A Multiscale Dissection of Decision-Making in Microbial Ecosystems

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Microbial ecosystems in nature are typically composed of hundreds or thousands of species, heterogeneously distributed in space and time. Interactions between these microorganisms help regulate the overall activity and functional outputs of these ecosystems. We have been applying the principles of quantitative biology to understand regulation in multispecies communities of microbes. By approaching ecosystem regulation at multiple length scales, ranging from transcriptional decisions made at the molecular level to the propagation of functional states through signal exchange, we aim to develop a predictive understanding of the collective properties of these diverse cellular networks.

Towards this goal, I will discuss our efforts to predict global regulatory outputs of individual cells in response to changes in environmental conditions, using iron-mediated regulation in Pseudomonas aeruginosa as a specific example. Regulatory decisions are modulated by multiple factors, and through a combination of theoretical approaches and the tools of synthetic biology to tune regulatory parameters of cells, we attempt to discover how information from multiple inputs is integrated at the promoter level.

Given the diversity of microbial communities in the real world, we then examined how such regulatory decisions at the single-cell level are shaped by local interactions between different species. At the ecosystem level, I will present theoretical and experimental results examining the ability of changes in regulatory states to be communicated via diffusible signals. We explored how ecosystem composition, the spatial distribution of cells, and the mechanistic details of communication pathways influenced the potential for global coordination of activity within the ecosystem.

Engineering Electron Transferring Proteins and their Assembly at Electronic Interfaces

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Many key cell functions are accomplished through complicated system of enzymes and redox carrier molecules that control electron and proton transport. Although significant number of these enzymes has been structurally characterized, the actual mechanism of redox catalysis is not always understood. Therefore we have adopted a different approach to address the structure-function relationship of oxidoreductases: we aim to uncover the assembly instructions required for function using smaller, simpler, more robust model proteins, maquettes. Our questions ask how many engineering elements are required to achieve a particular biological function, what are the individual biochemical and structural tolerances of these elements and how much of a protein infrastructure is consumed in accommodating the function. To start answering these questions, we have synthesized a set of amphiphilic maquettes. These maquettes transfer electrons across membranes, bind O2 and CO. They can be assembled on electro-active substrates through a variety of attachment strategies including simple adsorption, cysteine attachment to gold, histidine attachment to Ni-NTA, and click chemistry.

Automated Design of Enzyme-Driven DNA Circuits

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Molecular programming allows for the bottom-up engineering of biochemical reaction networks in a controlled in vitro setting. These engineered biochemical reaction networks yield important insight in the design principles of biological systems and can potentially enrich molecular diagnostic systems. The DNA-based polymerase-nickase-exonuclease (PEN) toolbox has recently been used to program oscillatory and bistable biochemical networks using a minimal number of components. Previous work has reported the automatic construction of in silico descriptions of biochemical networks derived from the PEN toolbox, paving the way for generating networks of arbitrary size and complexity in vitro. Here, we report an automated approach that further bridges the gap between an in silico description and in vitro realization. A biochemical network of arbitrary complexity can be globally screened for parameter values that display the desired function and combining this approach with robustness analysis further increases the chance of successful in vitro implementation. Moreover, we present an automated design procedure for generating optimal DNA sequences, exhibiting key characteristics deduced from the in silico analysis. Our in silico method has been tested on a previously reported network, the Oligator, and has also been applied to the design of a reaction network capable of displaying adaptation in one of its components. Finally, we experimentally characterize a novel, self-replicating trajectory of the enzyme that can be used to generate functional enzyme strands. The strong non-linearities in the degradation of active components caused by this unintended cross-coupling are shown computationally to have a positive effect on adaptation quality.