Rewiring the Specificity of Two-Component Signal Transduction System

By Jeffrey M. Skerker, Barrett S. Perchuk, Albert SiryaPorn, Emma A. Lubin, Orr Ashenberg, Mark Goulian, and Michael T. Laub
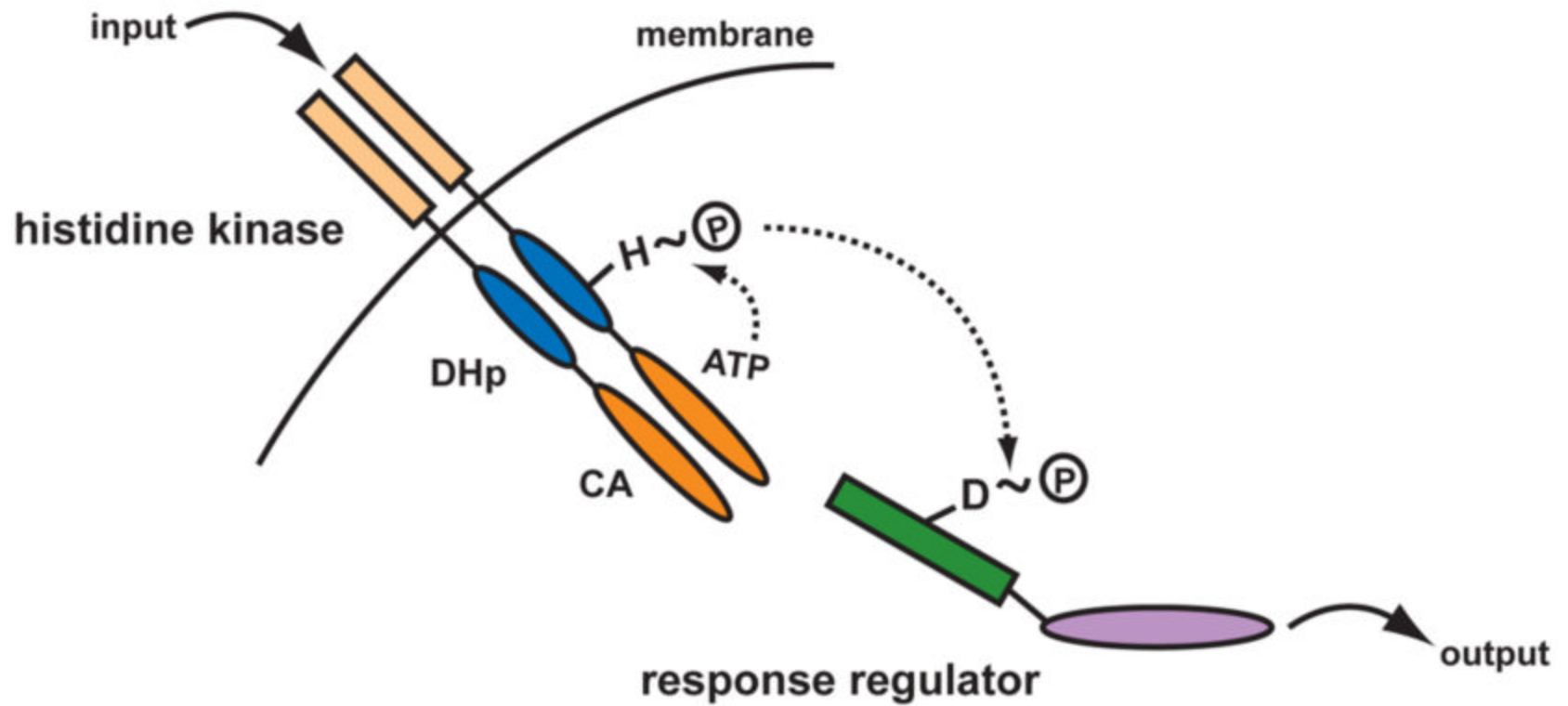
Cell (2008); 133(6); 1043-1054

