Alistair Boettiger

Department of Molecular & Cell Biology
University of California, Berkeley
alistair@berkeley.edu

Unique features of metazoan gene regulation provide robust cell fate decisions from stochastic components

The developmental patterning of multicellular organisms requires precise control on the scale of microns and chemical sensitivity on the scale of tens of molecules. The molecular system which regulates this careful construction however operates in an environment of substantial stochastic variation. We quantify this variation in the muscle fate-determination pathway in the early Drosophila embryo and study two evolutionary innovations by which the effects of such variation is minimized to allow very precise tissue specification.

The substantial degree of stochastic variation in the muscle differentiation process within clonal populations of embryos, can readily be unmasked by complimenting mild genetic and environmental stress. This results in a substantial array phenotypic differences in developmental patterning, from fully viable to lethal ones (despite identical genetic and environmental conditions). We have developed a collection of single-molecule imaging techniques with the ability to simultaneously detect hundreds of thousands of molecules to study the molecular sources of this variation. We apply these techniques to measure the contribution from independent regulatory sequences involved in regulation the key muscle determination factor, *snail*. We show that while individual sequences can be removed without destroying downstream developmental processes, such modifications do substantially increase variation in on the cellular and molecular level. Moreover, we find that this molecular scale variation in a random subset of embryos are such as to produce developmental defects. This work demonstrates that gene-regulatory mechanisms which appear redundant on scale of individual embryos in fact play a critical role for the fitness of the overall population. The regulatory features of the genome which allow multiple independent, spatially distributed regulatory sequences to control a common promoter of the same gene, critical for these effects we observe, is unique to metazoan genomes.

These measurements also provide some novel insights into the molecular mechanisms of regulating gene expression. In particular they suggest that for the response to muscle determination transcription factors, it is not the site occupancy of transcription factor at the regulatory site that is limiting for transcription, but the rate of interaction between the regulatory site and its cognate promoter. Using a mathematical abstraction of the gene regulation, we predict the maximum contribution from combining multiple regulatory sequences based on measurements from only the independent components. This work suggests that chromatin dynamics are a substantial source of noise in gene expression and molecular mechanisms which modulate chromatin interaction rates may function to substantially reduce the resulting variation.

We have also observed that the *snail* gene employs a recently characterized type of promoter common to multicellular organisms and rarely if ever found in single-cell species. This promoter binds polymerase in a non-tissue specific fashion, but only releases the polymerase to a productive elongation state in the presumptive muscle tissue. We use a combination of modeling and high resolution imaging techniques to show that this regulation of mRNA elongation instead of regulation of Pol II recruitment is a mechanism that further dampens the noise produced by molecular scale fluctuations. Joint work with Jacques Bothma, Michael Perry, and Michael Levine.

Paul François

Department of Physics
McGill University
paulf@physics.mcgill.ca

Stochasticity in evolution of biochemical reaction networks

I will describe an evolutionary procedure *in silico* that evolves small gene networks and selects for a desired dynamical behavior. I will show on several examples that, despite the intrinsic stochasticity in evolution itself, for the same function, selection often converges towards similar network dynamics (which can be still be implemented in different ways). This suggests a kinetic view of evolution, and I will relate this to the evolutionary phenomenon of parallel/convergent evolution.

Lingchong You

Biomedical Engineering & IGSP
Duke University
you@duke.edu

Using viral-mediated noisy gene expression to probe signaling dynamics in mammalian cells

Gene expression mediated by viral vectors is subject to cell-to-cell variability, which limits the accuracy of gene delivery. When coupled with single-cell measurements, however, such variability provides an efficient means to quantify signaling dynamics in mammalian cells. Here, we illustrate the utility of this approach by mapping the E2f1 response to MYC, serum stimulation, or both. Our results revealed an under-appreciated mode of gene regulation: E2f1 expression first increased, then decreased as MYC input increased. This biphasic pattern was also reflected in other nodes of the network, including the miR-17-92 microRNA cluster and p19Arf. A mathematical model of the network successfully predicted modulation of the biphasic E2F response by serum and a CDK inhibitor. In addition to demonstrating how noise can be exploited to probe signaling dynamics, our results reveal how coordination of the MYC/RB/E2F pathway enables dynamic discrimination of aberrant and normal levels of growth stimulation. Building on this concept, we are currently developing both computational and experimental methods to exploit these noisy signaling dynamics as quantitative cell phenotype.

