We formulate the hybrid approach to structure determination as an optimization problem, the solution of which requires three main components: the representation of the assembly, the scoring function, and the optimization method. The ensemble of solutions to the optimization problem embodies the most accurate structural characterization given the available information. The key challenges remain translating experimental data into restraints on the structure of the assembly, combining these spatial restraints into a single scoring function, optimizing the scoring function, and analyzing the resulting ensemble of solutions. To address these challenges, we are developing the Integrated Modeling Platform (IMP) (http://salilab.org/imp). IMP is designed to allow mixing and matching of existing modeling components as well as easy adding of new functionality. It supports a wide variety of assembly representations and input data. We will also provide infrastructure that encourages and supports contributions from other laboratories.
IMP will be illustrated by its application to the determination of the molecular architectures of the Nuclear Pore Complex and the 26S proteasome.

Minisymposium 1: Cellular Decision Making: Gene Networks and Evolutionary Dynamics

74-MiniSymp
A Model For Genetic and Epigenetic Regulatory Networks Identifies Rare Pathways For Transcription Factor Induced Pluripotency
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With relatively low efficiency, differentiated cells can be reprogrammed to a pluripotent state by ectopic expression of a few transcription factors. An understanding of the mechanisms that underlie data emerging from such experiments can help design efficient strategies for creating pluripotent cells for patient-specific regenerative medicine. We have developed a model for the architecture of the epigenetic and genetic regulatory networks which describes transformations resulting from expression of reprogramming factors. Importantly, our studies identify the rare temporal pathways that result in induced pluripotent cells. Further experimental tests of predictions emerging from our model should lead to fundamental advances in our understanding of how cellular identity is maintained and transformed.

75-MiniSymp
Complex Topology Rather Than Complex Membership Is a Determinant of Protein Dosage Sensitivity
Richard Oberdorf, **Tanja Kortemme**.
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I will describe a simple mathematical model of the relationship between protein interaction topologies and the sensitivity of biological responses to gene dosage and noise effects.
The 'balance hypothesis' predicts that non-stoichiometric variations in concentrations of proteins participating in complexes should be deleterious. As a corollary, heterozygous deletions and overexpression of protein complex members should have measurable fitness effects. However, genome-wide studies of heterozygous deletions in Saccharomyces cerevisiae and overexpression have been unable to unambiguously relate complex membership to dosage sensitivity. We have tested the hypothesis that it is not complex membership alone but rather the topology of interactions within a complex that is a predictor of dosage sensitivity. We develop a model that uses the law of mass action to consider how complex formation might be affected by varying protein concentrations given a protein's topological positioning within the complex. We find significant correlations between predicted sensitivity of complex formation to protein concentrations and both heterozygous deletion fitness and protein abundance noise levels. Our model suggests a mechanism for dosage sensitivity and provides testable predictions for the effect of alterations in protein abundance noise.

76-MiniSymp
Decision-Making in Bacteriophage Lambda: A View From the Single Phage
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Upon infection of an *E. coli* bacterium by phage lambda, a decision is made between a violent (lytic) pathway, leading to cell death and the release of hundreds of new phages; and a non-violent (lysogenic) pathway, in which the phage DNA gets integrated into the bacterial genome. This post-infection decision process serves as a paradigm for an environmentally-regulated genetic switch and has been put forward as an example of noise-driven bifurcation of cellular fate. By following viral infection at the level of individual phages and cells under the microscope, we demonstrate how deterministic and stochastic aspects of the decision-making process combine to yield the observed noisy phenotype. A fluorescently-labeled phage is used, in conjunction with fluorescent reporters for the alternative developmental pathways. We find that, for each individual infecting phage, the probability of lysogeny exhibits a threshold dependence on the density of viral genomes inside the infected cell. However, the final fate of the cell depends on the individual decisions of all infecting phages, in a way that renders the whole-cell decision noisier, the higher the number of infecting phages. We also find that moving from the single-cell to the population-averaged level does not add significantly to the apparent noisiness of the decision.

77-MiniSymp
Variability in Gene Expression Underlies Incomplete Penetrance in C. Elegans: Using Single Molecules To Study the Development of Single Cells
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Phenotypic variation is ubiquitous in biology and is often traceable to underlying genetic and environmental variation. However, even genetically identical organ-isms in homogenous environments vary, suggesting that random processes may play an important role in generating phenotypic diversity. Few studies, have ex-plored the impact of stochastic fluctuations in gene expression on phenotypic variation and cell fate decisions in multicellular organisms. In order to examine the consequences of gene expression variability in development, we explored intestinal specification in C. elegans, in which wild-type cell fate is invariant and controlled by a small transcriptional network. In contrast, cell fates in embryos with mutant skn-1, the first gene expressed in this network, are variable: while most mutant embryos fail to develop intestinal cells, some embryos nevertheless produce intestinal precursors. By counting transcripts in individual embryos, we show that mutations in skn-1 result in large variability in the expression of the downstream gene end-1, arising partly from misregulation of chromatin remodel-ing. end-1 expression is are subsequently thresholded during a critical time win-dow to produce an ON/OFF expression pattern of elt-2, the master regulator of intestinal differentiation. The loss of skn-1 activity eliminates redundancy in the network, making elt-2 activation particularly sensitive to variability in end-1 ex-pression. Although end-3 can also activate elt-2, deleting end-3 in wild-type ani-mals results in variability in levels and timing of elt-2 expression, suggesting that robust expression of the downstream target requires multiple transcriptional acti-vators and also hinting at subtle differences in the roles of putatively redundant elements in the network. Our results show that mutations in developmental net-works can expose otherwise buffered stochastic variability in gene expression, leading to pronounced phenotypic variation.

78-MiniSymp
FM Signaling in Single Cells
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Regulation of transcription factor localization allows cells to respond rapidly to extracellular signals. Although the molecular mechanisms of nuclear import and export have been examined, it remains unclear how localization varies among individual cells, and how dynamic changes in localization affect expression of downstream genes. In the presence of extracellular calcium, Crz1, the calcineurin responsive zinc finger transcription factor of Saccharomyces cerevisiae, is dephosphorylated and translocates into the nucleus. By observing the localization of Crz1-GFP fusion proteins using time-lapse microscopy, we found that Crz1 exhibited bursts of nuclear localization with a characteristic nuclear residence time of ~2 minutes. These bursts occurred in a stochastic fashion in individual cells and propagated to the expression of downstream genes, contributing significantly to fluctuations in gene expression. Strikingly, calcium concentration controlled the frequency, but not duration, of nuclear localization bursts. Using an analytic model , we find that the observed stochastic frequency modulation (FM) of localization bursts can enable cells to proportionally coordinate expression levels of multiple target genes by regulating the fraction of time a promoter is active, rather than tuning the level of activity itself. We experimentally confirmed this theory by showing that both natural and synthetic Crz1 target promoters are expressed proportionally across a wide range of calcium concentrations. Furthermore, we observe that many proteins exhibit localization bursts and show diverse dynamic behaviors. These results suggest that cells may utilize FM mode of regulation to control diverse cellular processes.