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cells by linking it to Lpp-OmpA, a protein created by fusing the first nine N-terminal amino acids of the lipoprotein (Lpp) to amino acids 46-159 of outer membrane protein A (OmpA), allowing fluorescent and magnetic particles to bind to the cell surface and serve as a reporter of synthetic gene network behavior. SNAP display on the surface of cells can be confirmed through microscopic observation and flow cytometry. We anticipate this new reporter will have broad impact fields ranging from cell wall and membrane biophysics to synthetic biology and material science.

2339-Pos Board B476
A Multiscale Dissection of Decision-Making in Microbial Ecosystems
James Boedicker.
Physics, University of Southern California, Los Angeles, CA, USA.
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 inside of cells, we attempt to uncover how information from multiple inputs is integrated at the promoter level.
Given the diversity of most microbial community 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.

2340-Pos Board B477
Engineering Electron Transferring Proteins and their Assembly at Electronic Interfaces
Bryan A. Fry, Chris Bialas, Zhenyu Zhao, Geetha Goparaju,
Christopher C. Moser, P. Leslie Dutton, **Bohdana M. Discher**.
Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, USA.
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 electrodic substrates through a variety of attachment strategies including simple adsorption, cysteine attachment to gold, histidine attachment to Ni-NTA, and click chemistry.

2341-Pos Board B478
Automated Design of Enzyme-Driven DNA Circuits
Tom F.A. de Greef.
Eindhoven University of Techology, Eindhoven, Netherlands.
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 unproductive sequestration of the exonuclease to phosphorothioate protected ssDNA 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.

2342-Pos Board B479
Coupling the Increase in Membrane Tension and the Synthesis of Phosphatidylserine in a "Smart" Artificial Cell
Kenneth Kwun Yin Ho, Victoria Murray, Jin Woo Lee, Allen Liu.
University of Michigan, Ann Arbor, MI, USA.
Assembling biological parts into a functional system using a bottom-up in vitro reconstitution approach offers the possibility of designing artificial cells with the ability of sensing and responding to external stimuli. An artificial platelet, which is programmed to mimic the functionality of a natural platelet, is our first test-bed for this design strategy. The greatest challenge of building the artificial platelet is the coupling of external mechanical stimuli to a biochemical response. We have reconstituted mechanosensitive channels (MscL) and phosphatylserine synthase (PSS1) into a liposome using cell free expression so that the liposome can sense an increase in membrane tension and allow influx of calcium ions, which serve as the second messengers to activate the synthesis of phosphatidylserine (PS). We used double emulsion template to generate lipid bilayer vesicles with encapsulation capability. We have also developed an in-house cell free expression technique for expressing MscL and PSS1. By encapsulating the cell free expression components inside the lipid vesicles, we have shown that the membrane proteins successfully insert into the lipid bilayer membrane and function properly. We also showed that the system can couple the increase in membrane tension and the synthesis of PS. The success of this work provides a great leap forward in achieving our ultimate goal, which is to build an artificial platelet.

2343-Pos Board B480
Light-Controlled Growth Factor-Mediated Signal Transduction
Kai Zhang.
Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
Growth factor-mediated signal transduction regulates critical cellular responses including cell proliferation, differentiation, migration, and apoptosis. However, direct and quantitative linkage between time kinetics of growth factor-mediated pathways and the cellular response is still lacking due to difficulties in perturbing the kinetics of intracellular signaling pathways. Here, we construct an optogenetic system that uses light to regulate growth-factor mediated signaling pathways. We show that various downstream pathways control cell fate determination differentially. Overall, light-controlled growth-factor signal transduction enables precise dissection of individual subsets of signaling pathways in cells.

2344-Pos Board B481
Patterned Biofilms for Engineering Microbial Consortia
Xiaofan Jin, David S. Glass, Ingmar Riedel-Kruse.
Bioengineering, Stanford University, Stanford, CA, USA.
Over the past decade, synthetic biology has developed increasingly robust gene networks within single bacterial cells, but relatively few systems have demonstrated engineered multicellular behaviour. In contrast, naturally existing terrestrial bacteria primarily live in complex surface-attached communities known as biofilms. Within these biofilms, multiple distinct microbial sub-populations form intricate spatial structures including colony co-localization and cell-cell co-aggregation. This spatial organization allows bacterial communities to achieve cooperative behaviours such as metabolic division-of-labour, and conversely, ecological interactions between different microbial subpopulations in turn influence the spatial patterning within the biofilm. Using optogenetic, metabolic, and cell-adhesion tools from synthetic biology, we are developing a biofilm culture platform that can generate optically patterned, metabolically interacting microbial communities. The platform will provide spatiotemporal control of both cell-surface and cell-cell attachment, tunable for both strength and specificity, as well as adjustable regulation of intercellular metabolic interactions. Taken together, these represent new tools to investigate how spatiotemporal patterning develops in biofilms and to engineer synthetic microbial consortia capable of complex tasks requiring biological division of labour.