Online Proceedings

POSTER SESSION 4

Balcony, Easel 92

4:00 PM to 6:00 PM

Stabilizing Self-Assembling Protein Cage for Use Towards Vaccine Design

Gargi Mukund (Gargi) Kher, Junior, Biochemistry
Mentor: Neil King, Biochemistry
Mentor: Karla-Luise Herpoldt, Bioengineering

Natural proteins often assemble into various complex geometric structures based on their interactions with each other. These structures can hold and transport "cargo" as well as display antigens, making them extremely useful in vaccine design. The King Lab at the University of Washington uses the way these proteins assemble to develop computational models that help them design novel self-assembling protein cages, or nanoparticles. These nanoparticles are then used to develop vaccines or treatments for diseases. Components of the designed protein cage can be modified and expressed individually before being assembled together into the nanoparticle. I am working on stabilizing one of these protein cages known as T33DN2, so it can be used towards creating a vaccine. T33DN2 is a tetrahedral cage comprised of two trimeric proteins known as T33DN2A and T33DN2B. When expressed individually through *E.coli*, DN2A is produced in a soluble form while DN2B is produced in a mostly insoluble form. T33DN2 is currently an unstable cage, as only the A component is expressed solubly. Soluble proteins are generally more stable and thus easier to work with than their insoluble counterparts. To increase the solubility of DN2B, I have been making mutations to specific amino acids in the DNA that produces this protein, as well as expressing and purifying this component to determine its stability.

POSTER SESSION 4

Balcony, Easel 91

4:00 PM to 6:00 PM

A Designed Self-Assembling Nanoparticle Vaccine for Parenteral Induction of Mucosal Immune Responses

Rose B Fields, Junior, Biochemistry
Mentor: Neil King, Biochemistry
Mentor: Karla-Luise Herpoldt, Bioengineering

Enteric diseases, or diseases of the Gastrointestinal (GI) tract, remain one of the most prevalent killers of children in sub-Saharan Africa. The most practical way to prevent such diseases is through vaccination, but antigens for enteric diseases need to be delivered directly to the GI tract to be most efficient, making vaccination difficult. Recent studies by the von Adrian group at Harvard University have found that both T and B cells are reprogrammed to home to the GI tract when they encounter retinoic acid, a metabolite of vitamin A. The King Lab at the University of Washington is working to develop a novel vaccine candidate using recently developed self-assembling protein nanoparticles, that can simultaneously package all-trans retinoic acid (ATRA) and multivalently display enteric antigens. Previous work has suggested that two cystine mutations to Cellular Retinoic Acid Binding Protein I (CRABP-I) create a disulfide bond as a result of the conformational change that CRABP-I undergoes when it binds ATRA. This disulfide bond would essentially lock ATRA into CRABP-I, reducing its dissociation constant in vivo and maintaining the gut-homing properties of the nanoparticle post-injection. In order to assess the efficacy of these cysteine mutations, I expressed two versions of CRABP-I, the wildtype protein with no cysteine residues, and a version with no cystine residues except for the two that create the disulfide bond. After establishing that these new CRABP-I mutants folded into the approximate shape of wildtype CRABP-I via circular dichroism, I designed and tested new assays that measured free thiol concentrations of each protein after binding ATRA, as well as free ATRA concentration overtime. This data will help us determine whether these two cystine mutations make a significant difference in the ATRA binding quality of CRABP-I, which could improve the immune response generated by our vaccine candidate.