Histidine Kinase

Introduction

Some substances that we want to detect cannot diffuse into the cell or they do not activate promoters. To test for these substances we want utilize the modularity of *E.coli*'s EnvZ/ompR two-component signaling system[1] by creating chimera proteins that detect the desired substance.

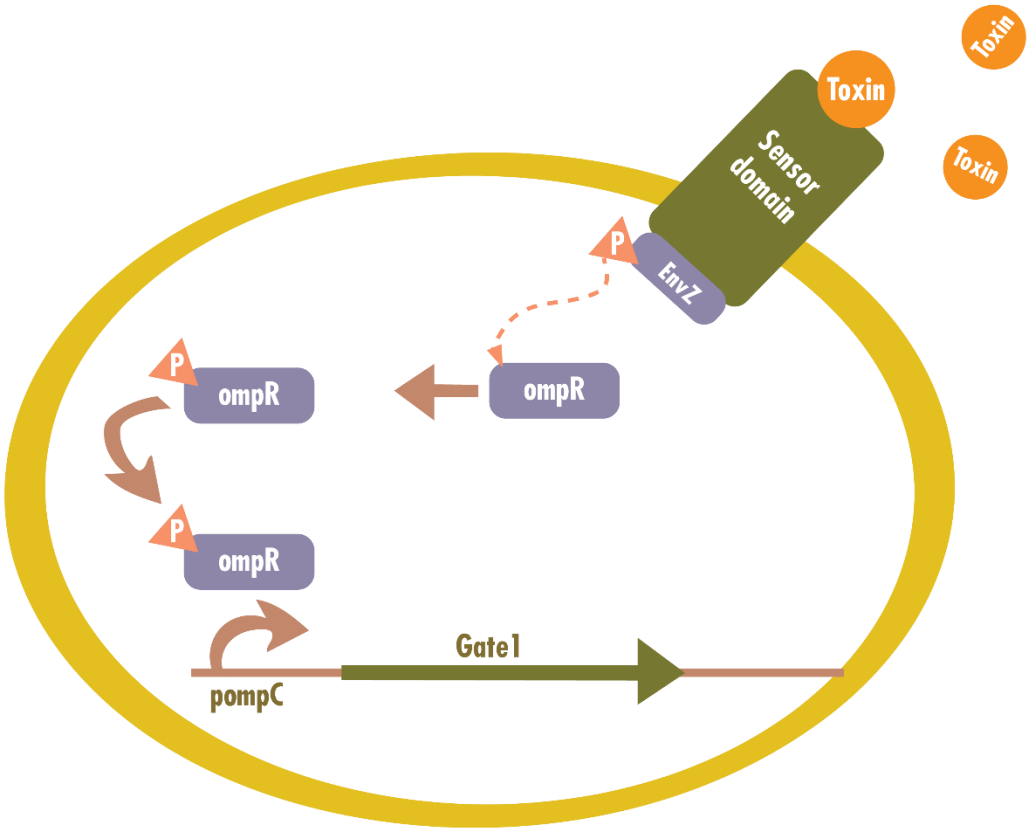

Figure 1: How a chimaera protein would use the EnvZ/ompR two-component signalling system to trigger our system

We first got the idea when we read about *E. coli* who were engineered to sense light (Levskaya, Chevalier, & Tabor, 2005). In this research, a chimaera protein was constructed from a photoreceptor and the EnvZ. We read up on chimaera proteins and

[1] EnvZ/ompR Two-component Signaling system (Forst & Roberts, 1994): Bacteria sense changes in their environment using two-component signaling systems. The EnvZ/OmpR system consists of an EnvZ histidine kinase protein with an external domain that senses osmolarity changes in the environment. At high osmolarity, the internal domain of the EnvZ autophosphorylates. The phosphorylated EnvZ then transfers its phosphate to the response regulator ompR. The phosphorylated ompR activates the promoter PompC which regulates production of membrane porin protein ompC. (Heyde, Laloi, & Portalier, 2000)

decided to test our system using Taz. Taz is a chimaera protein of the cytoplasmic domain of EnvZ fused with the sensory domain of the transmemebrane aspartate receptor (TAR) (Tabor, Groban, & Voigt, 2009). Figure 2 shows the structure of the Taz protein.

