Abstracts of Talks for
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Co-organizers: Christopher Rao and Lingchong You

Evolution through the lens of synthetic biology

Gabor Balaszi

We use synthetic gene networks to control stochastic gene expression. We develop a quantitative framework that connects stochastic expression of a drug resistance protein to cellular fitness, and further to cell population fitness. Using this framework, we gain insights into long-term genetic evolution by allowing cells with the synthetic gene network to evolve in well-defined environments for hundreds of generations.

A Control Theory Approach to Engineering Biomolecular Networks

Domitilla Del Vecchio

The past decade has seen tremendous advances in the fields of Systems and Synthetic Biology to the point that de novo creation of simple biomolecular networks, or "circuits", in living organisms to control their behavior has become a reality. A near future is envisioned in which re-engineered bacteria will turn waste into energy and kill cancer cells in ill patients. To meet this vision, one key challenge must be tackled, namely designing biomolecular networks that can realize substantially more complex functionalities than those currently available.

A promising approach to analyzing or designing complex networks is to modularly connect simple components whose behavior can be isolated from that of the surrounding modules. The assumption underlying this approach is that the behavior of a component does not change upon interconnection. This is often taken for granted in fields such as electrical engineering, in which insulating amplifiers enforce modular behavior by suppressing impedance effects. This triggers the fundamental question of whether a modular approach is viable in biomolecular circuits. Here, we address this research question and illustrate how, just as in many mechanical, hydraulic, and electrical systems, impedance-like effects are found in biomolecular systems. These effects, which we call retroactivity, dramatically alter the behavior of a component upon interconnection. We illustrate how, similarly to what is performed in electrical networks, one can reduce the description of an arbitrarily complex system by calculating equivalent retroactivities to the input. By merging disturbance rejection and singular perturbation techniques, we provide an approach that exploits the structure of biomolecular networks to design insulating amplifiers, which buffer systems from retroactivity effects. We provide experimental demonstration of our theory on a reconstituted protein modification cycle extracted from bacterial signal transduction and on a synthetic biology circuit in vivo.

Anticipating tipping points in biological populations: Cooperation, cheating, and collapse

Jeff Gore

Natural populations can suffer catastrophic collapse in response to small changes in environmental conditions, and recovery after such a collapse can be exceedingly difficult. We have used laboratory

microbial ecosystems to study early warning signals of impending population collapse. Yeast cooperatively breakdown the sugar sucrose, meaning that below a critical size the population cannot sustain itself. We have demonstrated experimentally that changes in the fluctuations of the population size can serve as an early warning signal that the population is close to collapse. The cooperative nature of yeast growth on sucrose suggests that the population may be susceptible to cheater cells, which do not contribute to the public good and instead merely take advantage of the cooperative cells. We confirm this possibility experimentally and explore how such social parasitism can reduce population resilience.

Design Principles of information processing in the Bacillus subtilis sporulation network
Oleg Igoshin

Starving B. subtilis cells cease their vegetative groth and execute a complex gene-expression program resulting in formation of stress-resistant spores. This program is initiated by the sporulation master regulator, Spo0A, that is activated by a phosphorelay network which transfers phosphoryl groups from the kinases (KinA-E) to Spo0A through intermediate phospho-transferases. To understand how this regulatory network processes information to make reliable cell-fate decisions we combine complementary approaches such as synthetic rewiring of the networks, single-cell microscopy, and mathematical modeling. To this end, we first decoupled sensing of starvation signals from decision making by artificially inducing the expression of the kinase KinA and thereby directly controlling the activation of Spo0A. We found that the induction of a threshold level of KinA results in a switch-like increase in the number of sporulating cells. We show that around this ultrasensitivity to KinA levels is created by a cascade of coherent feed-forward loops downstream of Spo0A. Our results also show that many cells initiate the sporulation program even below the KinA threshold but ultimately reverse these changes and do not form spores. Time-lapse microscopy of starving wild-type cells confirmed that activation of Spo0A and formation of asymmetric septa are reversible and only cells that activate the regulators controlled by feed-forwards downstream of Spo0A complete sporulation. Using our model we show that this reversibility of the sporulation program together with a feed-forward based decision switch allows the cells to minimize the effects of stochastic fluctuations in Spo0A activity on the cell-fate decision. Further, we investigated the design of the signal-sensing phosphorelay, by decoupling various positive transcriptional feedback loops in it. We found that these positive feedbacks ensure that the phosphorelay responds to starvation with a gradual increase in Spo0A activity. This gradual increase in Spo0A activity, in turn, ensures the proper temporal coordination of septation and chromosome segregation during sporulation.

