

Principles of Systems Biology, No. 23

This month: protein engineering (Mootha, Chica), synthetic biology (Khalil, You, Ting Lu, Timothy K. Lu), microbial ecology and evolution (Desai), side-stepping antibiotic resistance (Chandrasekaran), and mapping microbiomes (Borisy)

Genetic Tools for Studying Redox Metabolism in Living Cells

Zenon Grabarek and Vamsi K. Mootha, Department of Molecular Biology, Massachusetts General Hospital

Principles

Metabolic enzymes will selectively use either NADH or NADPH as a cofactor to carry electrons. Cells maintain NADPH in the reduced form (low NADP⁺/NADPH ratio) to drive anabolic processes and NADH in the oxidized form (high NAD⁺/NADH ratio) to drive catabolic processes. These ratios can change dynamically in response to metabolic or pathological factors, posing formidable experimental challenges. There are many tools for measuring NADH and NADPH in living cells but few for perturbation. We previously used a genetically encoded bacterial water forming NADH oxidase (LbNOX) (Titov et al., *Science* 352, 231–235) to perturb NAD⁺/NADH in human mitochondria. Now we have reengineered LbNOX to utilize NADPH exclusively (10⁸-fold change in specificity) (Cracan et al., *Nat. Chem. Biol.* 13, 1088–1095). This engineered protein (TPNOX) can be used with LbNOX, targeted specifically to mitochondrial matrix or the cytosol, to interrogate the relationship between redox pools in cellular compartments.

LbNOX and TPNOX may prove useful for examining redox compartmentalization in cellular processes.

What's Next?

LbNOX and TPNOX may prove useful for examining redox compartmentalization in cellular processes. They could be targeted to the nucleus to help evaluate the relative contributions of these cofactors to epigenomic control. LbNOX and TPNOX variants with altered K_m for NADH or NADPH or chemically/optically controlled variants could be used to probe rapid redox dynamics. Other applications include investigating mitochondrial dysfunction or the role of NADPH in fueling tumor metabolism and cancer growth.

Rational Design of Protein Dynamics: Making Proteins Dance

Roberto A. Chica and Natalie K. Goto, University of Ottawa

Principles

Proteins are dynamic molecules that often undergo structural changes in order to carry out the complex molecular processes required for life. In spite of this, established methods to design new proteins for biotechnological applications have focused on the creation of sequences that stably adopt a single structure, ignoring the potential for dynamics to facilitate function and create complex functionalities.

We developed a computational method for the rational design of protein dynamics called meta-multistate design, which we used to create proteins that undergo spontaneous exchange between two target structures. These proteins, named DANCERS (dynamic and native conformational exchangers), are stably folded and were confirmed to exchange between target conformational states on functionally relevant (i.e., millisecond) timescales. (Davey et al., *Nature Chemical Biology*, published online on October 23, 2017. <https://doi.org/10.1038/nchembio.2503>).

We ...create proteins that undergo spontaneous exchange between two target structures ...on functionally relevant (i.e., millisecond) timescales.

What's Next?

In order for engineered proteins of the future to access the diverse range of functional capabilities rivaling those found in nature (e.g., allosteric enzymes, motors, biosensors), it will be essential to design systems that are able to adopt all the necessary conformational states on functionally relevant timescales. Our meta-multistate design framework achieves this objective, paving the way to the design of more complex protein functions than previously possible.

Uncovering and Engineering the Protein Aggrogome

Gregory A. Newby, Broad Institute; Erinc Hallacli, Brigham and Women's Hospital; Szilvia Kiriakov and Ahmad S. Khalil, Boston University

Principles

Proteins can take on different conformations, sometimes causing them to aggregate in cells. Protein aggregation can participate in normal cell function or pathological disease, and neither role is well understood. We developed yTRAP, a genetic tool for high-throughput quantification and control of protein aggregation in live cells (Newby et al., *Cell* 171, 966–979). We applied it to yeast prions, which can aggregate to form conformation-based elements of inheritance. Using mutant screens and rational design, we identified prion alleles that induce or eliminate prions in a population. Harnessing these elements for synthetic biology, we engineered “anti-prion drives” to cure prions and a memory circuit that senses and remembers heat. Next, we constructed aggregation sensors for nearly all yeast mRNA-binding proteins, tested them, and learned that Hrp1, a cleavage factor complex member that regulates polyadenylation, is particularly prone to aggregation. A genome-wide screen revealed potential regulators of Hrp1 solubility and function.

...a genetic tool for high-throughput quantification and control of protein aggregation in live cells.