Tom Shimizu

Systems Biophysics Program
FOM Institute for Atomic and Molecular Physics
t.shimizu@amolf.nl

Experimental dissection of chemotactic signaling dynamics in live bacteria

In this talk, I will describe recent experimental efforts to quantitatively characterize the dynamics of the signaling pathway mediating chemotaxis in bacterial cells. In vivo FRET, complemented by theoretical modeling, has proven very powerful in uncovering the molecular mechanisms underlying this signaling system, which features precise adaptation, strong amplification, and rescaling of response sensitivity over a broad dynamic range. I will also discuss the experimental challenges in characterizing signal fluctuations at the single-cell level.

Nacho Molina

Computational Systems Biology Group
EPF Lausanne
nacho.molina@epfl.ch

Analysis of temporal reporter expression using a stochastic biophysical framework identifies kinetic models of gene activity

The intrinsic stochasticity in the dynamics of mRNA and protein expression has important consequences on gene regulation and on non-genetic cell-to-cell variability. In both prokaryotes and eukaryotes, gene transcription is believed to occur mainly during short and intense periods referred as to transcriptional bursts, interspersed by silent periods. However, in mammalian cells, the fine transcriptional kinetics of endogenous genes has not been characterized yet.

Here, we monitored transcription kinetics by single-cell time-lapse bioluminescence imaging of mouse fibroblasts expressing a short-lived luciferase reporter gene controlled by endogenous loci, circadian regulatory sequences, or artificial promoters. We analyze such signals using stochastic models that describe the three main processes of gene expression: gene activation, transcription and translation. Previous studies have mostly focused on describing the variability across populations and identifying the different sources of noise. Instead, we aim to reconstruct the temporal sequence of gene activity, mRNA and protein states from individual time traces. For this we developed a 3-layered Hidden Markov Model to describe gene activation, mRNA synthesis and protein translation. Deriving analytical approximations for the transition probabilities, we implemented decoding and estimation algorithms that enable us both to infer instantaneous gene activity status, mRNA, and protein copy number. Moreover the same method is used to learn the activation, synthesis and degradation rates defining the stochastic model, as well as to compute the uncertainty of the inferred trajectories.

We found that the bursting kinetics were highly gene-specific and provide stereotyped signatures of the insertion sites or regulatory sequences. More specifically, promoter architecture and cis-acting elements appear to play a dominant role in shaping transcriptional kinetics in mammals. Finally, our quantitative analysis allowed us to discover that transcription reactivation requires a minimum of two sequential steps constraining stochastic transcription events by refractory periods of gene activity.

Joint work with David Suter (University of Geneva), Benjamin Zoller (EPFL), Ueli Schilber (Geneva), and Felix Naef (EPFL).

Peter Swain

Centre for Systems Biology at Edinburgh
University of Edinburgh
peter.swain@ed.ac.uk

Identifying sources of variation in biochemical networks

Biochemical networks have two sources of stochasticity: intrinsic fluctuations, inherent in the biochemistry and enhanced by low numbers of molecules, and extrinsic fluctuations, generated by interactions of the system of interest with other stochastic systems in the cell or its environment. I will discuss the definitions of intrinsic and extrinsic stochasticity and their interdependencies. I will describe ways to model, simulate, and measure both types of fluctuations and illustrate how stochasticity can be important for understanding the "design" of some biochemical networks.

