Engineering bacterial two-component systems

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Two-component histidine kinase signal transduction systems (TCSs) are the largest family of multi-step signal transduction pathways and a treasure trove of genetically-encoded sensors for synthetic biology. Bacteria utilize TCSs to sense a remarkable range of chemical and physical stimuli in the environment, and respond by activating appropriate gene expression programs including virulence and antibiotic-resistance pathways. We have computationally identified over 25,000 non-redundant TCSs in bacterial genomes, with a typical organism containing several dozen. Despite the diversity and importance of these pathways, the vast majority remain uncharacterized. Major challenges are that most bacteria cannot be cultured in the laboratory, and that TCS output promoters are typically unknown or highly cross-regulated. We have developed a suite of technologies to overcome these challenges and dramatically improve TCS performance. For example, we have shown that pathway leakiness and dynamic range can be dramatically increased by optimizing the expression levels of the sensor kinase and response regulator proteins [1]. In addition, we have developed a general method for rewiring TCSs to synthetic output promoters, enabling these pathways to be ported between distantly-related host bacteria, converted into one-input/one-output sensors, and subjected to high throughput screens for input discovery [2]. Once functional, we have demonstrated that the input detection thresholds of TCSs can be tuned over more than two orders of magnitude by introducing mutations that specifically alter the secondary phosphatase activity of the sensor histidine kinase, enabling these sensors to be tailored to the application at hand [3]. We have deployed our methods to engineer bacteria that sense nitrate levels in soil, contaminants in seawater, and diagnose colon inflammation [4], among other applications.

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