Online Proceedings

POSTER SESSION 2

Balcony, Easel 135

2:30 PM to 3:30 PM

Demonstrating Functionality of Fluorogen-activating Protein Force Sensor

Colin Tan Sullender, Senior, Bioengineering
Mentor: Wendy Thomas, Bioengineering

Mechanical forces play a critical role in biology from cellular differentiation to the formation of blood clots. However, there are currently limited quantitative methods of measuring these intrinsic molecular forces. The development of fluorogen-activating proteins (FAPs) that are capable of allowing normally dark fluorogen dye molecules to fluoresce offers a unique possibility for the design of a novel force sensor with piconewton sensitivity. These proteins consist of two single-chain antibodies that must dimerize in order to bind and activate a fluorogen dye molecule for excitation. The force sensor itself consists of a single FAP with a flexible linker connecting the two chains such that they are held in close proximity to each other. In the presence of tensile mechanical force applied across the complex, the two antibody chains will be pulled away from each other, disrupting the binding of the fluorogen dye and subsequently decreasing the fluorescence intensity. The purpose of this project is to develop an in vitro testing system to demonstrate that the protein is responsive to applied forces. In order to apply tensile forces in a controllable manner, two high-affinity attachment tags (biotin and SNAP-tag) were added on the N and C termini of the complex. These ligands will be utilized to attach the force sensor to paramagnetic beads and a streptavidin-coated surface. Introducing a magnetic field will result in force simultaneously being applied across a large number of proteins and a detectable decrease in fluorescence intensity as the fluorogen dyes unbind. Positive confirmation of the relationship between fluorescence and force will validate the protein as a force sensor and allow for future development in implementing the probe in a cellular system.