

## **Engineering Chimeric Receptor Histidine Kinases**

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## Abstract

Bacteria have evolved a vide array of sensor histidine kinases for interacting and responding to their environment. Histidine kinases (HKS) detect a wide variety of stimuli including nutrients, changes in pH, antibiotics, and insa. Although there are over a million known HKS, only several hundred of them have been functionally characterized and the mechanisms for transmembrane signaling through these proteins are poorly understand in all but a few cases. We are developing a platform for the rapid, large-scale, multiplexed characterization of HK sensors in order to help answer fundamental questions about the biology of the organisms which employ them and provide valuable insights into how we can engineer these sensors for diverse biosennia acolications.

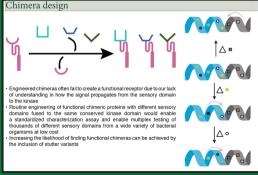
We leverage DropSymit gene synthesis to synthesize tens of thousands of chimein FM sensors and characterize their response across a multitude of stimuli and ligands; we have built a bioinformate loppiente to inform chimera design by retrieving sequences, annotating, and analyzing them. Using life-death selection our assay removes constitutively active HK chimeras and retrieves corresponding the corresponding DNA sequences for the sensors activated for each stimulus. We have designed and built a low-leakage suicide switch and a dual-antibiotic selection system, both with low except frequencies and genomically integrated into corresponding E. coil selection strains also having at least one native HK gene knocked out. This multiplexed assay should scale to tens-of-housands of sensors and help build a map of sequence-ligand relationships for these ubiquitous systems.

## Two-component systems (nutrients, metabolities, metabolit

- Detect a wide variety of stimuli and have been implicated in numerous downstream cellular processes including metabolic regulation, pathogenicity, chemotaxis, nodulation in rhizobla, stress response, nutrient transport, and cell division?
- Consist of a histidine kinase protein that detects a stimulus and transduces the signal through the membrane
  to a catalytic site on its kinase domain
- Upon stimulation the kinase phosphorylates a cognate response regulator protein capable of modulating gene expression by binding to a promoter proximal DNA sequence
- Most bacteria have genomes encoding about 50 TCS with some encoding hundreds<sup>3</sup>

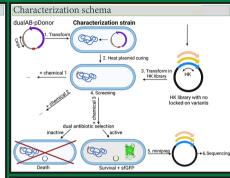
Environmental stimuli | pH, osmotic pressure, ionic strength, temperature | Nutrients, metabolites, | aspartate, matiose, galactose, ribose, fumarate, sucrose, citrate, nitrate, and other ligands | d-lactate, acetate, pyruvate, ubiquinone, quorum signaling molecules | Metal ions | Na, K, Mg, Fe, Cu, Ni, Co, Zn, Pb, Mn, Ag, Cd

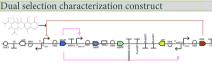
## Receptor histidine kinases domain TM linker domain dimerization & catalytic domains Highly modular proteins Many different domain architectures Typically a periplasmic. or extracellular, sensory domain flanked by two transmembrane domains one or more linker domains (HAMP, for example), and several domains involved in catalytic kinase function Alphafold-Multimer prediction of EnvZ homodimer



Pless C, Sitore AM, Lubock NB, et al. Multiplexed gene synthesis in emulsions for exploring protein functional landscapes. Science. 2018, 359 (6373):343-7

Whitworth DE, Cock PJA, Evolution of prolaryotic two-component systems: insights from comparative genomics, Amino Acids, 2009, 37(3):459-86 Whitworth DE, Genomes and knowledge - a questionable relationship? Trends Microbiol. 2008, 16(11):512-9





The dual selection characterization construct is made so cells where the histidine kinase is activated can be chosen either with life-death selection, or by screening for the presence of sfGFP



An assay was run using the dual selection characterization construct, and from the graph above, a qualitative difference can be seen in the amount of sIGFP produced when the system was under induction versus no induction