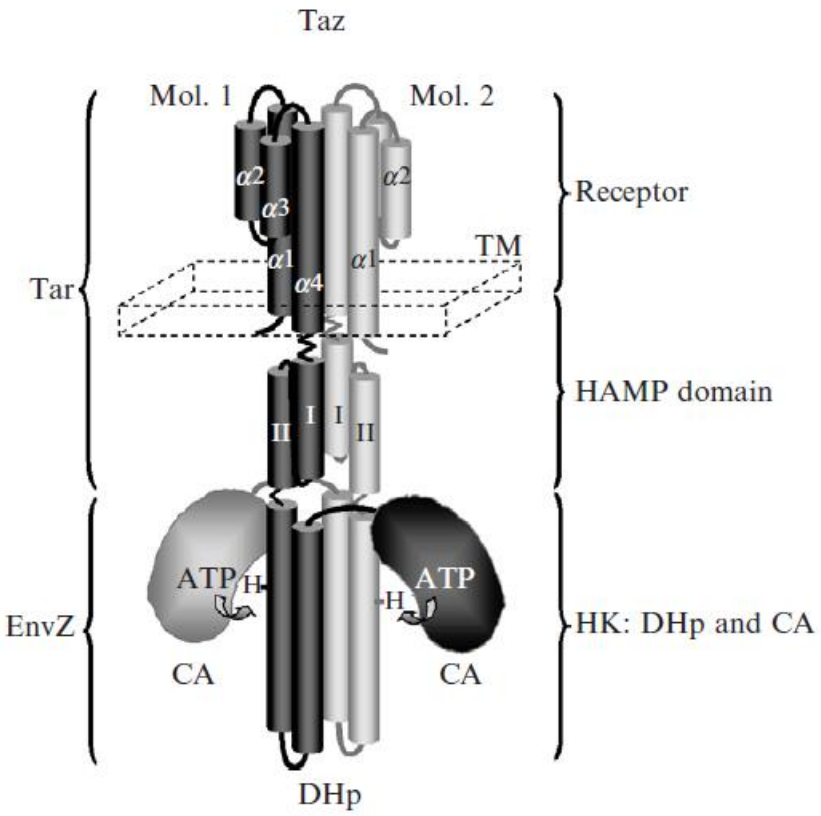

Figure 2: Schematic model of a Tar-EnvZ protein (Yoshida, Phadtare, & Inouye, 2007)

Taz Construct Status: Completed and Biobricked (BBa_K1343016)

We found biobrick Bba_C0082 (designed by Stephen Lee, Roshan Kumar, Joe Levine, Ziyan Chu) which contains the coding sequence for Taz. In order to use the Taz we needed to give it a promoter, RBS and terminator.

Ronen and Shira from our team gave us the plasmid pSB1AK3 with a double terminator (Bba_B0015 designed by Reshma Shetty) on it which they had used for gate 1. We did reverse PCR with extensions to add the promoter Pcat (Bba_I14033 designed by Vikram Vijayan, Allen Hsu, Lawrence Fomundam) and an RBS (Bba_B0034 designed by Vinay S Mahajan, Voichita D. Marinescu, Brian Chow, Alexander D Wissner-Gross and Peter Carr IAP) in front of the double terminator. After ligating the plasmid closed, we opened it up again between the RBS and the terminator using reverse PCR. We amplified the Taz from the Bba_C0082 biobrick using extensions to complement the promoter, RBS and terminator and did Gibson assembly to add it (see Figure 3). This created biobrick BBa_K1343016.

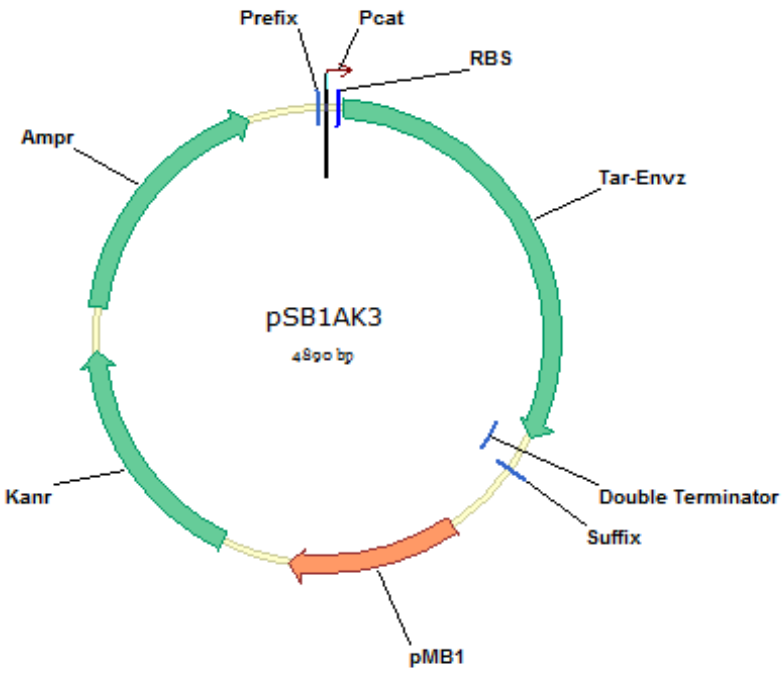

Figure 3: Taz under Pcat with RBS and double terminator (Taz construct) on plasmid pSB1AK3)