Presented by Jose R. Echevarria for 20.385
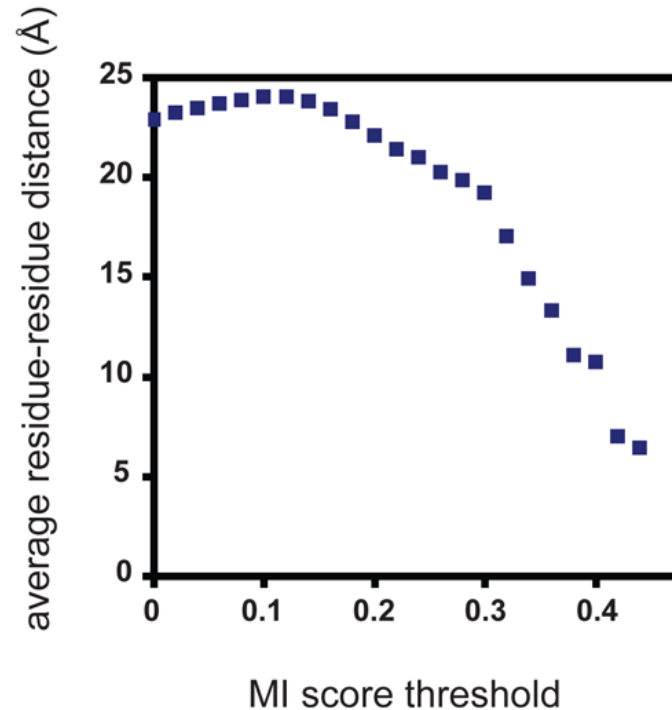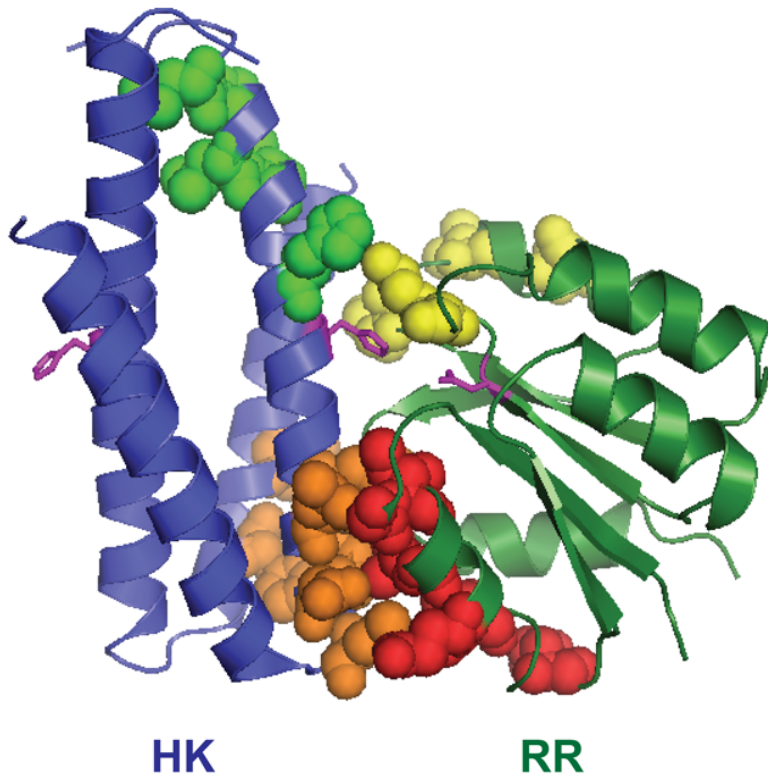
02-24-2010

Hypothesis

Could the Specificity of Histidine Kinases be determined by amino acids that exhibit a large degree of covariation.

If you vary a subset of amino acids of Histidine Kinases, could that modify the specificity of the Histidine Kinases.
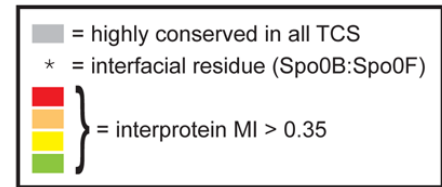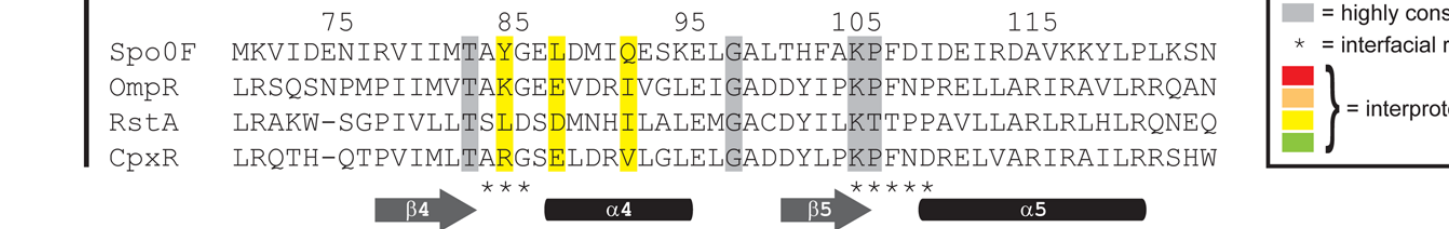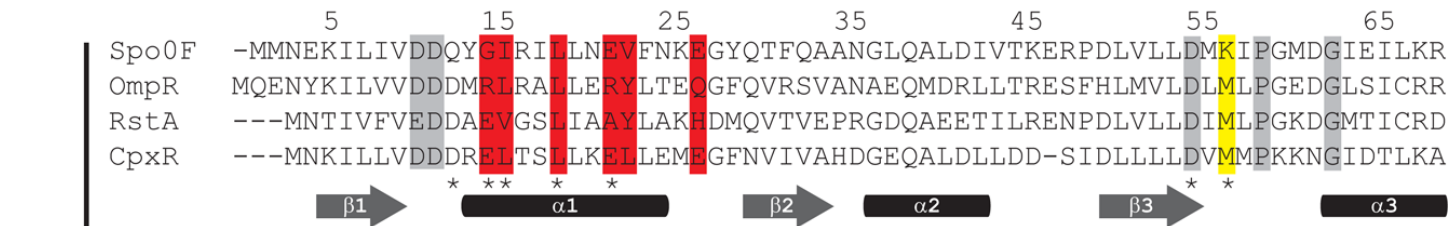
Histidine Kinase Diagram