André Estévez-Torres

Laboratoire de photonique et de nanostructures
Centre national de la recherche scientifique
aestevez@lpn.cnrs.fr

Engineering reaction networks outside the cell: reactions and reactors

Reaction networks with well-defined dynamics and topology are fundamental elements in the emergence of complex biological behaviors. Some of you engineer those networks inside cells using, and developing, the tools of synthetic biology. We lack of principles, however, for implementing similar networks outside the cell. The stakes are high, though. In the last century chemists have developed rules for engineering molecular architectures, such as drugs, dyes, or materials, that make our lives easier. The natural step to take now in chemistry is to learn how to construct (bio)chemical networks, which, I believe, would open exciting opportunities.

I will describe our ongoing efforts towards the engineering of such networks *in vitro*. First, I will attempt to give a chemist's point of view of the network description used in systems biology. Second, I will introduce the toolboxes that I think we need for engineering reaction networks with interesting functions outside the cell. Namely, a controllable set of reactions and a tunable reactor. Finally, I will show recent results of a nanoliter-scale continuous reactor for studying oscillatory reactions. Although stochasticity is not relevant to these experiments -there are 10^8 molecules in the reactor - it is crucial for future developments of in vitro networks as soon as smaller volumes – in vesicles for example - and lower concentrations (nM) are concerned.

Joint work with Jean-Christophe Galas and Ibtissam El Abdouni.

Robert Egbert

Department of Electrical Engineering
University of Washington
robere@u.washington.edu

Predictable tuning of gene networks with simple sequence repeats

With recent advances in genome manipulation methods and DNA synthesis and assembly techniques, synthetic biologists are building increasingly complex gene networks to understand or repurpose natural behaviors, and to program new ones. As engineered gene networks increase in complexity, our ability to implement the desired dynamics in living systems decreases. Even with proper circuit architectures, undesired behaviors may result from imbalanced gene expression levels, stochastic effects, or the strain background. Experimental systems generally need modification or tuning through multiple iterations, which can limit discovery and reduce efficiency. A comprehensive strategy for tuning complex gene networks must meet multiple criteria, including explorability, predictability, scalability, and evolvability. As a step towards more robust biological engineering, we have developed a tuning mechanism for bacterial gene networks that has promise as a comprehensive tuning strategy.

Our tuning mechanism, termed rbSSR, uses simple sequence repeats (SSRs) embedded in the spacer of the ribosome binding site (RBS). Variation in the number of nucleotides at the spacer, situated between the Shine-Dalgarno region of a gene's 5' untranslated region (5' UTR) and its initiation codon, has been shown to modulate the translation initiation rate of protein products. SSRs are tandem repeats of short DNA sequences found in all kingdoms of life that have much higher mutation rates than arbitrary sequences and are known to introduce variability and stochastic behaviors in populations. By placing these hypervariable SSR sequences in the RBS spacer, we can rapidly explore the parameter space of gene networks by constructing rbSSR libraries.

We show that the use of rbSSRs enables predictable fine-tuning of simple behaviors by building variable-repeat expression libraries of GFP and bistable switch circuits in *Escherichia coli*. We assembled multiple rbSSR-GFP libraries, each of which exhibits monotonically decreasing expression levels as the numbers of repeat units increase. To test the scalability of our tuning method, we added rbSSRs upstream each transcriptional repressor of a mutual-repression bistable switch and cloned an array of strains by varying the combinations of repeats. Changing the number of rbSSR repeat units for either repressor predictably shifts the dominant phenotype between unimodal populations in either of the two repression states, and, for some repeat combinations, balanced bimodal distributions. Finally, to assess the stability of rbSSR-encoded gene circuits and to explore their potential for *in vivo* optimization, we analyzed sequencing data from wild-type and mutator strains transformed with (A)15 rbSSR-GFP, passaged over 200 generations. We found that rbSSRs are surprisingly stable over this period in wild-type strains, while repeat drift in the mutator strain is detectable after fewer than 50 generations. With these results, we are now exploring how to use the high mutation rates of rbSSRs to optimize gene networks *in vivo*.