What's Next?

yTRAP screens can be employed to uncover genetic, chemical, or environmental factors influencing protein aggregation. Sensors could be expanded to study other classes of proteins from yeast, human, or other sources. We envision yTRAP will be ported to other cell types and even transgenic animals for live tracking of aggregation *in situ*. Finally, using programmable prions, more complex synthetic devices or memories could be engineered.

Toward Living Fabrication of Structured Materials

Yangxiaolu Cao and Lingchong You, Duke University

Principles

Nature is a master in fabricating structured materials consisting of living and non-living components from the bottom up, but such processes are not readily programmable. In a combination of gene-circuit engineering and precise control of porous membranes as growth substrates, we programmed bacteria to form a three-dimensional (3D) dome structure. The bacteria were then chemically fixed and served as a scaffold to assemble gold nanoparticles into domes that function as pressure sensors (Cao et al., *Nature Biotechnology*, published online on October 9, 2017. <https://doi.org/10.1038/nbt.3978>). These modules may be used for building more complex signal-processing systems, and the two-layer design strategy enables assembly of 3D structured materials that have well-defined physical and chemical properties.

A fundamental bottleneck is the still-limited capability of programming more-sophisticated spatial patterns in a predictable manner, using gene circuits.

What's Next?

Our work opens up several future possibilities in fundamental engineering design and potential applications. Since each bacterium has the gene circuit, which contains all the information to grow into the final structure, the living material could potentially be self-curing. A future extension of our study is to keep cells alive while programming structured materials, enabling a self-healing capability. Our fabrication process is modular and scalable by design, allowing many potential applications by plugging in different non-living materials or pattern-forming gene circuits. A fundamental bottleneck is the still-limited capability of programming more-sophisticated spatial patterns in a predictable manner, using gene circuits.

Integrative Thinking of Gene Circuits and Their Hosts

Ting Lu, University of Illinois at Urbana-Champaign

Principles

Synthetic gene circuits are programmed to create various novel functions in living systems. They possess enormous potential for applications in medicine, energy, and environment; however, the realization of these applications is hindered by a lack of effective tools for circuit design and optimization. Quantitative modeling is a natural choice for addressing the challenge, but its current “standard” scheme, which treats circuits as isolated entities, is oversimplified, often resulting in difficulties quantitatively describing circuit behaviors.

We recently constructed a gene-circuit modeling framework that explicitly integrates circuit dynamics with host physiology (Liao et al., *Nat. Microbiol.*, published online on September 25, 2017. <https://doi.org/10.1038/s41564-017-0022-5>). Involving dynamic resource allocation and multilayered coupling between circuits and the hosts, the framework is a fundamental step toward a quantitative and predictive understanding of gene-circuit behavior. The framework successfully captured and predicted a large set of experimental data concerning host physiology and gene overexpression. It also elucidated the enriched behaviors of a non-cooperative feedback circuit and a toggle switch and furthermore showed the potential translatability to multiple host species including *E. coli*, *S. typhimurium*, and *S. coelicolor*.

Involving dynamic resource allocation and multilayered coupling ..., the framework is a fundamental step toward a quantitative and predictive understanding of gene-circuit behavior.

What's Next?

The framework will benefit from considering circuit dynamics in complex physiological states and different host organisms. We envision that such efforts are critical to rationally, robustly, and efficiently create desired cellular phenotypes.

Synthetic Gene Circuits for Cancer Immunotherapy: Turning Cancer Cells against Themselves

Ming-Ru Wu,* Lior Nissim,* and Timothy K. Lu, Massachusetts Institute of Technology.

*These authors contributed equally

Principles

Immunotherapy has demonstrated robust efficacy in several clinical trials, but challenges, such as achieving localized yet effective anti-tumor activity, have remained. To address this problem, we designed a synthetic cancer-detecting gene-circuit platform to trigger cancer-specific and strong combinatorial immunotherapy from within cancer cells (Nissim et al. *Cell*, published online October 19, 2017. <https://doi.org/10.1016/j.cell.2017.09.049>) This Trojan horse-like approach has the potential to overcome key limitations in current immunotherapies, such as non-specific targeting of normal cells, tumor-mediated immunosuppression, and toxicities due to the systemic delivery of immunomodulators. Our modular circuit technology enables targeting of multiple cancer types and can trigger any genetically encoded immunomodulatory payload expression as therapeutic outputs. The circuits' cancer-targeting specificity was examined with cytotoxicity assays *in vitro*, and therapeutic efficacy was demonstrated *in vivo* using a disseminated ovarian cancer mouse model.

This Trojan horse-like approach has the potential to overcome key limitations in current immunotherapies, such as non-specific targeting of normal cells, tumor-mediated immunosuppression, and toxicities due to the systemic delivery of immunomodulators

What's Next?