Experimentation

Status: Success

To test the activity of the Taz construct we created, varying concentrations of L-aspartic acid were added to a culture of *E. coli* expressing the Taz construct on plasmid pSB1AK3 and RFP under the promoter PompC (biobrick Bba_M30011 designed by Natalie Kuldell) on plasmid pSB1C3. The experiment was based on Dundee iGEM 2013 team's experiment with ompC-GFP reporter construct (BBa_K1012005 designed by John Allan) (Harrison, 2013) and the research of Michalodimitrakis, Sourjik and Serrano who tested Taz expression using GFP under PompC regulation (Michalodimitrakis, Sourjik, & Serrano, 2005) (See Figure 4)

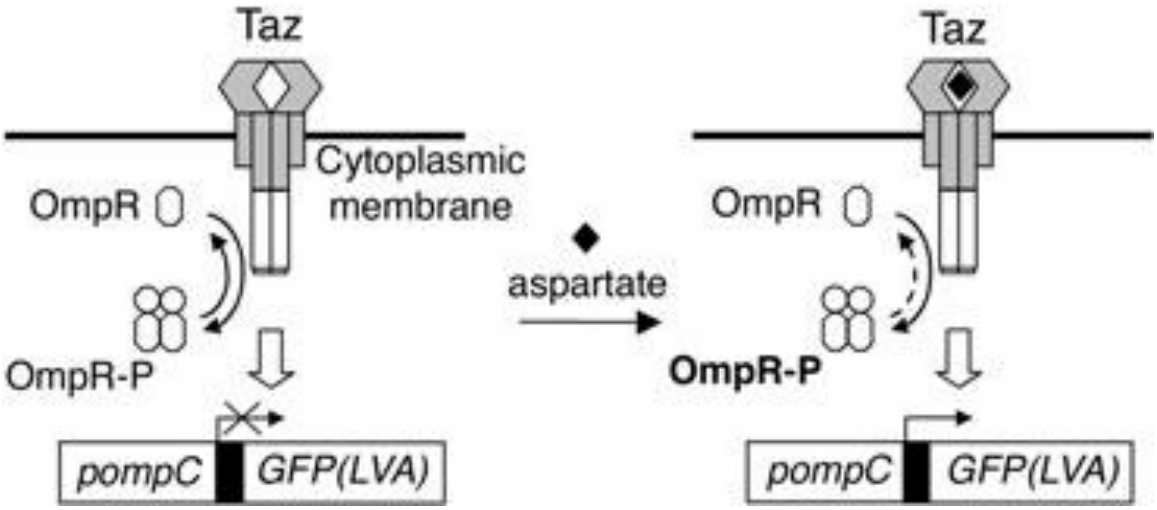

Figure 4: The chimeric Taz-ompR System as tested by Michalodimitrakis, Sourjik and Serrano (Michalodimitrakis, Sourjik, & Serrano, 2005)

Two isogenic strains of *E. coli* K-12, BW25113 (parent strain for the Keio collection[2]) and JW3367-3 (with ΔEnvZ mutation) were transformed with pSB1AK3 carrying our Taz construct (BBa_K1343016) and pSB1C3 carrying the BBa_M30011 reporter. The bacteria were cultured in growth media containing varying concentrations of L-aspartic acid. After two hours of growth the relative RFP fluorescence of the cultures was determined (fluorescence/OD).

(The E. coli strains were given to us by Lior Zelcbuch, Elad Hertz from Ron Milo's lab at the Weizmann Institute of Science)

OD was measured at 600nm.
Fluorescence excitation wavelength: 560nm
Fluorescence emission wavelength: 612nm

The goal was to compare the expression in the wild type and in the ΔEnvZ mutant. We expected that in the wild type the expression will be greater than in the mutant since the natural EnvZ/ompR system will cause expression of the RFP.

