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Synthetic Biology and Molecular Biotechnology

Session Moderator: Daniel Ratner, Bioengineering

022 JHN

1:15 PM to 2:45 PM

* Note: Titles in order of presentation.

Models for Cooperativity in Bacterial Antibiotic Resistance

Cody Christopher (Cody) Messick, Senior, Physics: Comprehensive Physics
Mentor: Paul Wiggins, Physics and Bioengineering

Bacterial antibiotic resistance is a rapidly growing concern for global health care. Typically, clinical antibiotics work by targeting essential pathways involved in bacterial metabolism or growth while leaving host cells unaffected. There are three basic mechanisms of bacterial resistance: (1) bacteria can chemically modify the antibiotic to a non- toxic form, (2) pump the antibiotic out of the cell, or (3) modify the enzyme or pathway targeted by the antibiotic. The second two mechanisms can be thought of as being selfish, as the mechanism doesn't directly help the community on a macroscale. However, the first mechanism helps the whole community, thus it can be thought of as cooperative. We hypothesize that the class of resistance mechanism, i.e. cooperative or selfish, is the primary factor that determines the geometry of colony morphology surrounding an antibiotic source. To test this hypothesis, we computationally simulated the diffusion of antibiotic on a plate inoculated with bacteria that express a cooperative resistance mechanism. Using insights gained from these simulations, we also experimentally explored this phenomena by imaging the growth of resistant E. coli cells in the presence of various antibiotics. In my talk I will discuss the results from the simulation and the experimental stage, as well as the current state of the analysis.

A Molecular Tether Design for von Willebrand Factor Protein Constructs

Tessa Olmstead, Senior, Bioengineering, Dance: Creative Studies
Mary Gates Scholar
Mentor: Wendy Thomas, Bioengineering
Mentor: Emilie Clemmens, Bioengineering

Affecting nearly 1% of the human population, von Willebrand Disease (VWD) is the most prevalent inherited bleeding disorder worldwide. VWD is caused by impaired platelet adhesion due to low plasma von Willebrand Factor (VWF) concentration, or dysfunction. The largest plasma glycoprotein, VWF's function is to mediate attachment of platelets to the exposed collagen on blood vessel walls at sites of injury. This interaction is sensitive to changes in flow forces or shear stress. To facilitate the study of VWF, the Thomas Lab is developing a single-molecule force measurement platform called magnetic tweezers; a tool capable of performing multiple single-bond force measurements in parallel. To use magnetic tweezers, VWF protein domains of interest are adsorbed to a glass surface on one end, and to a magnetic bead on the other. Electromagnets are then placed above the slide, applying an upward force to the VWF domains. The design and optimization of the VWF domain tethers is the focus of this research project. At present, a tethering scheme is being tested that utilizes the strength and longevity of the biotin-streptavidin non-covalent bond and the fimH lectin domain to mannose interaction. An alternative tethering scheme employing histidine tags instead of fimH is also being explored. While both types of tethers have been expressed successfully, tested in the magnetic tweezers, and shown to bind beads to the surface under force, recent data has shown that non-specific adhesion between protein-functionalized magnetic beads and the coverslip is an issue. Using immunoassays such as ELISA and the Western Blot, tether designs will be optimized using magnetic tweezers. With the capability to more accurately mimic physiologic conditions than immunoassays alone, coupled with the intrinsic ability to multiplex, the quantity and quality of data that could be acquired is impressive.

An Ultrasensitive Detector Protoype in *Escherichia coli*

Rahul Francis (Rahul) Brito, Senior, Bioengineering
Washington Research Foundation Fellow
Mentor: Eric Klavins, Electrical Engineering
Mentor: Rob Egbert, Electrical Engineering