Steps toward further clinical translation include designing optimized promoters for tumor targeting in human patients, identifying clinically relevant output combinations in additional tumor models, including humanized and syngeneic mouse models, and optimizing circuit delivery *in vivo*. In addition, this circuit platform has the potential to be adapted to study tumor immunology and to treat other type of diseases that require highly specific and localized immunomodulation.

Watching the Dynamics of Evolution over 60,000 Generations

Benjamin H. Good, University of California, Berkeley; Michael M. Desai, Harvard University

Principles

Evolution can be difficult to observe directly over long timescales. To overcome this problem, we took advantage of a well-preserved “fossil-record” from a long-term evolution experiment in *E. coli*, which covers more than 60,000 generations of evolution in 12 replicate populations (Good et al., *Nature* 551, 45–50). By sequencing archived populations at high coverage, we traced the dynamics of thousands of new mutations that rose and fell over this long evolutionary history.

These data reveal a surprisingly complex picture of evolution in this simple laboratory setting. Rates of adaptation remain high, even after tens of thousands of generations in the same environment. Many of the populations diversified into distinct “ecological units,” which were stable in the short term but shifted on longer timescales as they continued to acquire new mutations. By examining the identities of mutations across time and among populations, we also found evidence that new evolutionary paths are created by previous substitutions.

...spontaneous formation of ecology poses many challenges for existing population genetic models.

What's Next?

The spontaneous formation of ecology poses many challenges for existing population genetic models. Further theoretical work will be required to integrate these eco-evolutionary dynamics into a population genetic framework. Further empirical characterization will also be necessary to determine how ecology alters the fitness landscape for the two subtypes or whether they can continue to follow similar adaptive strategies in the future.

Microptosis: The Answer to Antibiotic Resistance?

Sriram Chandrasekaran, University of Michigan; Judy Lieberman, Boston Children's Hospital; Farokh Dotiwala, Wistar Institute

Principles

Killer lymphocytes eliminate intracellular pathogens in target cells using cytotoxic proteases called granzymes. How granzymes trigger death by cleaving protein targets in highly diverse pathogens is unclear.

Our proteomics screen identified the protein targets of human granzyme B in three evolutionarily diverse pathogens: *Escherichia coli*, *Mycobacterium tuberculosis*, and *Listeria monocytogenes*. Network-biology and comparative genomics analysis revealed that the granzyme B targets are highly conserved proteins that participate in critical protein synthesis and central metabolism pathways essential to the survival of most microbes.

Metabolic network modeling predicted that disruption of metabolic enzymes by granzyme B prevented microbial growth in over 100 different metabolic conditions. Pathogens are thus unable to survive this attack despite their extraordinary metabolic flexibility. *E. coli* did not become resistant even after exposure to granzyme B for 14 passages. We call this mechanism of cell death induced by granzymes in multiple microbial species *Microptosis* (Dotiwala et al., *Cell*, published online October 26, 2017. <https://doi.org/10.1016/j.cell.2017.10.004>).

...granzyme B targets are highly conserved proteins ...essential to the survival of most microbes....

What's Next?

We are now studying whether pathogens can evade this multi-pronged attack, how existing drugs interfere with this antimicrobial immune defense, and how these immune enzymes can selectively target vital proteins in pathogens. The bacterial enzymes cleaved by granzyme B are promising drug-targets for treating both aerobic and anaerobic pathogens.

Mapping Microbial Microbiomes

Gary G. Borisy, The Forsyth Institute; Jessica Mark Welch, Marine Biological Laboratory

Principles

Complex bacterial communities living within a host are functional equivalents of multicellular organs. Host organ anatomy had to be elucidated before its physiology could be understood. So, too, an understanding of microbial community function requires knowledge of its anatomy—mapping microbiomes amounts to knowing who is next to whom and who is next to what. Since microbes are small and their host habitats are finely structured, it is important that their spatial organization be mapped at the micron scale.

The technology best suited to acquiring micron-scale information is microscopy. Accordingly, we used a multiplexed fluorescence *in situ* hybridization strategy to visualize and identify individual bacterial cells within communities. We applied our approach to oral biofilms (Mark Welch et al., *PNAS* 113, E791–800) and humanized mouse gut (Mark Welch et al., *PNAS* 114, E9105–9114). In both instances, we found communities that were mixed at micron scales, although the two microbiomes differed dramatically in their degree of order and in their detailed arrangement.

...understanding of microbial community function requires ...knowing who is next to whom and who is next to what.

What's Next?

Micron-scale information will inform mechanisms of community interaction, community assembly, and emergent properties of the community. This will enable generation of hypotheses that can be tested. New tools will need to be developed to determine the dynamics of community assembly and interactions in living microbial communities. The next stage of experimentation will be to progress from anatomy to physiology.