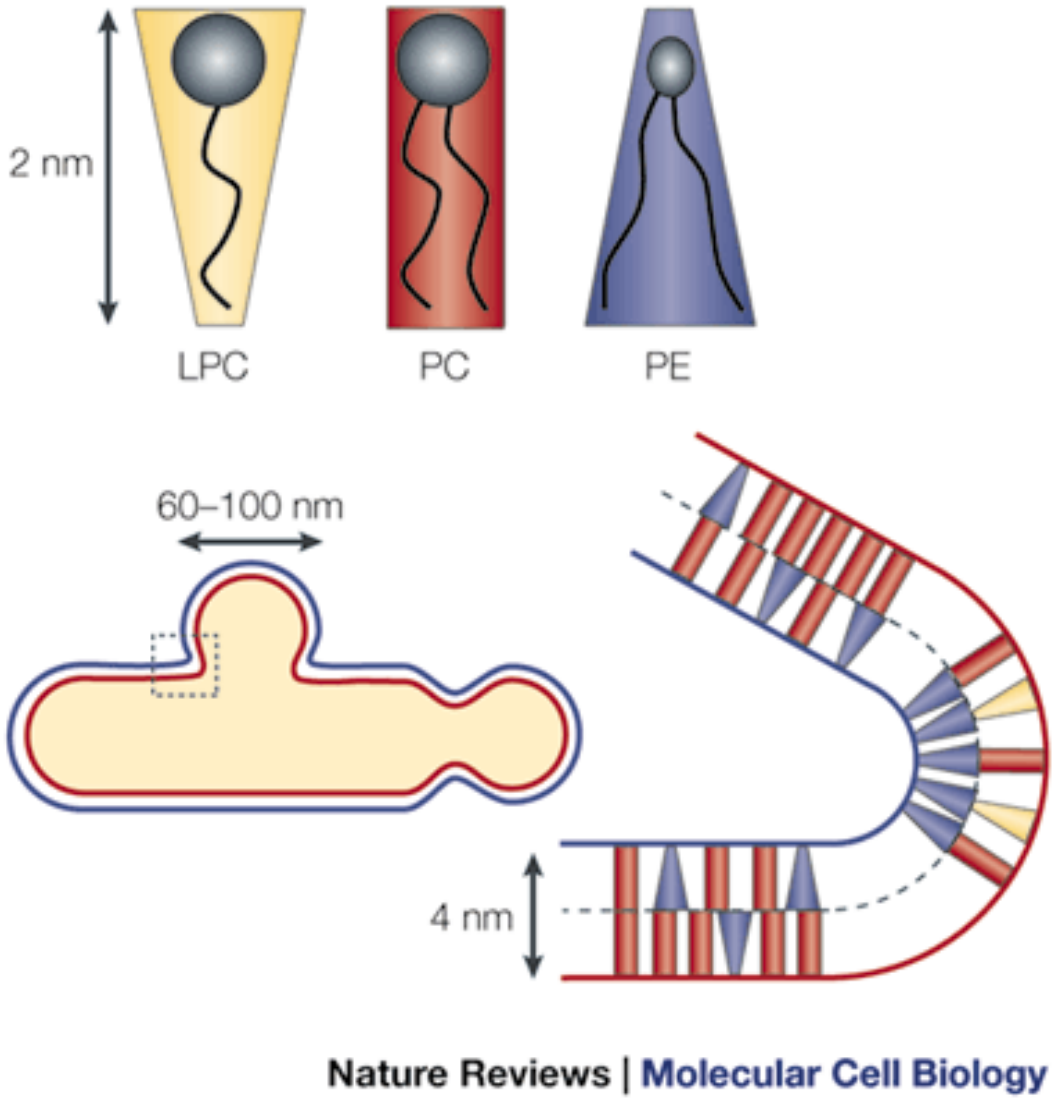


Syntaxin 1A and the inner leaflet lipid, PIP2

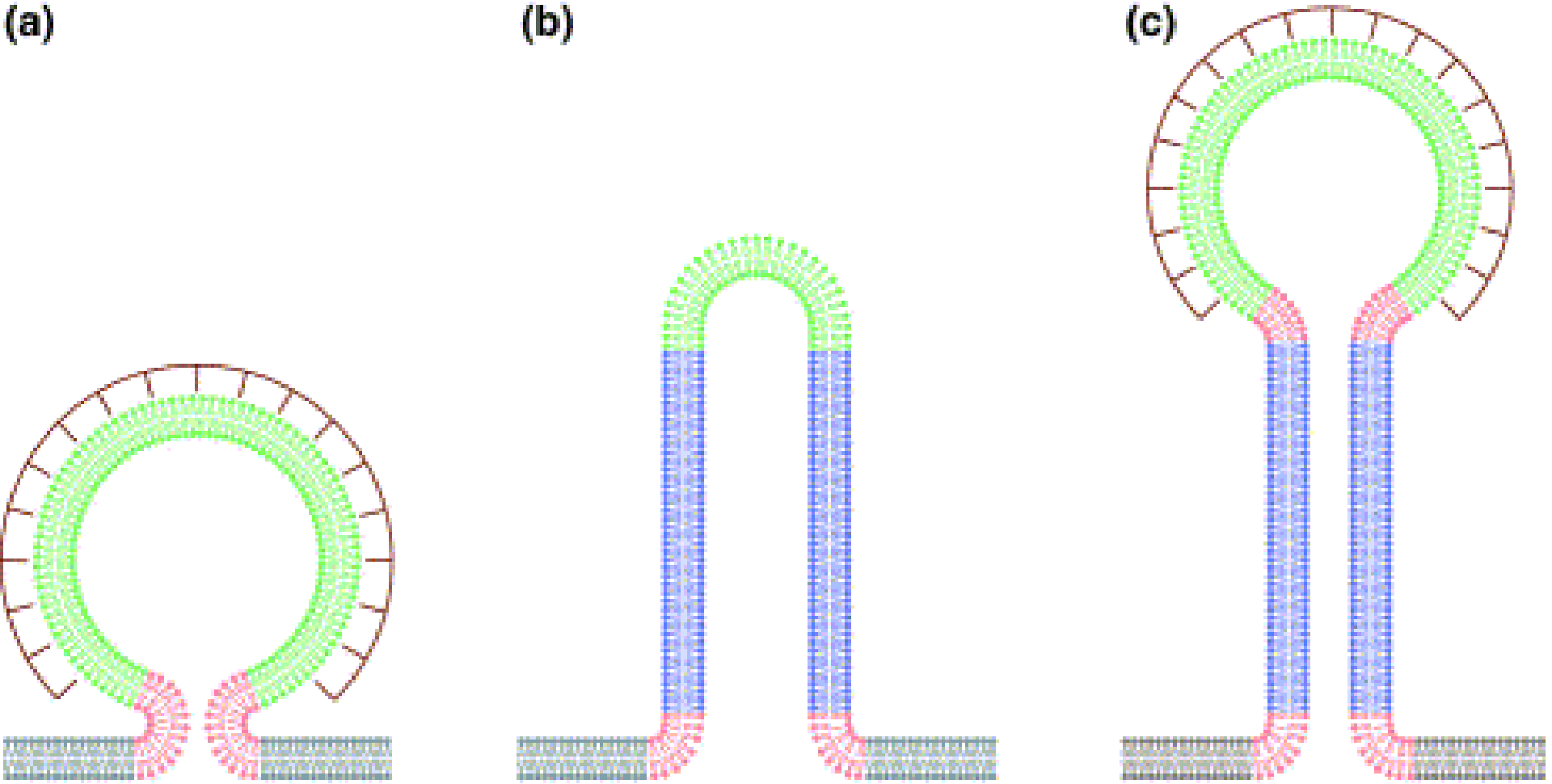
Membrane protein sequestering by ionic protein–lipid interactions
Reinhard Jahn
NATURE | VOL 479 | 24 NOVEMBER 2011



Cone-shaped & Reverse Cone-shaped Lipids Can Mediate Curvature



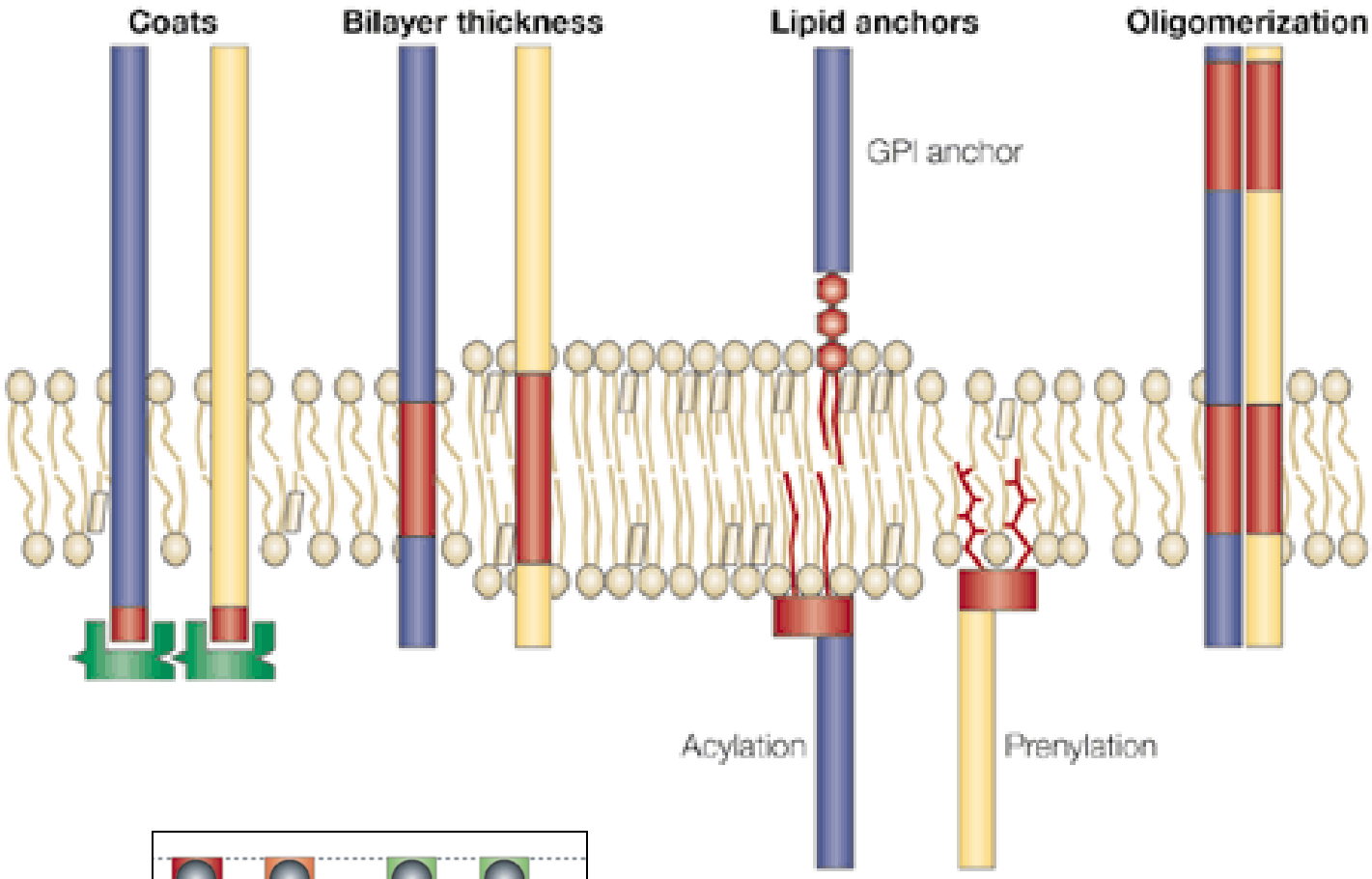
Vesicles & Tubules: Extremes in membrane curvature

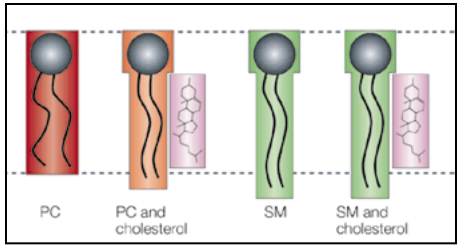


TRENDS in Cell Biology

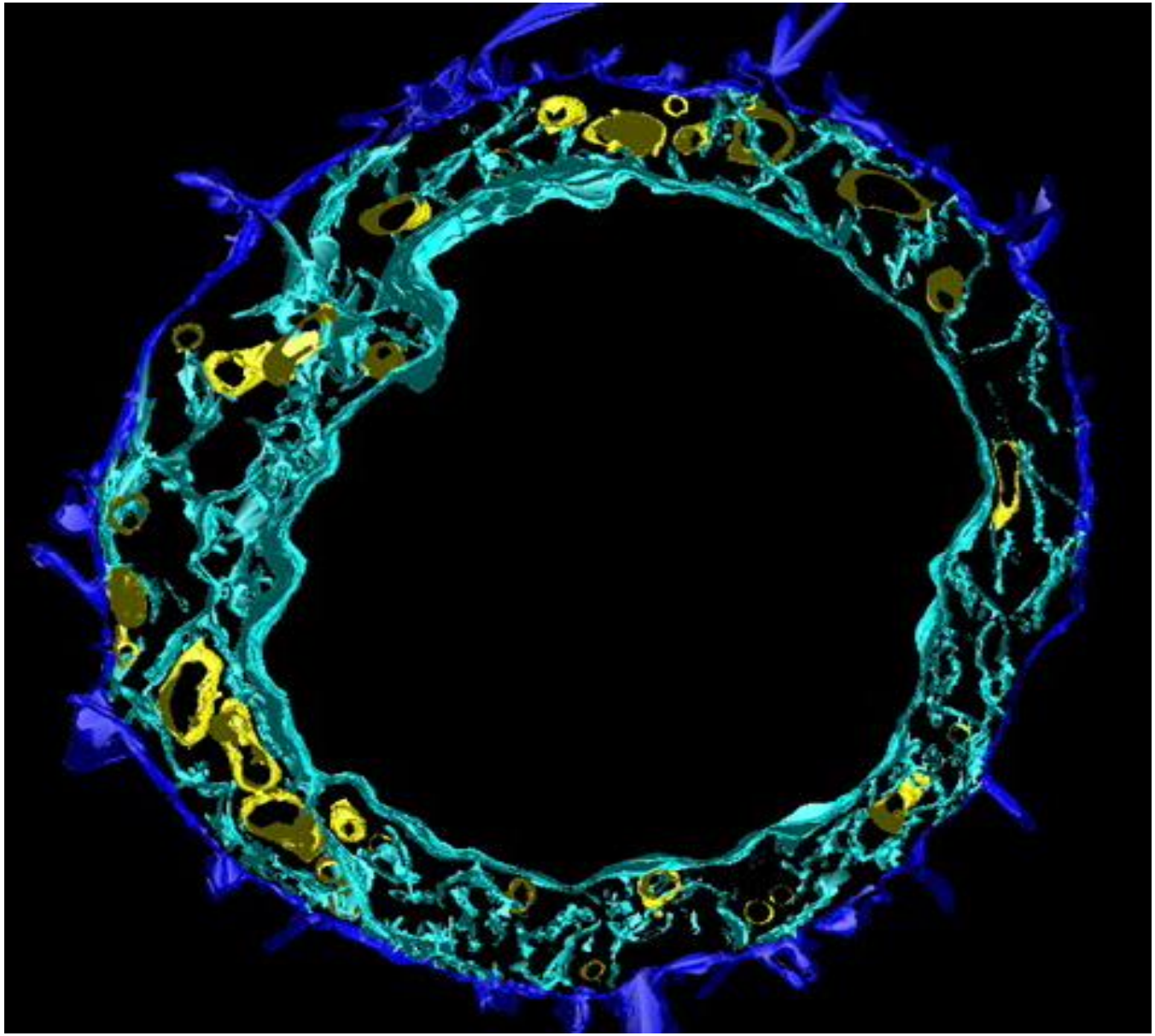
Other Concepts in Membrane Modeli

- **Fully saturated fatty acids in lipids pack tighter, since they don't have a "Kink"**
- **Myristate and palmitate are saturated acyl chains that can be covalently attached to proteins**
- **Prenyl groups are kinky**
- **Length of fatty acid chains in lipids and transmembrane domains in proteins influence membrane thickness and raft composition**



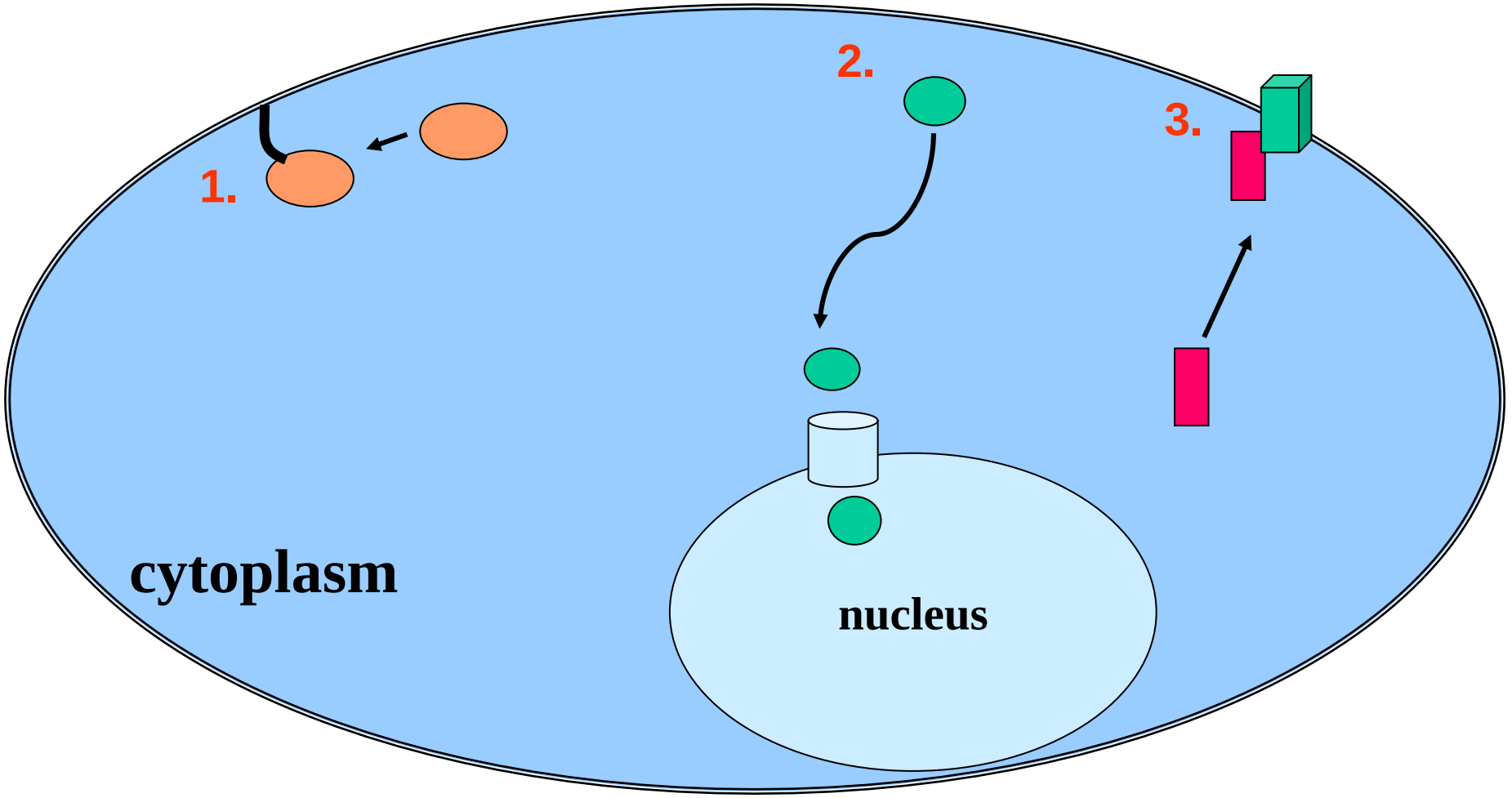


Signal Transduction in Cells Takes Place in Context of Complex Cell Geometry



Mazel et al., Biophysical J. 2009

Cellular Location Strategies Are Important for Signaling



Cellular responses are cell-type specific. For example, epinephrine stimulates different responses from lung or heart cells

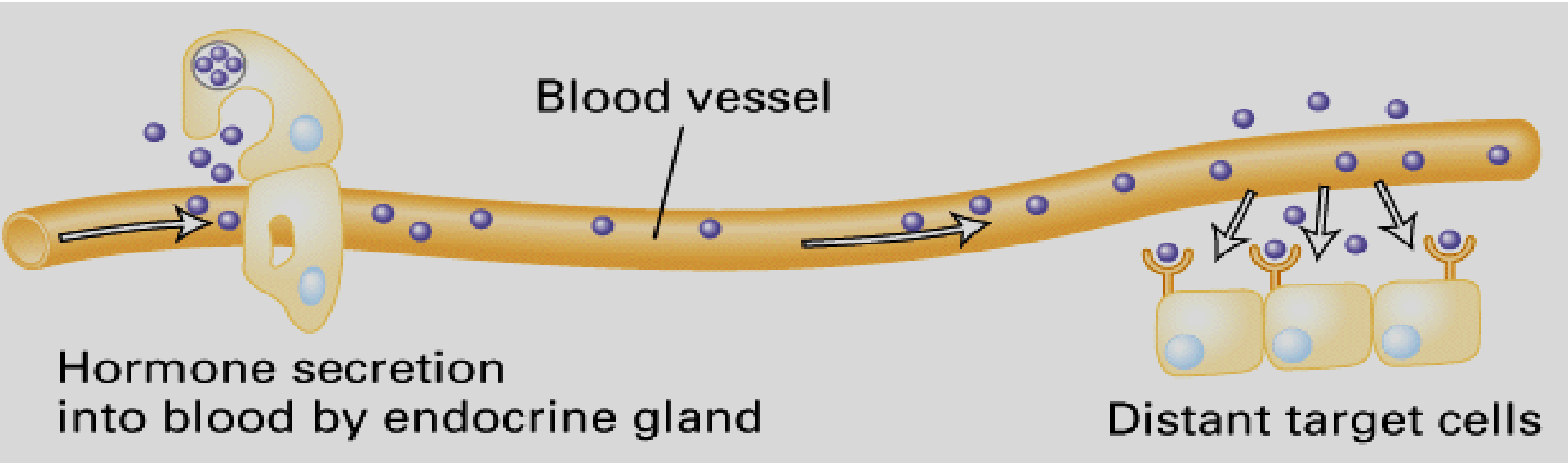
http://learn.genetics.utah.edu/

These concepts come up in every day life…Such as these bronchodilators that deliver epinephrine or other β2 agonists

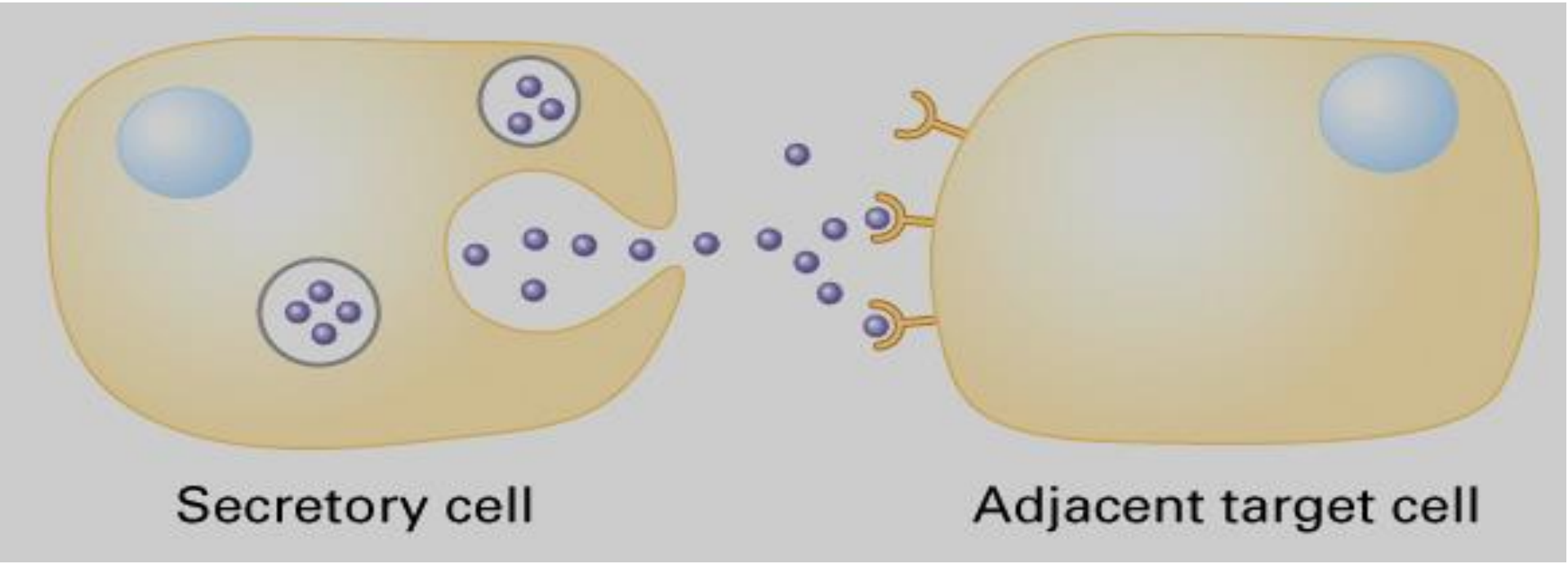
Signaling proteins are the most common drug targets. They underlie many fundamental disease mechanisms.