Yannick Rondelez

LIMMS/CNRS-IIS, Institute of Industrial Science
University of Tokyo
rondelez@tokyo.ac.jp

In vitro minimal universal networks

The concept of dynamic chemical network is increasingly recognized as the best framework to describe biological processes. Such networks occur at every stage of the biochemistry of living organisms, from their most basic metabolism, to elaborate computational scheme embedded for example in gene regulatory networks.

Compared with this strong shift toward the study of cells as complex integrated systems, it may seem surprising that, up to very recently, there existed no method to build similar networked dynamic architectures *in vitro*. Man-made chemistry simply did not provide such tools, and it was just impossible to connect rationally out-of-equilibrium chemical elements in a network to obtain, say, an oscillator or a switch.

Three toolboxes for this purpose have now been experimentally demonstrated. Each presents a class of generalized reactions that can be connected to each other and are sufficiently complex. These three toolboxes use DNA as the programmable element. One is purely chemical and the two others are based on enzymatic reactions. They allow, at least theoretically, the assembly of elements in arbitrary topologies and hence the building on demand of any dynamic behavior or computation.

Besides their interest as precursor in the move toward information processing in wet medium, such simple *in vitro* model systems may provide a great tool to better understand biological networks: they can be used to reproduce the biological system-level architecture in a tube, with the following advantages over *in vivo* artificial systems:

•A well controlled, reproducible environment
•The possibility of quantitative analysis and modeling, because all the reactions rates and thermodynamic constants are experimentally accessible
•An increased tunability and versatility, because the conditions can be systematically altered, or the network topology can be rearranged very simply.

I will first show how it is possible to assemble a minimal set of enzymatic catalysts to generate such a universal programmable environment. This provides the wet equivalent of the hardware. The topology of the targeted network (the software) can then be enforced in the tube by encoding this topology within the sequences of a few small single strand DNA strands. The system then runs autonomously, creating and destroying small oligomers (the data) according to the rules that have been implemented. I will describe some recent achievements, starting from simple logic elements (gates), up to toggling toggle switches and various oscillators.

I will also try to show what can be the benefits of this *in vitro* learning-by-doing approach to address basic questions in biology. One example may be the discussion around the design rules and the degree of modularity of genetic regulatory networks. Another future possibility will be to extend these approaches to the experimental study of stochastic phenomena and their consequences on the dynamic of such molecular dynamic networks.

Gabriele Lillacci

Center for Control, Dynamical Systems and Computation
University of California, Santa Barbara
gabriele@engr.ucsb.edu

Efficient model selection in discretely observed stochastic chemical reaction networks

The model selection problem, that is picking the model that best explains an experimental data set from a list of candidates, arises frequently when studying unknown biological processes. In this talk, we describe a new method for model selection in stochastic chemical reaction networks using measurements from flow cytometry. A distinctive feature of our proposed approach is its ability to perform statistically significant selection using a very small number of Monte Carlo simulations of the candidate stochastic models. After reviewing the theory associated with our procedure, we introduce the model selection algorithm and we demonstrate it on an example drawn from molecular biology.

Gürol Süel

Department of Pharmacology/Green Center for Systems Biology
University of Texas Southwestern Medical Center
gurol.suel@utsouthwestern.edu

Cellular decision-making in the context of population dynamics

How do cells execute decisions to cope with and survive under environmental conditions? My laboratory focuses on understanding how the dynamics of genetic circuits comprised of interactions between genes and proteins allow cells to govern decision-making. Interestingly, we find that stochastic fluctuations that are inherent to the biochemical reactions within genetic circuits can allow cells to cope with unpredictable environmental conditions. In addition, since cells have the ability to alter their own environment, the decisions at the single-cell level can depend on the context of the population. I will be presenting our attempts to understand these problems.