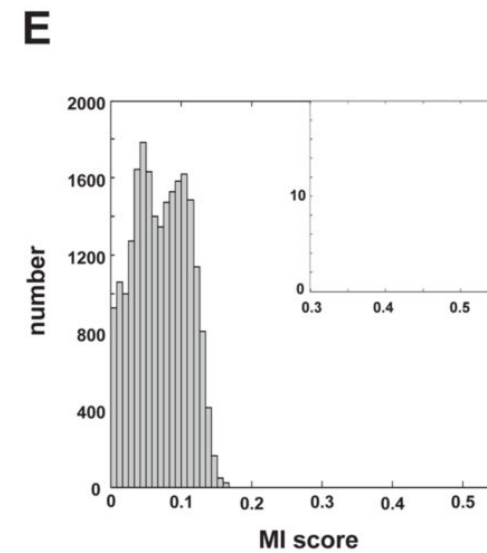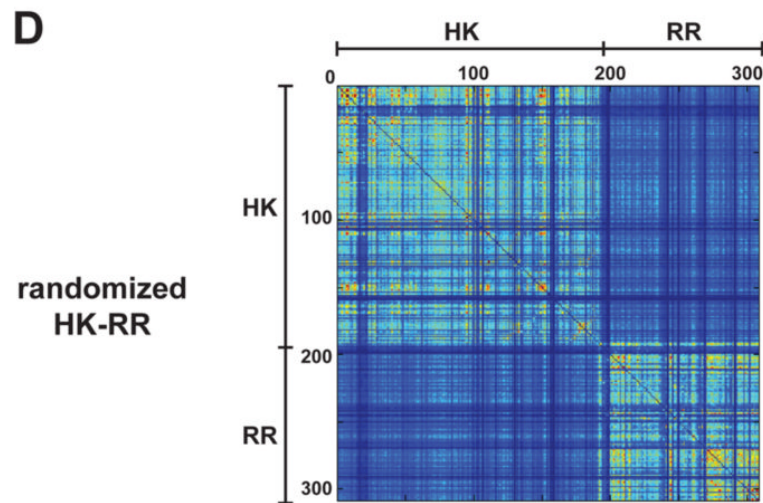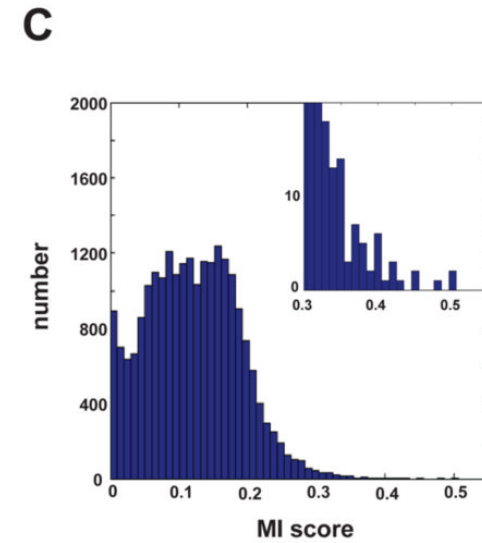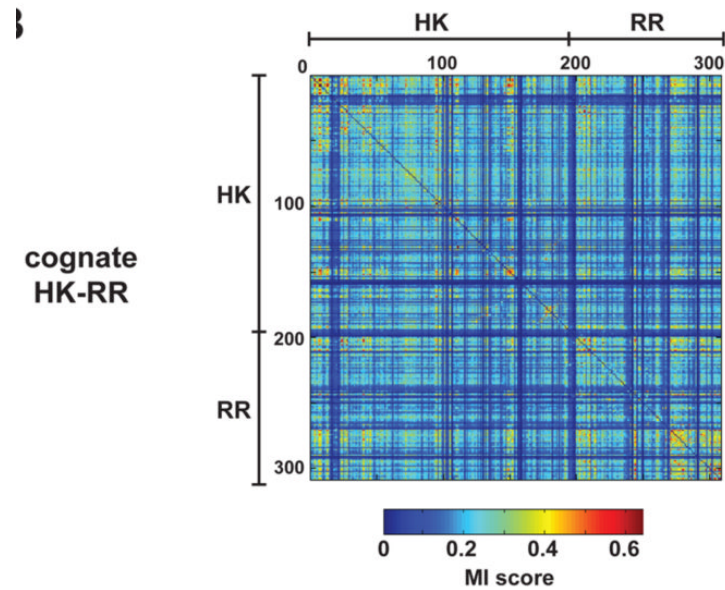# Structural Analysis based on Histidine Phosphotransferase