Signaling Up Close and Far Away

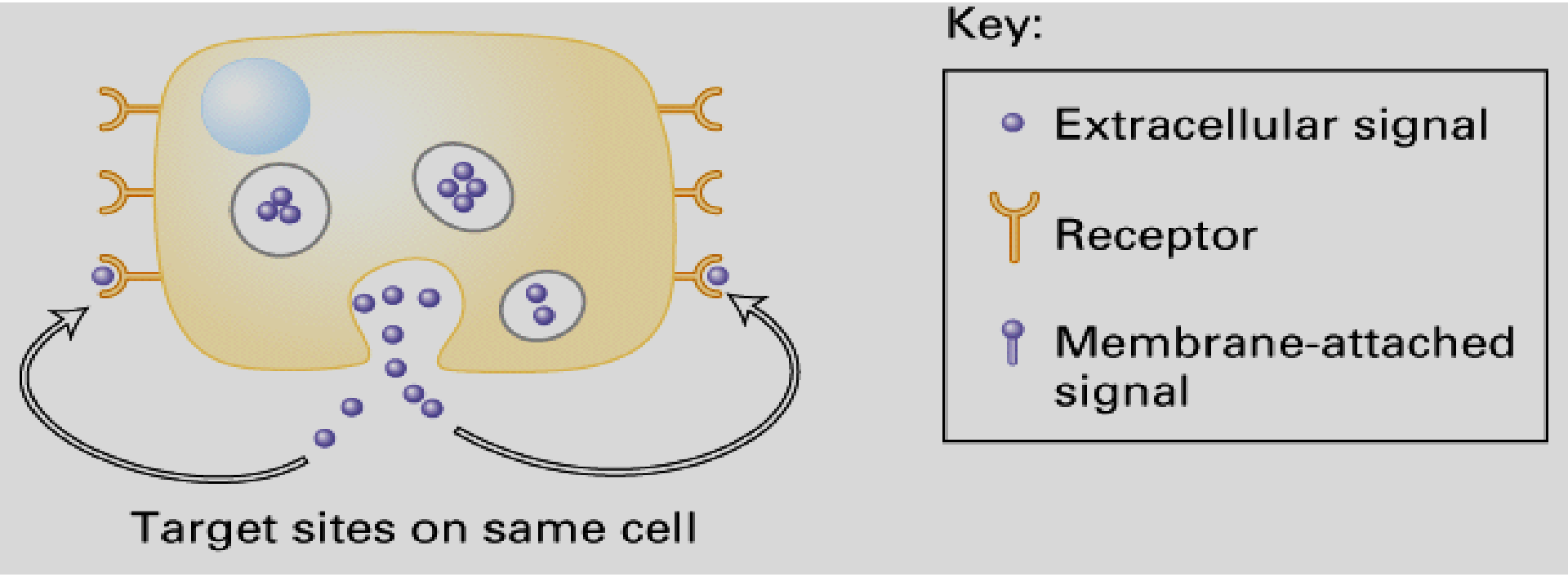
ENDOCRINE SIGNALING



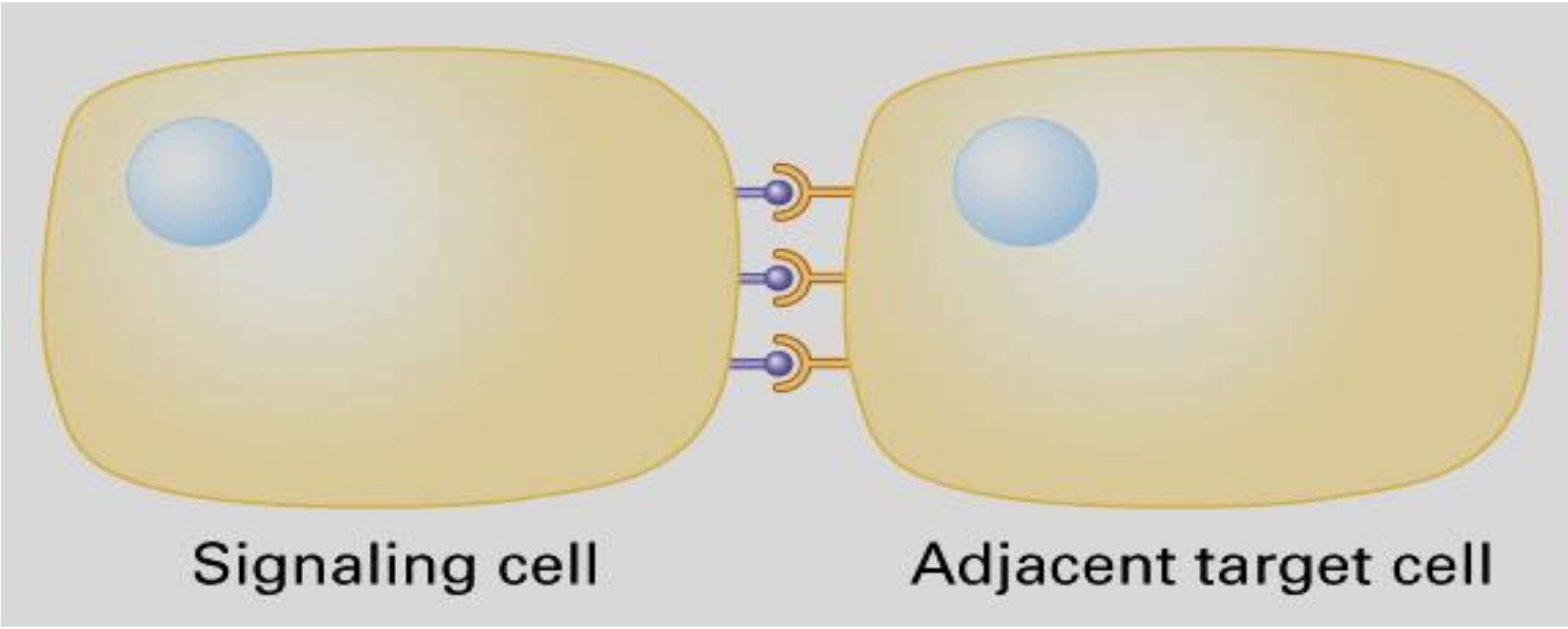
PARACRINE SIGNALING



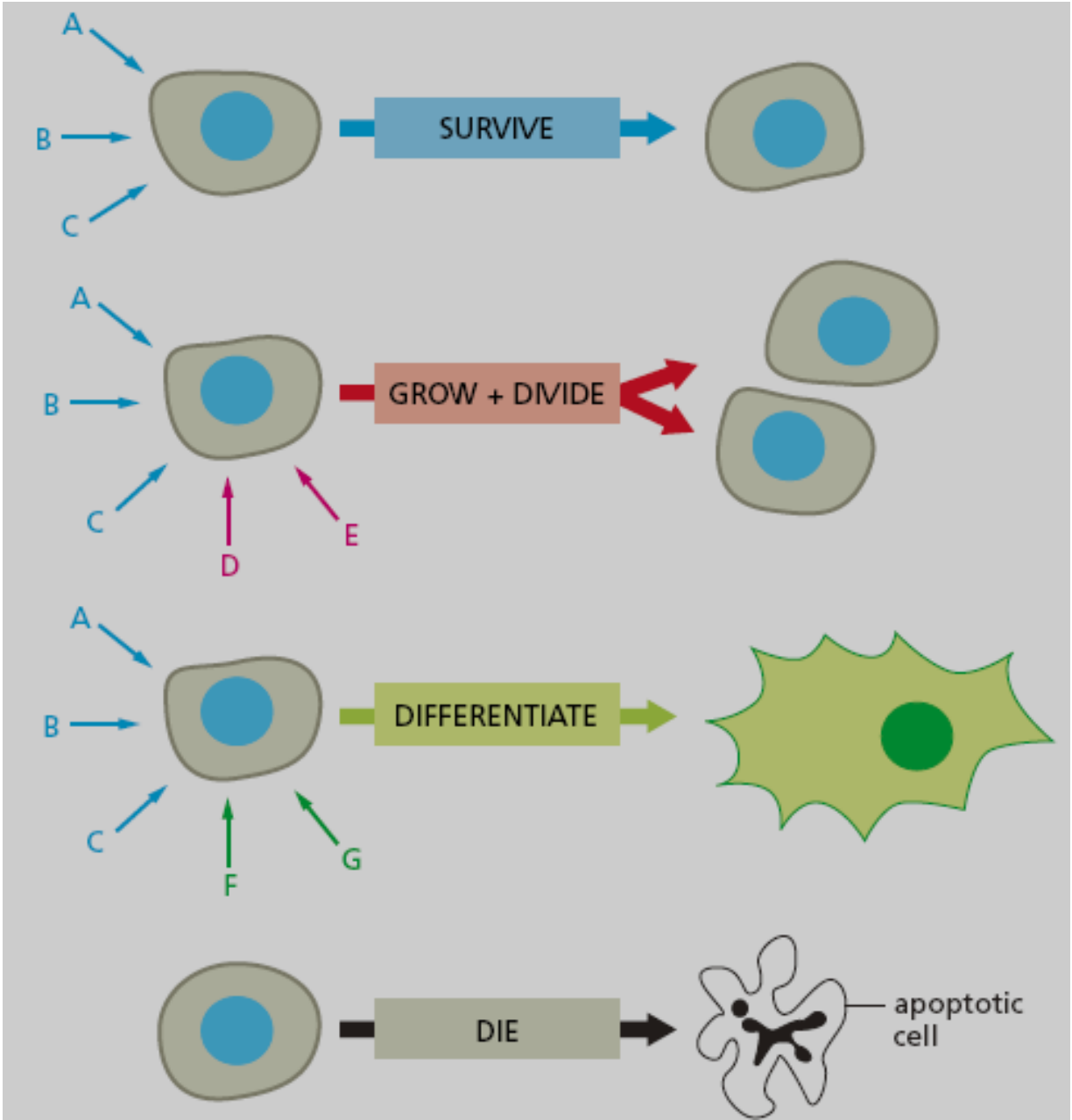
AUTOCRINE SIGNALING



ADHESION-BASED SIGNALING

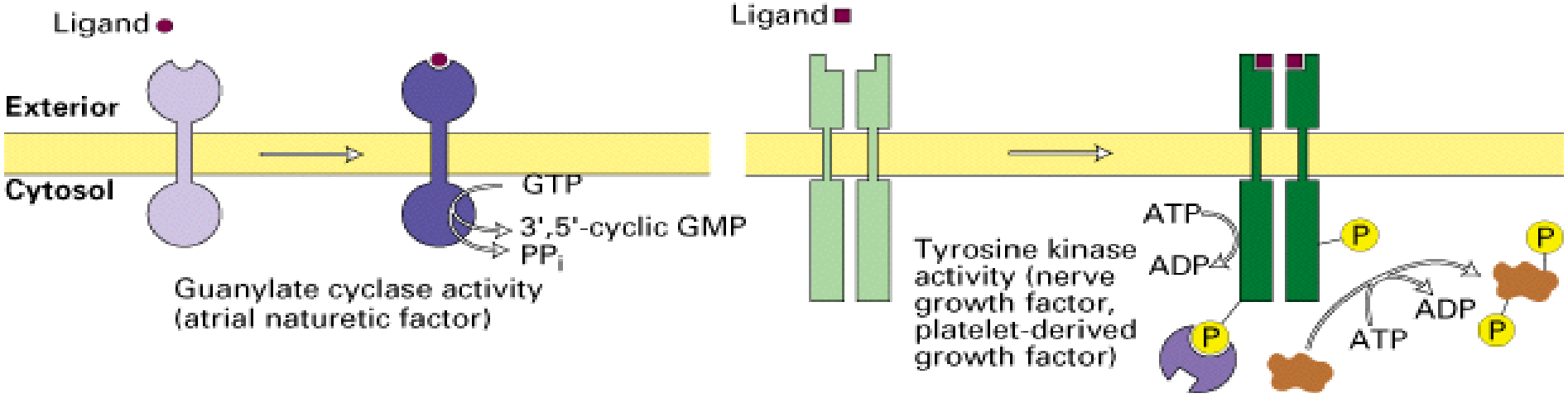


Some outcomes of cell signaling

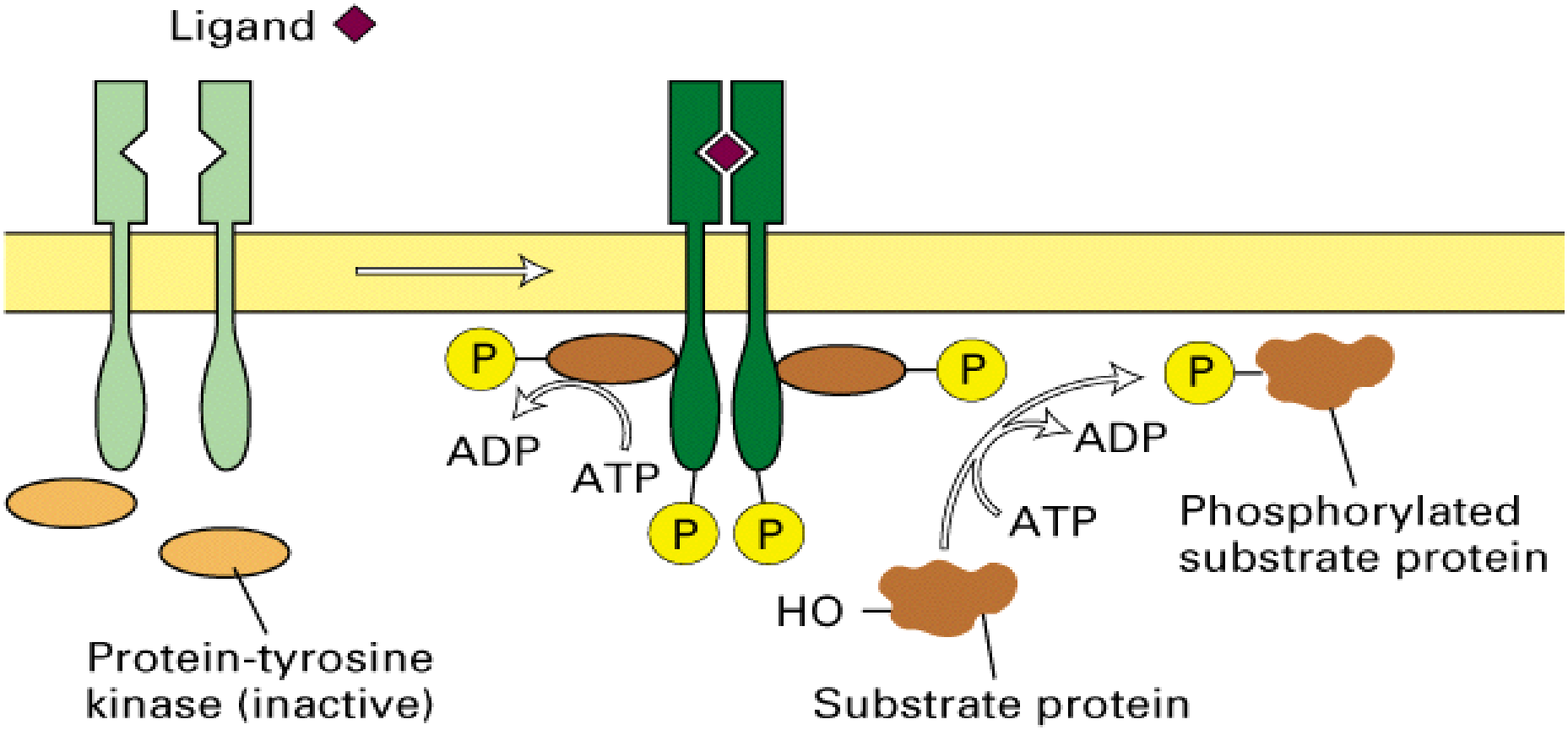


Types of Signaling Receptors

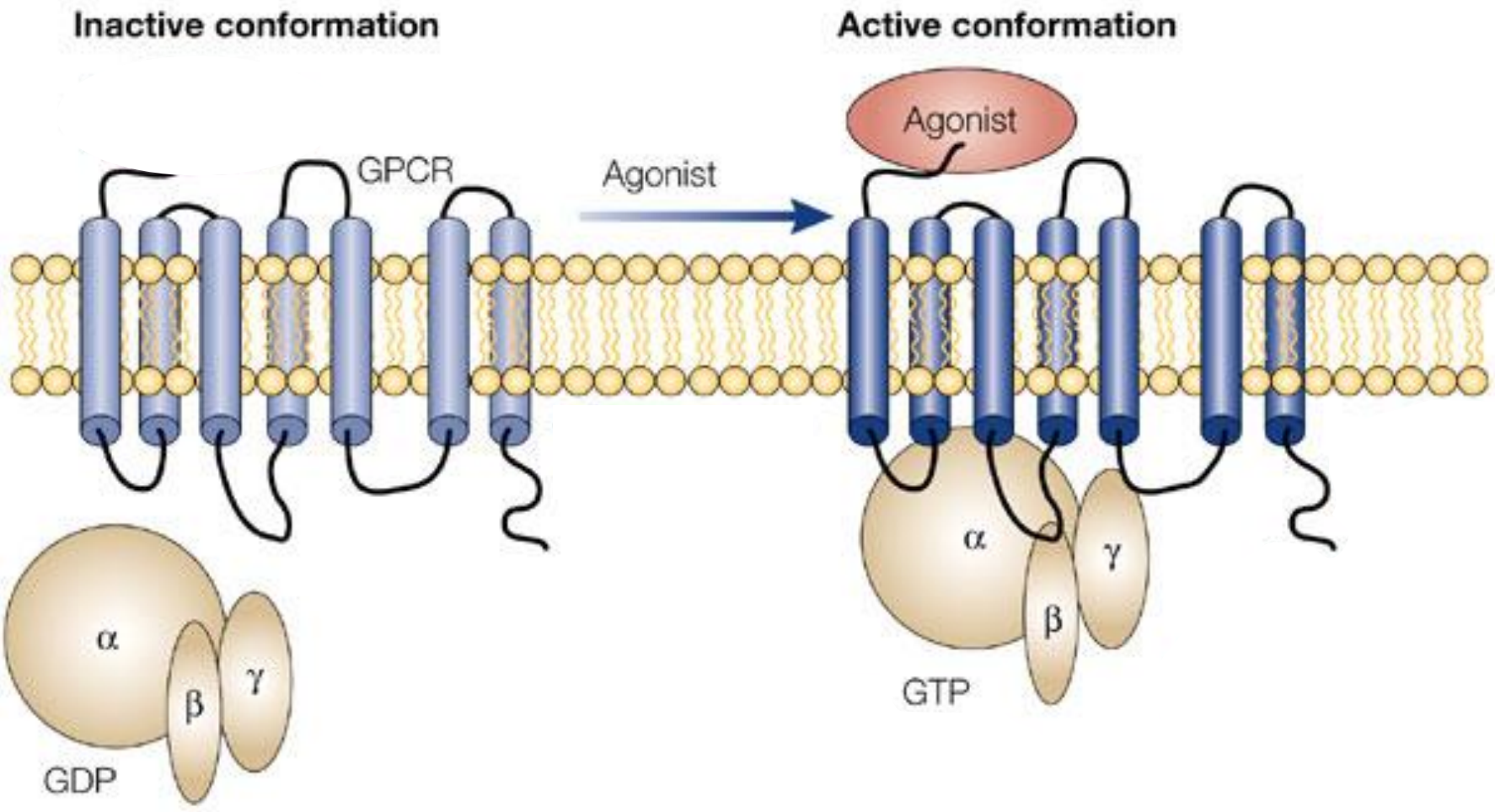
Receptors with Intrinsic Enzymatic Activity



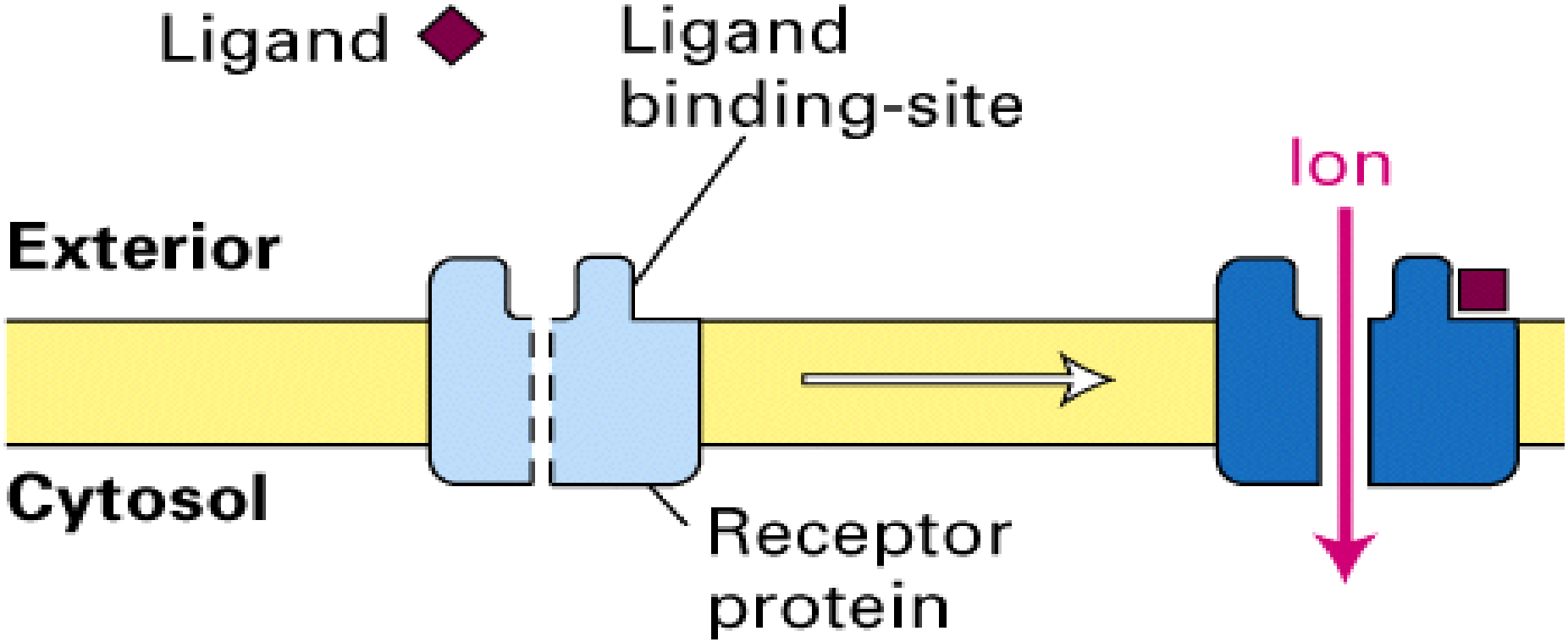
Receptor Cytoplasmic Tails Recruit Cytosolic Kinases & Phosphatases.