Rosalind Allen

School of Physics
University of Edinburgh
rallen2@ph.ed.ac.uk

Competition between mRNAs for a limited pool of ribosomes

Ribosomes are crucial components of the cellular protein production machinery. In cells, many mRNA molecules compete for a finite number of ribosomes. Using a simple theoretical approach combined with stochastic simulations, we explore the implications of this competition for protein production at a whole-cell level. In particular, we propose a mechanism that effectively buffers the free ribosome pool, making it independent of fluctuations in mRNA-number and total amount of ribosomes. This mechanism is based on the hopping dynamics of ribosomes on mRNA: a few mRNA molecules with high affinity for ribosomes can act as reservoir of free ribosomes. Our results may have significant implications for cells´ robustness to fluctuations in ribosome number and ability to respond independently to multiple demands on the ribosome pool.

Pablo Meyer Rojas

Computational Biology Center
IBM Thomas J. Watson Research Center
pablo.meyerojas@gmail.com

Metabolic regulation and enzyme localization in cells

Regulation of cellular metabolism is controlled by the expression level and activity of the enzymes composing the reactions but is also constrained by the structure of biochemical networks. The major element missing from theories explaining the regulation of metabolic networks, such as Metabolic Control Analysis and Flux Balance Analysis, is to show which of the many occurring regulatory events are determinant in controlling metabolic function in a cell. This is due in part to the lack of single-cell level information on the dynamics of these reactions. I will here show data on the organization of enzymes from a specific biochemical pathway in *B. subtillis* and then extend this analysis to genome-level organization of biochemical networks in *E. coli* and then eukaryotic cells.

Narendra Maheshri

Department of Chemical Engineering
Massachusetts Institute of Technology
narendra@mit.edu

Molecular and kinetic analysis of *FLO11*: a *cis*-encoded epigenetic switch

Analyzing the dynamics of motifs that recur within gene regulatory networks has aided in understanding their function. Positive feedback is a motif whose dynamics confers a switch-like response and bistable, epigenetic states whose memory is encoded in a *trans* factor. This (and other) behaviors often ascribed to network motifs may also occur at a single promoter because of complex protein/nucleic acid interactions, but less is known about how these interactions dictate dynamics.

Here, we describe the regulation of the *FLO11* gene in *S. cerevisiae*, which encodes a cell-wall adhesion protein whose expression leads to a distinct morphology and flocculation in liquid cultures. Under certain conditions, *FLO11* expression is bimodal – with single cells exhibiting heritable “ON” and “OFF” expression states. We show that bimodal *FLO11* expression is because its 3 kb promoter functions as a *cis*-encoded epigenetic switch, as two copies of *FLO11* within the same cell stochastically and independently. We integrate how *trans* factors affect the kinetics of these slow transitions with molecular and biochemical analysis including ChIP measurements of chromatin status and binding of transcription factors, the role of two ncRNA’s within the promoter, and 3C measurements. This approach reveals that the formation of two distinct DNA loops correlate with the two epigenetic states and they are required for stability. Our work suggests that cooperative interactions between loop formation, ncRNA transcriptional status, and chromatin state provide the strong stability. Finally, we show how the population distribution of gene expression from the *FLO11* promoter is easily evolvable: insertion of a single binding site within different regions of the promoter leads to large range of transition rates.

Joint work with Leah Octavio (M.I.T., Whitehead Institute for Biomedical Research) and Gerry Fink (Whitehead Institute).

James Werner

Center for Integrated Nanotechnologies
Los Alamos National Laboratories
jwerner@lanl.gov

