Whitepaper for NSF Workshop on Biologically-Enabled Wireless Networks

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Research Interests

My lab is interested in developing fundamental platforms for biological circuit design, including frameworks for memory and processing. For example, memory is a key component for the persistent recording of signals in biological systems and achieving circuits capable of higher-order processing. We have pioneered a modular design for heritable, stable, and interoperable memory devices called Single Invertase Memory Modules (SIMMs) (Figure 1)[1]. At the core of a SIMM is a recombinase that can invert DNA between two oppositely-oriented cognate recognition sites. Upon expression of the recombinase by an upstream promoter, the entire SIMM is inverted between the recognition sites, representing the flipping of a digital bit. Due to the inverted orientation of the recombinase gene with respect to the upstream promoter, further expression of recombinase protein ceases and DNA orientation is fixed. This design stores heritable memory information in the physical orientation of DNA. Stability is conferred by sandwiching the recombinase gene between its own recombinase recognition sites. Interoperability is achieved by the natural ability of recombinase proteins to recognize and focus their activity upon specific DNA sequences in their cognate recognition sites. This design is independent of host organisms and can be triggered bidirectionally via exogenous or endogenous signals. There are >100 bidirectional recombinases that can be used in SIMMs[2]. Memory devices such as SIMMs can be used to encode more complex circuits, such as digital-to-analog converters and analog-to-digital converters[3]. Such designs may be useful for biocomputation and realizing biologically enabled wireless networks.

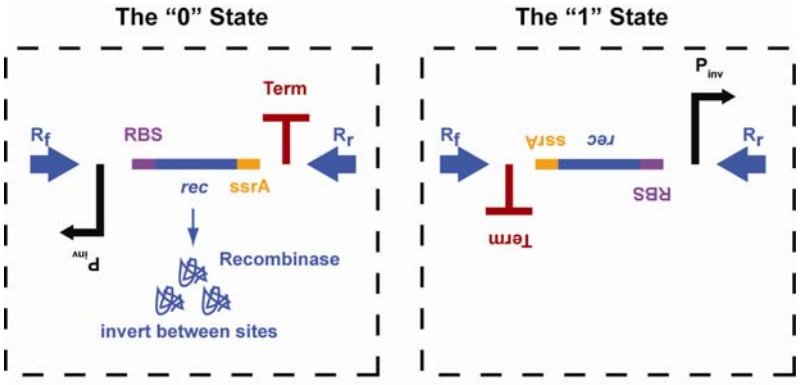

Figure 1. The SIMM design is composed of opposing recombinase recognition sites (R_f and R_r) which contain between them an inverted promoter (P_{inv}), a synthetic ribosome-binding-sequence (RBS), a recombinase gene (*rec*), an *ssrA*-based degradation tag, and a transcriptional terminator (Term). A SIMM maintains memory based on DNA orientation, which is inverted when the recombinase is expressed, enabling us to define two distinct memory states. Upon flipping, the originally inverted promoter (P_{inv}) becomes upright, enabling the expression of downstream genes as outputs.

Current State of the Art and Important Research Directions

Progress in synthetic biology is currently limited by several major factors, including 1) underdeveloped *in silico* models and design tools, 2) expensive and slow DNA synthesis and assembly technologies, 3) inadequate platforms for interoperable, extensible, and modular parts, and 4) poor understanding of chassis-circuit interactions. There are ongoing efforts to resolve these issues but there is much still to be accomplished. For example, researchers have achieved impressive feats of DNA synthesis and assembly[4] but such feats still require significant investments of time and capital that are incompatible with a rapid design cycle. In addition, most biological circuits to-date have been designed using *ad hoc* parts rather than extensible well-characterized devices[5]. The next decade of synthetic biology will focus on these important and challenging problems, with the promise of establishing the field as a true engineering discipline.

1. Friedland, A.E.*, et al.* Synthetic gene networks that count. *Science* **324**, 1199-1202 (2009).
2. Groth, A.C. & Calos, M.P. Phage integrases: biology and applications. *J Mol Biol* **335**, 667-678 (2004).
3. Lu, T.K., Khalil, A.S. & Collins, J.J. Next-generation synthetic gene networks. *Nat Biotechnol* **27**, 1139-1150 (2009).
4. Gibson, D.G.*, et al.* Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome. *Science* **329**, 52-56 (2010).
5. Purnick, P.E. & Weiss, R. The second wave of synthetic biology: from modules to systems. *Nat Rev Mol Cell Biol* **10**, 410-422 (2009).