The gratuitous growth bistability of antibiotic resistant bacteria
Minsu Kim, Emory

Bacterial cells expressing antibiotic resistance can grow at antibiotic concentrations that would otherwise inhibit the growth of wild type cells. When the resistance-expressing cells are exposed to antibiotics, the degree of resistance expression is expected to change via global growth-mediated effects, resulting in a positive feedback between the growth-inhibiting effect of the antibiotics and the cells' ability to resist the antibiotics. Consistent with this positive feedback effect, *E. coli* cells expressing antibiotic resistance are found to exhibit an abrupt transition from rapid growth to non-growth at a threshold antibiotic concentration. Below the threshold, the population consisted of a mixture of growing and non-growing

cells. Quantitative modeling of known interactions between the antibiotics and the expression of antibiotic resistance can quantitatively account for various experimental observations.

Bacterial Surface Defense Against Environmental Threats
Meta Kuehn

The bacterial cell surface plays a major role in bacterial interactions with the environment and it is well-established that the bacterial exterior serves a protective barrier. For example, oxidation, heat shock, antimicrobial peptides (AMPs), and bacteriophage are ubiquitous threats to bacterial viability in nature, and these attacks commonly modify or interact with the bacterial cell surface. In the case of Gram-negative bacteria, that outer surface is composed primarily of outer membrane protein and lipid. However, bacterial surface components are also found as secreted membranous spheres known as outer membrane vesicles (OMVs) whose functions have only been recently studied. OMVs have been shown to play beneficial roles in pathogenesis, colonization, toxicity, and horizontal gene transfer. Our work has demonstrated that OMVs can also act as an envelope stress response and as decoys to allow bacterial survival at the cost of producing the OMVs. OMV production increases survival during stress, and OMVs inactivate AMPs and essentially irreversibly bind phage and resulting in a lowered efficiency of infection. However, in the course of these studies, we also discovered that not only are phage-OMV complexes protective for the OMV donor species, but contact with the complexes induced phage activation in a completely different species, ultimately leading to lysis of that species. These studies demonstrate how the OMVs derived from the bacterial surface can be both protective and stimulatory in response to environmental threats.

Directed evolution of biosynthetic pathways using synthetic integrons
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Technologies that enable the rapid construction and optimization of novel and complex functions in biological systems are a central goal of the synthetic biology community. However, assembling multigenic functions such as engineered biosynthetic pathways currently requires laborious iterative approaches and detailed knowledge of both host biology and “part” function, since such exogenous genes and their products often interact strongly with one another and with native functions in the host cell. Such challenges complicate efforts to engineer metabolism in microbes and plants and consequently restrict the scope of projects that are currently attempted. Here, I will present a novel technology platform that aims to address this need by enabling the continuous directed evolution of biosynthetic pathways to dynamically optimize their function. As part of an international consortium, we are developing this technology using a mechanism that is inspired by bacterial integrons, which are dynamically rearranging arrays of gene cassettes found in many microbial genomes. I will describe our work to date enabling the generation of diverse synthetic integrons, or syntegrons, which are the basis of this directed evolution approach. The syntegron platform contains many “levers” that may be varied to alter the conditions under

which directed evolution proceeds, and I will also describe our development of computational testbeds that enable the characterization and design of this new class of directed evolution experiments.

Adaptive collective responses of E. coli to spatial confinement
Andre Levchenco

Bacterial cells can frequently grow within highly spatially confined environments, and under some circumstances, can actively seek them. Such environments can results in considerable risks to cell and colony survival. What adaptive strategies exist to protect cells from physical and nutritional stresses they can face? In this talk, I will review examples of 'intelligent' cell responses, including intercellular communication, spatial self-organization and biofilm formation that appear to have evolved in response to the self-imposed uncertainty in environmental conditions. I will demonstrate how we analyze them using a combination of synthetic biology, mathematical modeling, micro-fabrication of novel analysis platforms and other methods as a way to unravel complex underlying mechanisms.