G-protein coupled Receptors

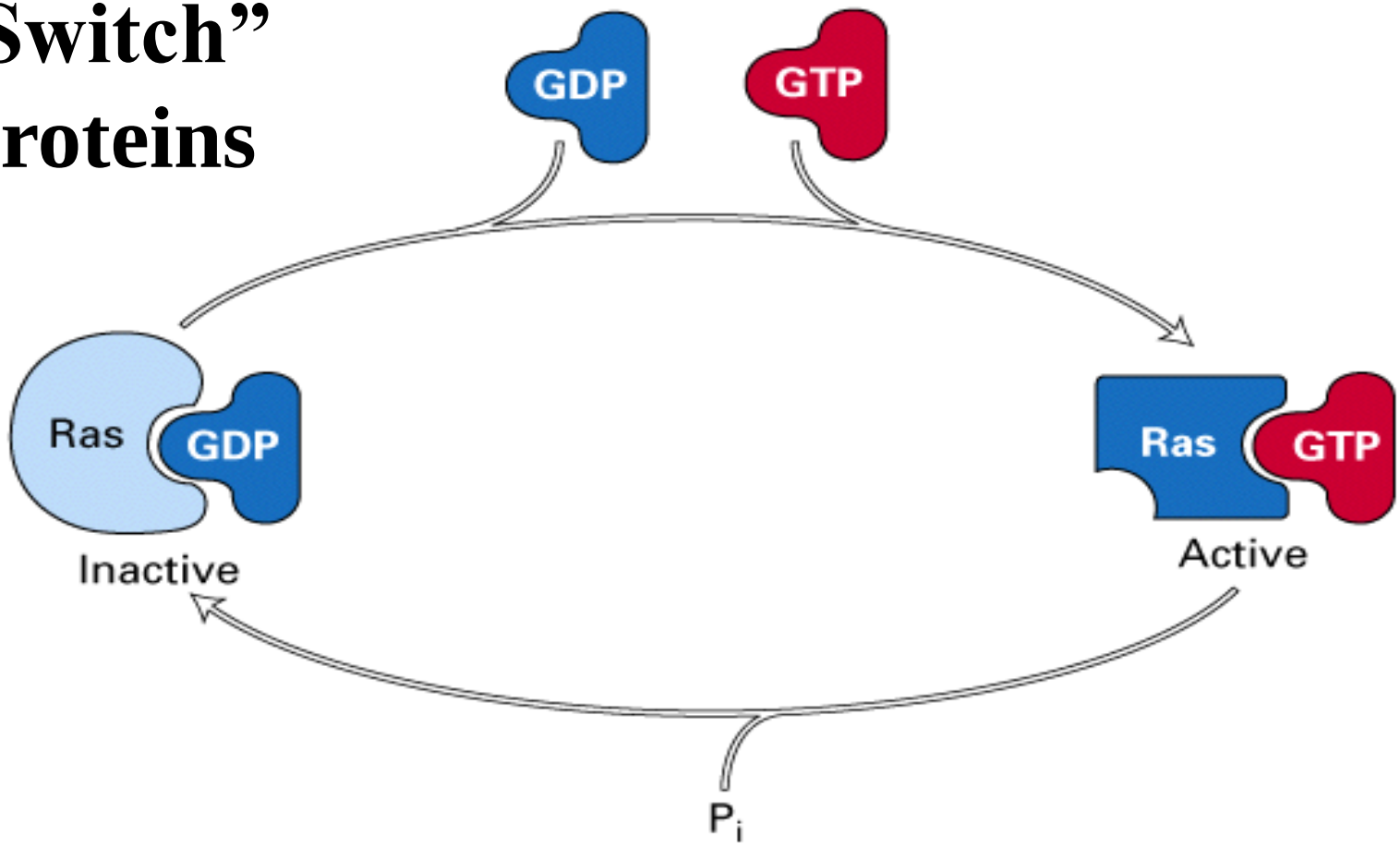


Ion Channel/Receptors



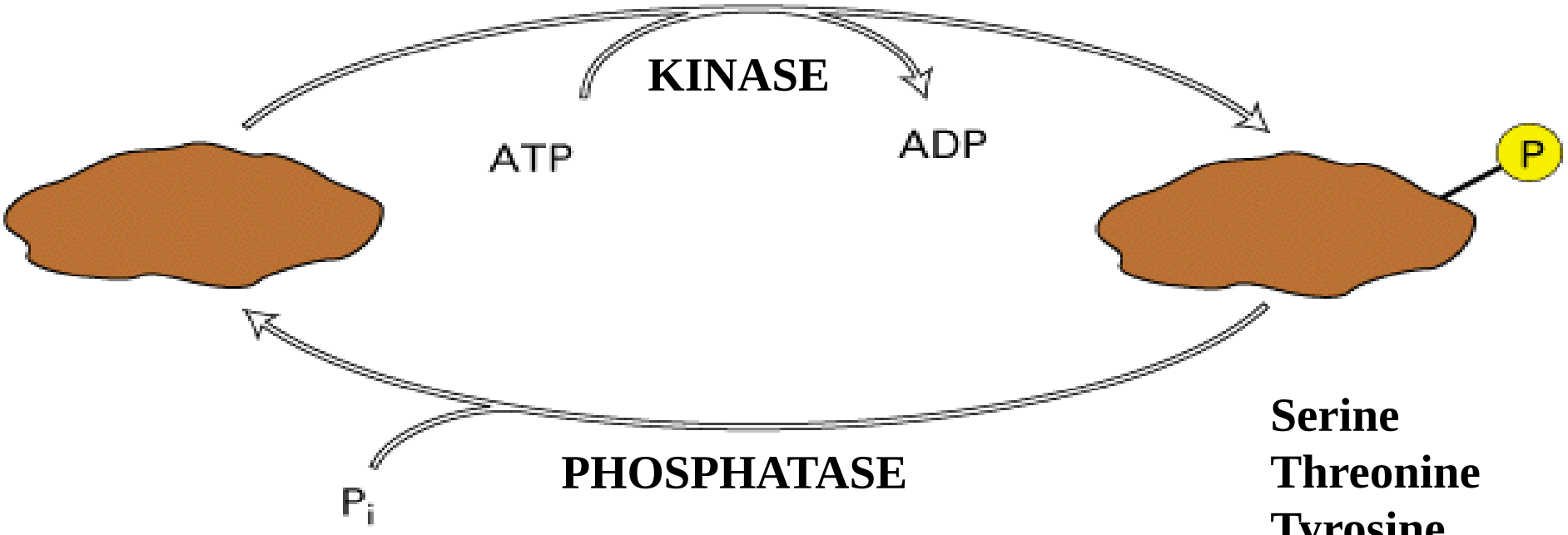
BASIC MECHANISMS

GTPase "Switch" Proteins



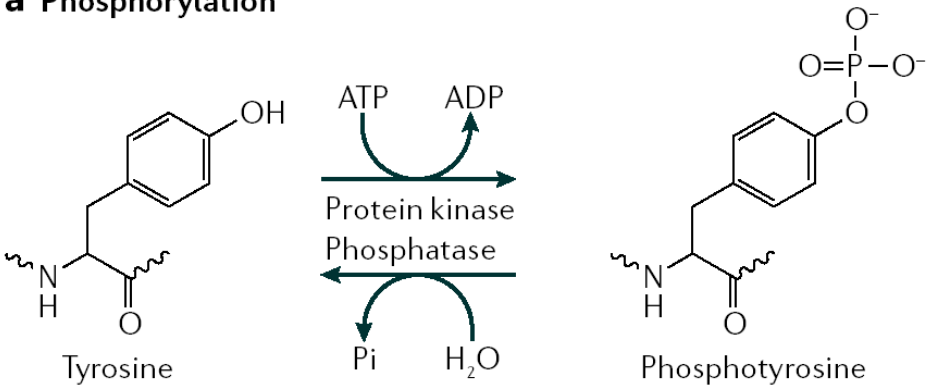
BASIC MECHANISMS

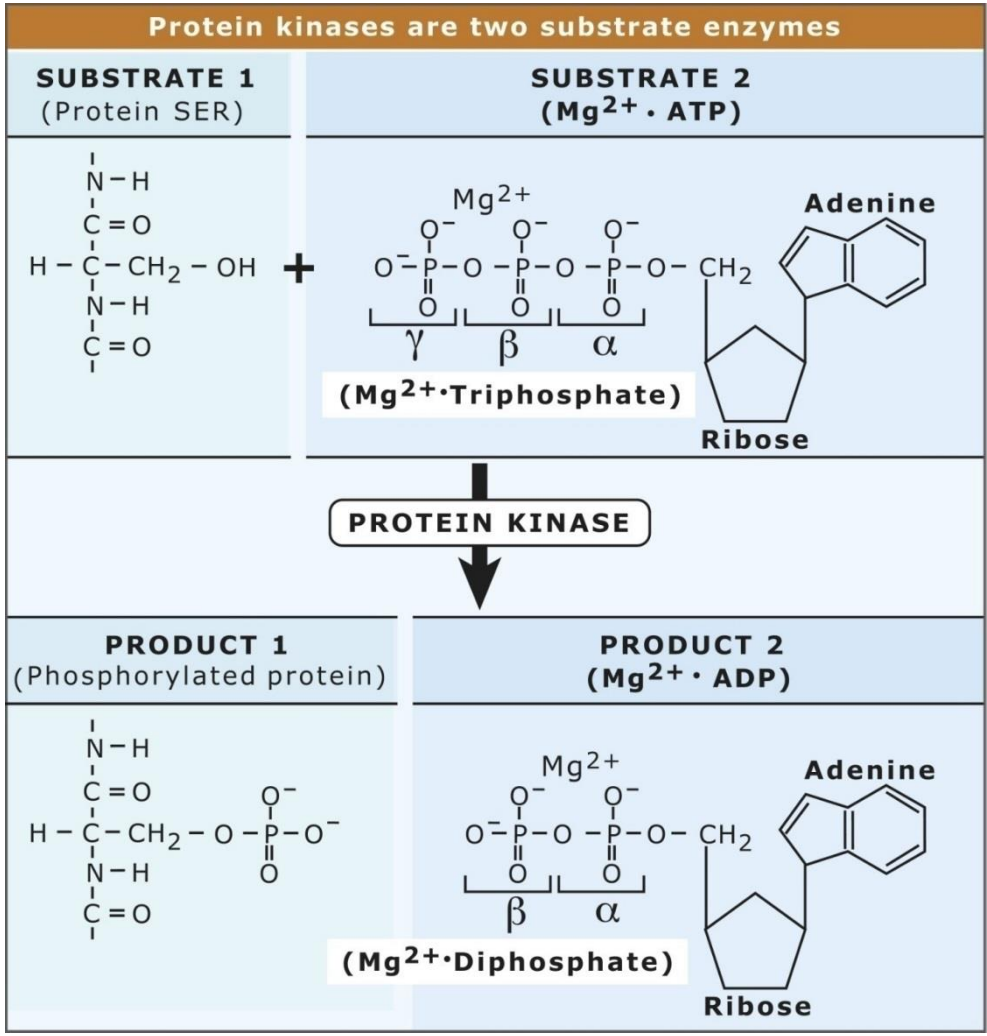
Protein Phosphorylation & Dephosphorylation



Serine
Threonine
Tyrosine

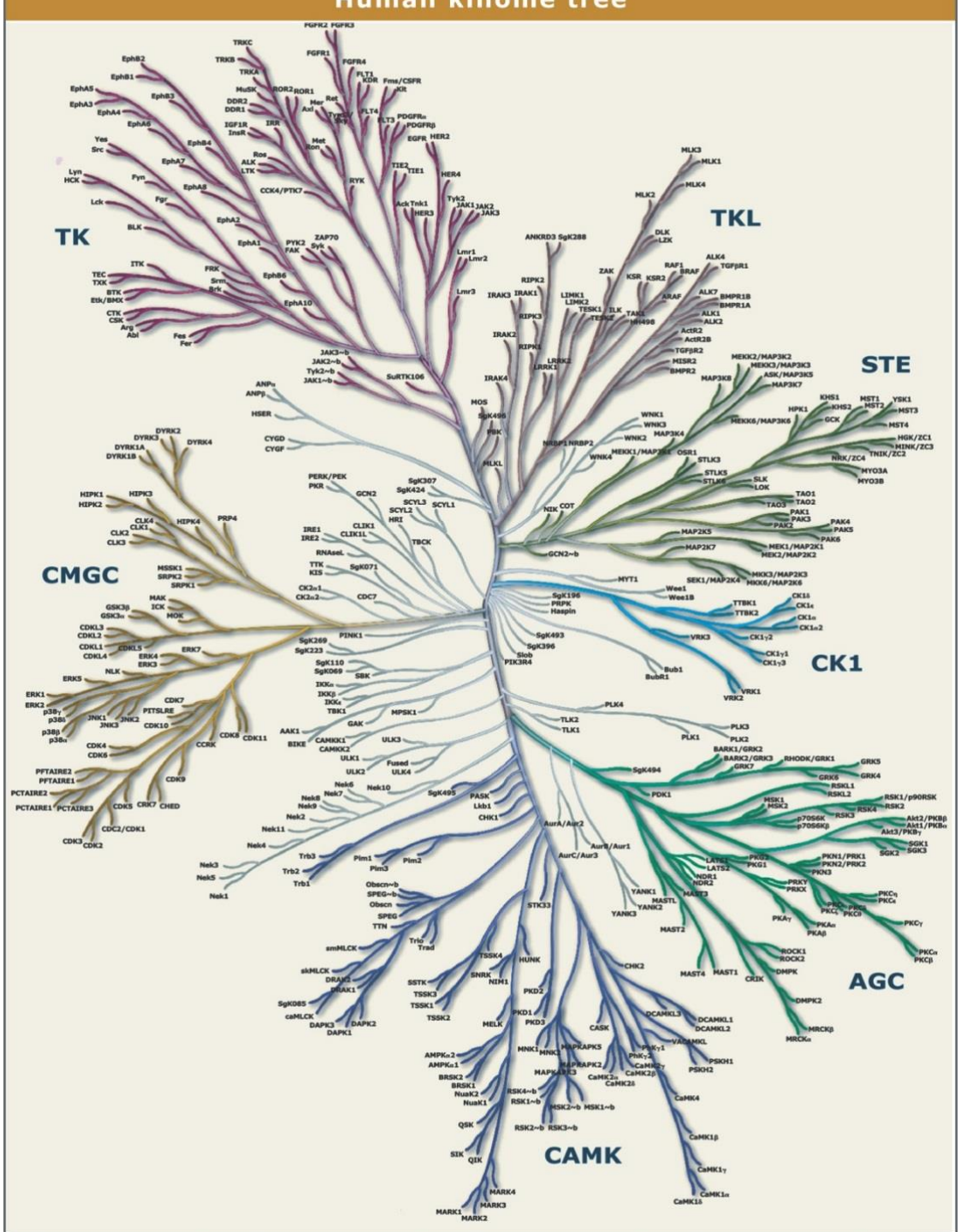
a Phosphorylation



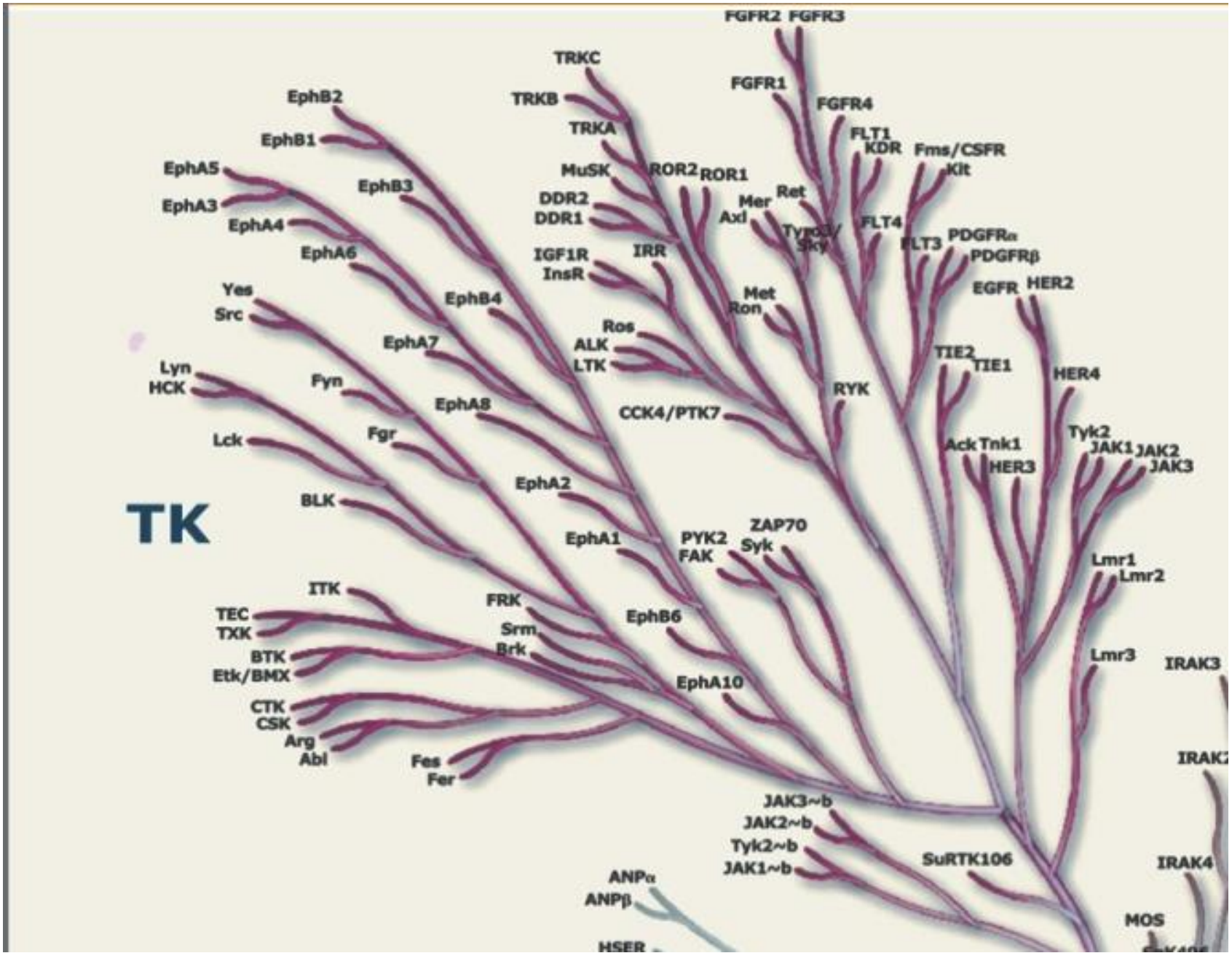


From Cell, Lewin Ed,
Jones & Bartlett publishers

KINOME

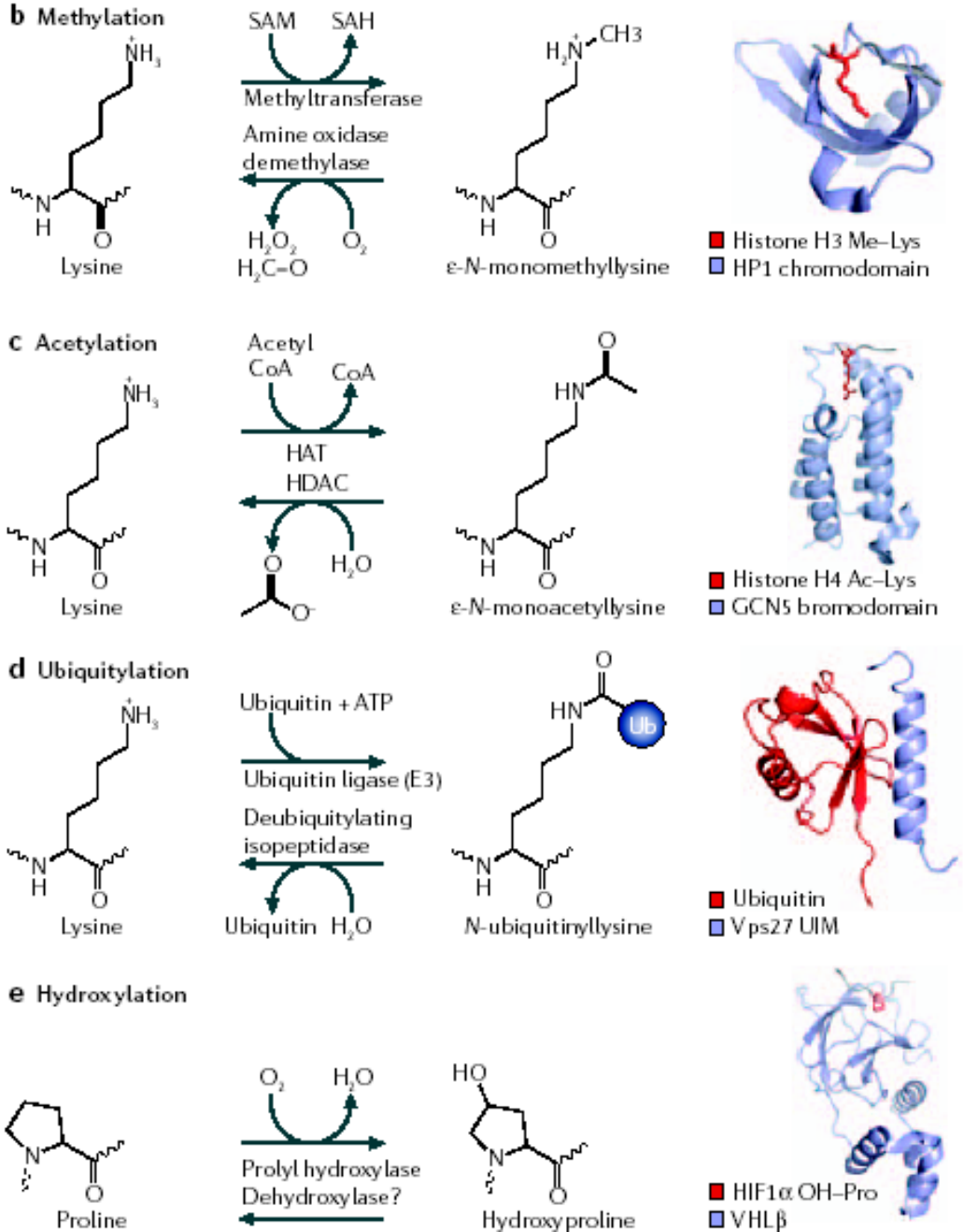


The tyrosine kinases represent just 1 branch



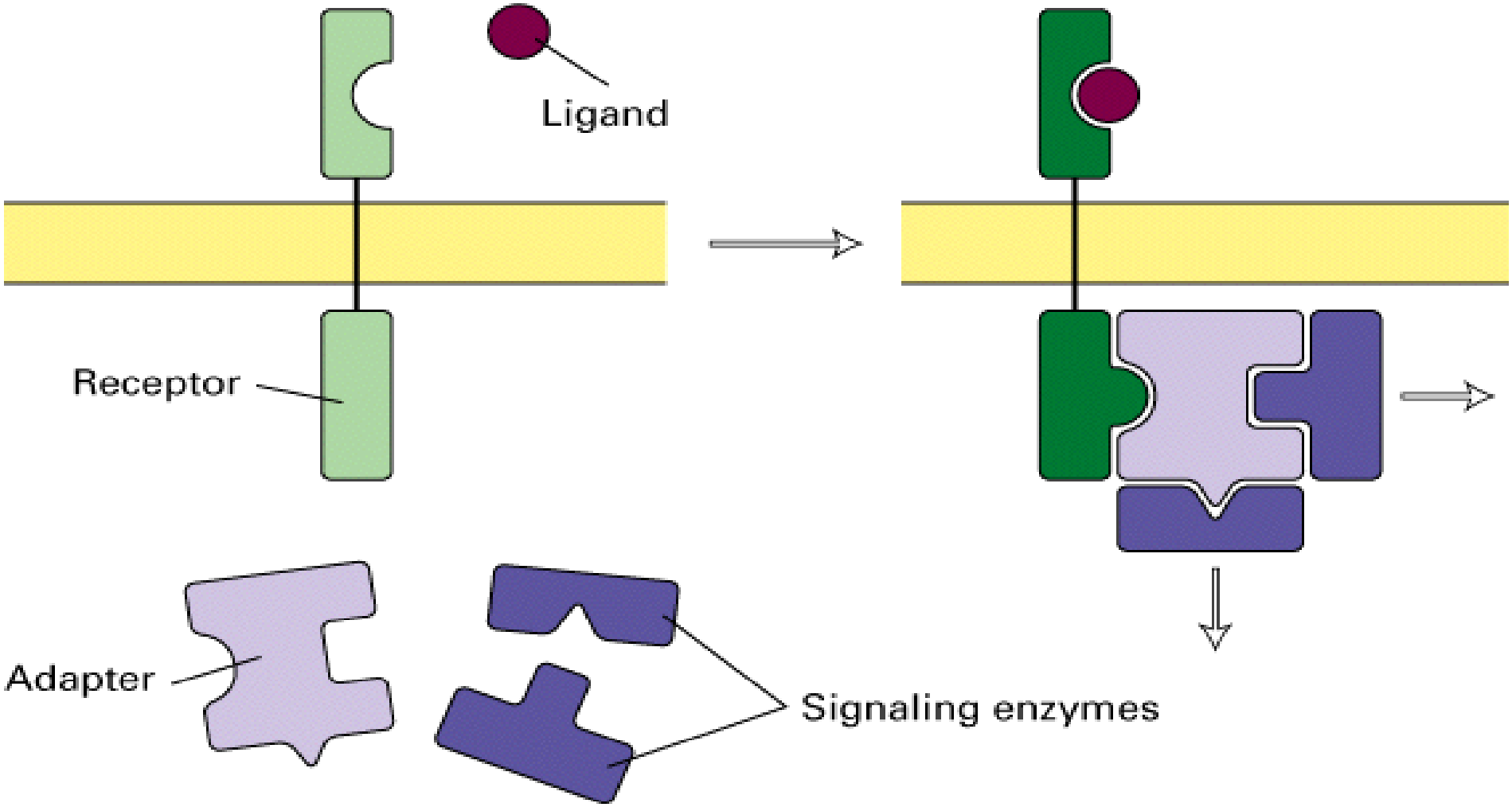
Other post-translational modifications

- Methylation
- Acetylation
- Ubiquitylation & sumoylation
- Hydroxylation



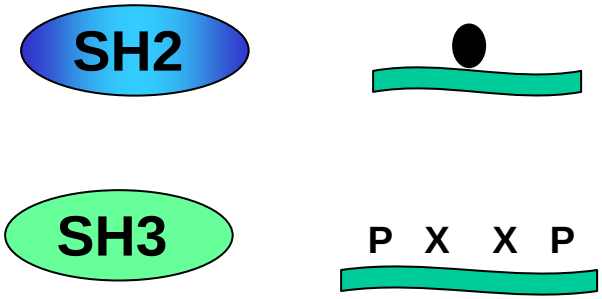
BASIC MECHANISMS

Macromolecular Assembly Using "Motifs"



Motifs Bind to Other Motifs in Proteins. WHAT?!

	Stucture	Counterstructure
Src homology 2-domain	SH2	Phospho-tyrosine
Src homology 3-domain	SH3	P X X P Proline-rich stretch

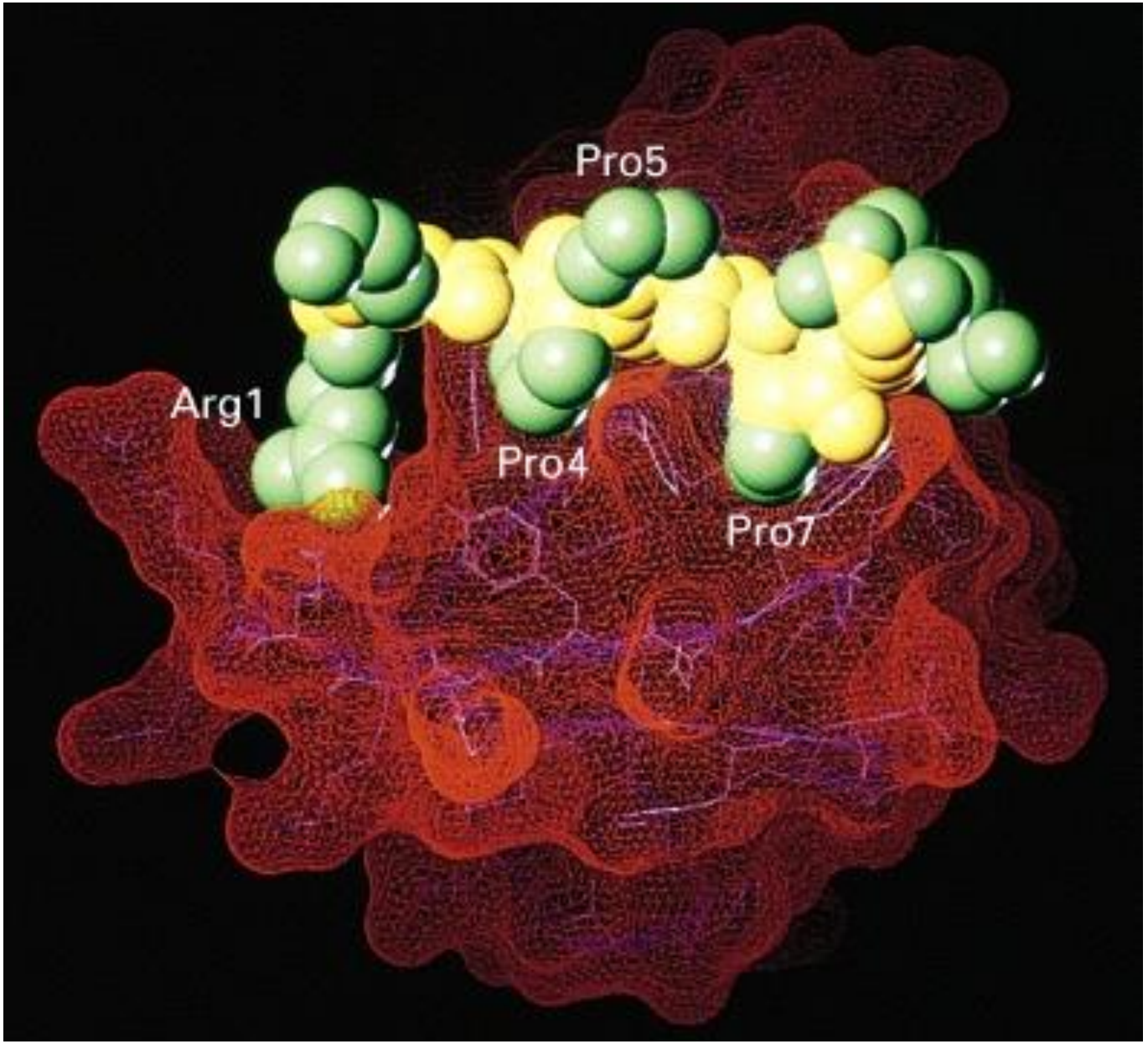


There are dozens, if not hundreds, of other examples.

