**Dual Polarization Interferometry for Small Molecule Drug Development Against Francisella tularensis**

Sarina Nawim, CSU East Bay, Bioscience and Biotechnology Division, Lindsey Evans and Brett Chromy

Lawrence Livermore National Laboratory,

Tularemia, also known as rabbit fever, is an infectious disease caused by the gram-negative bacterium *Francisella tularensis*. Transmission to animals and humans occurs via several routes, including ingestion of contaminated water, or inhalation of contaminated dusts or aerosols, tick bites, and skin contact with infected animals. Because of its highly pathogenic and opportunistic nature, *F. tularensis* is a likely candidate for use as an airborne biological weapon. For this reason, developing a vaccine and identifying appropriate treatments are critical to effective bioterrorism preparedness and response. It may exist in nature within encysted amoeba, which may represent a similar survival strategy within human macrophages. To facilitate antimicrobial drug development, a study of the encystment process was carried out. One of the proteins discovered during this study was Rep 24, a novel Franciscella cytosine protein. In this current study the interaction between Rep 24 and a possible interactive substrate, JPM-565, is observed. This interaction is detected through the optical sensing technique known as Dual Polarization Interferometry (DPI). Immobilized Rep 24 is layered on a thiol chip, followed by a wash of JPM. Changes in the refractive index of the molecules, as measured by DPI, demonstrate binding events between the two. Data from binding experiments are later used for quantitative measurements of size, density and mass. Future work includes investigation of additional *Francisella tularensis* proteins and screens against small molecules that may bind and abrogate function, leading to countermeasures against *Francisella tularensis*. 

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**Introduction:**

*Francisella tularensis* is the causative agent of the infection Tularemia, also known as Rabbit Fever. It is theorized that a naturally-occurring reservoir exists for *F. tularensis* in environmental amoeba. Aided by a rapid encystment process, amoeba remain viable for long periods of time, which allow them to contaminate the surrounding soil and water. Once in the soil and water, *F. tularensis* can be transferred to ticks and other animals.

*Francisella tularensis* is a gram-negative bacteria, which is highly infective. Between 10-50 bacteria are enough to cause disease in humans. In addition to being highly infective, it is a likely candidate for use as a biological weapon.

Developing vaccines and identifying appropriate treatments are important for bioterrorism preparedness and response. In this study, the interaction between *F. tularensis* proteins Rep 24 and Rep 24 mutant, with a small molecule JPM-565 is being investigated. JPM-565 could act as an inhibitor to block the active site on the enzymes. This interaction is detected through the optical sensing technique known as Dual Polarization Interferometry (DPI). Immobilized Rep 24 and a non-functional Rep 24 mutant is bound to a thiol chip, followed by a wash of JPM. Changes in the refractive index of the chip surface, as measured by DPI, demonstrate binding events between the two. Data from binding experiments are later used for quantitative measurements of size, density and mass.

**Methods:**

**How DPI works**

- Light travels through both waveguides to create an interference pattern.
- Shifts in the RI due to the binding of molecules on the chip surface, changes the interference pattern.
- DPI is being investigated. JPM-565 wash -565 could act as an inhibitor to block the active site on the enzymes. This interaction is detected through the optical sensing technique known as Dual Polarization Interferometry (DPI). Immobilized Rep 24 and a non-functional Rep 24 mutant is bound to a thiol chip, followed by a wash of JPM. Changes in the refractive index of the chip surface, as measured by DPI, demonstrate binding events between the two. Data from binding experiments are later used for quantitative measurements of size, density and mass.

**Results:**

- After JPM-565 injection to the chip surface, binding was observed. Data analysis confirmed this observation with increases in RI and density.
- After JPM-565 was exposed to the adhered protein surface, binding was anticipated to occur between the protein and small molecule. However, the data from this experiment did not confirm this hypothesis. Future work will include repeating the experiment using a derivative of JPM, JPM-Oct, with an attached acid ring.

**Discussion:**

Stable binding occurred between Rep 24 and the surface of the thiol chip. Data analysis confirmed this observation with increases in RI and density. After JPM-565 was exposed to the adhered protein surface, binding was anticipated to occur between the protein and small molecule. However, the data from this experiment did not confirm this hypothesis. Future work will include repeating the experiment using a derivative of JPM, JPM-Oct, with an attached acid ring.

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