Residues do not perfectly correlate with contact residues of the Spo0B:Spo0F model
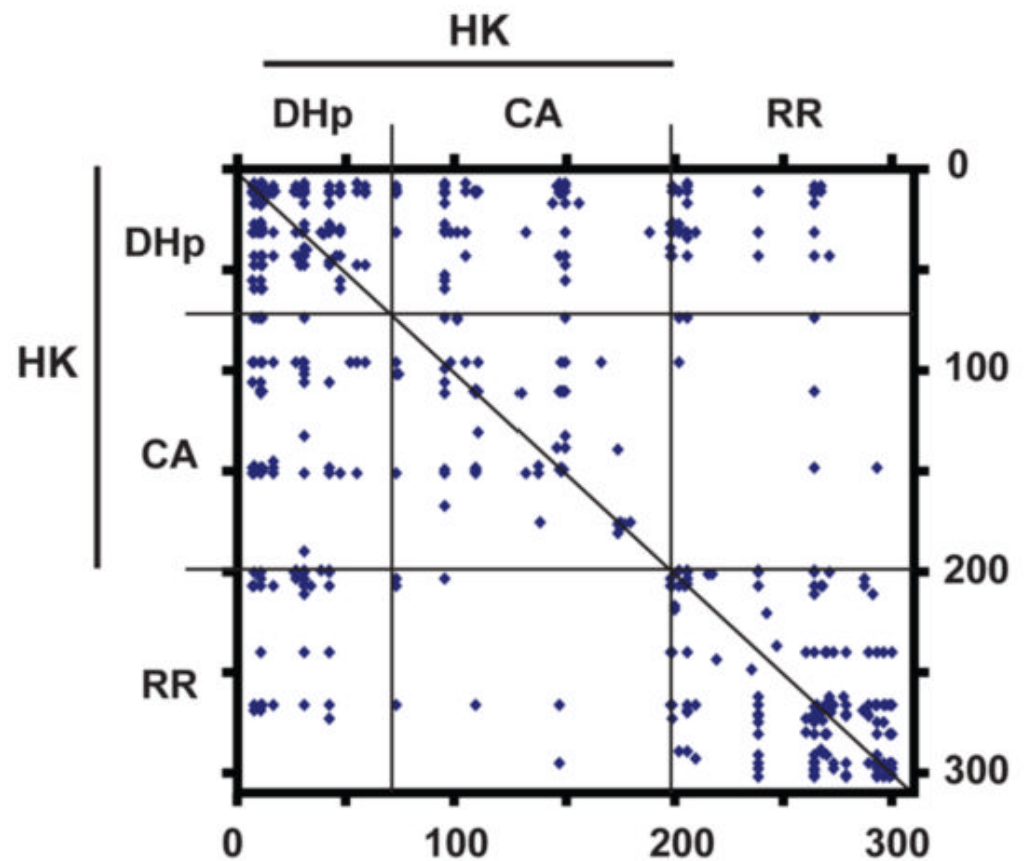
Primary Alignments
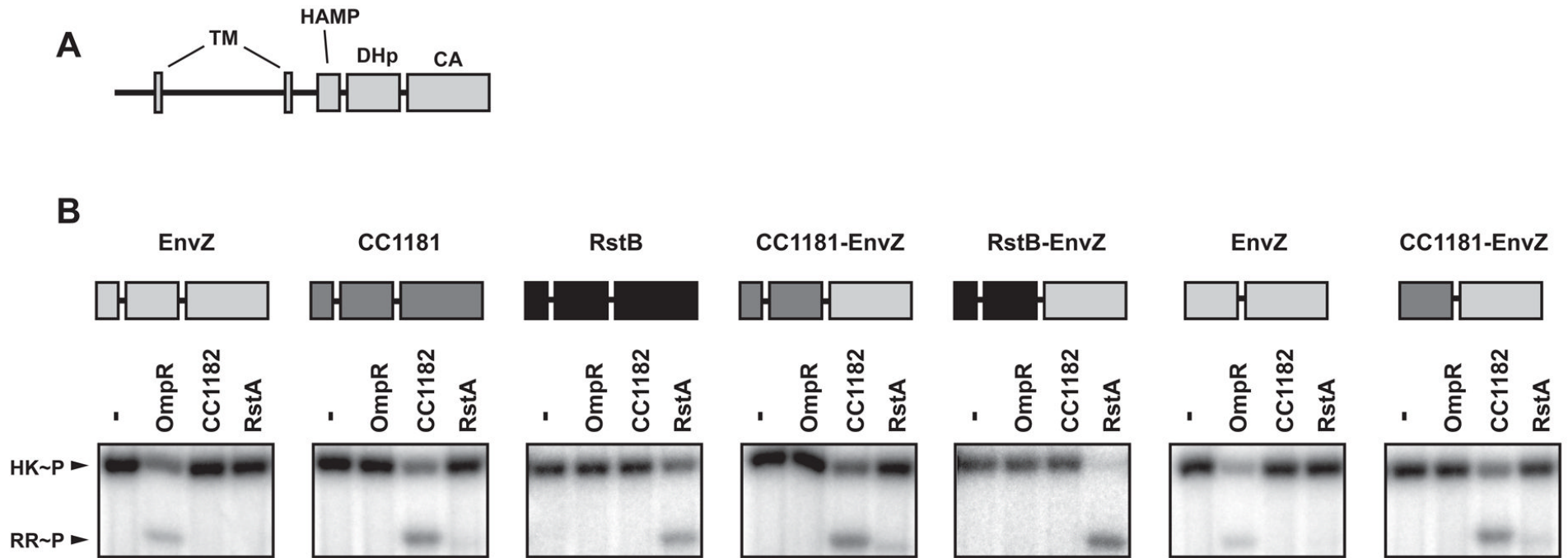
Search for covarying residues

