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

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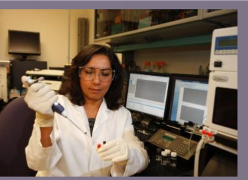


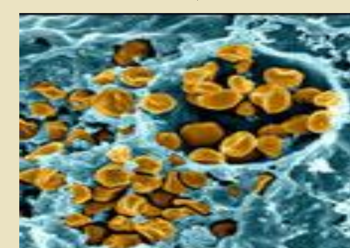
Tularemia, also known as rabbit fever, is an infectious disease caused by the gram-negative bacterium *Francisella tularensis.* Transmission to animals and humans occurs via several routes, including ingestion of contaminated water, or inhalation of contaminated dusts or aerosols, tick bites, and skin 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 Francisella cysteine protease. 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.*

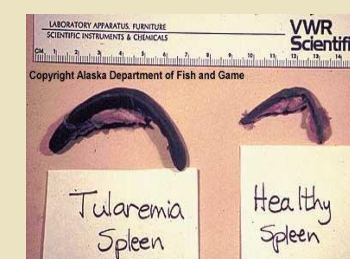
Introduction:

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 tics and other animals.

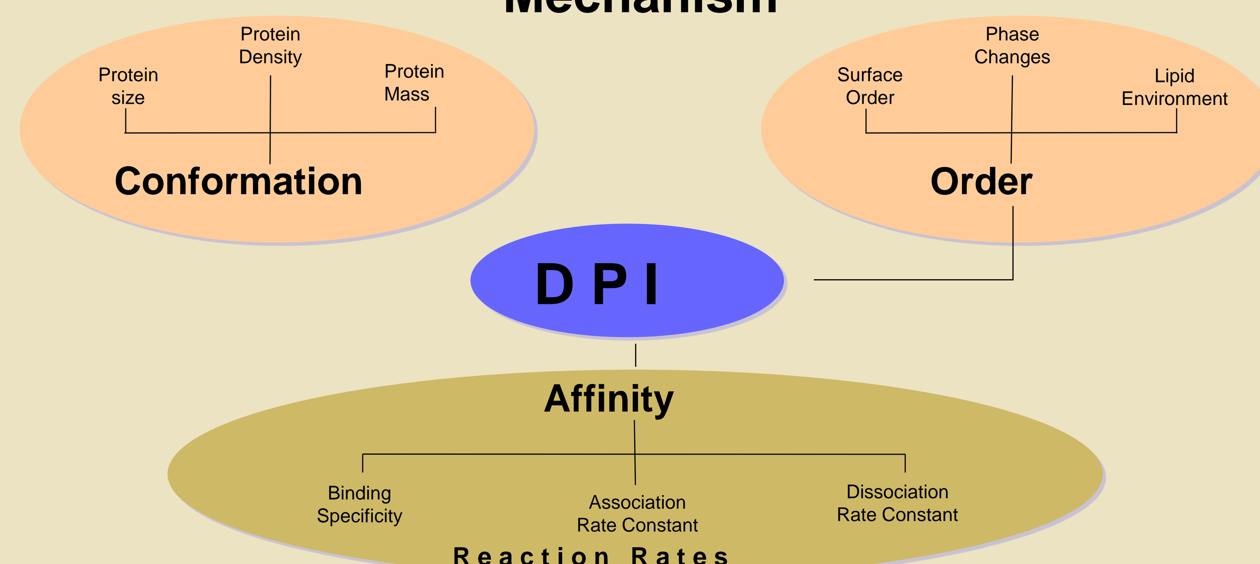
Francisella tularensis is a gram-negative bacteria, which is highly infective. Between 10-50 bacteria are enough to cause disease in humans. In addition to being highly infective, it is a likely candidate for use as a biological weapon.

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.