SH3 domain bound to Proline-rich peptide

Peptide Backbone

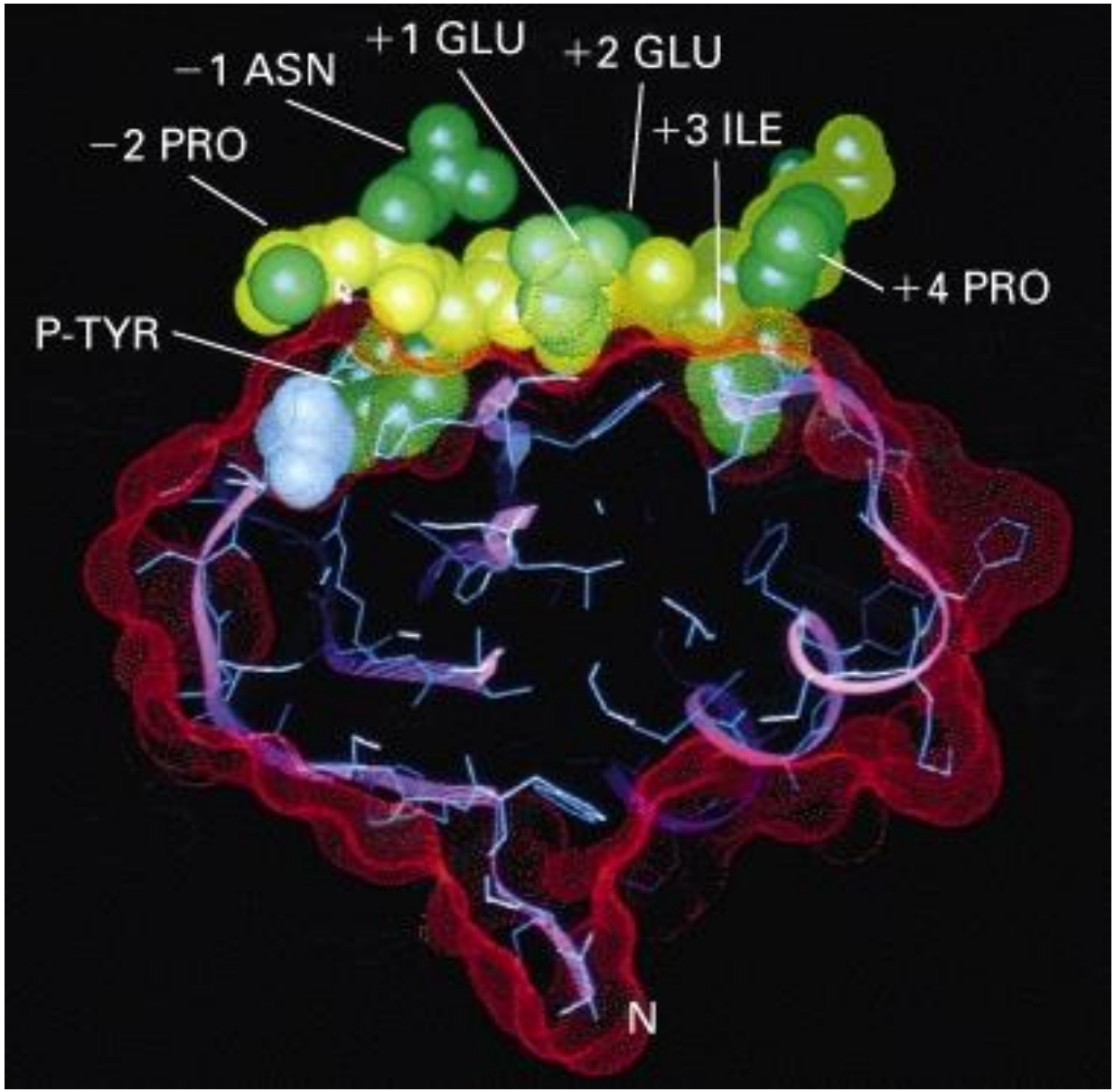
Side chains



SH2 Domain bound to Phospho-Tyr in Peptide

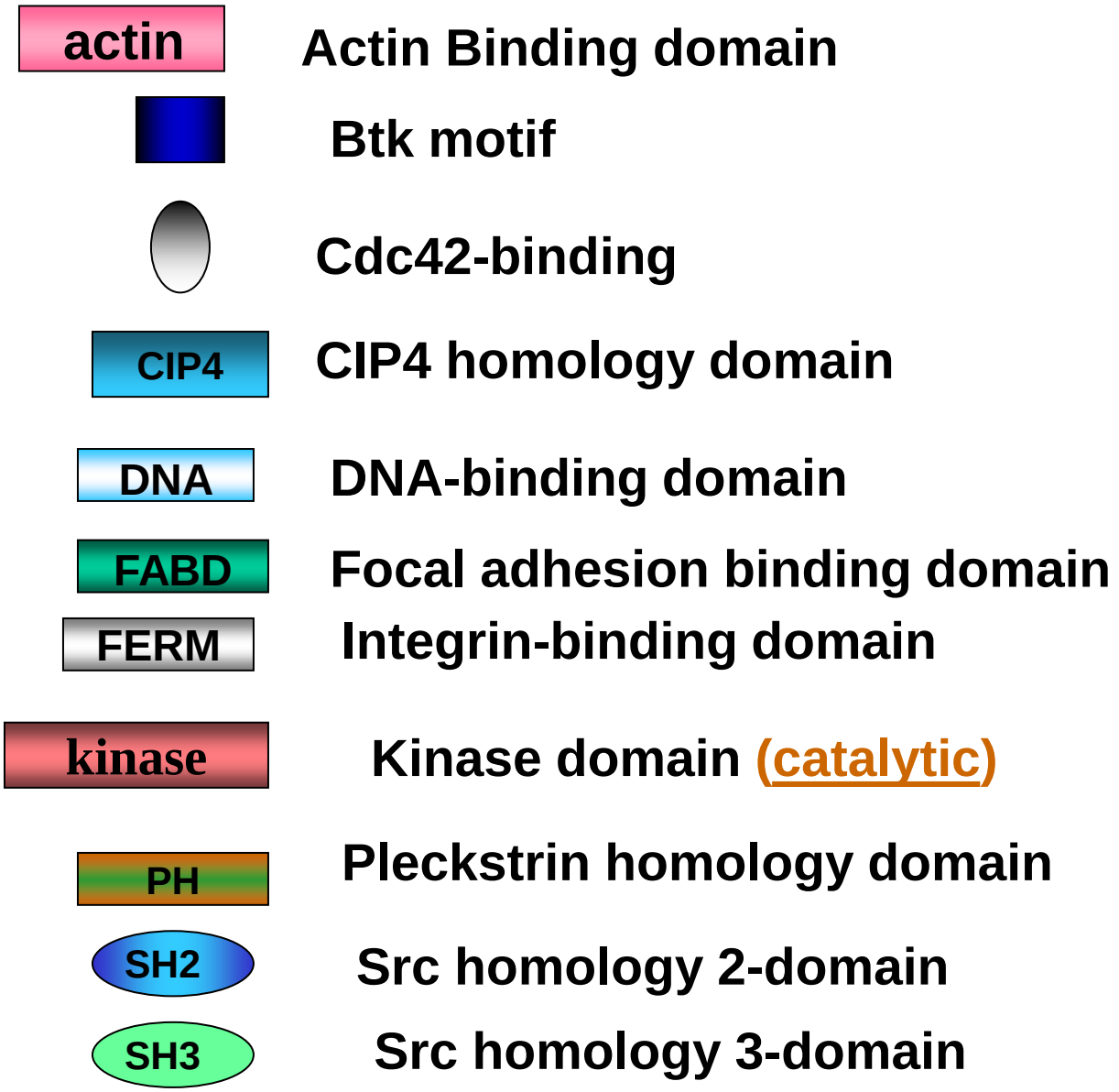
Peptide Backbone

Side chains

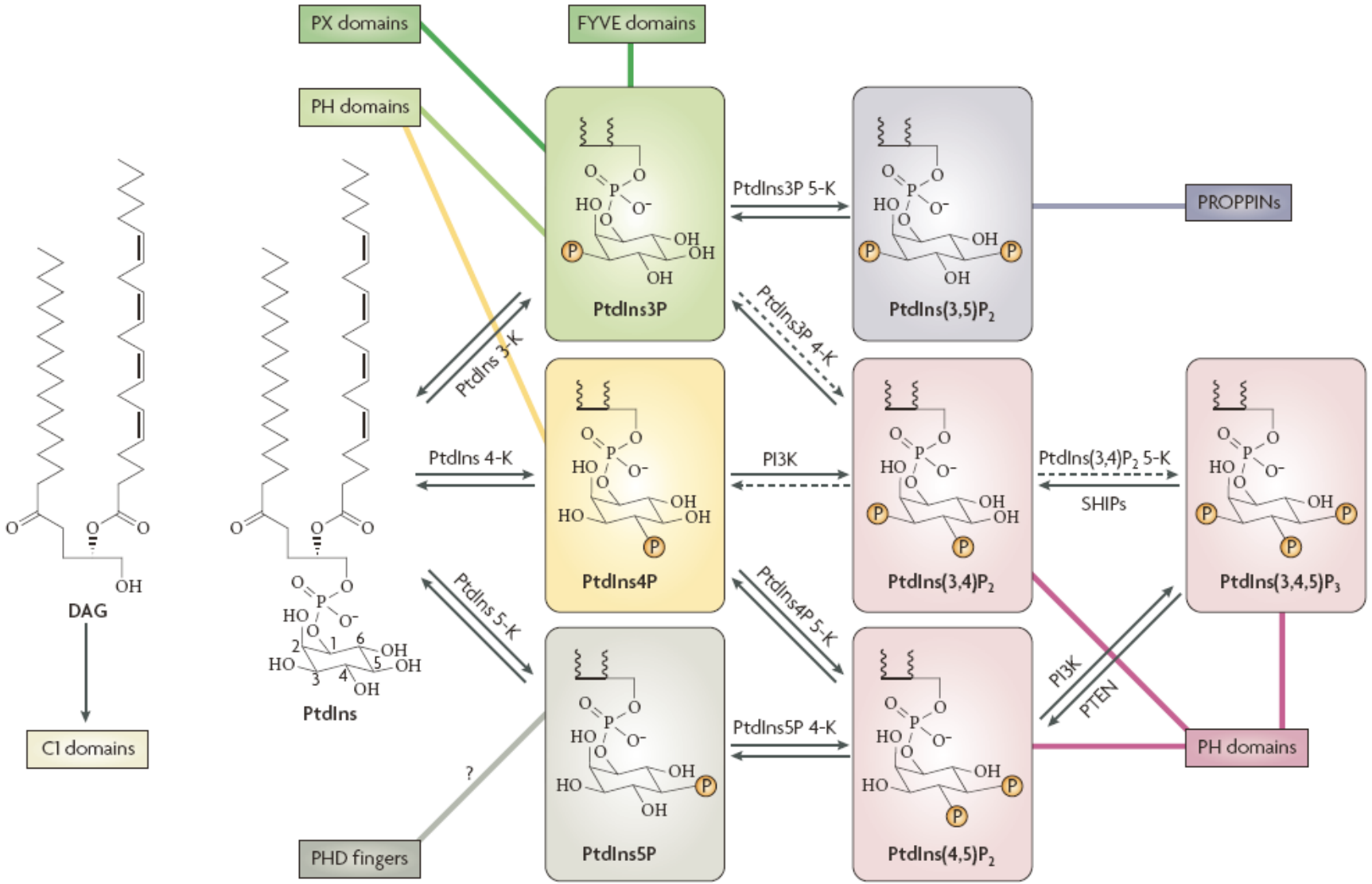


Do you experience MOTIF "Madness"?

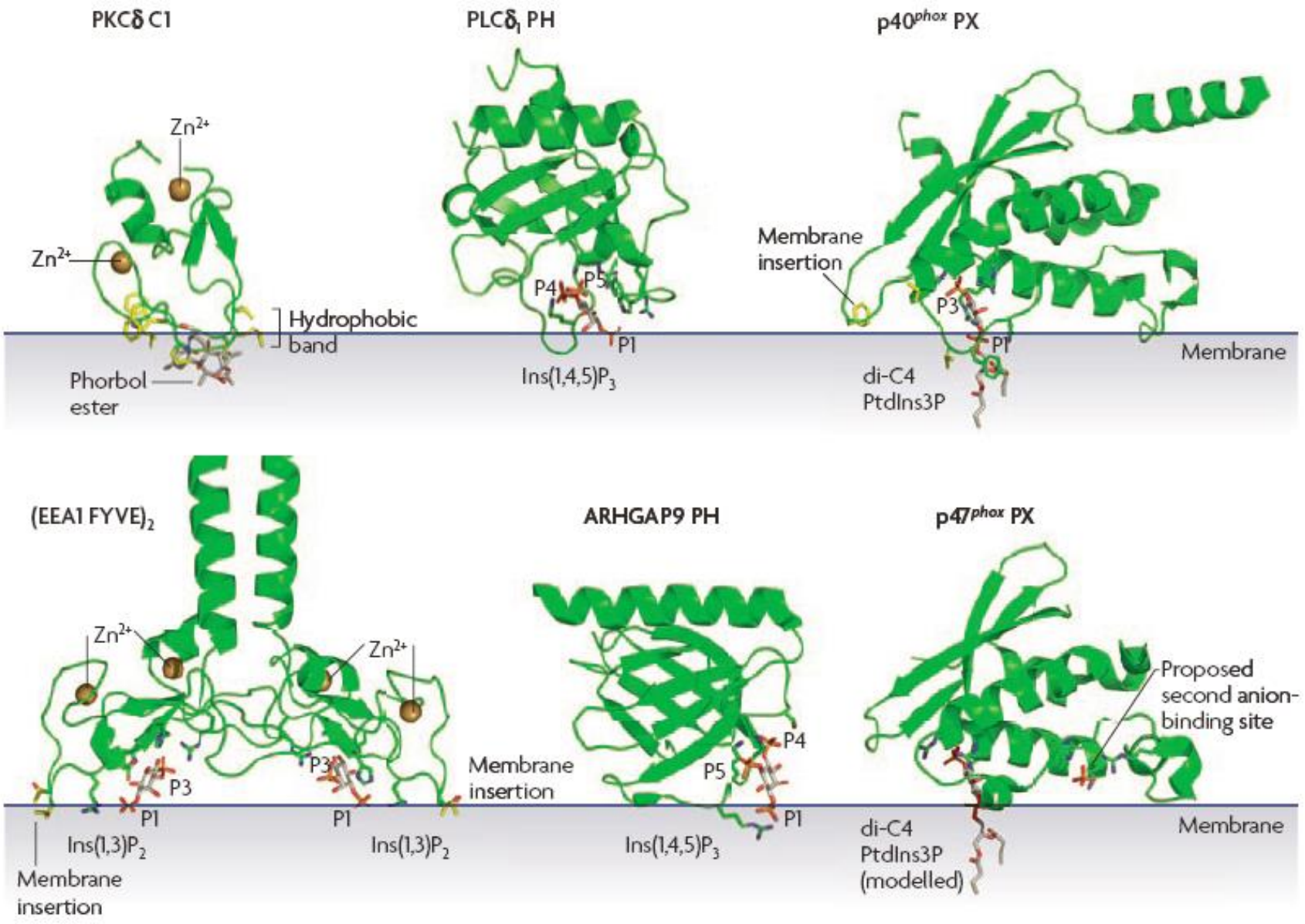
Alphabet Phobia?



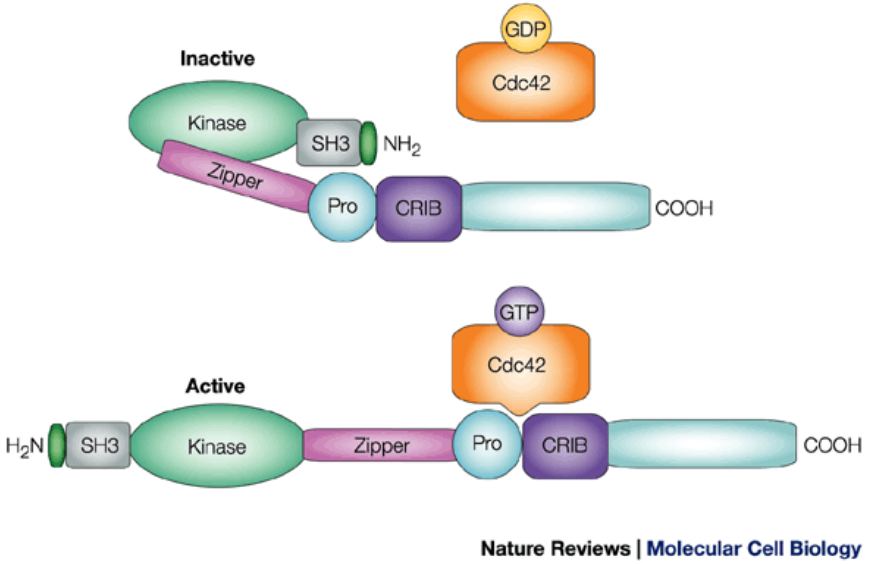
Some Protein Motifs Bind to Features on Lipids. WHAT?!



Structures of target-specific phospholipid binding domains



Signaling proteins often have multiple motifs – these building blocks offer multiple opportunities for macromolecular assembly



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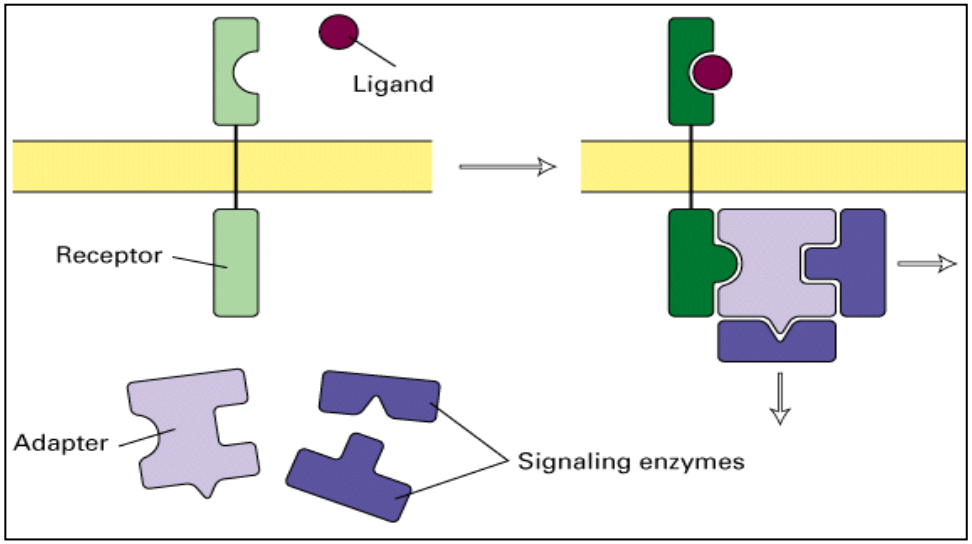
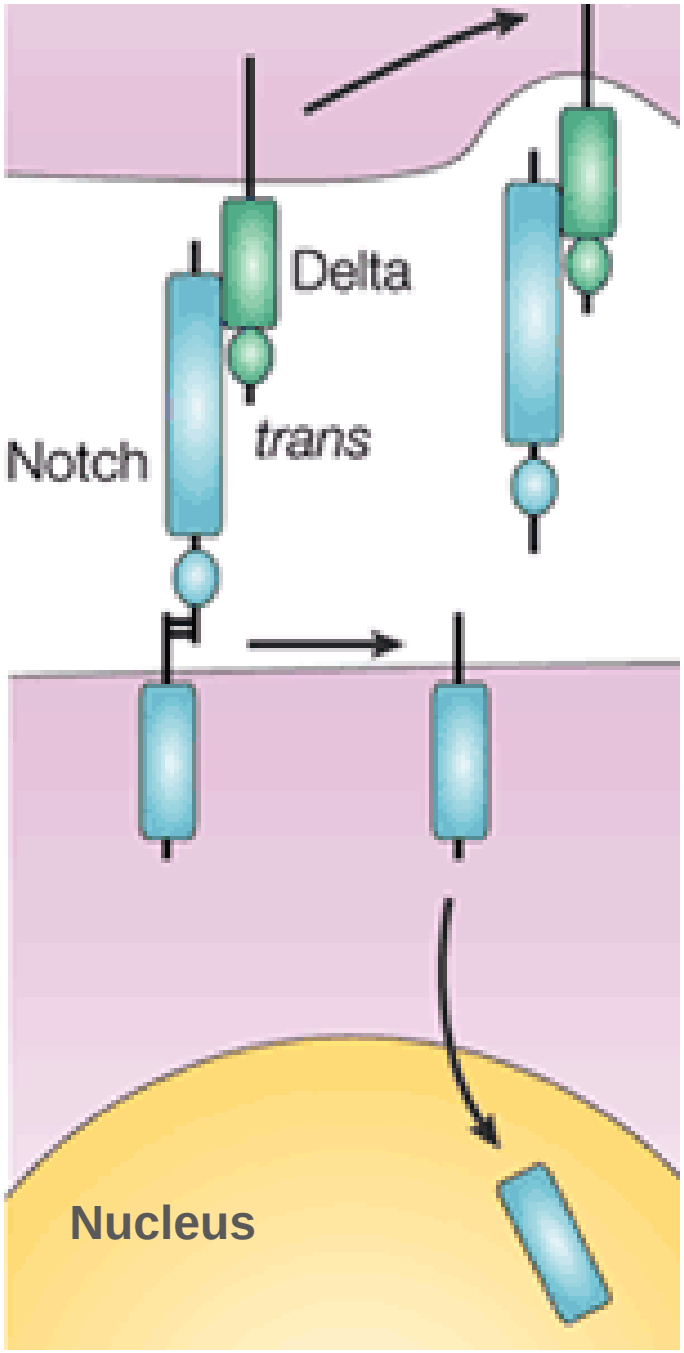


BASIC MECHANISMS

Proteolytic Processing to Generate New Signaling Moiety …

Example: Notch

In this scheme, new synthesis must occur to restore competency to previous levels.



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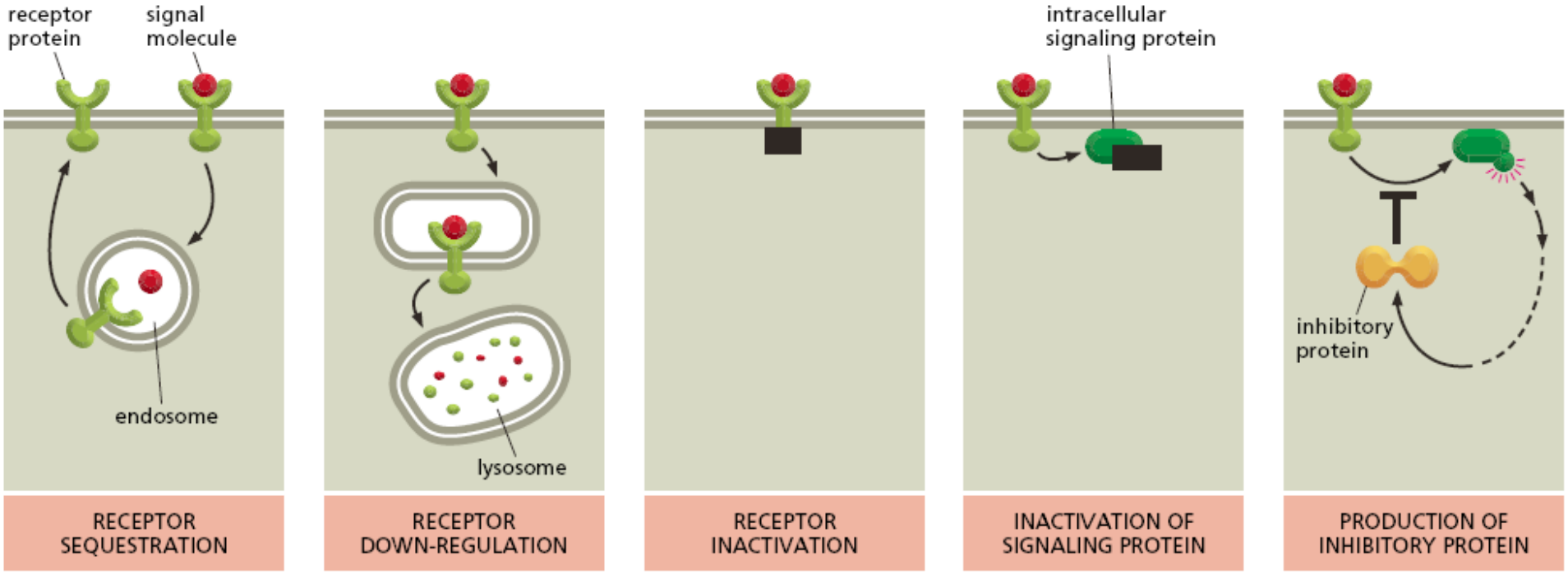
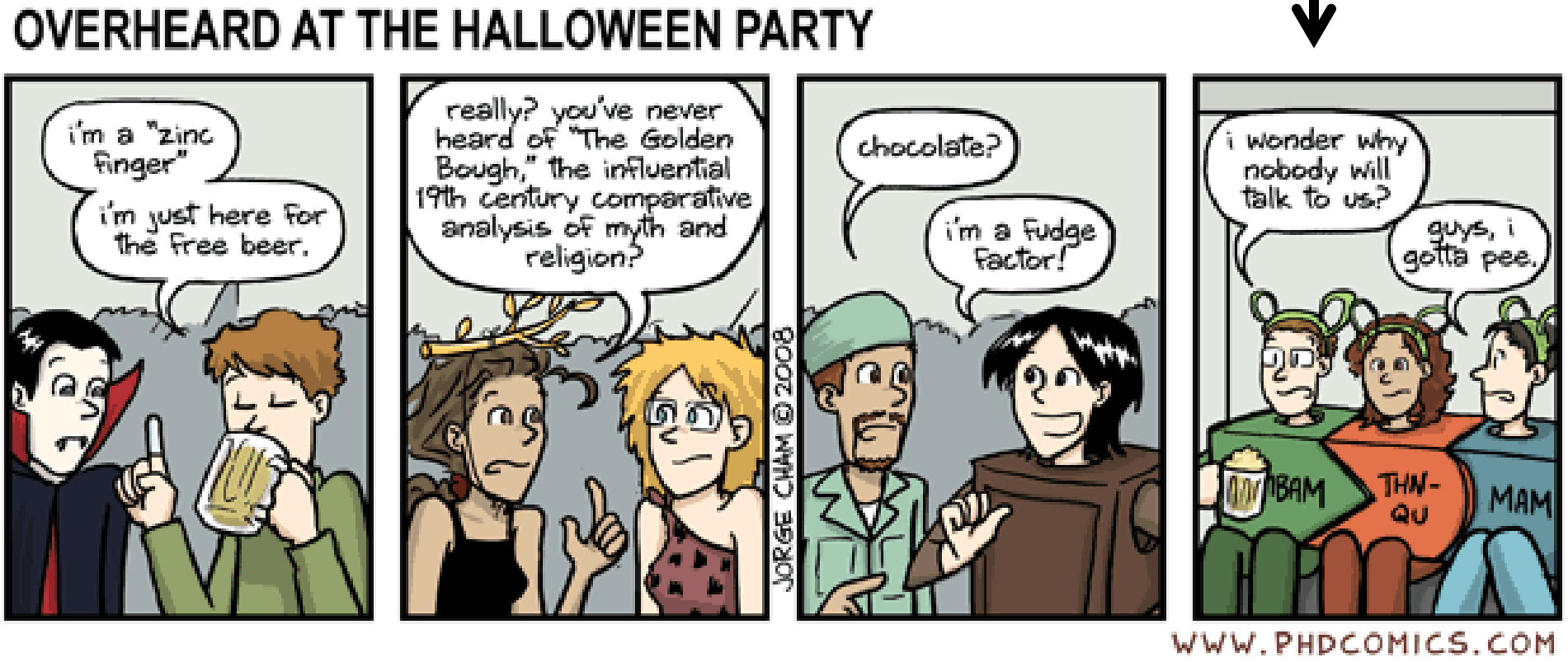
BASIC MECHANISMS

Most signaling events are tightly controlled.

