Co-crystallization of a Histidine Kinase with Newly Discovered Inhibitors

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Histidine Kinases as a Drug Target: Advantages and Challenges

Antibiotic resistant bacteria are a clear threat to global health. The drugs that have been used for decades to fight bacteria are no longer as effective as they once were, indicating that new therapeutics are needed. Two component systems (TCS) are often involved in the acquisition of antibiotic resistance.[1] A model of a TCS is shown in **Figure 1**. In the search for alternative ways to fight microbes, histidine kinases (HKs) are a viable drug target; they are unique to and common in bacteria.[1] The Carlson group previously conducted a high-throughput screen to identify inhibitors of the HK, HK853 (*Thermatoga maritima*), to identify inhibitory compounds that interact with their ATP-binding domain.[2] Crystallization of this histidine kinase in the presence these inhibitors will give invaluable information regarding the structure and behavior of the binding site and help with the development of a strategy to fight microorganisms by targeting histidine kinases. **Figure 2** shows an example of the type of useful image that will be obtained using x-ray crystallographic analysis.

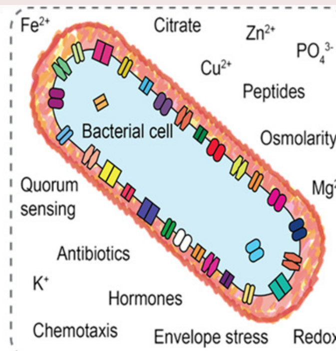


Figure 1.[3] Model of a bacterial cell with numerous two component systems. This cell uses outside signals to drive its cellular processes.

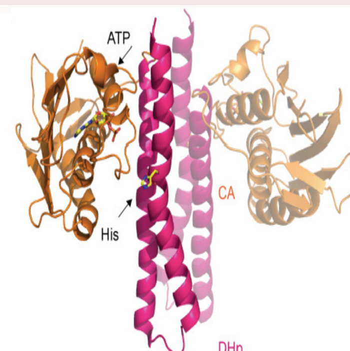


Figure 2.[3] A 3-D model of the histidine kinase protein obtained following crystallization. This protein is a possible drug target and the subject of these studies.

Histidine Kinase Overexpression and Purification

In this project, HK853 was tagged with a His-tag to facilitate purification following production in *E. coli*. This tag needs to be removed prior to crystallization as it can affect the crystal packing of the protein of interest. Cleavage of the tag was performed with tobacco etch virus (TEV) protease followed by purification of the protein using fast protein liquid chromatography (FPLC). The overall process is shown below.

Agar Plate → Liquid Culture → Sonicator → FPLC

Step 1. Bacteria Plating: Rosetta *E. coli* overexpressing HK853 were plated onto LB agar media and incubated at 37 °C. The same process was performed using an *E. coli* strain that overexpresses TEV protease.

Step 2. Liquid Cultures: A single bacterial colony was transferred from the LB agar plate into a 100 mL liquid culture and a subsequent 1 L culture. The 1 L culture was induced overnight to overexpress the histidine kinase protein.

Step 3. Centrifugation and Cell Lysis: The induced bacterial cultures were centrifuged and the pellet collected. The pellet was resuspended in lysis buffer and lysed using a sonication wand. The resulting liquid was centrifuged and the supernatant containing protein collected.

Step 4. Purification: Purification of the overexpressed protein was performed using nickel affinity and size exclusion chromatography columns on an FPLC system (nickel affinity only for TEV). Protein purity was confirmed by SDS-PAGE gel analysis.

Step 5. TEV Reaction and Purification: After pure TEV protease and pure HK853 were obtained, TEV was added to HK853 to cleave the His-tag from HK853. This product of this reaction was purified using nickel affinity chromatography. The purification was confirmed by SDS-PAGE gel analysis.

Crystallization of Cleaved Histidine Kinase

The pure, cleaved protein was used for crystallization trials since an ordered crystal lattice is necessary to perform x-ray diffraction analysis effectively. The goal of this project was to optimize the conditions for crystallization of HK853 before addition of inhibitors.

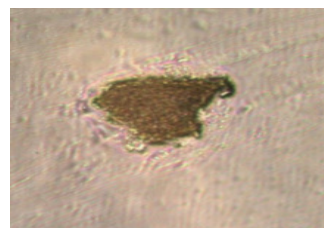


Figure 3. Example of protein aggregation that constitutes a failed crystallization attempt. Crystallization conditions: 10% PEG 6000, 3 μg protein, and 0.1M sodium citrate at pH 5.6.

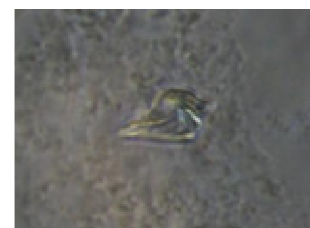


Figure 4. Example of protein crystal that constitutes a partial crystallization success. Crystallization conditions: 15% PEG 1050, 3 μg protein, and 0.1M sodium citrate at pH 5.6.

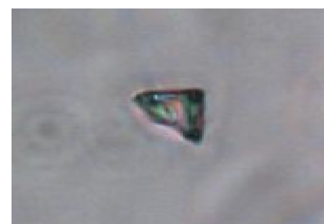


Figure 5. Example of protein crystal that constitutes a partial success that was further optimized. Crystallization conditions: 1.7 M $(NH_4)_2SO_4$, 2 μg protein, and 0.1M sodium citrate at pH 5.7.

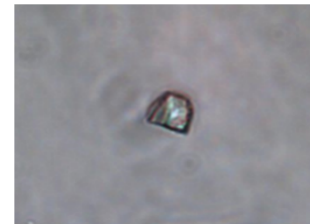
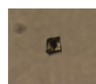
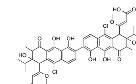


Figure 6. Example of protein crystal that constitutes a partial success to be even further optimized. Crystallization conditions: 1.7 M $(NH_4)_2SO_4$, 3μg protein, and 0.1M sodium citrate at pH 6.7.

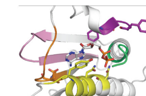
Future Goals



Further Optimization



Co-crystallization with Inhibitors (Walkmycin pictured above[3])



X-Ray Diffraction Analysis[3]

References:

1) Gao, R.; Stock, A. M. *Annu. Rev. Microbiol.* **2009,** *63*, 133-54.
2) Wilke, K.E.; Francis, S.; Carlson, E. E. *ACS Chem. Biol.* **2015**, 10, 328-35.
3) Wilke, K. E. Chemical Probes for Histidine Kinase Protein Profiling and Inhibitor Discovery. Ph.D. Thesis, Indiana University, Bloomington, Indiana, 2014.

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