Parameter estimation from live cell siRNA data predicts gene function from dynamics

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***Short Abstract* — Biological signaling systems rely on ubiquitous feedback and cross-talk involving numerous signaling proteins, posing a challenge to identify specific roles of putative regulators. Here we show that molecular roles can be assigned using a mechanistic model to deconvolute live single cell data of Ca2+ signaling into specific activities and states that are difficult to measure otherwise. An siRNA screen of 250 putative regulators showed that shifts in parameter estimates away from control cells explicitly predict gene function at the mechanistic level and revealed novel regulators of calcium signaling that were confirmed in direct assays.**

***Keywords* — parameter estimation, calcium signaling, high-throughput screening, live-cell imaging, cell signaling models.**

I. Background

THE unifying goal of systems biology and cell signaling research is to establish a comprehensive quantitative framework for understanding cellular dynamics and decision making. Mass action models in the form of ordinary differential equations promise to be the language of choice because they are mechanistic representations of molecular processes. The challenges to systems biology with this approach, however, include uncertainty in model parameters [1] and model structure, also because feedback and cross-talk may be implemented in nature by a multitude of unknown regulators.

II. Results

We show that a dynamic model of intracellular calcium signaling can be employed to infer changes to specific internal states and activities that occur due to an siRNA perturbation. A rapid perturbation protocol was devised to elicit cytosolic Ca2+ trajectories that were informative with respect to the parameters of a mechanistic model. This differential equations model was able to tightly reproduce experimental data from individual cells despite high cell-to-cell variability of the response trajectories. Intriguingly, parameter estimation mapped the effects of siRNA perturbation of known protagonist molecules faithfully to changes in corresponding model parameters. In other words, we found that parameter shifts in this model induced by siRNA perturbation are accurate and explicit predictions of protein function. This allowed us to screen an siRNA library targeting 250 putative Ca2+ regulators for specific effects on model parameters. Previously known regulators stood out from the siRNA distributions along the expected parameter axes. Moreover, unexpected and highly relevant regulators of two distinct molecular processes were confirmed in direct assays and will be discussed.

III. Conclusion

A cell signaling model based on differential equations was used to deconvolute time-resolved single cell data into internal states and activities that are difficult to measure in direct assays. Information in trajectories of individual cells can be aggregated despite significant cell-to-cell variability. Our results demonstrate how parameter estimation can increase the resolution of a cell signaling assay, accelerating the path to discovery.

References

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