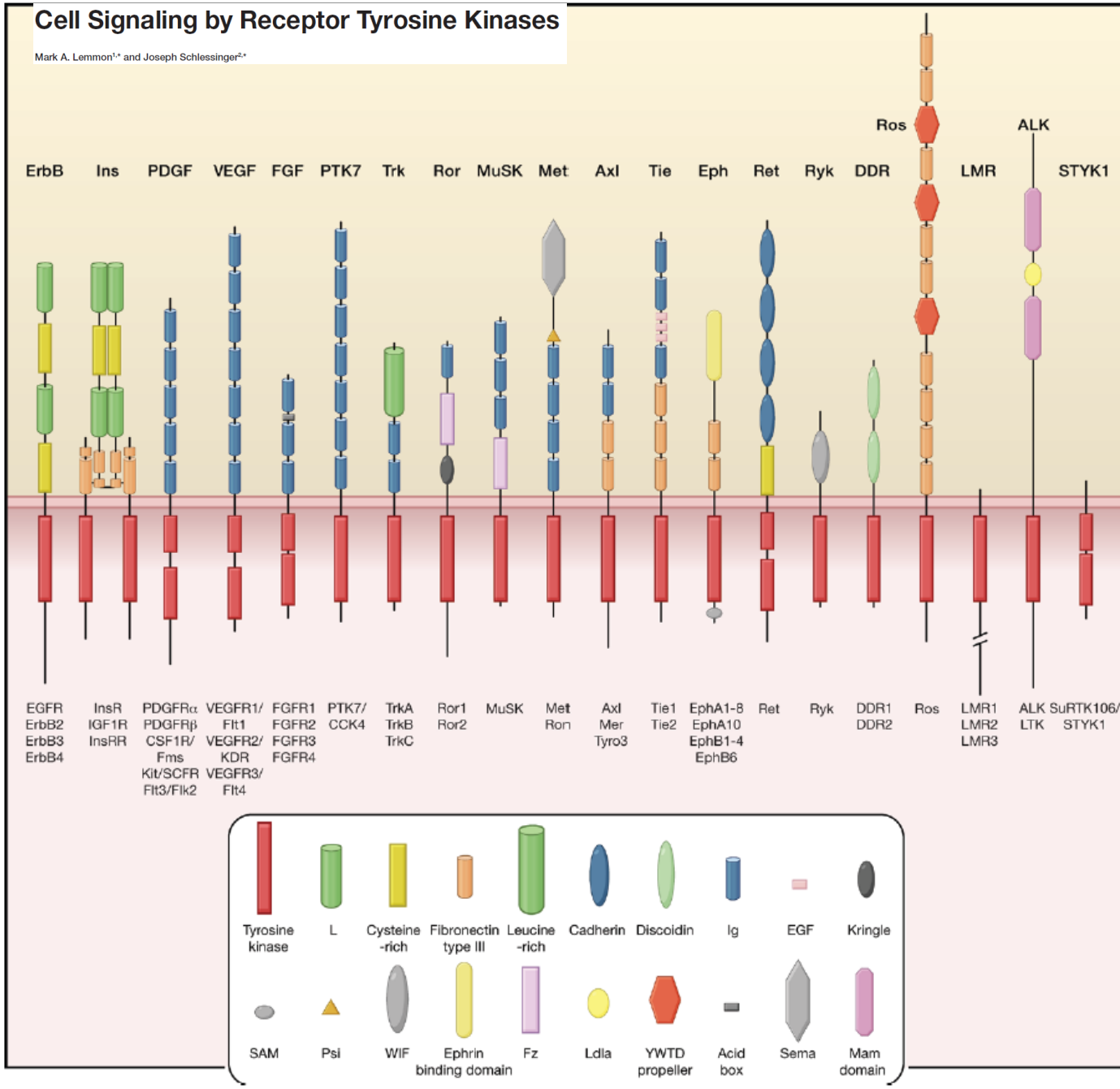


5 min Stretch Break

These former q-bio students have “Motif Madness”

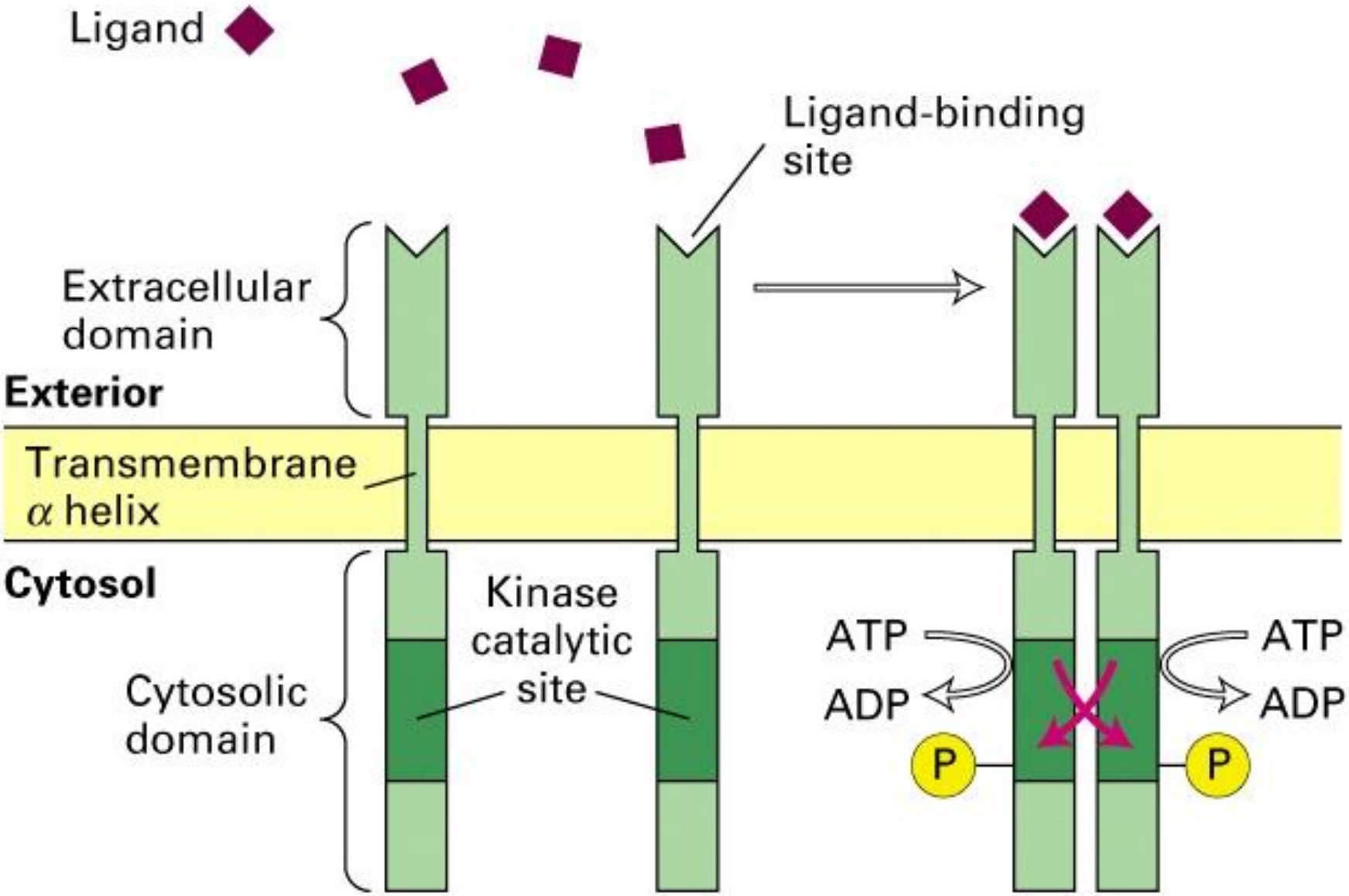


Example: Receptors with Intrinsic Tyrosine Kinase Activity.

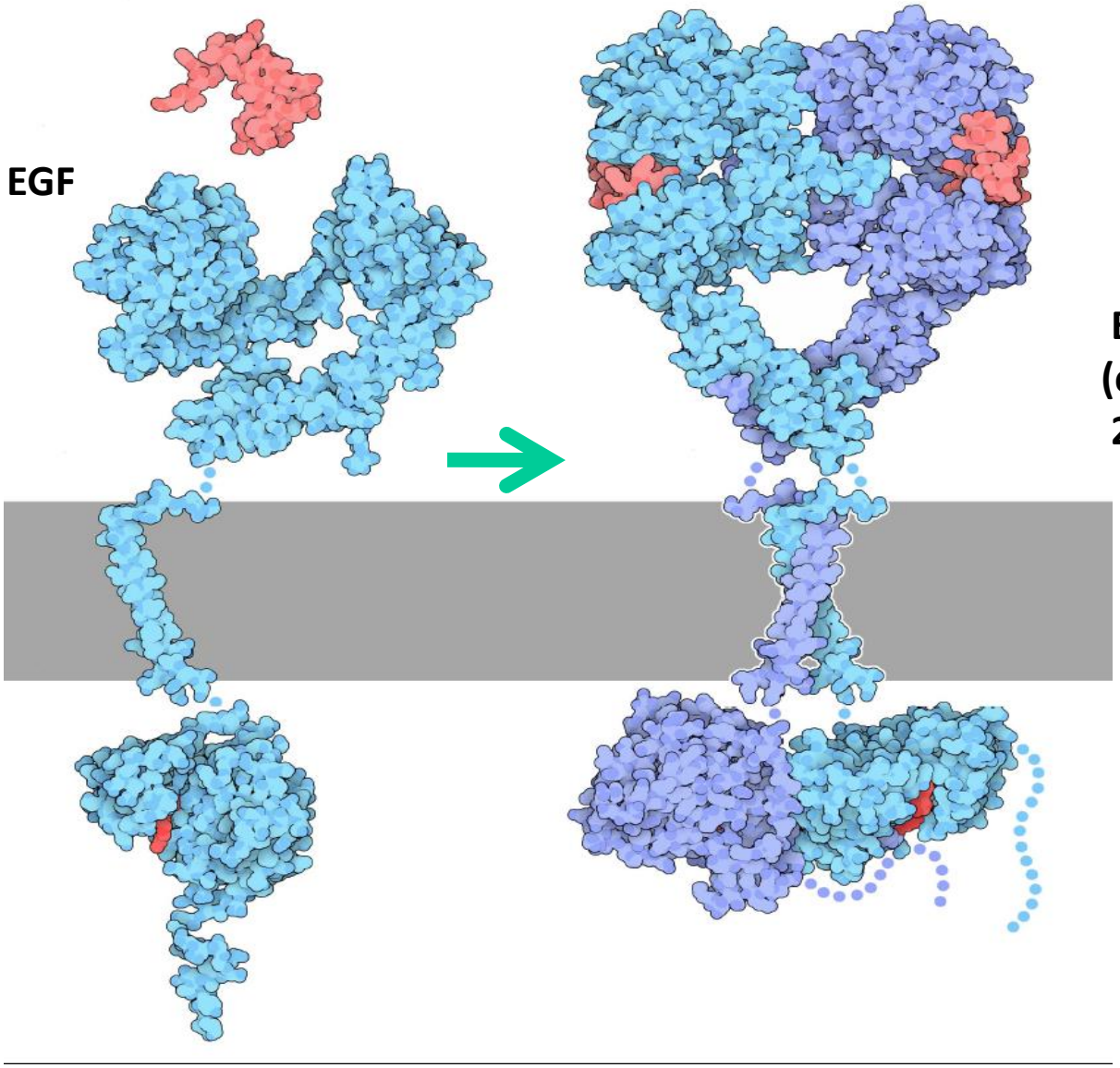


Motif Madness

Cell *141*, June 25, 2010 ©2010 Elsevier Inc.


Ligand
Ligand-binding site
Extracellular domain
Exterior
Transmembrane α helix
Cytosol
Kinase catalytic site
Cytosolic domain
ATP
ADP
ATP
ADP
P
P

More than a Cartoon – structural look at EGFR homodimer

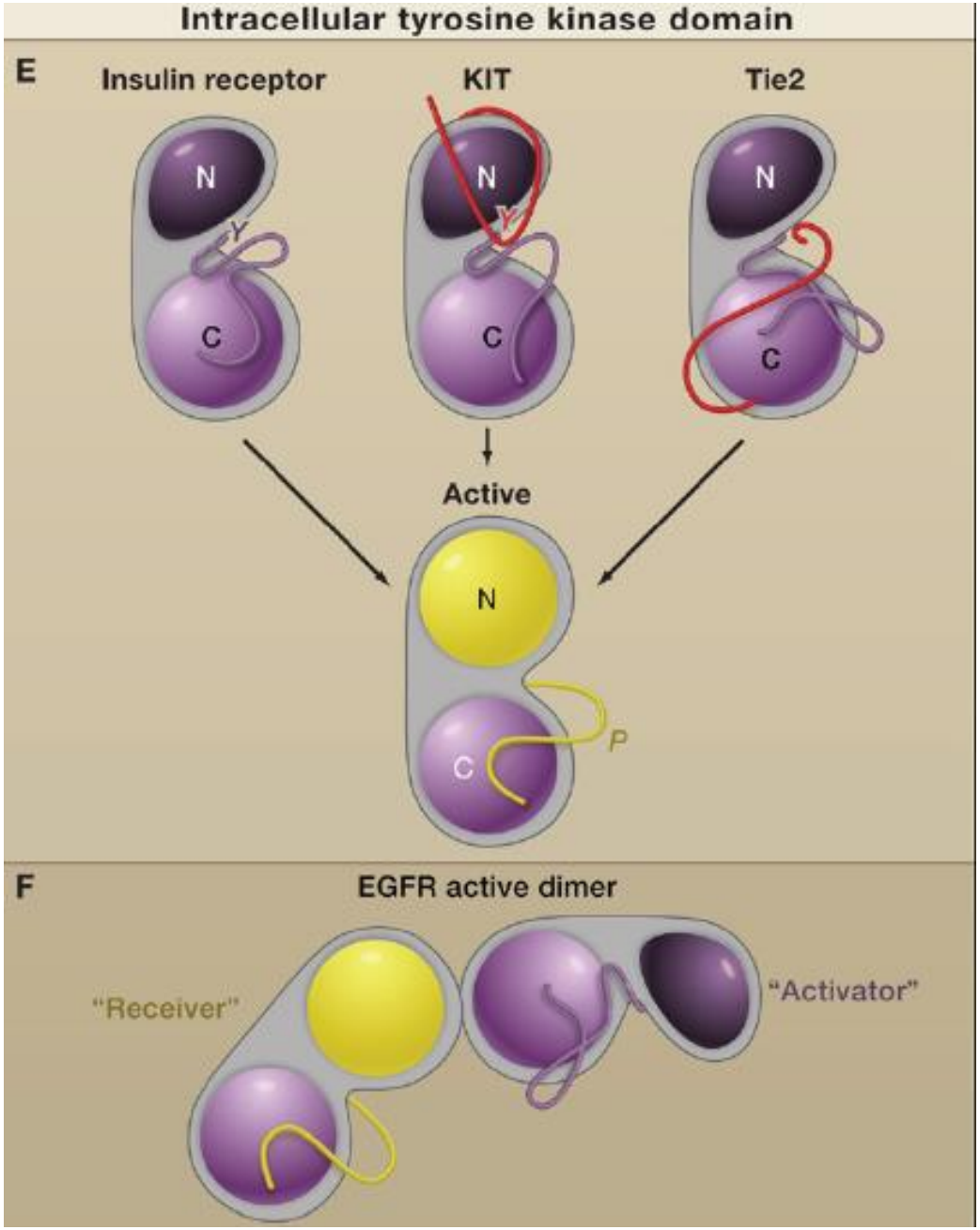


RCSB PDB
PROTEIN DATA BANK

Variation is not just "outside."

Dimerization leads to kinase activation by different mechanisms:

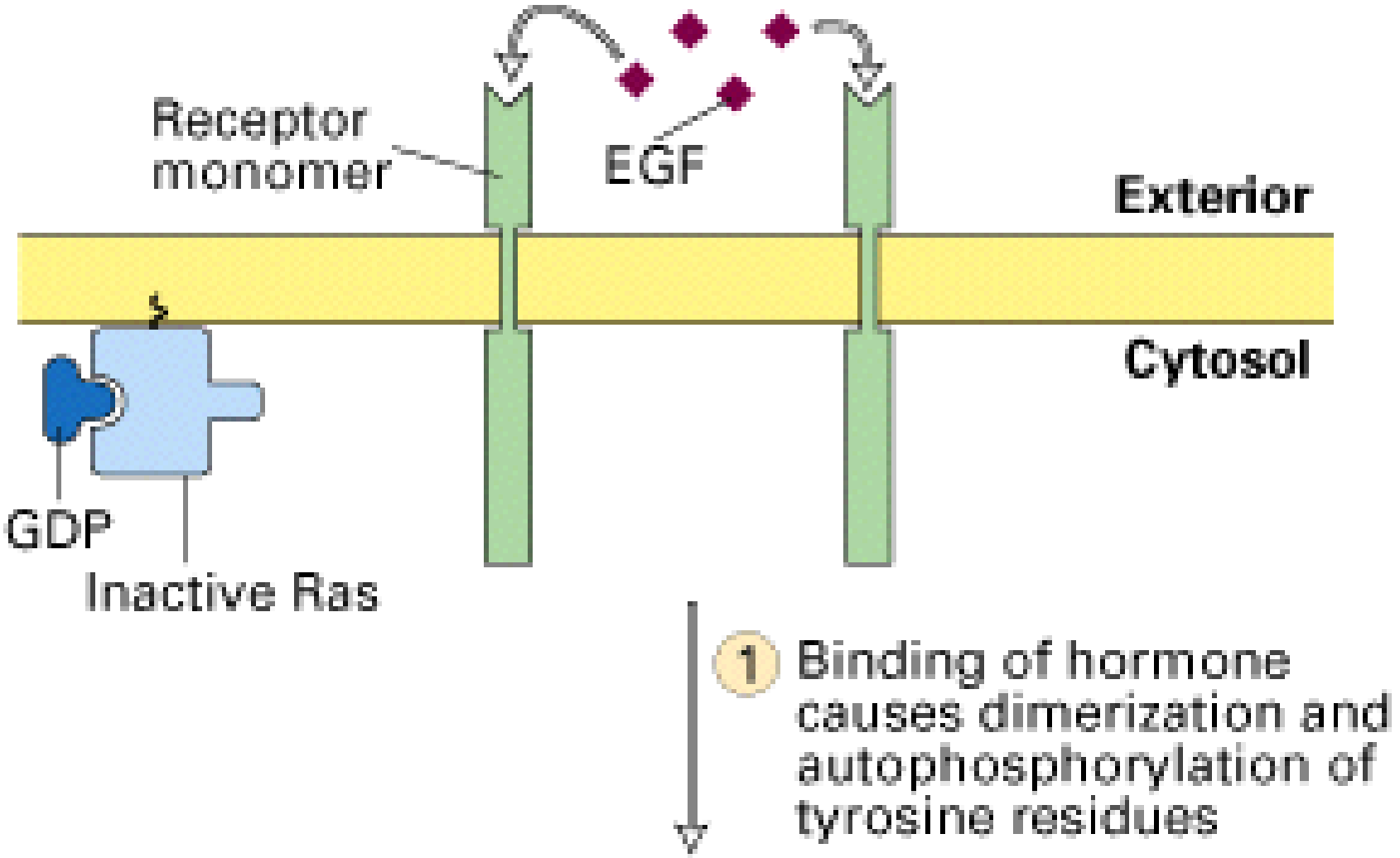
- **Displacing regulatory "loops" that auto-inhibit activity**
- **Allosteric, asymmetric interactions (N-lobe of 1 partner activates C lobe of other partner)**



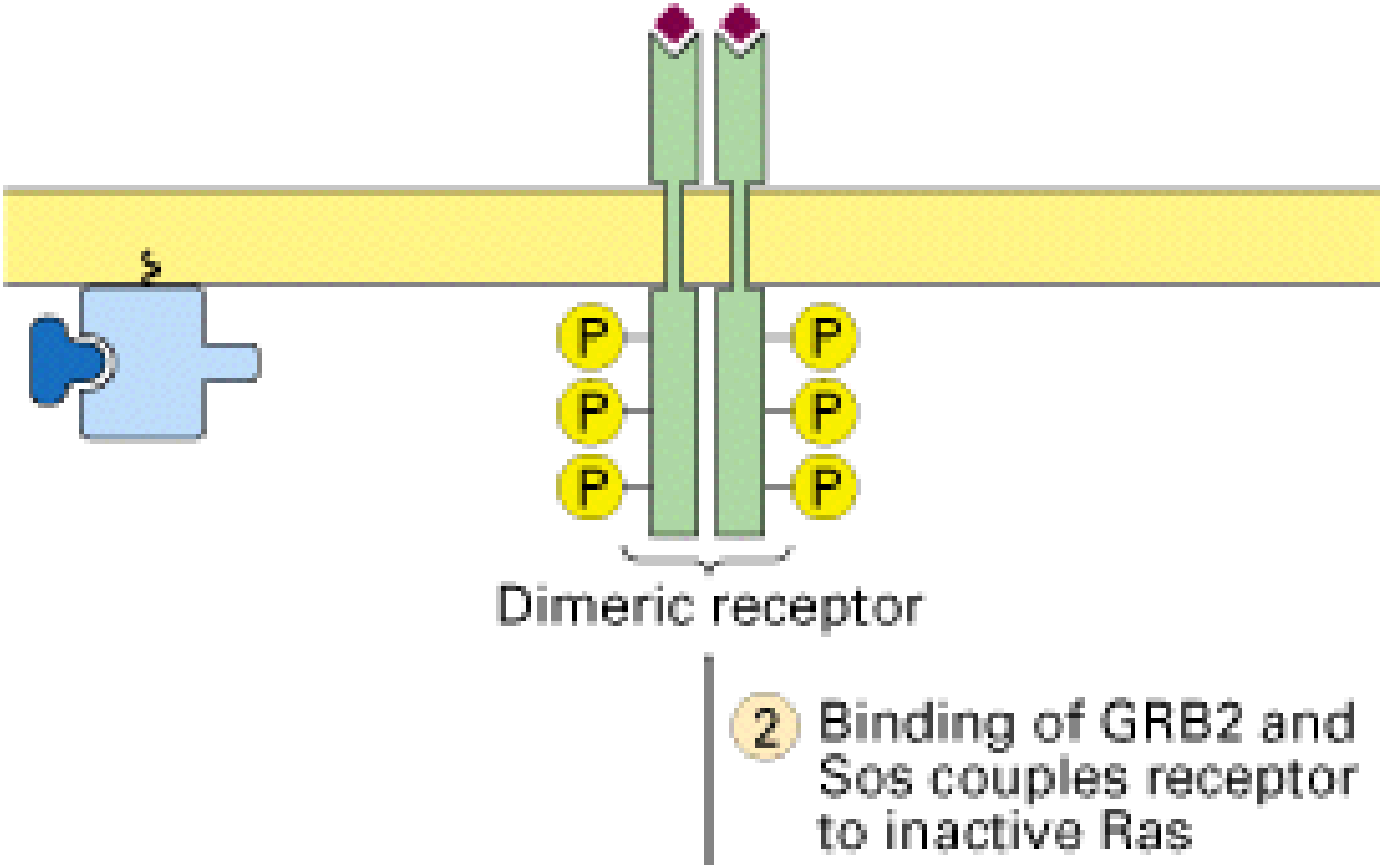
Cell Signaling by Receptor Tyrosine Kinases

Mark A. Lemmon[1,*] and Joseph Schlessinger[2,*]

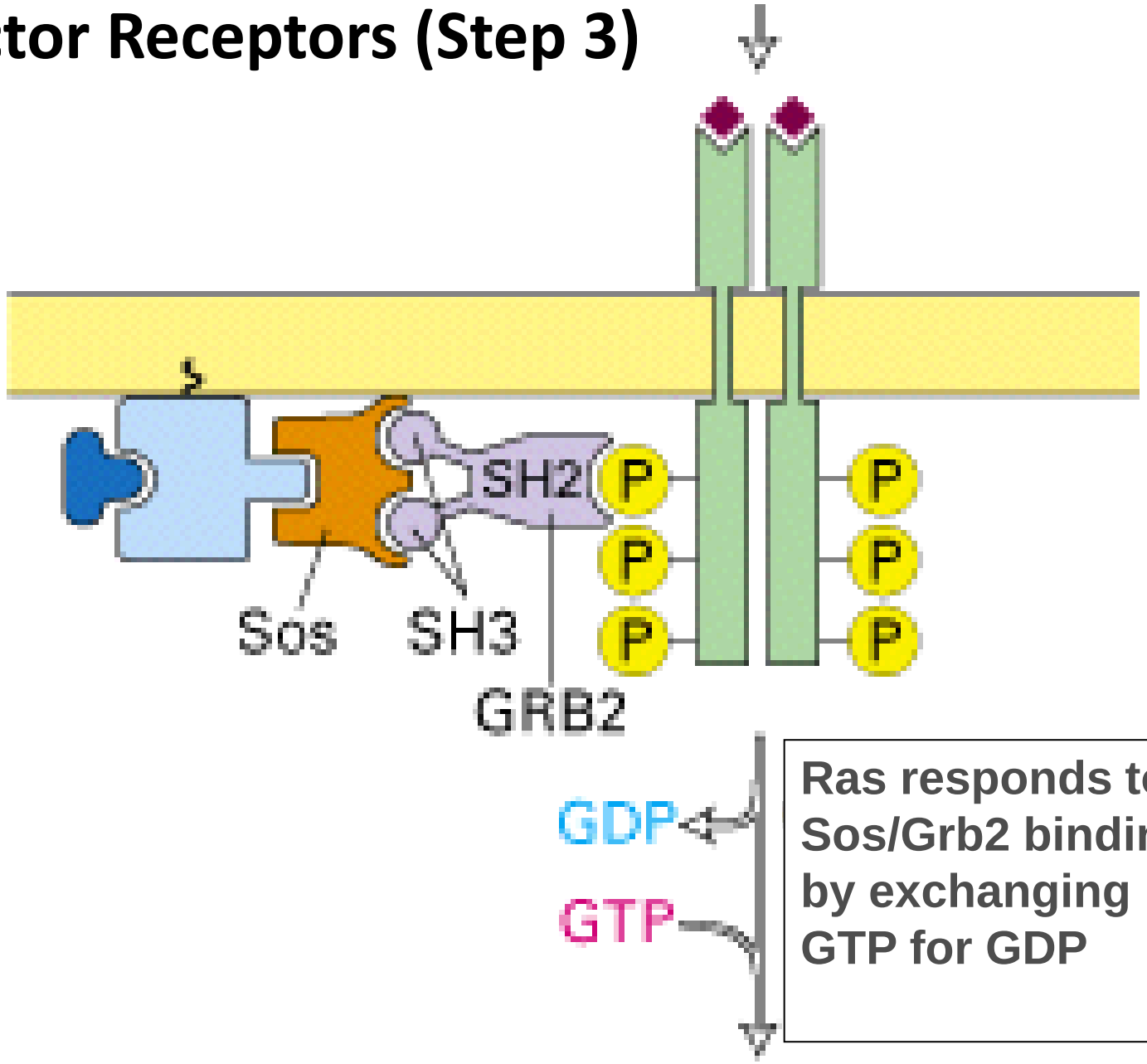
Activation of Ras By Growth Factor Receptors (Step 1)



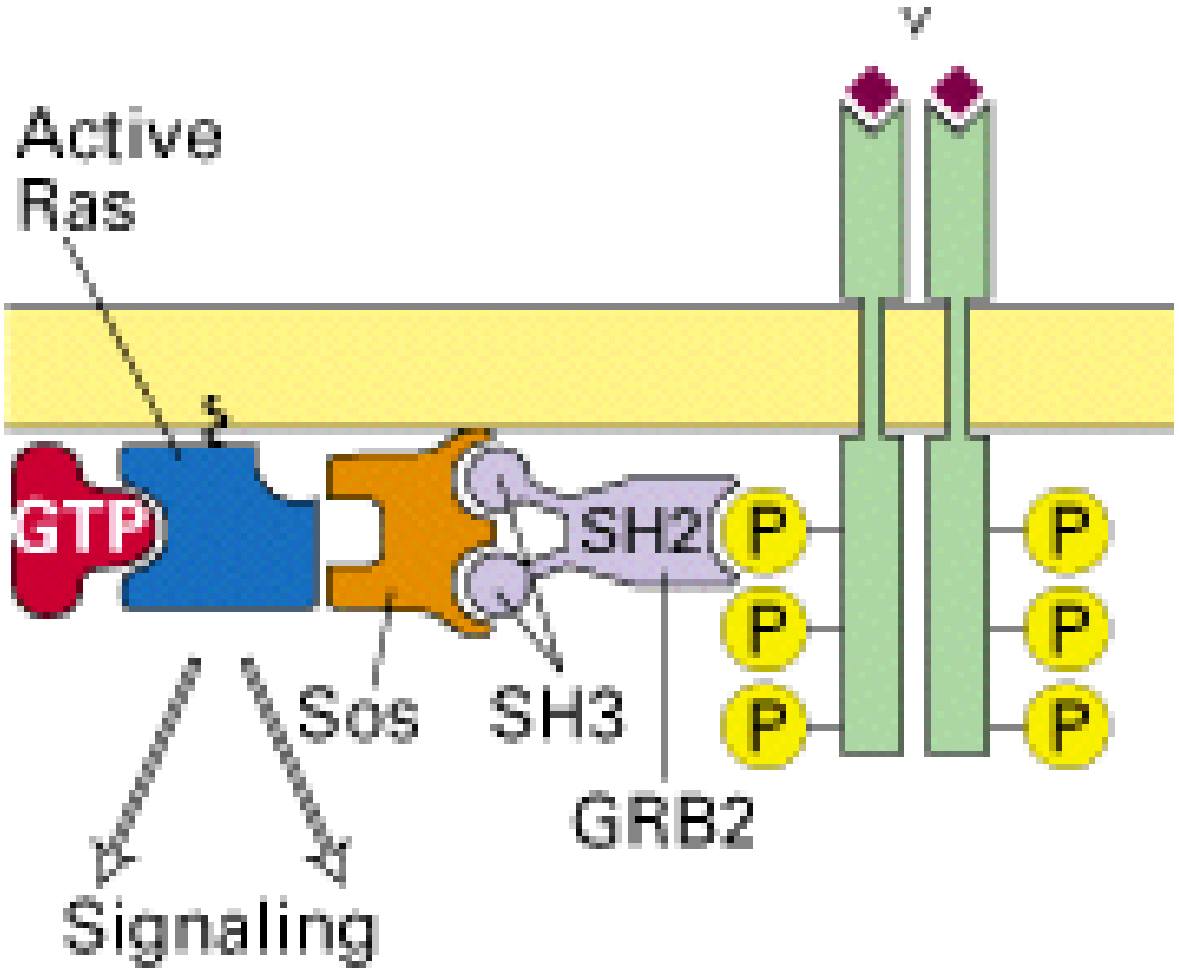
Activation of Ras By Growth Factor Receptors (Step 2)