Search for Covarying Residues

- 43 amino acids found with an MI greater than .35
- 36 of them included the dHp domain of histidine kinase and it's response regulator
- Formula for MI:

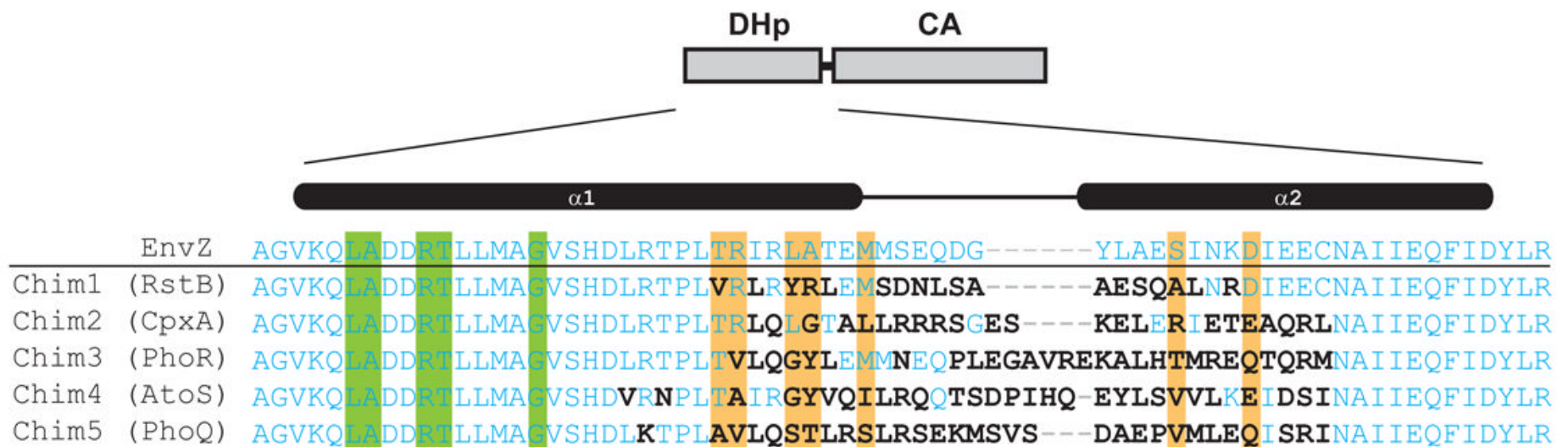$$\sum_{j=1}^{n}\sum_{k=1}^{m} p_{jk}\log\frac{p_{jk}}{p_{j}q_{k}}$$
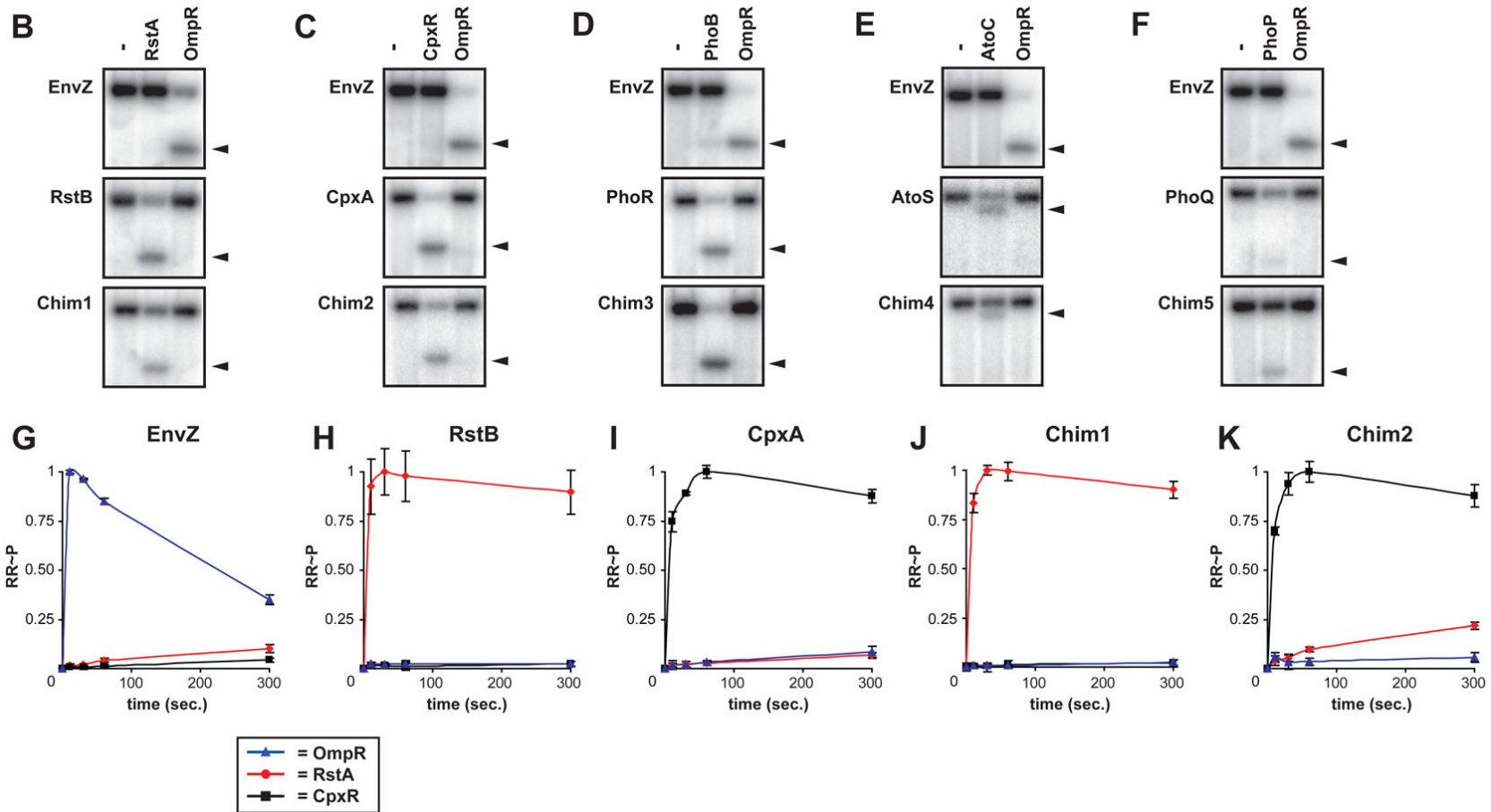
DHp Domain is Critical

