Understanding the structural basis of small molecule inhibitors of *M. tuberculosis* DosS

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**Background**

- **Tuberculosis** (TB) is an infectious airborne disease caused by *Mycobacterium tuberculosis*, that becomes dormant under hypoxia.
- Therapeutics have proven to be ineffective when treating dormant TB.
- The DosS/DosR regulatory system is responsible for the upregulation of the dormancy genes.
- The regulatory system can be inhibited by introducing small molecule inhibitors that bind to the GAF-A domain of the DosS sensor.

**Expression and purification of wild-type GAF-A domain**

- Protein was purified using immobilized metal affinity chromatography (IMAC).
- The eluted protein was reacted with TEV protease to cleave off His-Tag. The sample was then purified again to separate His-Tag from cleaved GAF-A protein.

**Crystallization of GAF-A to prepare seed stock**

- Purified GAF-A protein was crystallized using the hanging drop crystallization method.
- GAF-A crystals obtained were used to create a seed stock to aid in crystallizing inhibitor-bound GAF-A.

**Crystallization of inhibitor-bound GAF-A**

- Two inhibitors synthesized by the Bhagi-Damodaran lab were tested out.
- Inhibitor was added to GAF-A and incubated; the absorbance was measured periodically to monitor the shift in Soret peak and determine whether the inhibitor was bound to the protein.
- Once the inhibitor was bound, crystal trays for the inhibitor-bound protein were set using prepared seed stock. Crystal trays were observed over a period of 3 weeks.

**Methodology**

**Expression and purification of wild-type GAF-A domain**

- SDS-PAGE was used to analyze purity of protein and to determine whether TEV reaction was successful.

**Crystallization of GAF-A to prepare seed stock**

- SDS-PAGE analysis of TEV protease cleavage efficiency. Expected mass difference of 2 kDa was approximately seen.

**Results**

**Expression and purification of wild-type GAF-A domain**

**Crystallization of inhibitor-bound GAF-A**

- Obtained inhibitor-bound crystals will be sent to a synchrotron and diffracted in order to determine the overall protein structure. We are looking to see how the inhibitor binds to GAF-A and affects the overall protein structure.

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