Activation of Ras By Growth Factor Receptors (Step 3)



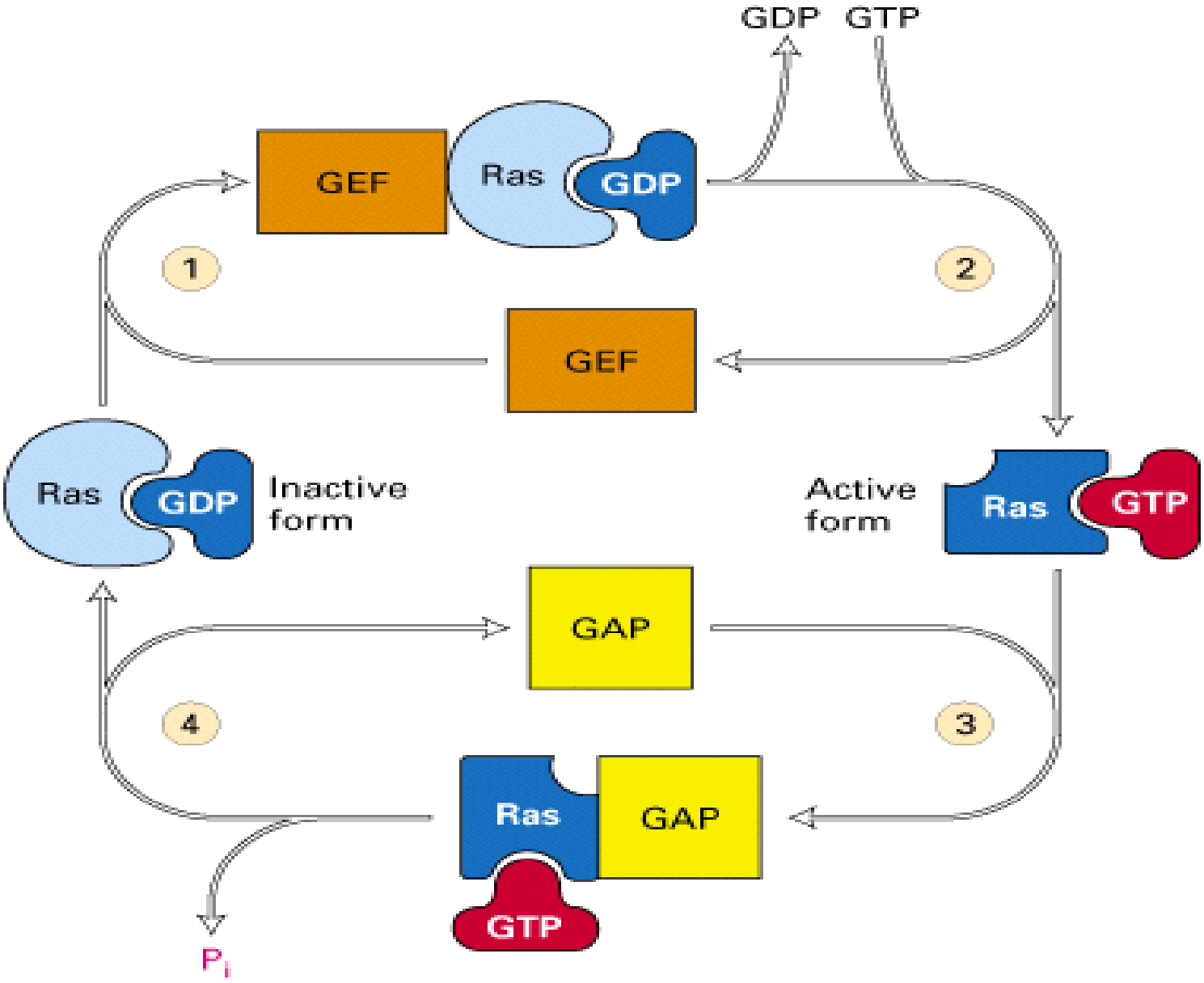
Activation of Ras By Growth Factor Receptors (overview)



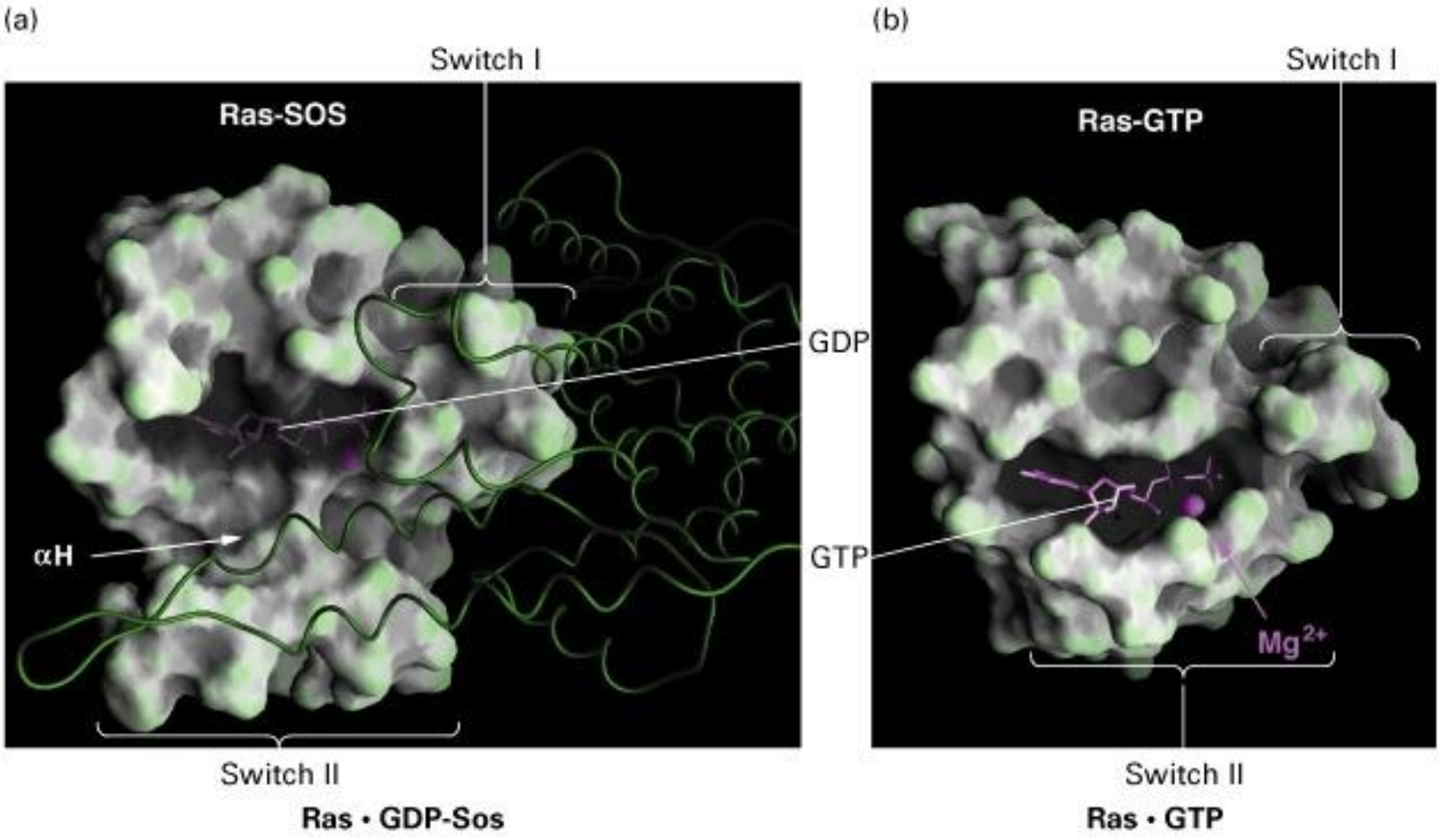
<u>Use of 3 elements of signaling</u>

1. **Phosphorylation**
2. **Macromolecular Assembly**
3. **GTP "switch"**

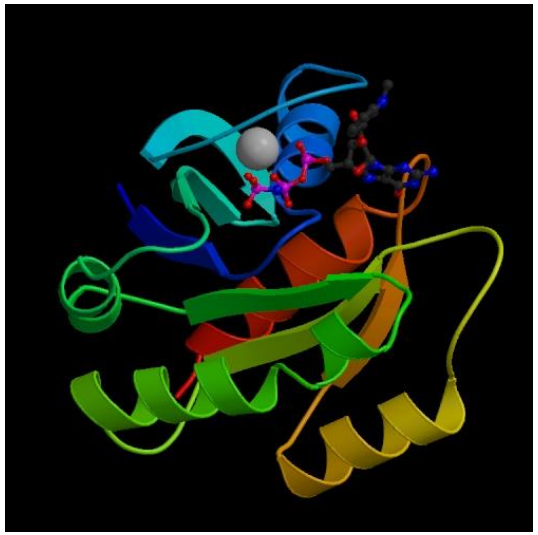
The Complex Ras Cycle



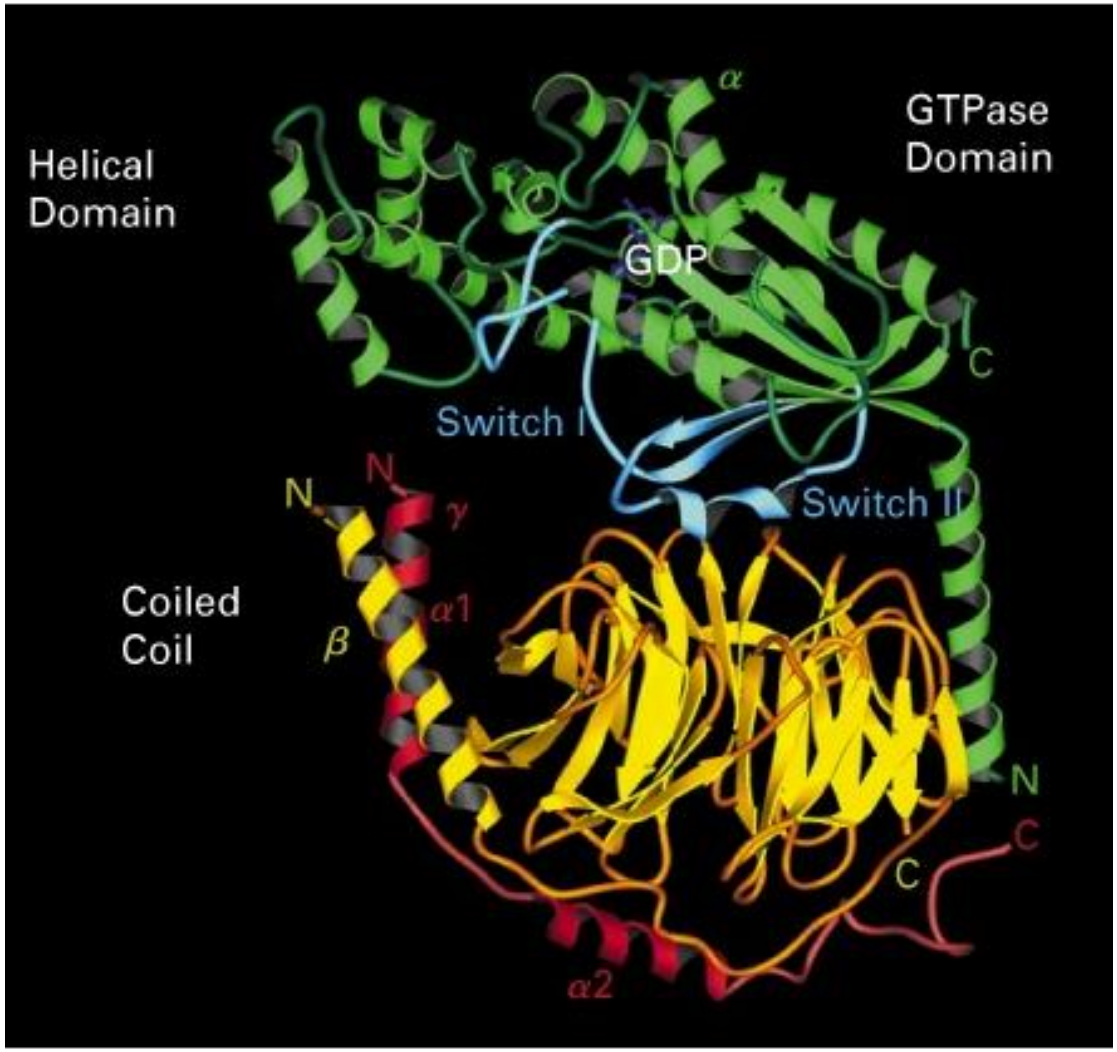
The conformational change is easy to spot.



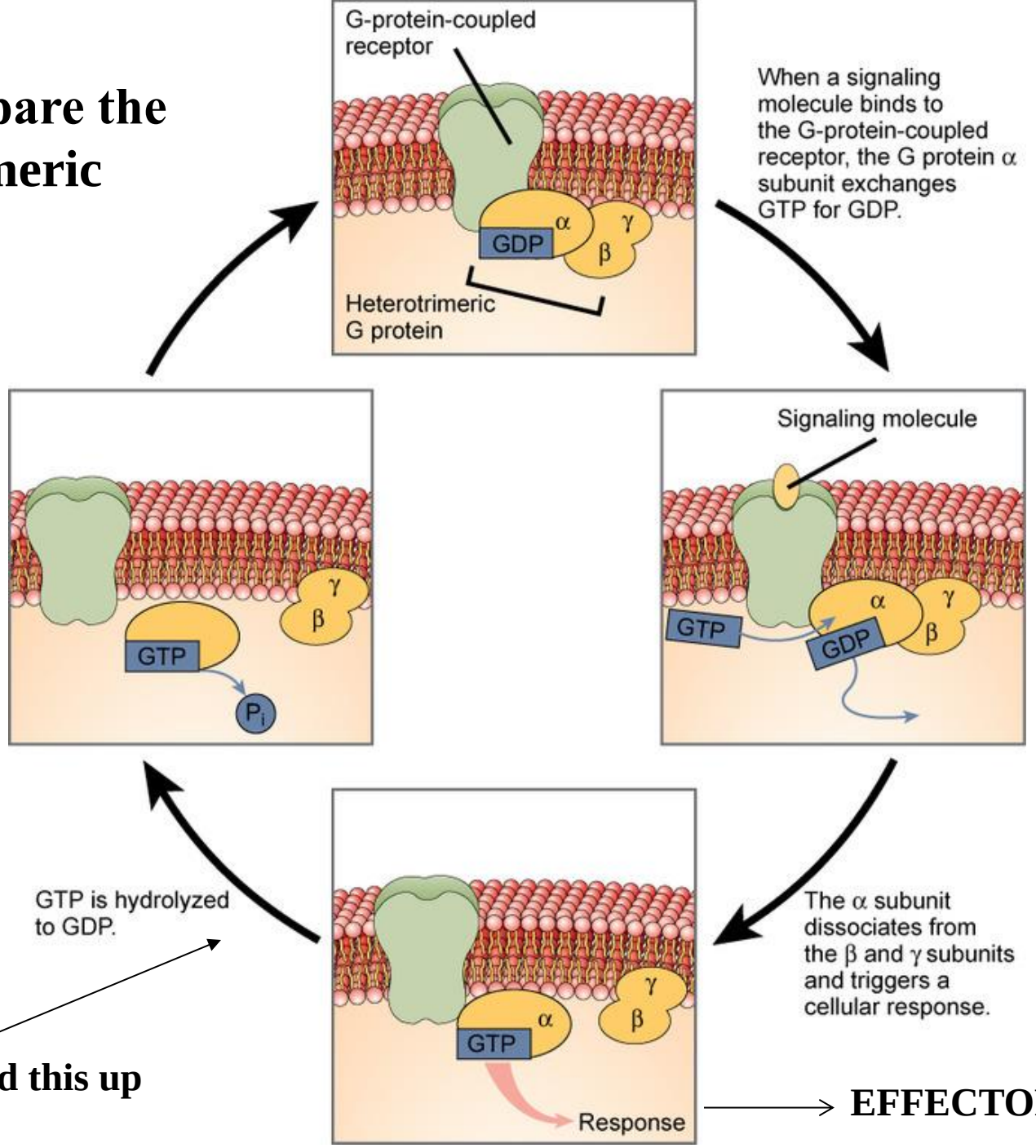
Ras (“little” G)



heterotrimeric G (“big” G)



Let's compare the Heterotrimeric Cycle ….

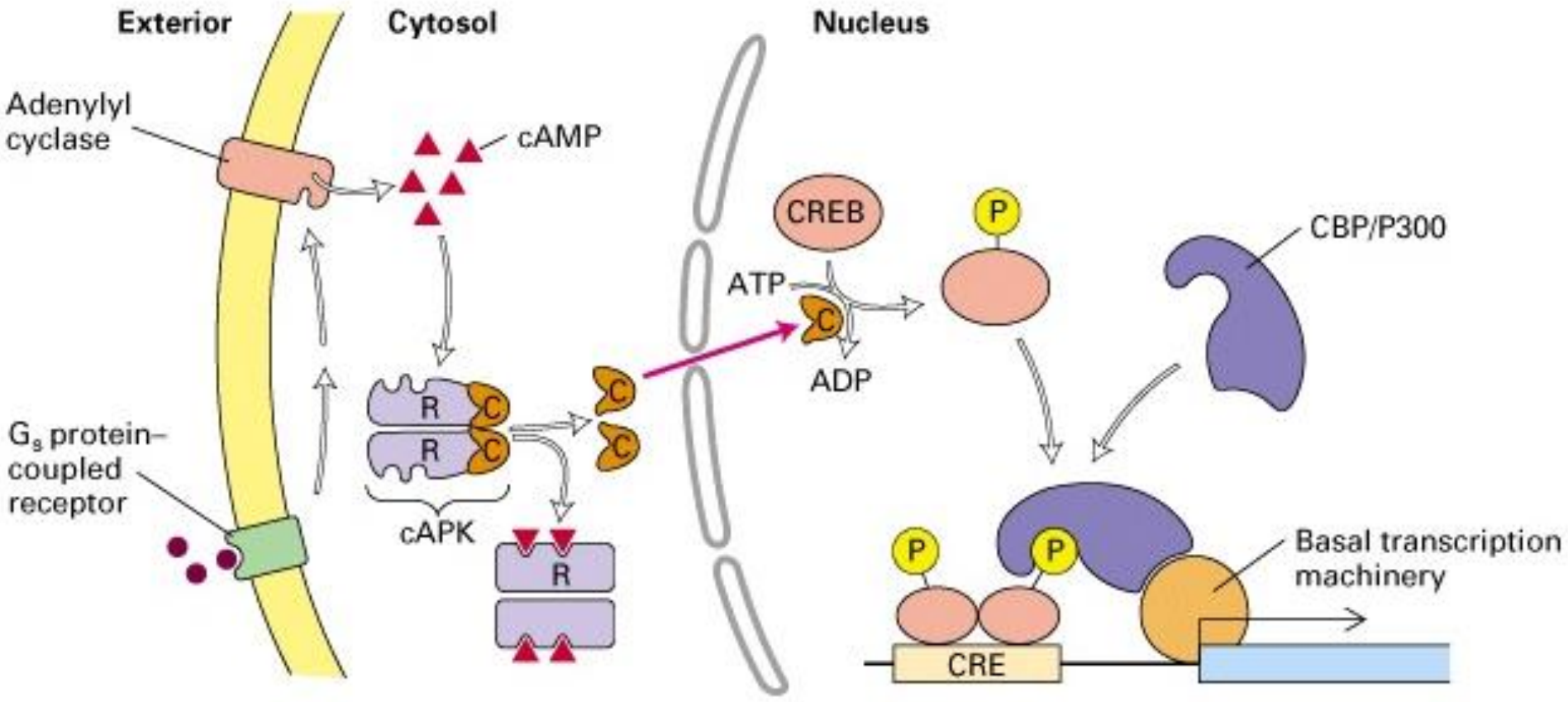


RGS proteins speed this up

→ **EFFECTOR**

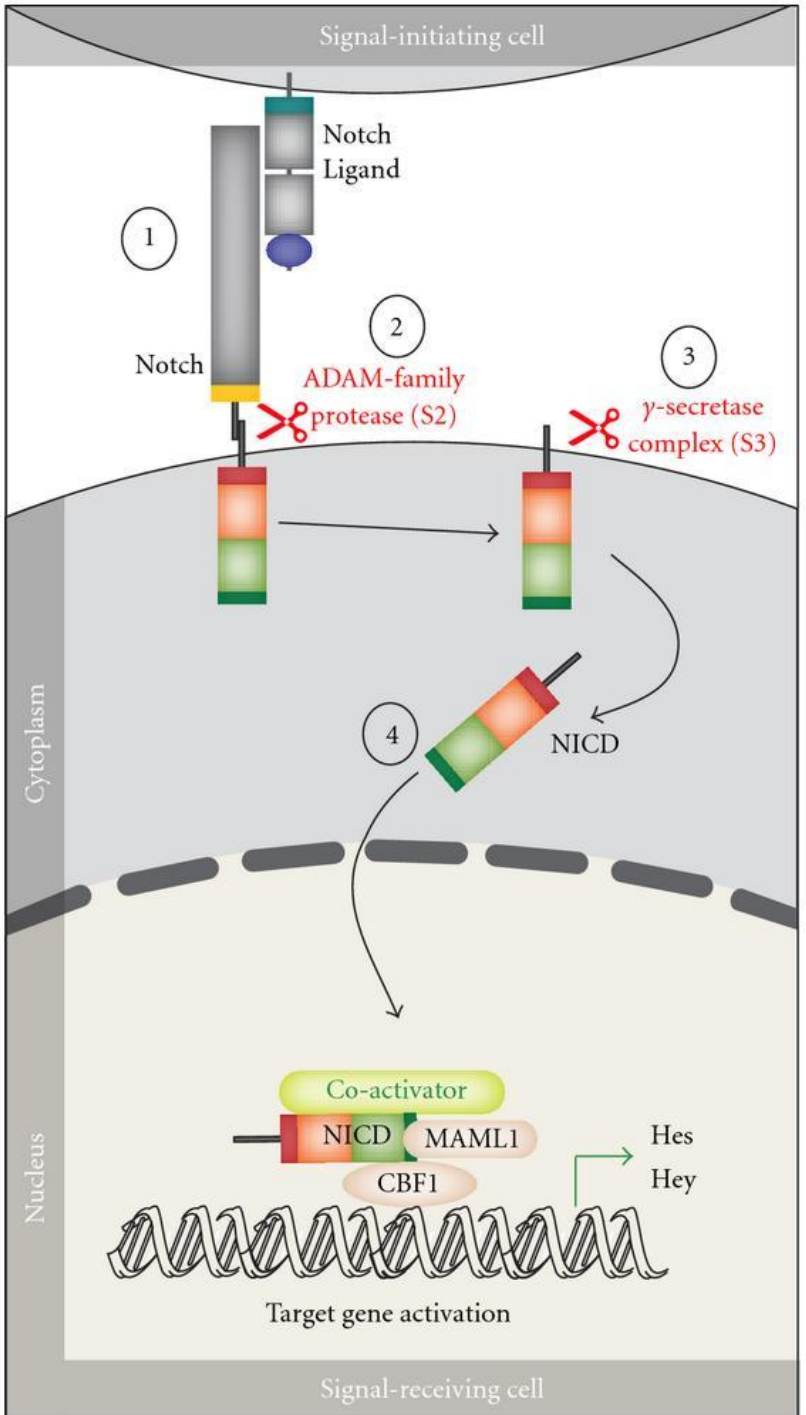
Boundless.com

Activation of CREB Transcription factor thru Gs ► Adenylate cyclase ► PKA



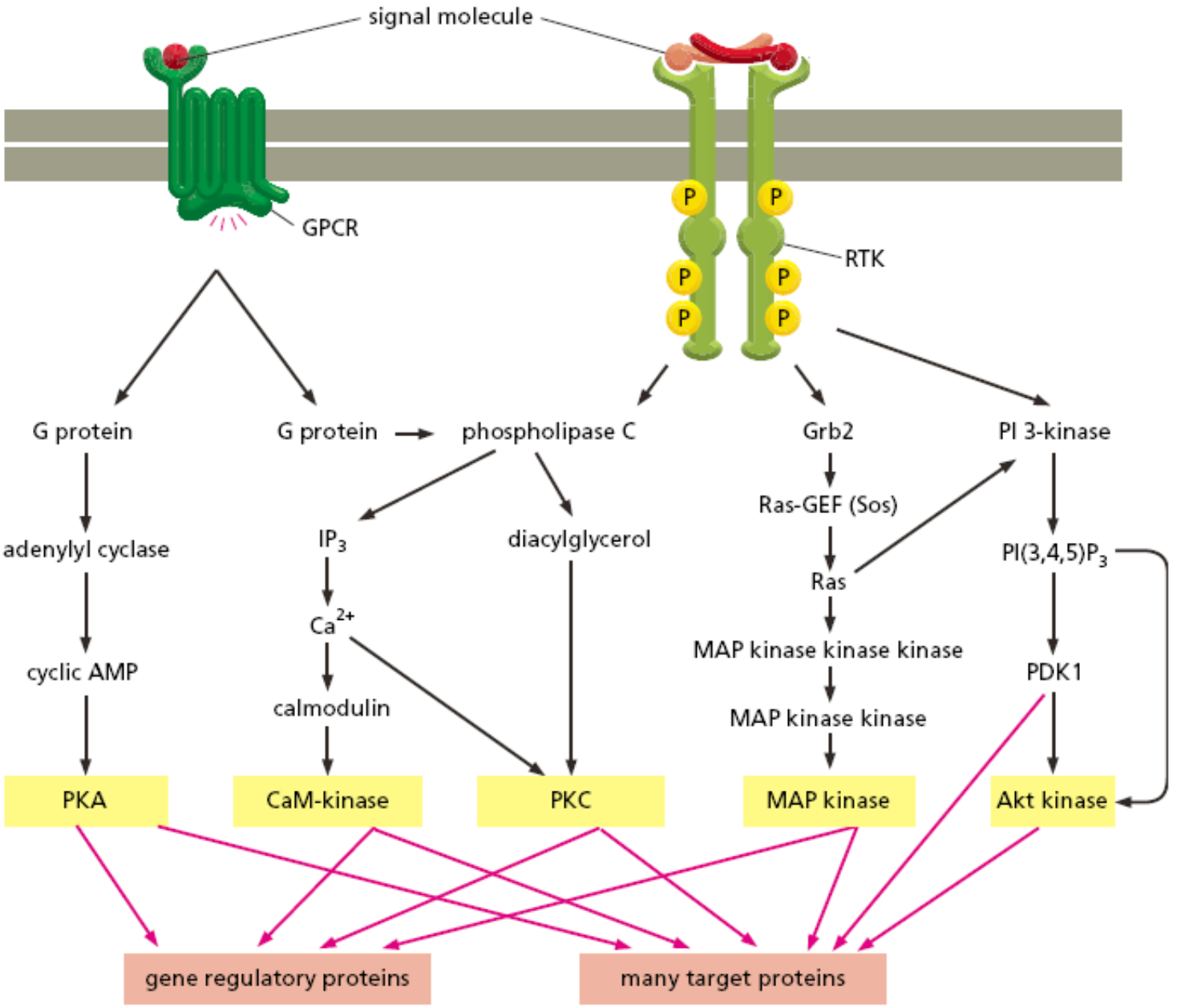
Closer Look:

