TITLE: Determining the interaction between CT228 and MYPT1 in *Chlamydia trachomatis*

Chlamydia trachomatis is a human pathogen responsible for an array of diseases with a substantial medical impact. *C. trachomatis* infections are the most commonly reported bacterial sexually transmitted infections, with 3 million cases estimated annually in the United States[1]. Even after the clearing of a *C. trachomatis* infection, there is an observed correlation of complications in females: pelvic inflammatory disease, scarring of the fallopian tubes, tubal infertility, chronic pelvic pain, and ectopic pregnancy[2-4]. There is also an increased risk of cervical cancer. Despite the longevity of health concerns, after the clearing of a chlamydial infection there are fundamental gaps in our understanding of *Chlamydia* pathogenesis, particularly in regard to the mechanisms used to manipulate host proteins for intracellular survival and growth.

Chlamydia is an obligate intracellular pathogen that can only survive inside of a host cell. To do this, *Chlamydia* manipulates many host signaling processes for survival and replication. One of the host proteins identified to be recruited during infection is myosin phosphatase[5] which is a host protein known to regulate myosin. This interaction is highly significant since changes in myosin regulation are critical for the development and proliferation of cancer cells[6-8]. In fact, *Chlamydia* is the first bacterial pathogen identified to manipulate myosin phosphatase during infection, and does so via the chlamydial protein CT228. Understanding the interaction between CT228 and MYPT1 may shed significant insights into how a *Chlamydia* infection may lead to increased rates of cervical cancer.

The aim of this proposed project is to identify the region of CT228 that interacts with MYPT1 using a yeast-two hybrid system. The **hypothesis within the proposed project is that some truncations in CT228 will be deficient in interacting with MYPT1 in the yeast-two hybrid system.**

The proposed project is to generate four CT228 truncations (each truncation will remove 10 amino acids from the end of CT228) and then clone the mutations into the yeast-two hybrid system. My proposed mentor's lab has already generated three of the CT228 truncations. My goal will be to generate the last truncations of CT228. This will be done by amplifying the gene with specially designed primers using polymerase chain reaction (PCR) amplification and cloning into the bait yeast-two hybrid vector. All CT228 clones (prey), and a previously generated MYPT1 clone (bait), will be transformed into yeast and assessed for interaction. If the prey interacts with the bait, then we will see blue yeast colonies on restrictive media. If any of the truncations of CT228 (baits) do not interact with prey, we will not see any growth on restrictive media.

It is anticipated that the proposed project will be completed in one semester and contribute to a published manuscript. These experiments will help us identify how *Chlamydia* interacts with MYPT1. Knowing the specific region of CT228 that interacts with MYPT1 will help us understand how *Chlamydia* manipulates myosin, and in turn provide insight into long-term consequences of chlamydial infections, such as cervical cancer.