7 Residue Chimera Construct

Mutants with varying levels of homology to the EnvZ DHp domain.

Holds 7 Specificity – determining residues at the C- terminal, N-terminal portion of helix 2, and a connecting loop.

More Chimera Testing

K$_{cat}$/K$_m$ Analysis

Summary of Kinetic Preference for Wild-Type, Chimeric, and Mutant Kinases

Histidine Kinase	Kinetic Preference: RstA versus OmpR[a]	Kinetic Preference Relative to EnvZ	Kinetic Preference: CpxR versus OmpR[b]	Kinetic Preference Relative to EnvZ
EnvZ	0.0031	1	0.0013	1
RstB	2566	8×10^5	—	—
Chim1	1408	5×10^5	—	—
Mut4	206	7×10^4	—	—
Mut5	94	3×10^4	—	—
CpxA	—	—	296	2×10^5
Chim2	—	—	690	5×10^5

[a] Specificity constants (k_{cat}/K_M) were measured for each kinase relative to the response regulators RstA and OmpR. The kinetic preference value reported is the ratio of these two constants.

[b] Specificity constants (k_{cat}/K_M) were measured for each kinase relative to the response regulators CpxR and OmpR. The kinetic preference value reported is the ratio of these two constants.
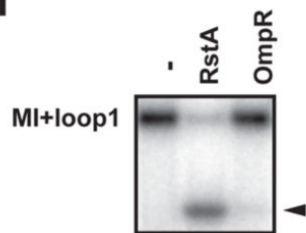
Mutation of Residues and Analysis
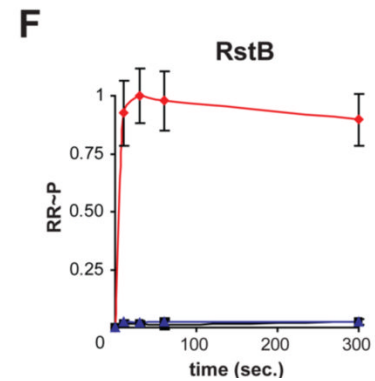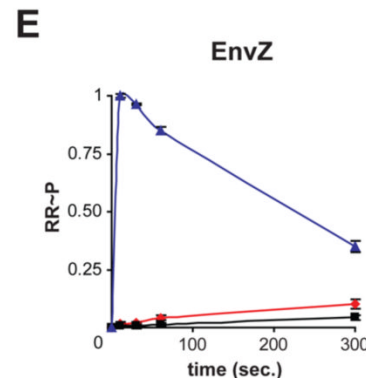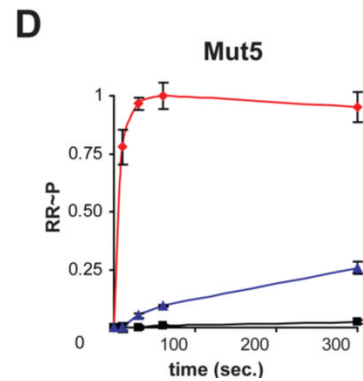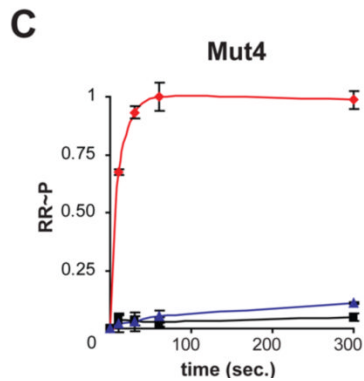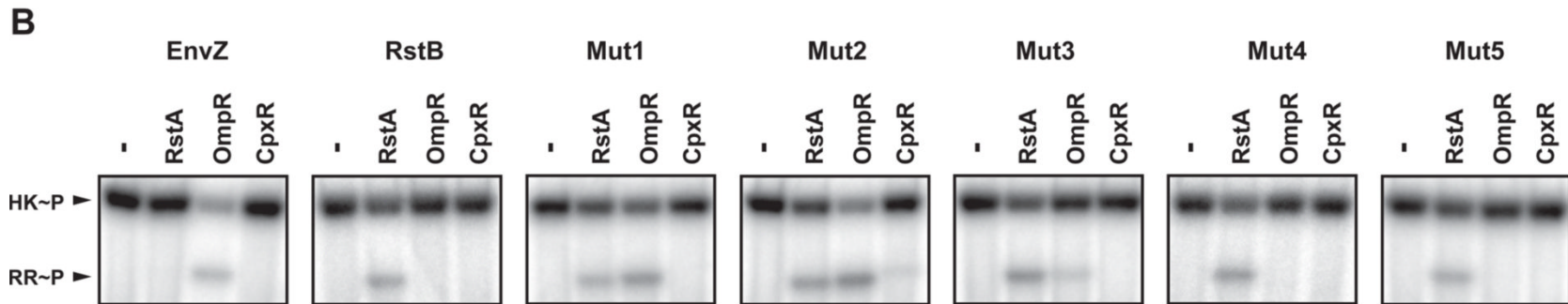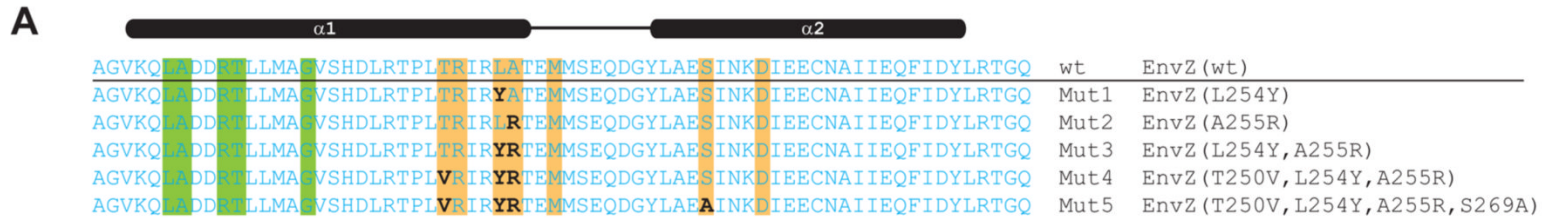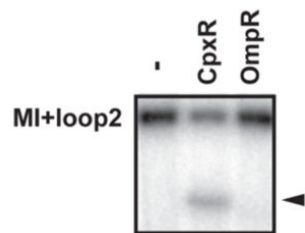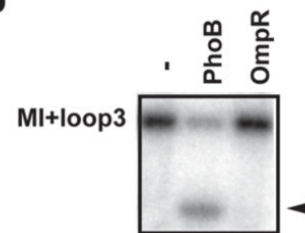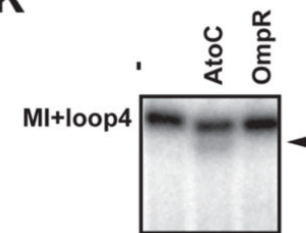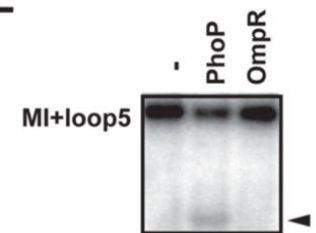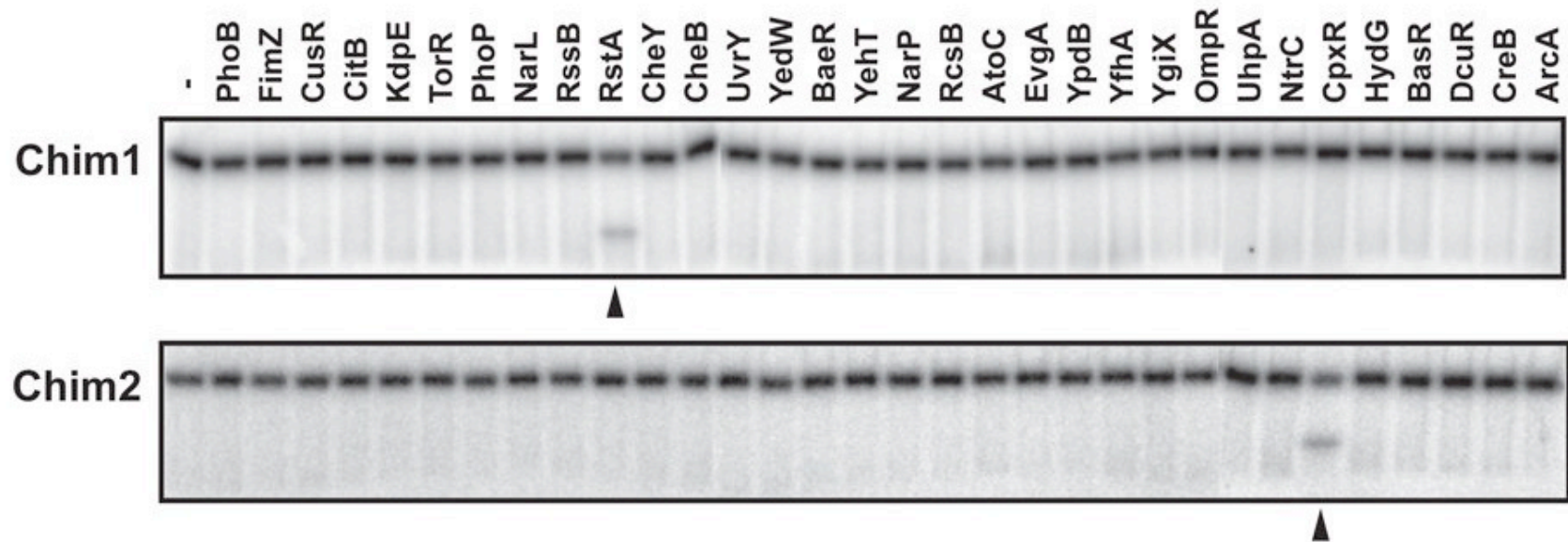
New Mutants containing Loop