[2] Keio collection: A research group from Keio University in Japan made a set of single-gene deletions of all non-essential genes in the *E. coli* K-12 strain BW25113 using the lamda red technique. (Baba, Ara, Hasegawa, Takai, & Okumura, 2006)

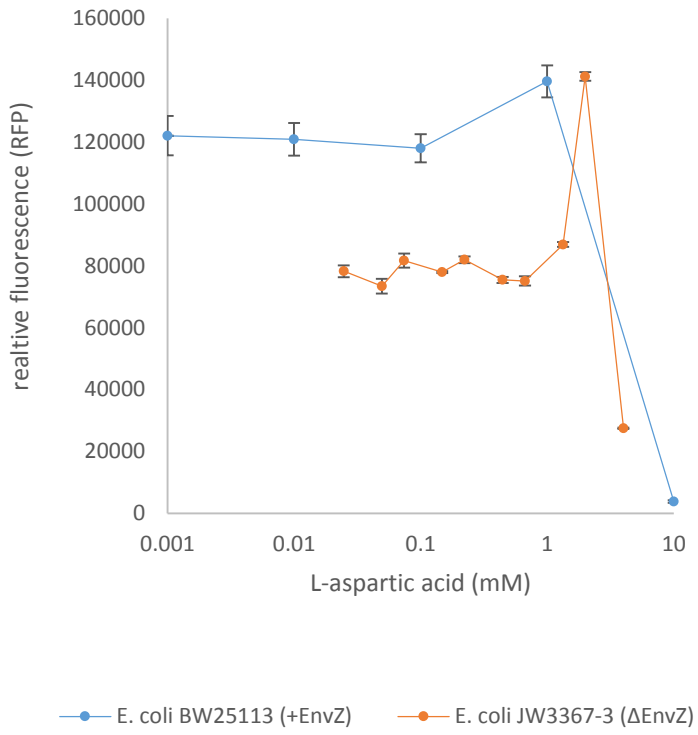

Figure 5: Relative fluorescence dependent on L-aspartic acid concentration (mM)

Figure 5 shows that at low concentrations of L-aspartatic acid (below 1mM), there is a steady, constant expression of the reporter in both the wild type and the mutant strain. The relative fluorescence observed by the mutant strain (ΔEnvZ) is lower than that of the parent strain. This is because the natural histidine kinase protein which detects a high level of osmolarity in the cells environment (EnvZ) is present in the wild type. This contributes to phosphorylation of the ompR. The low level of fluorescence observed in the mutant strain could be due to another mechanism (such as an acetyl phosphate dependent mechanism) which phosphorylates the ompR, leading to activation of the PompC promoter.

The mutant shows an increase in relative fluorescence is observed at 1.33mM L-aspartic acid, with a peak at 2mM followed by a drop in relative fluorescence at 4mM. The drop in relative fluorescence in both the mutant and wild type indicates a toxic concentration level.

In the mutant strain, the relative fluorescence increases 202% in comparison to the basal level. This occurs over a narrow concentration range, which reflects the sensitivity of the two-component signaling system. This sensitivity is critical for our system to be able to function as a low-concentration detector.

References

Baba, T., Ara, T., Hasegawa, M., Takai, Y., & Okumura, Y. (2006). Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular Systems Biology*, 1-11.

Forst, S. A., & Roberts, D. L. (1994). Signal transduction by the EnvZ-OmpR phosphotransfer system in bacteria. *Research in Microbiology, 145*, 363-373.

Harrison, K. (2013). *Reporter ompC-GFP*. Retrieved from Toximop: http://2013.igem.org/Team:Dundee/Project/ReporterOmpC

Heyde, M., Laloi, P., & Portalier, R. (2000). Involvement of Carbon Source and Acetyl Phosphate in the External-pH-Dependent Expression of Porin Genes in Escherichia coli. *Journal of Bacteriology, 182*(1), 198-202.

Levskaya, A., Chevalier, A. A., & Tabor, J. J. (2005). Engineering Escherichia Coli to see light. *Nature, 438*(24), 441-442.

Michalodimitrakis, K. M., Sourjik, V., & Serrano, L. (2005). Plasticity in amino acid sensing of the chimeric receptor Taz. *Molecular Microbiology, 58*(1), 257–266.

Tabor, J. J., Groban, E. S., & Voigt, C. A. (2009). Performance Characteristics for Sensors and Circuits Used to Program E. coli. In S. Y. Lee (Ed.), *Systems Biology and Biotechnology of Escherichia coli* (pp. 401-439). Springer Science+Business Media B.V.

Yoshida, T., Phadtare, S., & Inouye, M. (2007). The Design and Development of Tar-EnvZ Chimeric Receptors. *Methods in Enzymology, 423*, 166-183.