Synthetically designed anti-defense proteins overcome barriers to bacterial transformation and phage infection

ABSTRACT

Bacterial defense systems present considerable barriers to both phage infection and plasmid transformation. These systems target mobile genetic elements, limiting the efficacy of bacteriophage-based therapies and restricting genetic engineering applications. Here, we employ a de novo protein design approach to generate proteins that bind and inhibit bacterial defense systems. We show that our synthetically designed proteins block defense, and that phages engineered to encode the synthetic proteins can replicate in cells that express the respective defense system. We further demonstrate that a single phage can be engineered to carry multiple anti-defense proteins, improving infectivity in bacterial strains with multiple defense systems. Finally, we show that plasmids that express synthetic anti-defense proteins can be introduced into bacteria that naturally restrict plasmid transformation. Our approach can broaden host ranges of therapeutic phages and can improve genetic engineering efficiency in strains that are typically difficult to transform.

Engineering Reliability in Synthetic Biology: Sequence Design, Evolutionary Incentives, and Protein Architecture

ABSTRACT

Engineered biological systems can lose function at multiple levels: DNA sequences may contain instability hotspots, burdensome expression can favor non-producing cells, and combining proteins into new architectures can compromise molecular function. My doctoral research develops computationally guided approaches for improving reliability across these connected levels.

The Evolutionary Stability Optimizer (ESO) identifies and redesigns mutational and epigenetic hotspots while preserving gene-expression constraints. STABLES addresses the selective pressure against heterologous production by coupling a gene of interest to an essential endogenous gene, making loss of production costly to the host. Machine learning, biophysical modeling, and sequence optimization guide construct design. In yeast, STABLES fusion constructs produced approximately five times more cumulative human proinsulin than an unfused baseline.

The fusion architecture used by STABLES also highlights a broader protein-engineering problem: linker suitability depends on the specific proteins it connects. My current work develops a context-aware framework for linker design using natural-linker datasets, curated experimental benchmarks, and structural metrics focused on preserving the adjacent proteins. The framework is intended to support fusion-protein design across diverse applications.

Together, these projects form a broader research program in which reliability is treated as an explicit design objective at the sequence, evolutionary, and protein-architecture levels.

Modular Scalable Synthetic Gene Circuits for Complex Functions Within Minimal Computational Layers in Human Cells

ABSTRACT

Engineering mammalian cells to execute complex genetic programs remains a significant challenge in synthetic biology. Synthetic gene circuits typically implement sophisticated programs through cascaded computational layers. However, these architectures require numerous orthogonal parts, increase genetic payload, and deplete cellular resources, thereby limiting functionality and scalability. We present a modular design framework for engineering scalable gene circuits that execute complex functions within fewer computational layers. The platform integrates orthogonal trans-splicing-based AND gates, native-synthetic hybrid promoters for tunable regulation, and synthetic microRNAs that implement inhibitory logic. Using this approach, we engineer complex circuits, including a three-input combinatorial logic gate, a half adder, a full adder, and a dynamic 3-to-1 multiplexer with a dedicated Selector Overload Status output, generated only when both selector inputs are activated. By minimizing computational layers while maintaining functionality, this strategy addresses scalability barriers in gene circuit engineering and expands applicability in biomedicine, biotechnology, and fundamental biology.

Synthetic microbial carbon fixation for sustainable food and chemicals production

ABSTRACT

Engineering synthetic autotrophy in non-native microbial hosts offers a promising route towards sustainable bioproduction and an opportunity to uncover principles of metabolic design. In my PhD, I use an *Escherichia coli* strain that we engineered and evolved to grow autotrophically as a model system.

The strain uses a synthetic Calvin cycle and a formate-based energy module, providing a platform to study synthetic autotrophy and develop CO2-based production powered by renewable energy. We first showed that autotrophic growth, previously achieved only after prolonged adaptive evolution, can be reconstructed using only three additional mutations. My current work investigates how *E. coli* adapts to support synthetic autotrophy, revealing design principles involving carbon-flux distribution, separating reducing-power supply from energy generation, and accounting for non-enzymatic physiological constraints that can limit engineered metabolism. In parallel, I have contributed to collaborative efforts to translate synthetic one-carbon metabolism toward sustainable production. These included coupling autotrophic growth to photoelectrochemical formate generation, linking renewable energy conversion to microbial CO2 fixation, and demonstrating C1-based bioplastic production in E. coli.

We have also introduced a bacterial CO2 -concentrating mechanism and are evolving the strain toward autotrophic growth in ambient air, while developing a genome-integrated strain as a more stable platform for future bioproduction. The broader goal is to make major metabolic transitions more predictable and easier to engineer and to advance microbial production from CO2 and renewable energy.