Art of war against quorum-sensing mediated cooperation -- lessons from synthetic gene circuits
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Quorum sensing (QS) plays a critical role in controlling diverse physiological functions in bacteria. This includes cooperative bacterial actions such as secretion of exoenzymes, toxins, and biofilm forming compounds that are responsible for pathogen growth and virulence. Inhibition of QS has thus been recognized as a potentially effective strategy to inhibit pathogen growth and virulence. Intuitively, this strategy may avoid the undesirable consequence of antibiotics that directly inhibit bacterial growth – wide use of antibiotics has been blamed for the rising antibiotic resistance crisis. To examine the consequences of QS inhibition strategies, we established a synthetic bacterial population where QS mediates the production and secretion of a cooperative public good. We show that inhibition of QS can lead to the selection of more cooperative bacterial variants. As a result, such treatment can increase the virulence of the overall population. In contrast, we find that directly inhibiting the public good can destabilize bacterial cooperation and reduce virulence in the overall population. Our results have implications for designing effective treatment strategies against bacterial pathogens aimed at QS-mediated cooperation.

Time dependence of force development in a localized bacterial adhesin
Setayeshgar, Sima

Bacterial attachment to surfaces is an important first step in the formation of collective populations and microbial biofilms. Bacteria attach to surfaces using biopolymers, with remarkable adhesive properties in variety of environments. Using {\it Caulobacter crescentus} as a model system, I will discuss joint experimental and theoretical study of the dynamics of force development in the holdfast, an

approximately $10^{-2} \, \mu{\rm m}^2$ patch of polar adhesive. {\it C. crescentus} synthesizes its holdfast adhesin during the differentiation of the motile swarmer cell into a sessile stalked cell. Previous work reported an overall holdfast adhesion strength greater than 68 ${\rm N/mm^2}$. Using Dynamic Force Spectroscopy, it is shown that the initial adhesion force is significantly weaker, suggesting the existence of a curing process. We show that the holdfast adhesion process is time-dependent and involves transformations at multiple time scales. We develop a multistep kinetic model of adhesin-surface binding coupled to bulk diffusion of the adhesin with the holdfast that reproduces the observed time dependence and strength of adhesion. I will discuss the potential advantages of such a curing process in cell-surface attachment, as well as other as other related aspects of the transition from motile to sessile state.

Evolution of incipient cooperation: pleiotropic mutations can confer "win - win" phenotypes directly benefiting both self and partner

Wenying Shou

Inter-population cooperation, in which two genetically-isolated populations promote each other's fitness at a cost to self, can be found in diverse forms of mutualism. Extant inter-population cooperation could have evolved over millions of years, making it difficult to retrace their evolutionary trajectories. Here, we used an engineered microbial system to examine how incipient cooperation between populations could evolve. The system is composed of two reproductively-isolated S. cerevisiae strains: a red-fluorescent strain that requires lysine and releases adenine and a green-fluorescent strain that requires adenine and releases lysine. The ancestral coculture is viable, i.e. able to grow from low to high density in the absence of adenine and lysine supplements, only if the initial total cell density exceeds a minimum "viability threshold." All cocultures rapidly evolved to improve viability by reducing the viability threshold, and evolved was sufficient for this viability improvement. Deep-sequencing and phenotypic analyses of evolved revealed mutations that directly benefited the mutants by allowing them to grow better under the lysine-limited cooperative environment. Surprisingly, these mutations also directly benefited the partner by increasing the adenine release rate. This "win-win" phenotype was solely due to the pleiotropic nature of mutations because it also rose in monocultures evolving under lysine limitation. Thus, pleiotropy can promote incipient cooperation between populations by coupling partner-serving phenotype to self-serving phenotype.

A synthetic-biology approach to understanding bacterial programmed death and implications for antibiotic treatment

Yu Tanouchi

Abstract: Programmed death is often associated with a bacterial stress response. This behavior appears paradoxical, as it offers no benefit to the individual. This paradox can be explained if the death is 'altruistic': the sacrifice of some cells can benefit the survivors through release of 'public goods'. However, the conditions where bacterial programmed death becomes advantageous have not been unambiguously demonstrated experimentally. Here, we determined such conditions by engineering tunable, stress-induced altruistic death in the bacterium Escherichia coli. Using a mathematical model, we predicted the existence of an optimal programmed death rate that maximizes population growth under stress. We further predicted that altruistic death could generate the 'Eagle effect', a counter-intuitive phenomenon where bacteria appear to grow better when treated with higher antibiotic concentrations. In support of these modeling insights, we experimentally demonstrated both the optimality in programmed

death rate and the Eagle effect using our engineered system. These findings fill a critical conceptual gap in the analysis of the evolution of bacterial programmed death, and have implications for a design of antibiotic treatment.