Time-resolved three-dimensional molecular tracking in live cells

We report a method for tracking individual quantum dot (QD) labeled proteins inside of live cells that uses four overlapping confocal volume elements and active feedback once every 5 ms to follow three-dimensional molecular motion. This method has substantial advantages over three-dimensional molecular tracking methods based upon charge-coupled device cameras, including increased Z-tracking range (10 μm demonstrated here), substantially lower excitation powers (15 μW used here), and the ability to perform time-resolved spectroscopy (such as fluorescence lifetime measurements or fluorescence correlation spectroscopy) on the molecules being tracked. In particular, we show for the first time fluorescence photon antibunching of individual QD labeled proteins in live cells and demonstrate the ability to track individual dye-labeled nucleotides (Cy5-dUTP) at biologically relevant transport rates. To demonstrate the power of these methods for exploring the spatiotemporal dynamics of live cells, we follow individual QD-labeled IgE-FcεRI receptors both on and inside rat mast cells. Trajectories of receptors on the plasma membrane reveal three-dimensional, nanoscale features of the cell surface topology. During later stages of the signal transduction cascade, clusters of QD labeled IgE-FcεRI were captured in the act of ligand-mediated endocytosis and tracked during rapid (950 nm/s) vesicular transit through the cell.

Diego Ferreiro
Department of Protein Physiology
Universidad Nacional de Quilmes
diegulise@gmail.com

Repeat proteins: folding in parts, functioning as wholes

Repeat proteins are believed to be ancient folds. Instead of being formed by 'apparently random' amino-acid sequences, repeat-proteins are made up of tandem arrays of similar 20~40 amino-acid stretches that usually fold-up in elongated architectures. Quasi-one dimensional, these non-globular folds are stabilized only by interactions within each repeat or between adjacent repeats, with no obvious contacts between residues distant in sequence. Their 'biological function' is usually attributed as mediating specific protein-protein interactions, with a versatility for recognition paralleled to that of antibodies. Still, most of the natural repeat-proteins display strong couplings between distant functional sites, suggesting that specific binding is intimately coupled to strong structural transitions. Repeat proteins, by virtue of their inherent symmetries both in primary sequence and three dimensional structures, stand as remarkable models where the 'sequence-codes-folding-codes-function' hypothesis can be quantitatively evaluated. How many variables are needed to describe the 'functionally important motions'? What are these and how to find them? Can they be traced back to the amino-acid sequences? I will discuss how the uncomplicated topology of repeat-proteins may facilitate the functional mapping of protein folding dynamics.

Gabor Balazsi

Department of Systems Biology
University of Texas M.D. Anderson Cancer Center
gbalazsi@mdanderson.org

From genotype to phenotype: Selection and memory of noisy gene expression variants

Gene expression is the biological process that actualizes the organismal phenotypes encoded within the genome. By estimating the gene expression mean, traditional biological measurement techniques provided important insights into the genotype-phenotype connection. Recently it became clear that cells with identical genomes exposed to the same environment can differ dramatically in their gene expression and phenotype, and the phenotypic importance of gene expression variation (noise), in addition to the mean, is now well established. Still, the role of further cell population level gene expression characteristics remains unclear.

We utilized synthetic gene circuits in *Saccharomyces cerevisiae* to address this question. Cell populations carrying different gene circuits controlled the expression of a fluorescent reporter that also protected the cells from the antibiotic Zeocin. Using these synthetic systems, we found that gene expression characteristics other than the mean and variance, including the non genetic memory of protein expression could explain the remaining fitness difference. Moreover, we found that the cost associated with various protein expression levels strongly affected the shape of gene expression distributions in clonal cell populations, implying that the intracellular biochemical kinetics of individual cell lineages was insufficient to describe population level phenotypes.

The emerging picture is that noise forces cells to constantly explore a complex gene expression landscape, similar to the one proposed by C. H. Waddington. While exploring this gene expression landscape, cells are also forced to move on a non-genetic version of Sewall Wright's fitness landscape, and face varying levels of selection through the instantaneous fitness associated with their gene expression. A true understanding of the genotype-phenotype connection requires integrating the movement on these two different landscapes.