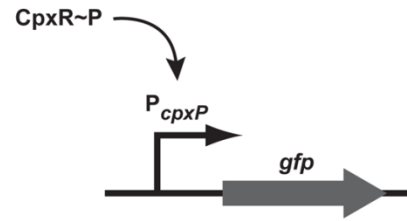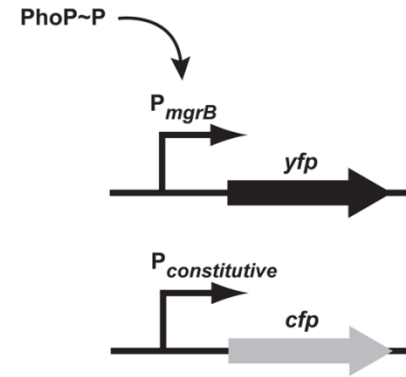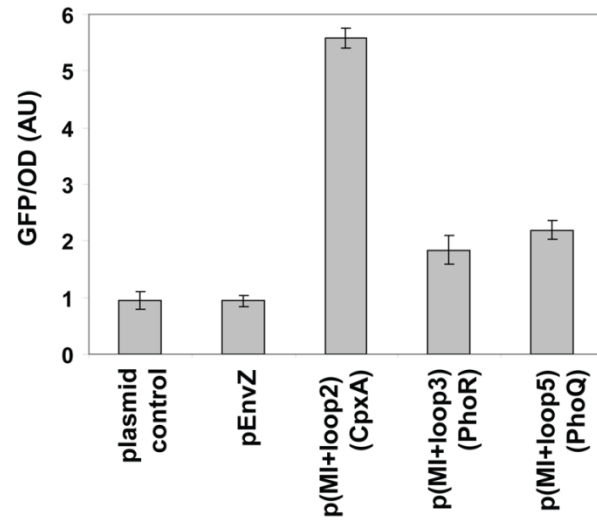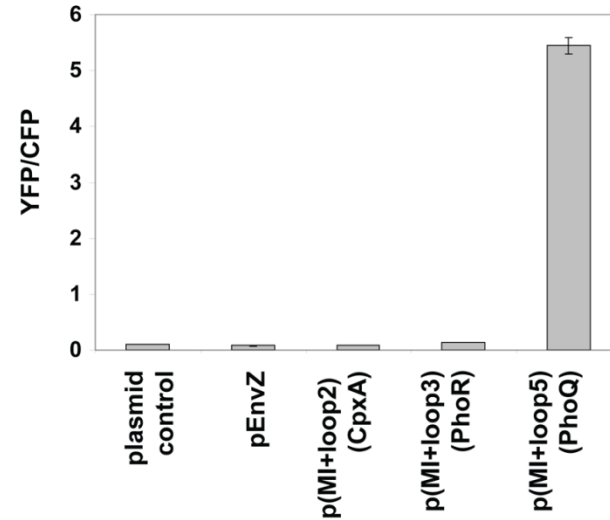# Well behaved Chimeras

In Vivo Testing

Critique

Key Assumptions

❖ Phylogeny is greatly reduced by screening for genomes with less than 90 % identity.

❖ Covariance determines the amino acids that would be more deterministic in histidine specificity.

Pit-Fall

❑ Need a combination of approaches for better understanding.

Significance

- Proof of the possibility to rewire the specificity of input systems by modifying a small number of residue.

- Model through which we can add complexity and apply to other biological systems *in vivo.*

- Cross-talk Suppression between the CpxA-CpxR and EnvZ-OmpR Two-Component Systems in E. *coli; Albert Siryaporn & Mark Goulian*