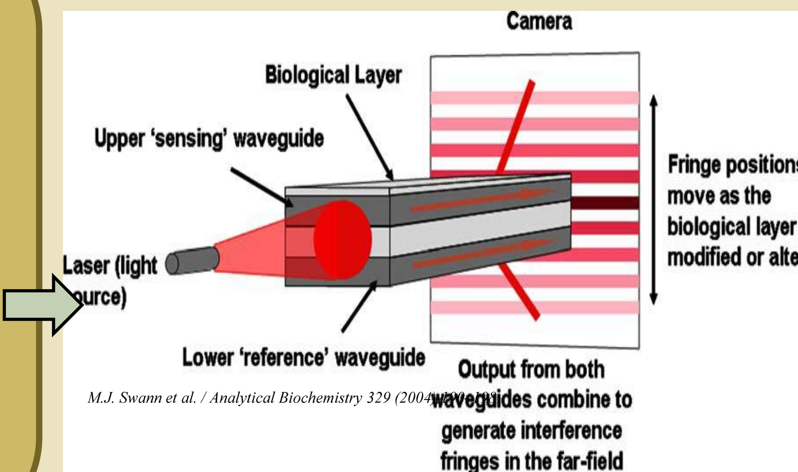
Mechanism



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
- The AnaLight® 4D records changes in RI. Data is entered into analysis software, which will resolves the size, density and mass of the molecules .

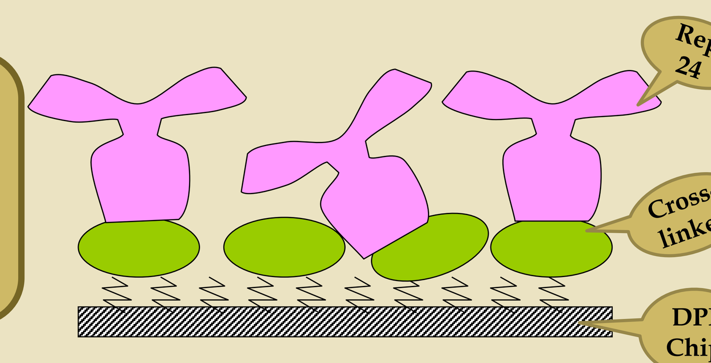


- Prepare buffer: Phosphate Buffered Saline (PBS). Degas buffer 15 minutes and exchange instrument fluidics.

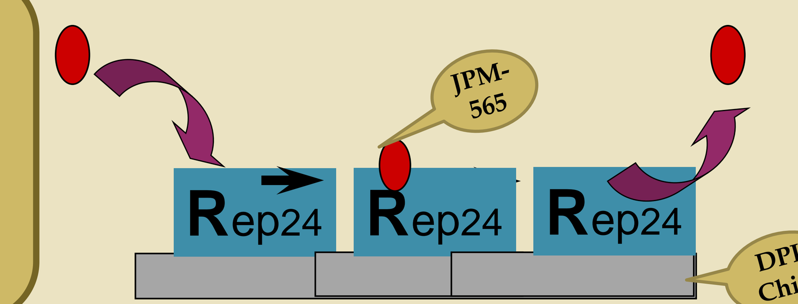
During Experiment

- Calibrate DPI with 80% ethanol and distilled water: standards with known refractive index.

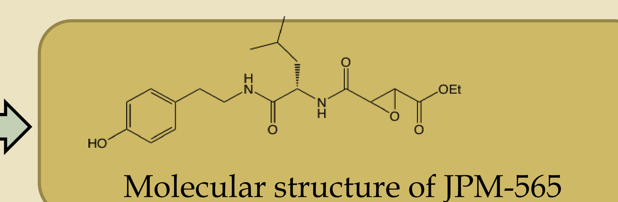
- Coupling of GMBS (cross-linker) and thiol chip surface to increase the stability of the immobilized protein



- Inject Rep 24 and Rep 24 Mutant, diluted in PBS buffer, over the chip surface to bind to GMBS
- Casein injections fill gaps on the chip surface to block non-specific binding