Adaptive virtues of bacterial suicide
Martin Tchernookov, Ilya Nemenman
Emory University

While active, controlled cellular suicide (autolysis) in bacteria is commonly observed, it has been hard to argue that autolysis can be beneficial to an individual who commits it. We propose a theoretical model that predicts that bacterial autolysis is evolutionarily advantageous to an individual and would fixate in physically structured environments for stationary phase colonies. We perform spatially resolved agent-based simulations of the model, which predict that lower mixing in the environment results in fixation of a higher autolysis rate from a single mutated cell, regardless of the colony's genetic diversity. We argue that quorum sensing will fixate as well, even if initially rare, if it is coupled to controlling the autolysis rate. The model does not predict a strong additional competitive advantage for cells where autolysis is controlled by quorum sensing systems that distinguish self from nonself. These predictions are broadly supported by recent experimental results in B. subtilis, S. pneumoniae, and other bacteria.

Landscape Theory and Cell Fate Decision Making
Jin Wang

We developed a general non-equilibrium landscape theory for studying the global stability and function of the cellular networks. The key ingredient is that the dynamics is determined by both the landscape gradient and the non-equilibrium curl flux. We provided a global picture and applied the concept to quantify the cellular network dynamics such as budding yeast cell cycle, stem cell development, and bacteria lambda phage fate decision making process. We found that the dynamical paths do not pass through the saddle point or transition state and the kinetic rate is not determined by the conventional transition rate theory or Kramer's rate formula. These features are critical in addressing the global function of the underlying cellular network dynamics.

Social Evolution in Bacteria
Stuart West, Department of Zoology, Oxford

I will review the basics of social evolution theory, as applied to microbes. What does it mean for a trait to be social? How do we test if traits are social? What are the implications? I will emphasise the opportunities being opened up for work in this area by synthetic biology methods.

Engineering Complex Dynamics Using Synthetic Gene Networks
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Synthetic gene networks can be constructed from bottom up with desired properties. However, constructing predictable gene networks with desired functions remains a challenge. It is because of

unpredictability of the assembled networks and the lack of well-characterized components. Here we present the bottom-up and diversity-based approaches that combine promoter synthesis and mathematical modeling to quickly construct gene networks with desired properties. Promoters with random strength diversities were synthesized and characterized. When coupled with mathematical modeling to simulate the network at a system level, promoters that are optimal for the intended functions were selected before the actual network assembly, without the need for post-hoc modifications. This approach was first demonstrated in yeast by constructing negative feed forward loop networks. Then the method was used to produce a synthetic gene network that acted as a timer, tunable by component choice. We also developed a stochastic model and predicted that the timer network can also induce random cell fate determination upon metabolic switch. This prediction is then verified experimentally using FACS and fluorescence microscopy. Finally, ongoing research on gene regulation networks that are responsible for high dimensional multistability will be presented to lead into discussions of future synthetic gene circuits.

Gene regulation at the single molecule level

Jie Xiao, Johns Hopkins Univerisity

Precise gene regulation is essential for the growth and development of all organisms. In prokaryotes, gene regulation primarily occurs at the level of transcription. Transcription factors (TFs) are protein molecules that bind to DNA and determine when, where and at what level a gene is transcribed. In addition, DNA loops mediated by TFs also play important roles in gene regulation. However, many TFs are expressed at low levels and are thus difficult to detect. Further, labeling TFs with fluorescent proteins can potentially disrupt their regulatory functions. Here, we developed a novel strategy, Co-Translational Activation by Cleavage (CoTrAC), to monitor the stochastic expression of a TF, λ repressor CI, in its natural regulatory context in live *E. coli* cells at the single-molecule level. We further develop the CoTrAC strategy to enable the simultaneous monitoring of the real-time interplay of two TFs that mutually repress each other. Finally, we develop an *in-vivo*super resolution method to visualize the formation of DNA looping mediated by the λ repressor CI in live *E. coli* cells and correlate DNA looping with transcription regulation by CI. The set of novel methods we developed in this work will open a wide door for quantitative investigations of the working of gene regulatory networks .