Eldon Emberly

Department of Physics
Simon Fraser University
eemberly@sfu.ca

Stochastic processes involved in organizing space inside bacteria

Spatial organization plays a significant role in many bacterial processes from chemotaxis to the segregation of chromosomes. In some processes, active mechanisms are required to create the spatial patterns whereas in others, structure formation can be aided significantly by entropic forces. Recent experimental and theoretical work has shown the potential importance of these forces in driving polar patterning of aggregating cytoplasmic proteins in bacterial cells. The entropic force that drives polar localization is generated by the bacterial chromosome. Besides polar localization of proteins, does the entropy of the chromosome aid the spatial organization of other bacterial processes? As an extension of the work on the polar localization of proteins, I will present data and simulations on the localization of diffusing plasmids inside bacterial cells and the crossover from localization to diffuse behaviour as a function of plasmid size. As a second example, entropic forces likely play an organizing role during sporulation in the bacteria, *Bacillus subtillis*. During sporulation, the division ring forms at 1/4 the cell length, contracts to form the much smaller spore, and then a fully replicated chromosome is packed into the much smaller cell. The motors that generate the force necessary to pull the replicated chromosome into the spore require the DNA to be in a particular ordered configuration. I will present results showing how the contraction of the division ring spontaneously organizes the DNA in the pore, allowing the motors to assemble and hence function. Lastly, I will discuss the implications of such entropic effects in the light of recent experimental work on replication, segregation and division in protocells.

Attila Becskei

Department of Chemical & Biomolecular Engineering
Institute of Molecular Life Sciences, UZH
attila.becksei@imls.uzh.ch

Evolution of feedback circuits by dynamical wiring

Cellular networks are rewired during evolution by strengthening or weakening the binding interactions in order to develop new regulatory schemes. We studied the functioning of parallel feedback loops that arise from evolutionary gene duplication. The GAL1 and GAL3 paralogs enhance their own expression mediated by the Gal4 transcriptional activator, which binds to multiple and single sites in the respective promoters. The expression of these genes is decreased when the number of activator binding sites is reduced. This can, paradoxically, boost retroactively the positive feedback loop. The stochastic bursts in basal expression determine the relative strengths of direct and retroactive effects. These determinants, along with fast mRNA decay rates, set the cellular memory and the speed of adaptation in the galactose regulon. Our results indicate that nonlinear stochastic transcriptional responses enable feedback loops to evolve functions contrary to what is dictated by the interaction strengths in the primary circuitry.

Gregor Neuert

Departments of Physics and Biology
Massachusetts Institute of Technology
gneuert@mit.edu

Systematic identification of signal-activated stochastic gene regulation

Understanding and predicting how cells sense and respond to their environments is a key goal of systems biology. Although new experimental and computational methodologies have elucidated many signal transduction pathways and gene regulation mechanisms, this goal remains elusive particularly when it comes to making quantitative predictions for the phenotypic diversity of single cell dynamics. To better understand and predict gene regulatory responses for these complex networks, we propose a comprehensive system identification and validation approach. First, we developed a quantitative assay to measure single-molecule expression of endogenous mRNA at high temporal resolution in individual cells. Second, we developed an efficient and flexible computational approach that captures the discrete, time varying, and stochastic nature of transcriptional regulation. Third, we integrated these experimental and computational approaches within a novel hierarchical system identification framework, which involves several clearly defined rounds of analysis, prediction, experiment design, and validation. We applied this approach to the osmotic stress response pathway of *Saccharomyces cerevisiae* and uncovered the best gene regulatory model among several thousand automatically generated model hypotheses. Using parameter identification and cross-validation analyses, we select a final model (both mechanisms and parameters) that is complex enough to match the observed single-cell/single-molecule data but simple enough to avoid over-fitting and thereby retain predictive power. The identified model describes several novel dynamical features in transcriptional regulation, including multi-step activation, low-pass filtering, kinetic proofreading and signal-modulated duration of gene expression. Furthermore, we found that kinetic proofreading, mRNA degradation and transcription dynamics are robust to environmental and/or genetic conditions. In addition, the selected model provides accurate quantitative predictions at diverse experimental perturbations, which extend well beyond the training dataset. In particular, we are able to predict the active transcription state of the gene - a new experimental feature that was never considered in our model identification procedure. This approach is not specific to any gene, pathway or organism, and may lead to new insight into inducible transcriptional networks in organisms ranging from yeast to human.