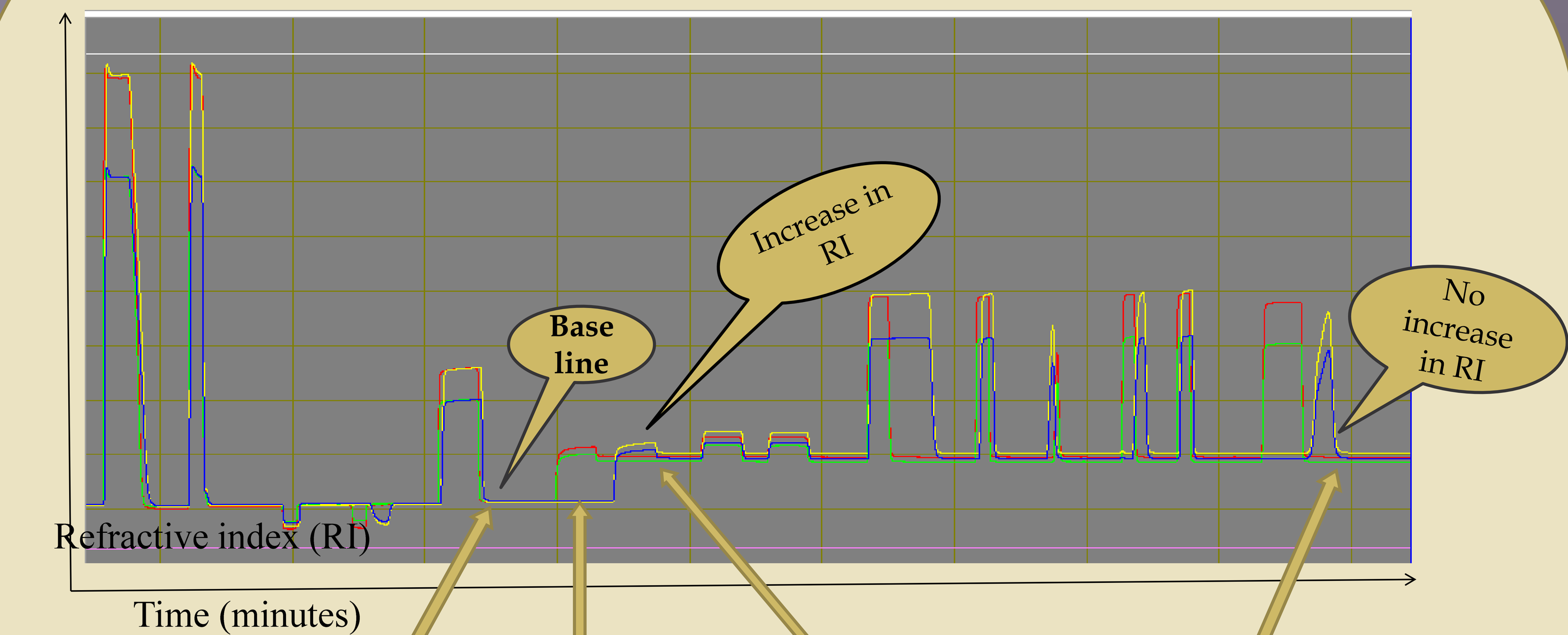
- Immobilized proteins are exposed to a JPM-565 wash



Molecular structure of JPM-565

•Data is continuously collected in the course of the experiment and displayed as shown to the right. Once the experiment is finished, points of interest are marked on the graph for data analysis followed by accurate measurements for refractive index, thickness, mass, and density.

Results:



	Before Protein injection	After Protein injection	After Mutant Protein injection	After JPM-565 injection
RI	1.4412	1.465 ±.002508	1.4636±0.004304	1.4638
Thickness (nm)	0.1846	2.299	2.5226	2.2839
Mass (ng/mm2)	0.1079	1.6446	1.7856	1.6187
Density (g/cm2)	0.5843	0.7154	0.7078	0.7088

Refractive index, thickness, mass, and density are increased compared to the baseline, after protein was injected on to the surface of the thiol chip. No increases were detected after a JPM-565 injection to the chip surface.

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-Oet, with an attached acid ring.

Acknowledgments: Amy Rasley,Brent Segelke , Shelly Corzett, Jane Bearinger and Lawrence Livermore Lab

Work Sites:
http://www.cdc.gov/Tularemia/
http://www.unbc.ca/nlui/wildlife_diseases_bc/tularemia.htm

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