Predicting and Expanding the Operational Envelope of Genetic Circuits

Hamid Doosthosseini

A single bacterium can detect diverse inputs with extreme sensitivity, including chemicals (some in the nanomolar range), electrical signals, mechanical stresses, and more. This is all encoded by ~2 megabytes of genetic data stored in a cell of femtoliter size. On a per-gram basis, this equates to roughly ~10^{17} sensor proteins and 10 million terabytes of genetic data. This genetic programming enables bacteria to survive in extreme environments, adapt to environmental changes, and chemically modify their surroundings. These capabilities can be leveraged to address pressing challenges, e.g. in sustainability, biomanufacturing, or healthcare, however, this requires engineering genetic circuitry that accurately computes these inputs and responds reliably. A key obstacle is designing circuits for diverse, non-model organisms beyond the well-studied lab strains. Typically, genetic circuit design relies on Boolean logic, host-specific components, and iterative tuning. In addition to being time-consuming and costly, this limits its scalability in size and in range of host organisms. To address these limits, we develop predictive modeling, AI-designed protein components for non-boolean circuits, and printed multi-cellular circuit boards, enabling more robust and adaptable biological computation.

First, we develop a data-driven framework for predictive modeling that automates genetic circuit characterization without requiring prior knowledge of the host organism. By integrating RNA sequencing and ribosome profiling, this approach fully recapitulates circuit behavior, identifies undetected faults, and accurately predicts dynamic and stochastic responses. Applied to a combinatorial logic circuit, the model identifies crosstalk interactions, predicts transient glitches, and establishes parameter robustness thresholds, enabling systematic debugging and circuit optimization.

Second, we incorporate completely synthetic, AI-designed protein binders into genetic circuit design, overcoming limitations of transcription-based, Boolean logic. By fusing protein-binders with inteins and extracytoplasmic function (ECF) σ factors, we connect protein-based logic to transcriptional inputs and outputs in a living cell. We build fuzzy logic gates and demonstrate they function in *E. coli* with over 50-fold dynamic range using a readily scalable set of AI-designed protein components.

Third, we address size constraints of single-cell circuits and costliness of cell engineering. We develop a multi-cellular gene circuit architecture based on Pass Transistor Logic (PTL) and quorum-sensing communication. By engineering a minimal set of non-model strains (*P. agglomerans*) to process molecular signals that diffuse in their surroundings, we implement logic based on colony arrangement rather than genome editing. Using acoustic liquid handling for precise spatial patterning, we construct fundamental arithmetic and logic circuits, including switches, demultiplexers, and adders, by printing cells as living circuit boards.

Together, these advances offer a robust set of tools to engineer scalable and adaptable genetic circuits in non-model organisms. By addressing three fundamental challenges, this work establishes a framework for simple and fast design of complex circuitry in a host-agnostic manner.

Thesis Supervisor: Christopher A. Voigt

Daniel I.C. Wang Professor
Head, Department of Biological Engineering