NOTCH pathway

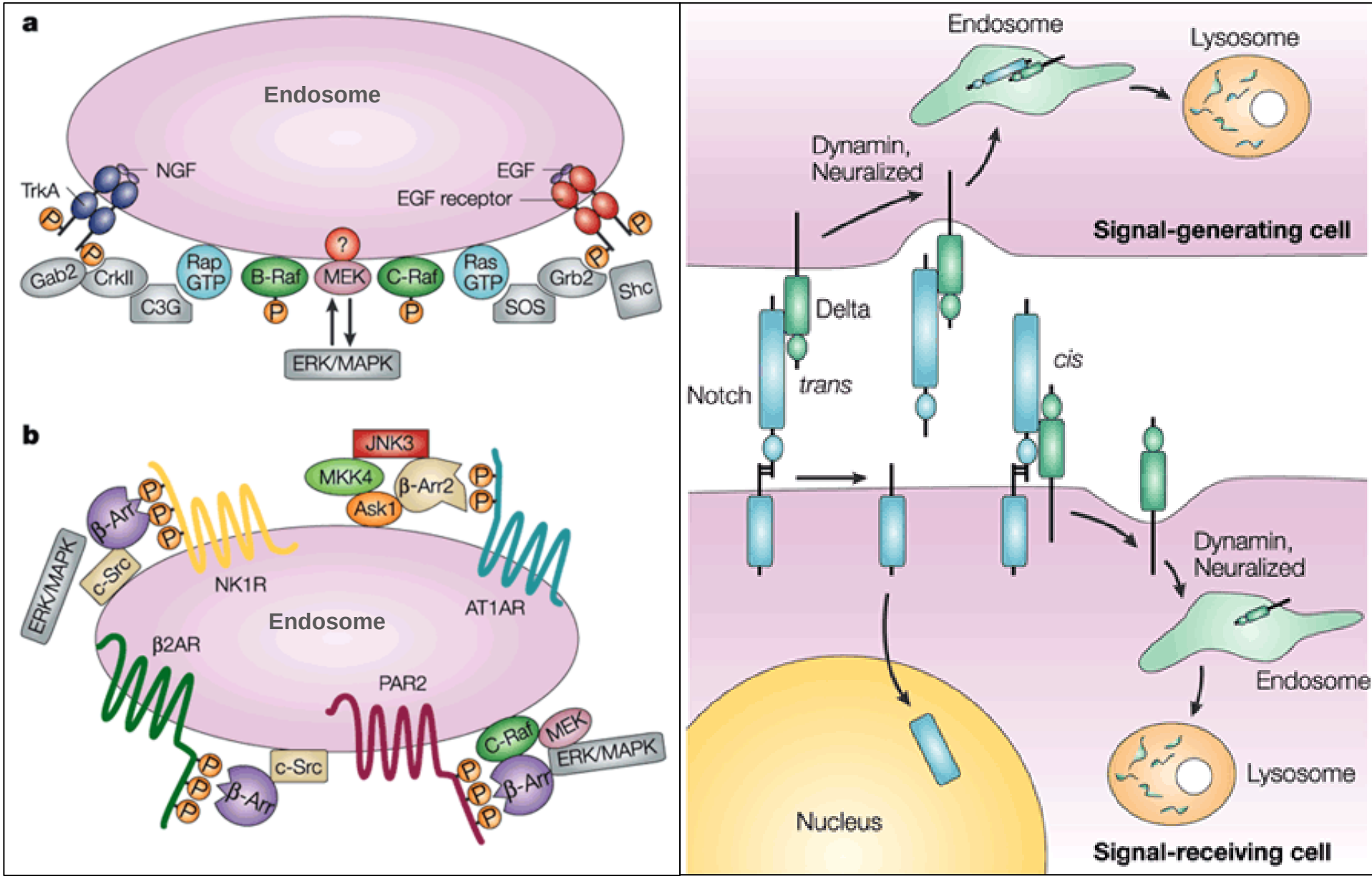


http://www.hindawi.com/journals/bmr/2011/570796/

Pathway Cross Talk is Often Complex



Intracellular Trafficking Pathways Often Impact Signaling – Does this aspect need to be in Your Model?



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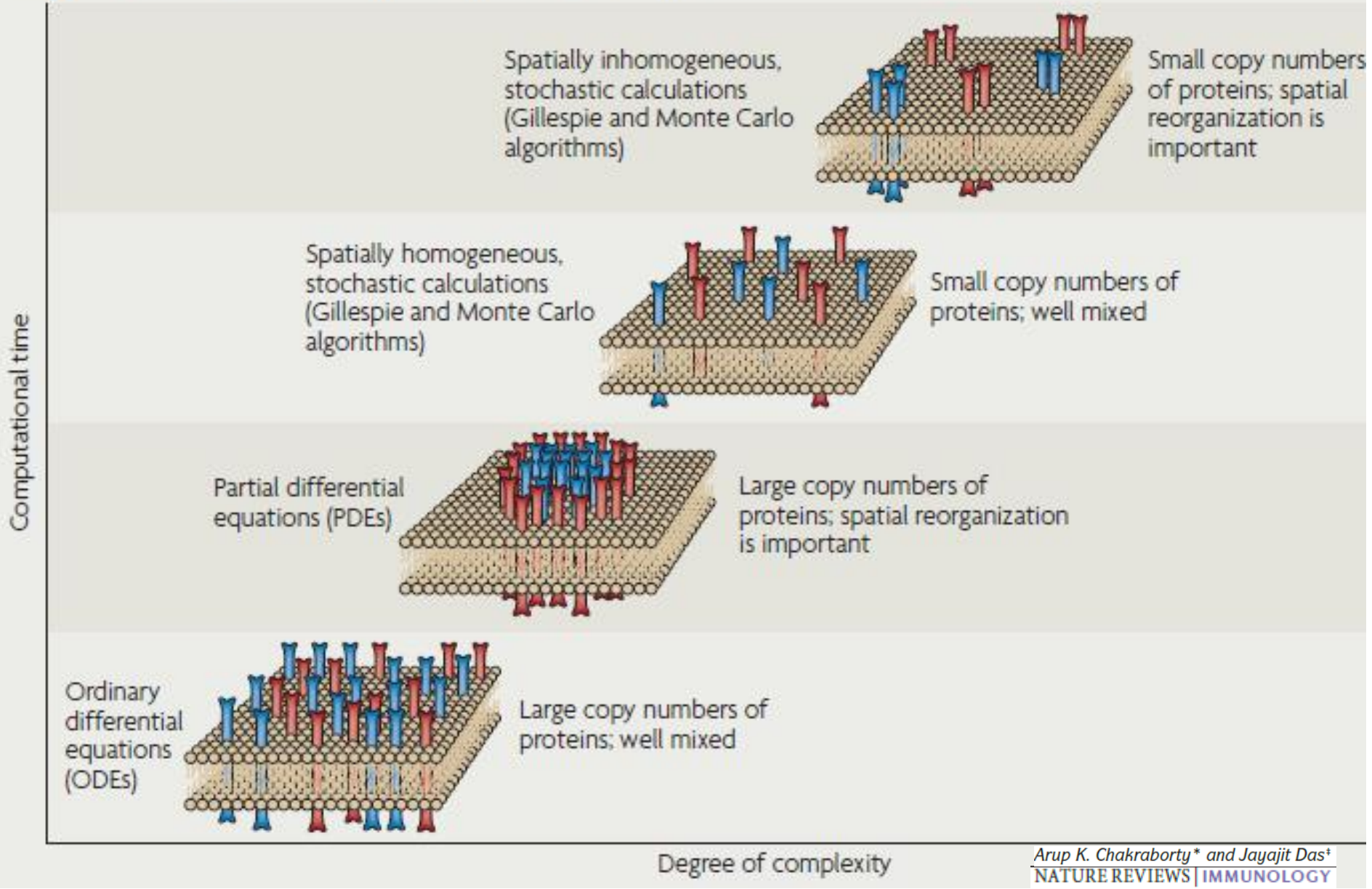
Mathematical Modeling: Methods and Challenges

Box 1 | **Types of theoretical and computational approaches**



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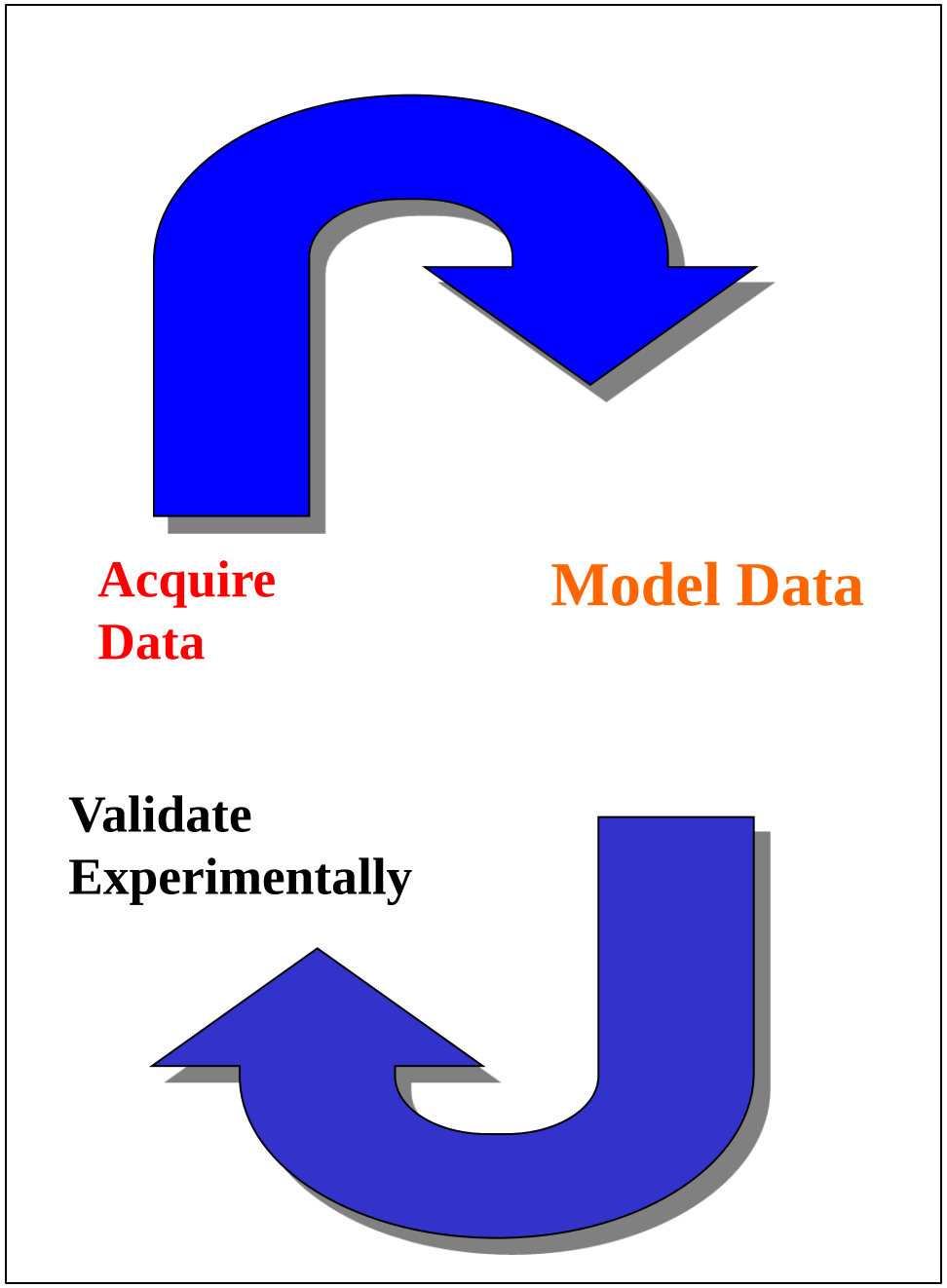
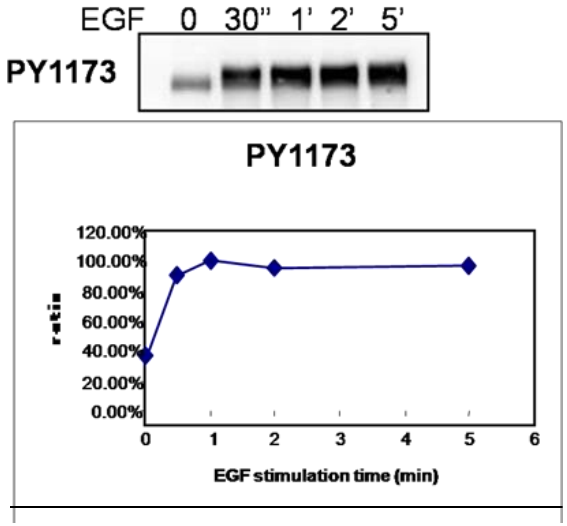


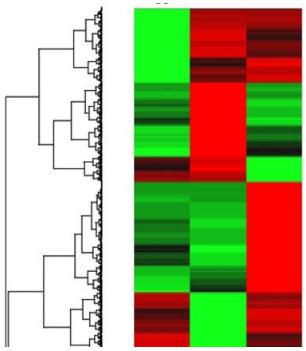
MODEL INTEGRATION

- Is biology important?
- Model yield new insight or have predictive power?
- Is it feasible to get the parameters and measurements you need?
- Is the problem multi-scale?
- Which modeling approach will work best?
- Is the system "well mixed"?
- Do you need to consider spatial aspects? If so, how complex is the geometry? Will simple compartmental models do?
- Interdisciplinary team?

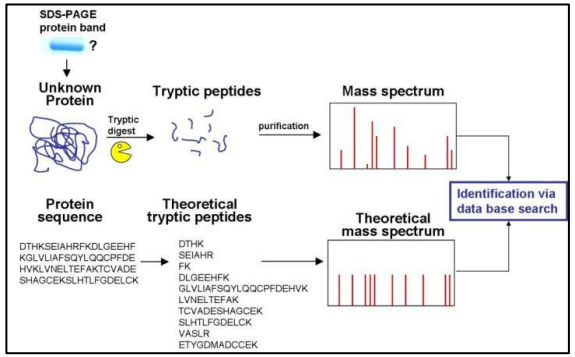
Data Collection – what's needed? Can it be calibrated?



- Western Blotting
- Difficult to calibrate

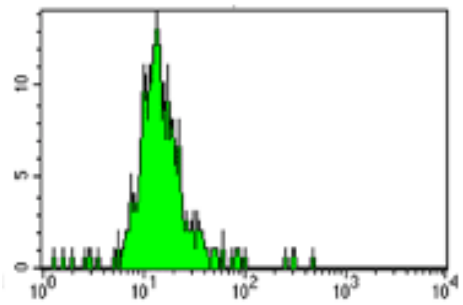


Genomics

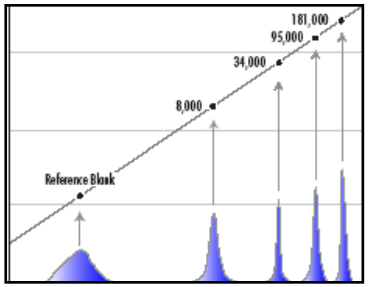


Proteomics

flow cytometry



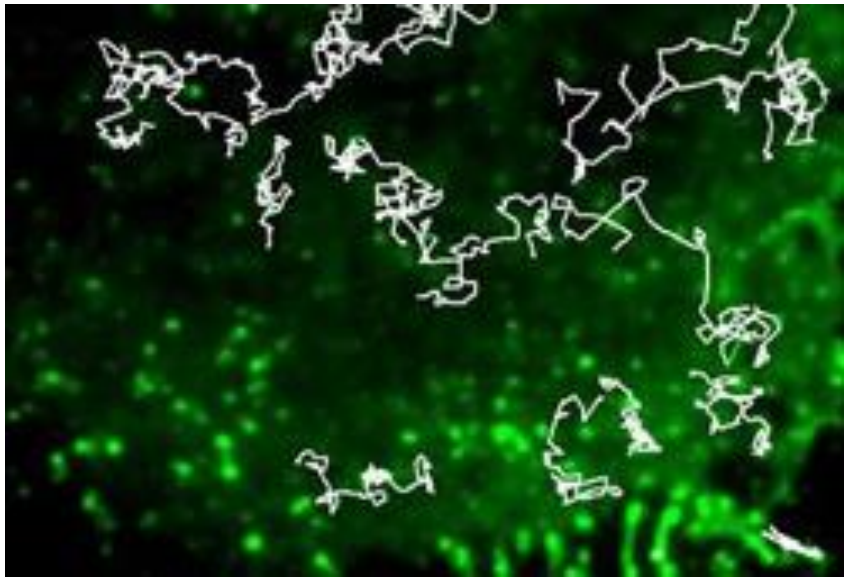
Fluorescence-based quantitative technique
(Example: monoclonal antibody binding to cell surface or intracellular target protein)

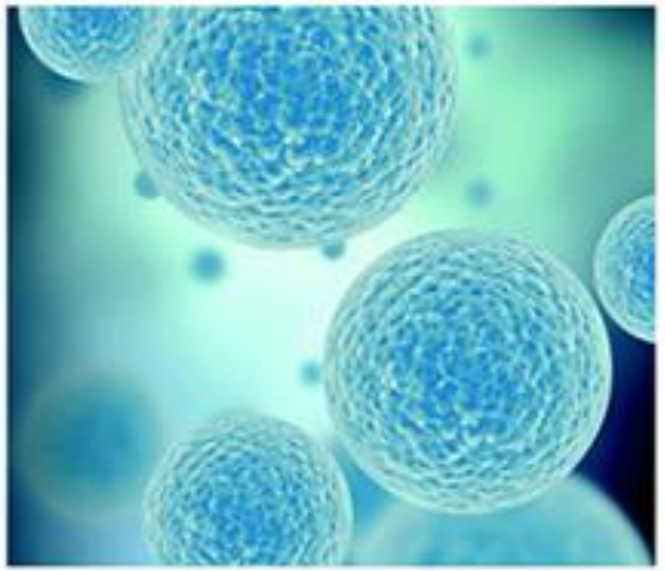


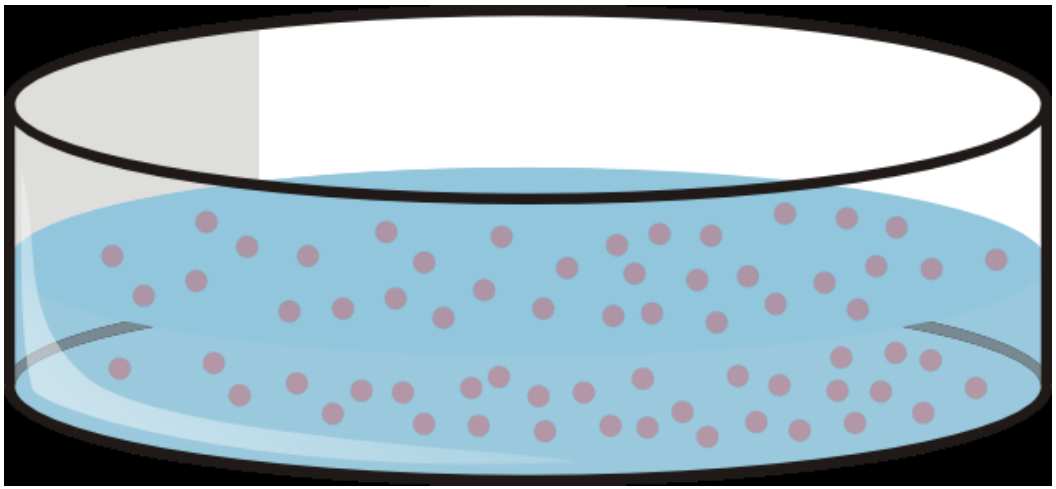
Fluorescence calibration
(mAb binding to bead standards)

High throughput screening

Single Molecule …. Single Cell? Or Population Measures?





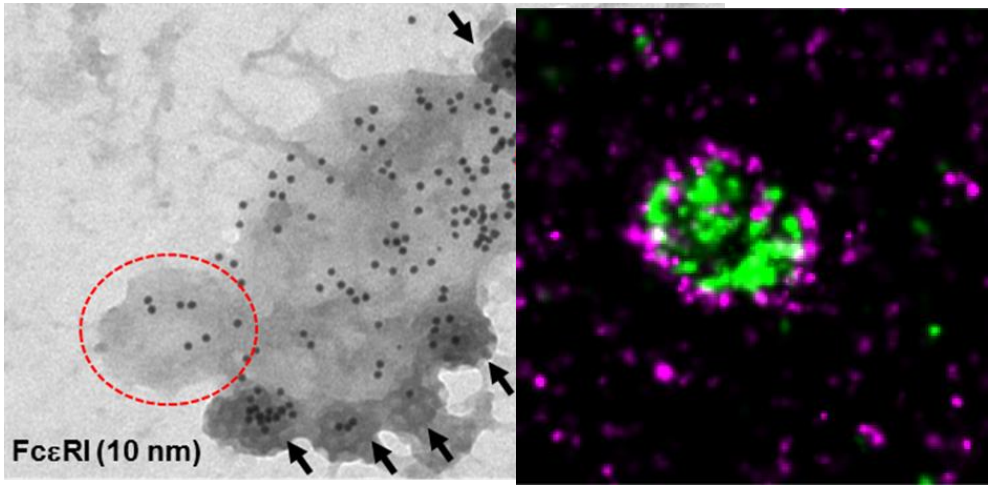


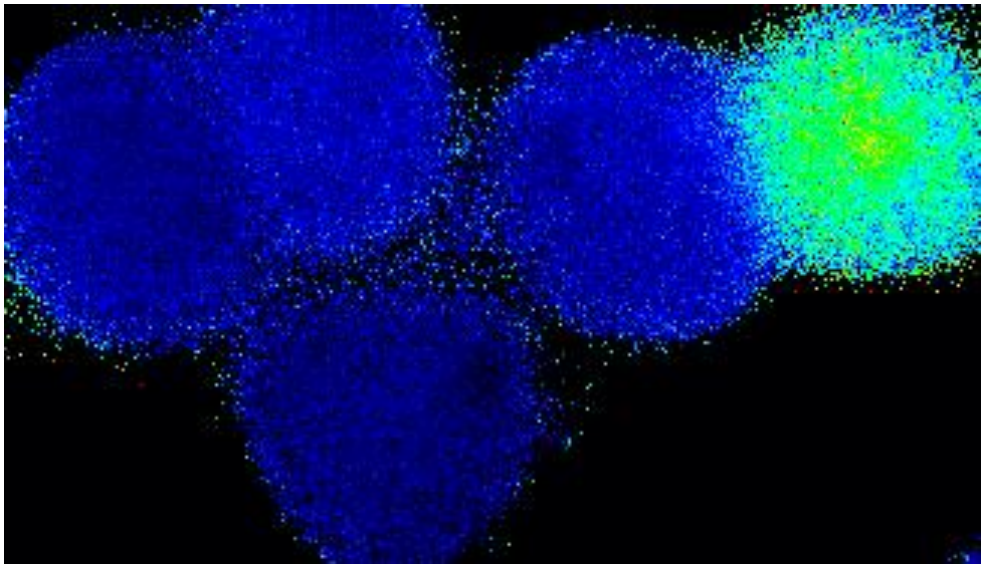
Microscopy: Quantitative or Qualitative?

• EM & other fixed protocols offer “snapshot” views of membrane organization, cells.

• Fluoresence-based, live cell imaging techniques are needed for time resolved measurements of protein-protein, protein-lipid interactions and diffusion rates. (FRET, FRAP, correlation spectroscopy, single particle tracking, etc). Some qbio students will get some hands on experence with SPT and SR techniques. And more lectures will introduce these methods – and the “noisy” but highly quantitative data they generate.

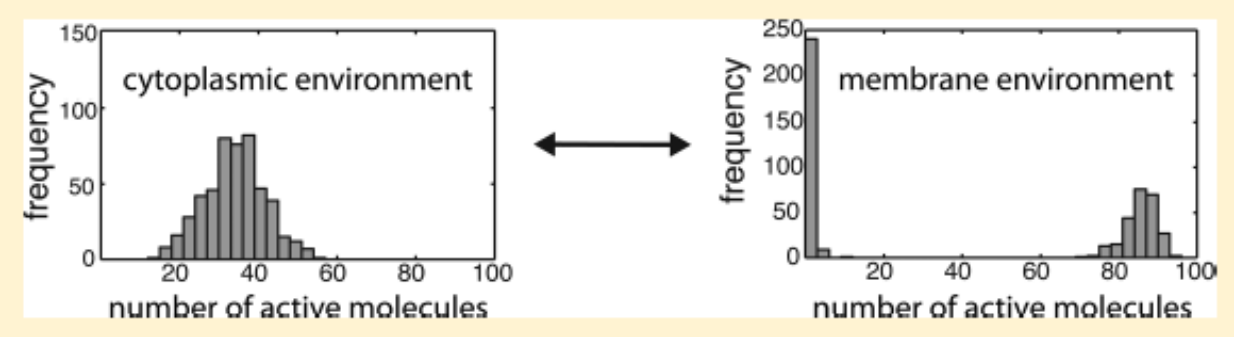
• No single technique provides all the data a modeler may need

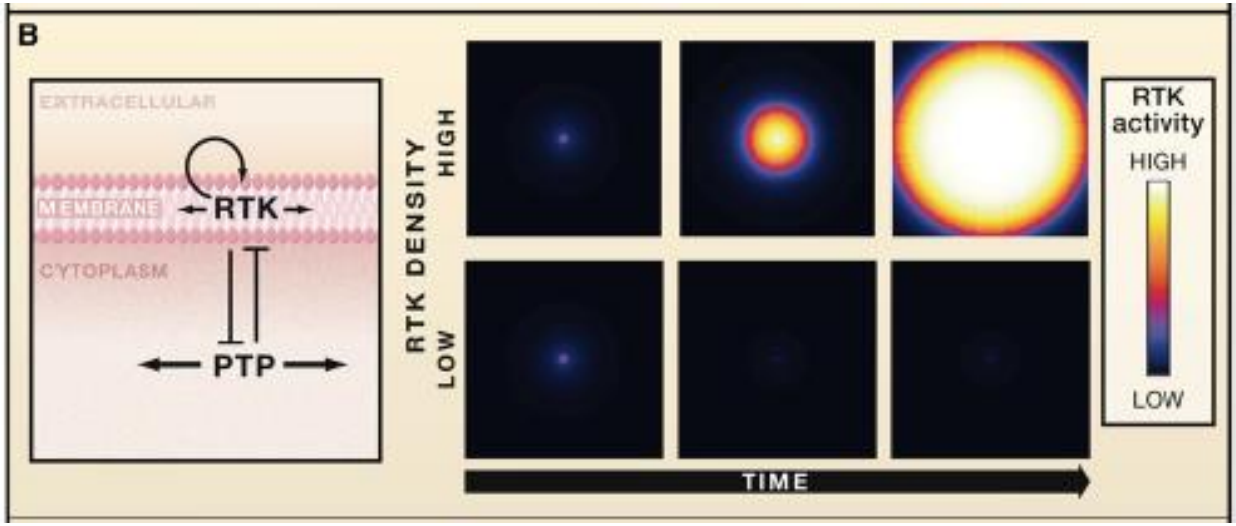




Let's look at examples of driving questions for computational approaches ….