While timely and specific diagnosis can enable optimal treatment of patients, modern-day diagnostics are not optimized for the needs of low-resource settings due to: 1) low sensitivity, 2) slowness in providing results, and 3) dependence on electricity, chemical reagents, and trained clinicians, at a high cost. The result is frequent poor diagnosis, which can increase the rate of negative health outcomes such as patient mortality and evolution of drug resistant pathogens. There is therefore a pressing need for diagnostics that are sensitive, accurate, rapid, and inexpensive. The field of synthetic biology holds much promise in this area due to the ease and affordability of genomic re-engineering and replicating of single-cell organism like *Escherichia coli* and *Saccharomyces cerevisiae*. To address this need and opportunity, we have engineered a prototype detector in E. coli that utilizes two DNA-based modules to sense a low concentration of target analyte and produce a large output response. Module 1 detects small concentrations of target analyte by switching cells from an ON to OFF state and Module 2 significantly amplifies an output signal by enabling ON cells to grow rapidly. By seeding these cells in an environment with a non-detector strain that grows faster than OFF cells but slower than ON cells, we hope to demonstrate that this genetic logic enables a detector strain that conditionally and significantly takes over the population after detection event. This approach holds much promise for a multiplexed diagnostic, as different strains of detector cells sensing a specific pathogenic marker could be co-cultured, with the result that only the cells that detect a target analyte grow. As a cell that is a diagnostic could be easily replicated and simple to use, this approach could be extremely feasible for a low-resource setting.

Social Dynamics of a Synthetic Cooperative *E. coli* System

Melissa Delaine (Melissa) Arnold, Senior, Biology (General)
Mentor: Benjamin Kerr, Biology
Mentor: Sonia Singhal, Biology

In this project, we explore conditions that are critical for *de novo* evolution of cooperation and altruism in a bacterial system. Previous theoretical work has shown that there is a competitive advantage to defection and selfishness, but the ubiquity of cooperation in nature suggests that cooperation may be adaptive. Here, we aim to uncover environments and genetic conditions that actually promote greater cooperation. We use an engineered cooperative strain of *Escherichia coli*. Through a bistable genetic switch, single cells have the capability to be either a producer cell that makes the cellulase enzyme to break down cellulose, or a consumer cell that eats the byproducts of cellulose breakdown. Our first aim is to characterize the social dynamics of this synthetic system and determine if a social dilemma is in fact occurring—i.e., is there a cost to producing cellulase, and can cheaters that do not produce cellulase displace the cooperators that do? Our second aim is to evolve the system over many generations and analyze how it changes. Uncovering the conditions favoring higher cooperation (e.g., greater production of cellulase) provides insight into how cooperation can evolve and how populations circumvent social dilemmas. We hope to use evolution as a tool to tune the synthetic genetic circuit for maximal cellulose breakdown. On a broader scale, these conditions that favor cooperation may have practical applications in waste degradation and biofuel production.

Methods & Algorithms Refinement for Rapid, Ultra-Low-Cost, Targeted DNA Sequencing

Evan August (Evan) Boyle, Senior, Microbiology, Biochemistry
NASA Space Grant Scholar, Washington Research Foundation Fellow
Mentor: Jay Shendure, Genome Sciences

New DNA sequencing technologies have increased the throughput of sequencing by several orders of magnitude, reaching upwards of 50 GB of data per day on a single instrument. However, advancing our understanding of how genetic variation impacts human health and disease depends on studies that survey very large numbers of individuals, and as such may only be economical by analyzing specific subsets of the genome. This is complicated by the fact that traditional methods of preparing DNA samples for sequencing do not allow selection for such candidate genes. One solution is the use of "capture by circularization" by molecular inversion probes (MIPs). MIPs allow dozens of genes to be targeted and amplified simultaneously; however, individual MIPs suffer from notoriously variable performance relative to one another, which results in uneven coverage of target regions. Prior work highlighted the potential for statistical analysis to improve MIP design and selection, inviting further testing. After performing a large scale sequencing run using an unbiased set of 12,000 MIPs, logistic regression was used to build a model capable of scoring MIPs prior to synthesis. New design software incorporating this model into the selection process was developed and tested by designing MIPs to over 60 new genes. Analysis of the resulting sequence data demonstrated the successful in silico identification of challenging genomic regions and a reduction in the number of gaps in coverage, which should support the use of MIPs in future large scale studies.