Joint work with B. Munsky (Los Alamos Natl. Lab.), R.Z. Tan (MIT), M. Khammash (University of California, Santa Barbara), and A. van Oudenaarden (MIT).

Ophelia Venturelli

Biochemistry & Molecular Biophysics
California Institute of Technology
opheliav@caltech.edu

Characterizing bimodality, bistability and cellular memory in biomolecular circuits

Cell fate determination is influenced by epigenetic effects also known as cellular memory. In this talk, I will discuss two ways that cells can transmit information over multiple generations. Passive memory is transient and requires stochastic fluctuations in a cell population. Active memory is persistent over time and requires active regulation which I will argue can only be generated by multistability. The galactose regulatory network has the potential to produce two discrete expression states in the same environment. We argue that this bimodality arises from bistability and seek to understand what key molecular interactions are necessary. Surprisingly, we find that transcriptional feedback is not necessary for bistability and identify a minimal circuit that displays the capacity for bimodality. Using a model, we propose a mechanism to generate bistability in the modified circuit.

Elisa Franco

Department of Mechanical Engineering
University of California, Riverside
efranco@ucr.edu

Analysis, design and in vitro implementation of robust biochemical circuits

The functionalities of every living organism are wired in the biochemical interactions among proteins, nucleic acids and all the other molecules that constitute life's building blocks. Understanding the general design principles of this "hardware of life" is an exciting and challenging task for modern bioengineers. In this talk, I will focus on design rules to achieve robustness and modularity in molecular networks. Experimental verifications of such rules is carried out using in vitro transcriptional circuits, a minimal analogue of cellular genetic networks.

The first problem I will consider is flux control, which is an important feature for the correct performance of biochemical systems. I will describe a simple model problem where two reagents bind stoichiometrically to form an output product. In the absence of any regulation, imbalances in the reagents production rates can cause accumulation of unused molecules, and limit the output flow. To match the reagents flux, robustly with respect to the open loop rates, I will propose the use of negative or positive feedback schemes that rely on competitive binding. Such schemes are modeled through ordinary differential equations and implemented using transcriptional circuits.

The second topic will be the functional robustness of interconnected networks: molecular devices characterized in isolation may lose their properties once interconnected. This challenge will be illustrated with a case study: a synthetic transcriptional clock will be used to time conformational changes in a molecular nano-machine called DNA tweezers. Mass conservation introduces parasitic interactions that perturb the oscillator trajectories proportionally to the total amount of tweezers “load.” To overcome this problem, we can use a genetic switch that acts as a buffer amplifier, achieving signal propagation and at the same time reducing the perturbations on the source of signal.

ADDENDA

Andreas Hilfinger

Department of Systems Biology
Harvard Medical School
andreas_hilfinger@hms.harvard.edu

Separating intrinsic from extrinsic fluctuations in dynamic biological systems

Species within biochemical networks fluctuate due to the intrinsic randomness of individual events as well as the extrinsic influence of changing environments. This combined effect is often too complex for effective analysis, which is why many studies make simplifying assumptions, for example ignoring either intrinsic or extrinsic effects to reduce the number of model assumptions. I will discuss how two identical and independent reporters embedded in a shared fluctuating environment identify intrinsic and extrinsic noise terms that under certain circumstance correspond to the noise contributions predicted by stochastic models of only intrinsic or only extrinsic mechanisms. For relatively broad classes of systems, the extrinsic noise from dual-reporter methods can be rigorously analyzed using models that ignore intrinsic stochasticity, whereas the intrinsic noise can be rigorously analyzed using models that ignore extrinsic stochasticity only under very special conditions that rarely hold in biology. Rigorously dissecting the origin of fluctuations within biochemical network will thus require additional experimental approaches.

Additional Participants

Nils Becker	FOM Institute for Atomic and Molecular Physics	n.becker@amolf.nl