How does the 2D membrane environment influence the behavior of Signaling Networks?



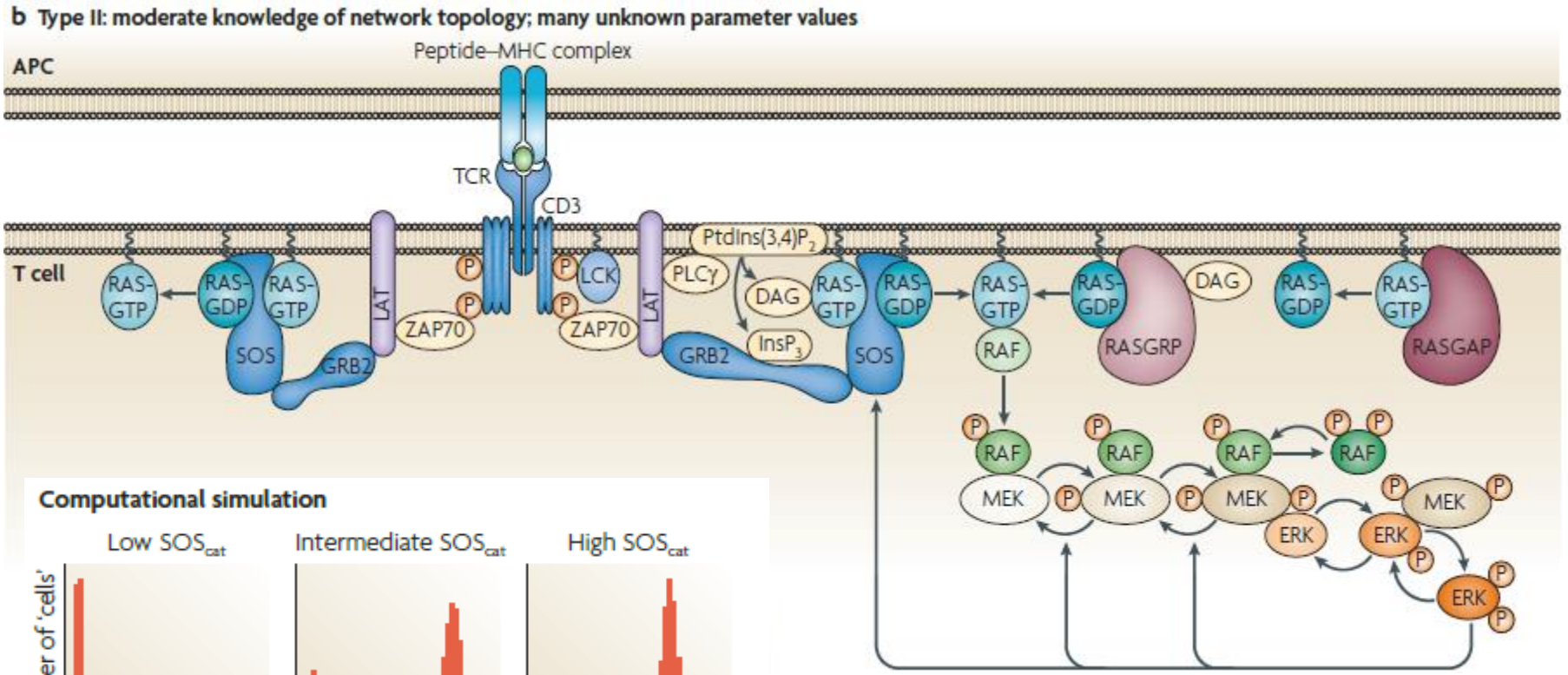


Bistability, which is dependent on rebinding, density, mobility, shape of confinement area

Top: Abel, Groves, Weiss, Chakraborty,
J. Physical Chemistry, 2012

Bottom: Grecco, Schmick & Bastiaens, Cell, 2011

What is Effect of Signal Initiation & Propagation Assemblies in 2D?



Pairing computation with experimentation: a powerful coupling for understanding T cell signalling

Arup K. Chakraborty and Jayajit Das†*

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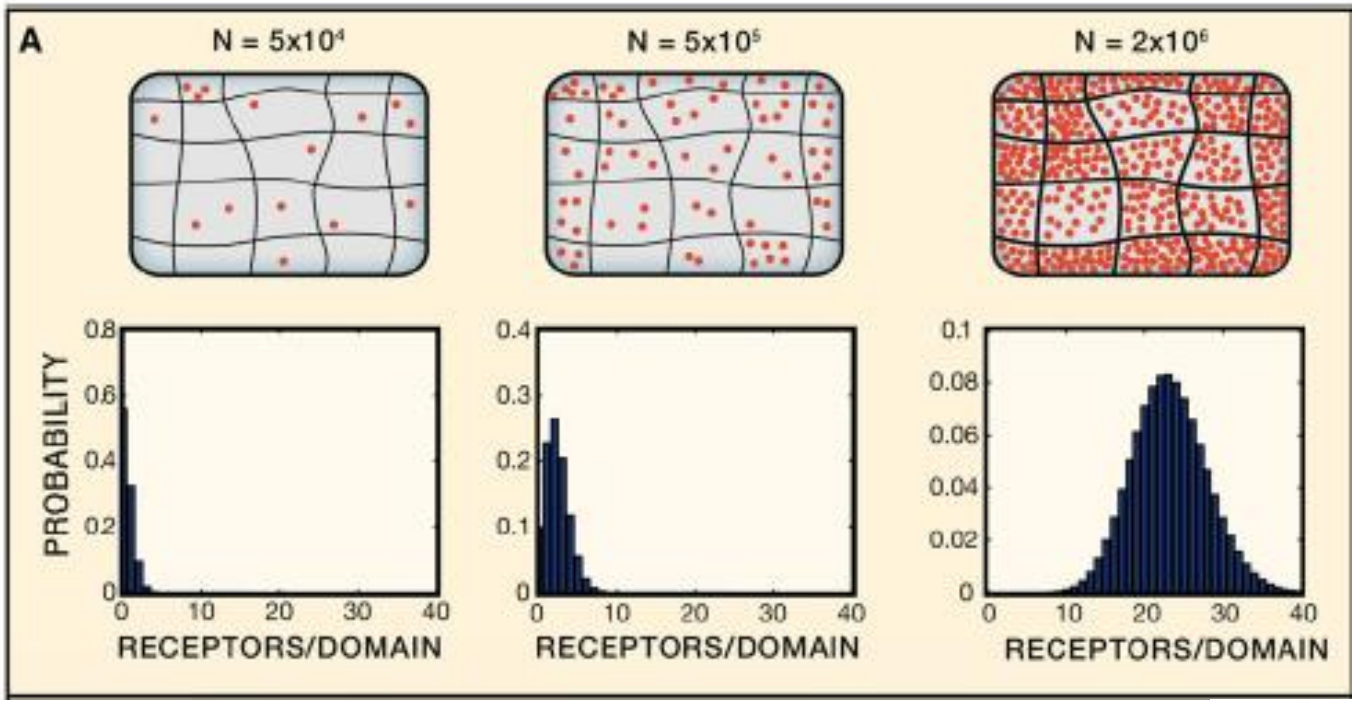
Do changes in density or distribution influence the behavior of Signaling Networks?



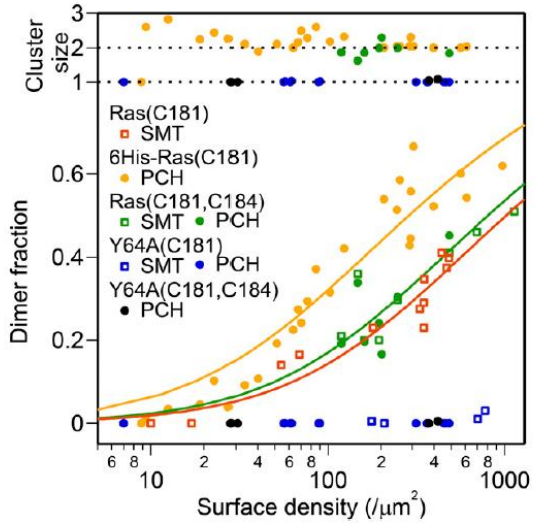
Signaling from the Living Plasma Membrane

Hernán E. Grecco,[1] Malte Schmick,[1] and Philippe I.H. Bastiaens[1,2,*]

Cell *144*, March 18, 2011

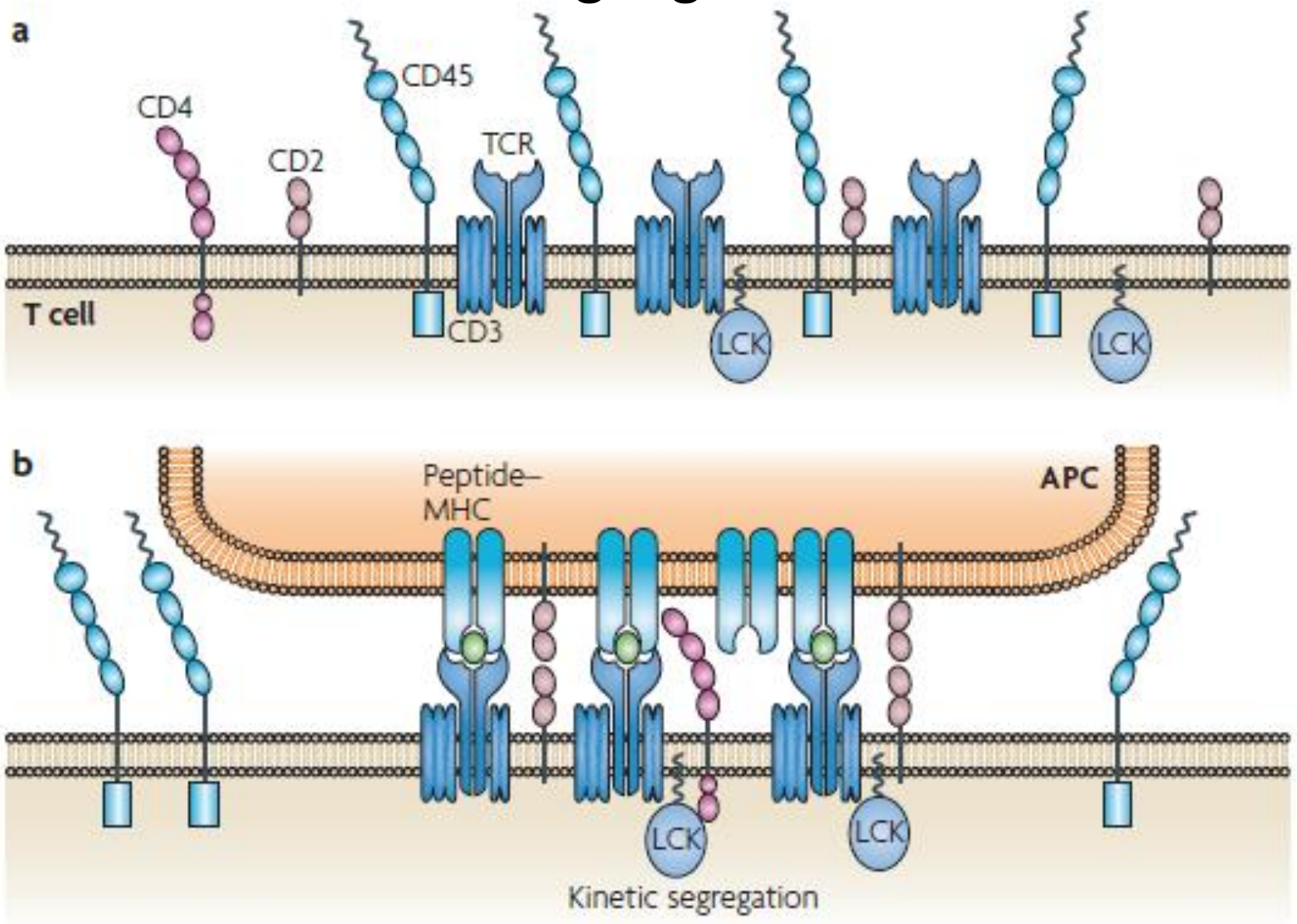
Surface-Density Dependency of H-Ras dimerization

PNAS | February 25, 2014



Spatial Organization for Controlling Signaling?

Kinetic Segregation Model



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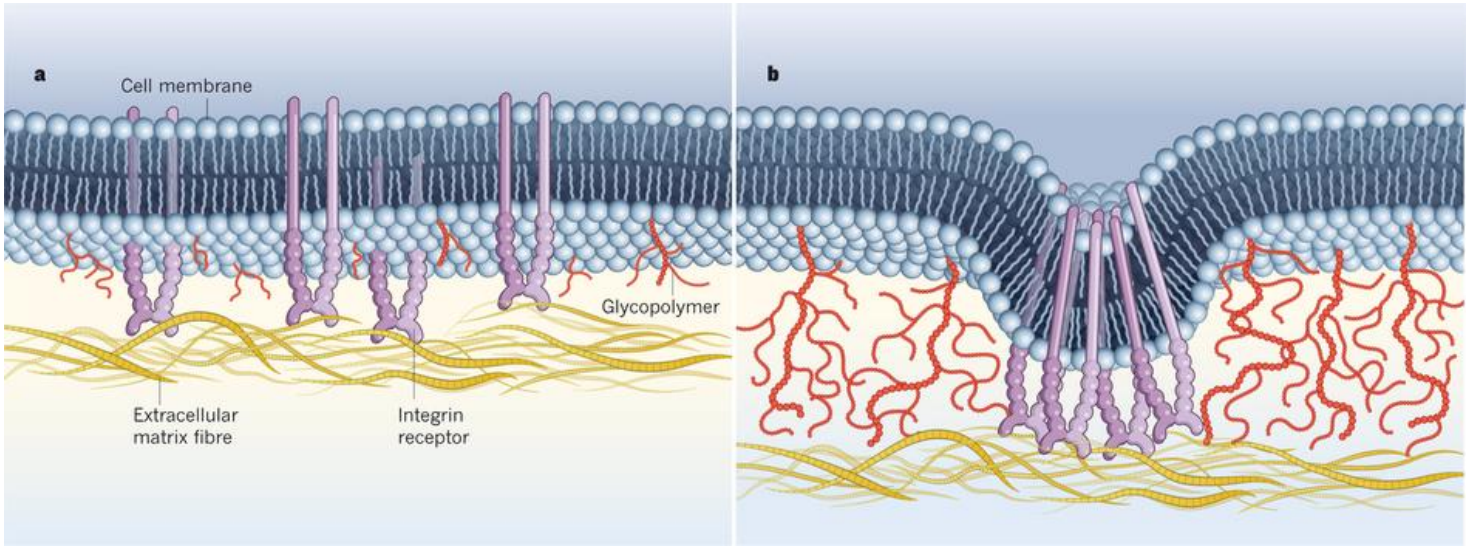
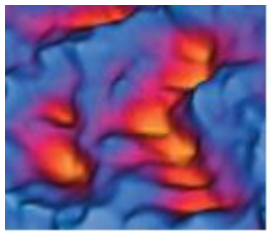
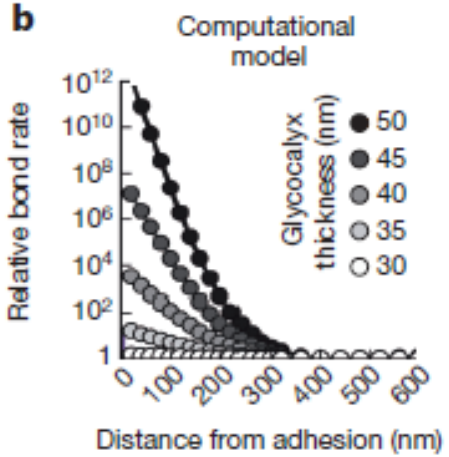
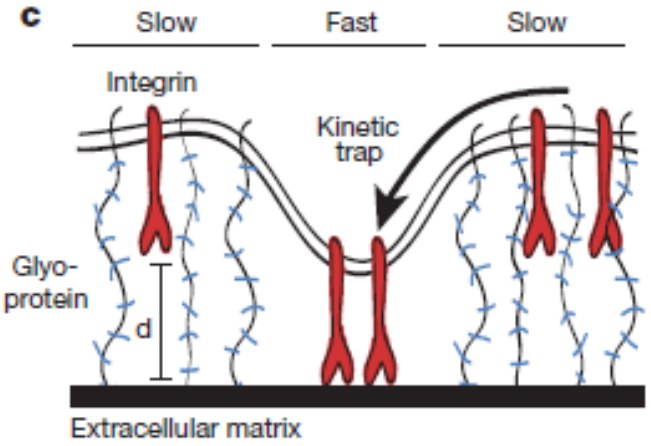
Spatial Organization for Controlling Signaling?

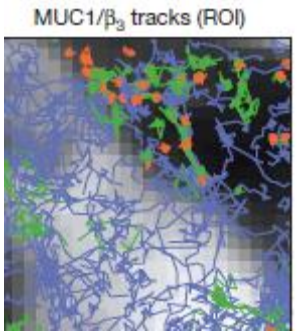
Kinetic Trapping









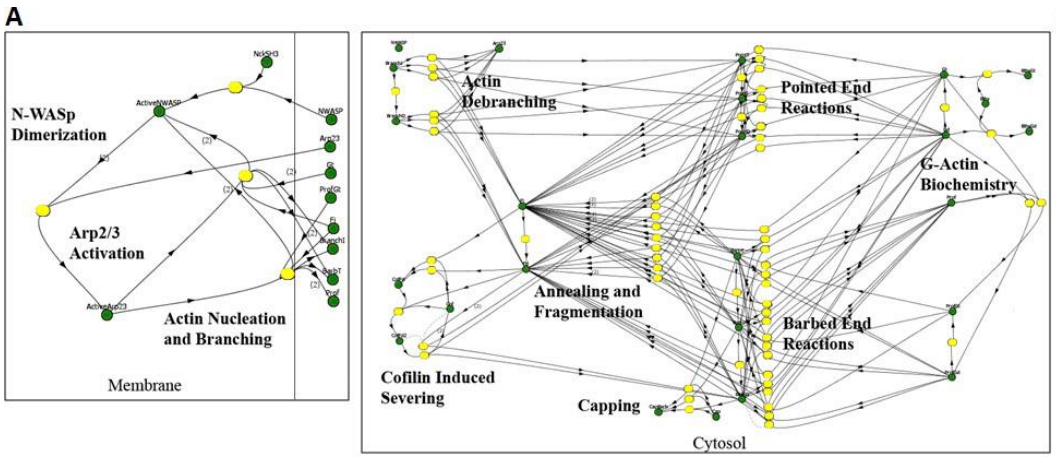


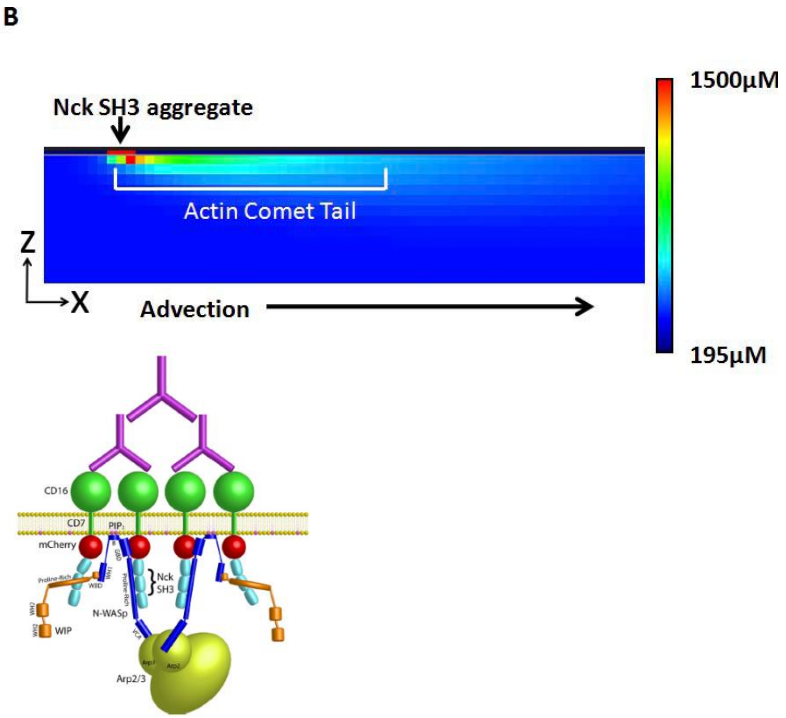
The cancer glycocalyx mechanically primes integrin-mediated growth and survival

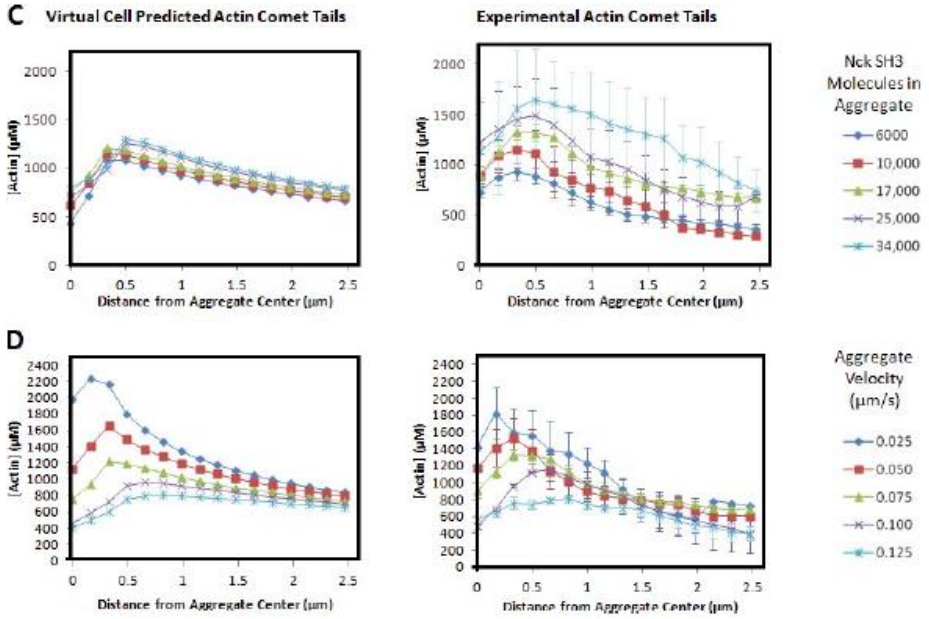
Valerie M. Weaver

NATURE | VOL 511 | 17 JULY 2014

Spatial Organization for Controlling Signaling?
Reaction Nucleation, 3D Cytosol Spread







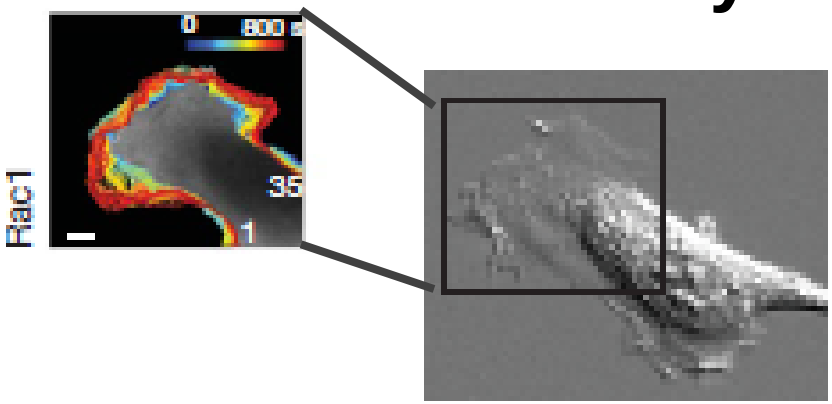
Virtual Cell Simulation

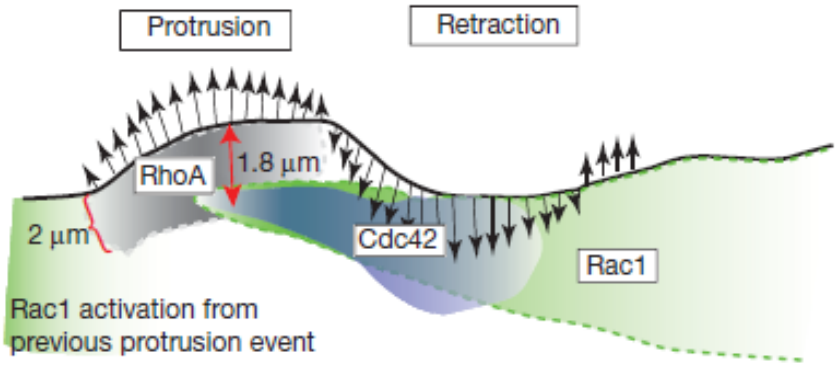
Stoichiometry of Nck-dependent actin polymerization in living cells

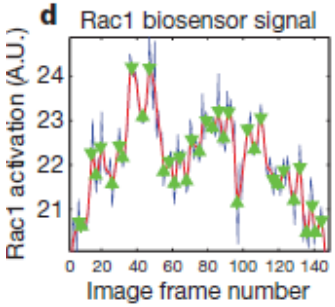
J. Cell Biol. Vol. 197 No. 5

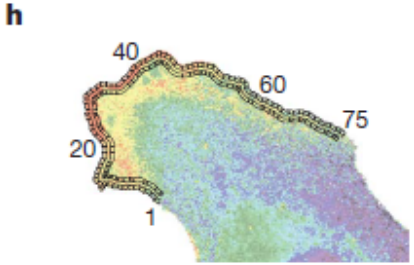
3D Spatial Organization for Controlling Signaling?

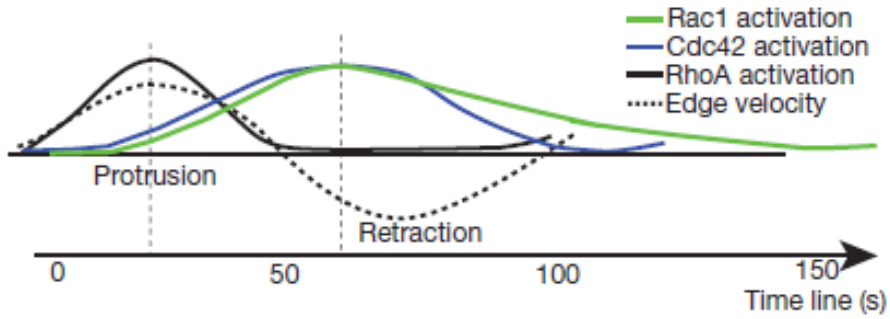
Polarity & Cell Migration

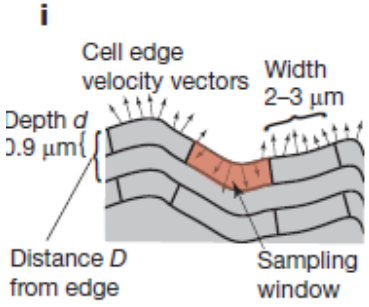


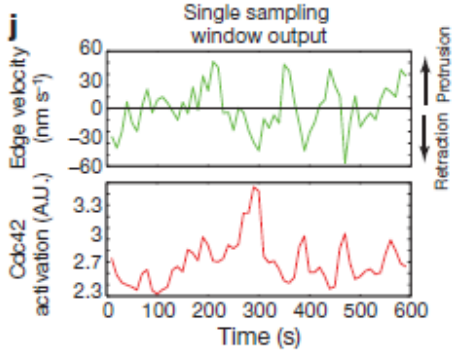












Coordination of Rho GTPase activities during cell protrusion

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Adapted from a TIBS cartoon from 1994.