SESSION 1R

SYNTHETIC BIOLOGY AND MOLECULAR BIOTECHNOLOGY

Session Moderator: Daniel Ratner, Bioengineering
022 JHN
1:15 PM to 2:45 PM
* Note: Titles in order of presentation.

A Molecular Tether Design for von Willebrand Factor Protein Constructs

Tessa Olmstead, Senior, Bioengineering, Dance: Creative Studies
Mary Gates Scholar
Mentor: Wendy Thomas, Bioengineering
Mentor: Emilie Clemmens, Bioengineering

Affecting nearly 1% of the human population, von Willebrand Disease (VWD) is the most prevalent inherited bleeding disorder worldwide. VWD is caused by impaired platelet adhesion due to low plasma von Willebrand Factor (VWF) concentration, or dysfunction. The largest plasma glycoprotein, VWF's function is to mediate attachment of platelets to the exposed collagen on blood vessel walls at sites of injury. This interaction is sensitive to changes in flow forces or shear stress. To facilitate the study of VWF, the Thomas Lab is developing a single-molecule force measurement platform called magnetic tweezers; a tool capable of performing multiple single-bond force measurements in parallel. To use magnetic tweezers, VWF protein domains of interest are adsorbed to a glass surface on one end, and to a magnetic bead on the other. Electromagnets are then placed above the slide, applying an upward force to the VWF domains. The design and optimization of the VWF domain tethers is the focus of this research project. At present, a tethering scheme is being tested that utilizes the strength and longevity of the biotin-streptavidin non-covalent bond and the fimH lectin domain to mannose interaction. An alternative tethering scheme employing histidine tags instead of fimH is also being explored. While both types of tethers have been expressed successfully, tested in the magnetic tweezers, and shown to bind beads to the surface under force, recent data has shown that non-specific adhesion between protein-functionalized magnetic beads and the coverslip is an issue. Using immunoassays such as ELISA and the Western Blot, tether designs will be optimized using magnetic tweezers. With the capability to more accurately mimic physiologic conditions than immunoassays alone, coupled with the intrinsic ability to multiplex, the quantity and quality of data that could be acquired is impressive.

POSTER SESSION 4

MGH 241, Easel 140
4:15 PM to 5:45 PM

Developing Magnetic Tweezers for Multiplexed Single Molecule Force Measurements to Quantify Biomechanical Function of Von Willebrand Factor

Hani Jason (Hani) Mahmoud, Senior, Bioengineering
Mary Gates Scholar
Mentor: Wendy Thomas, Bioengineering
Mentor: Emilie Clemmens, Bioengineering

Single-molecule force spectroscopy is a powerful tool for studying mechanical forces on proteins and other biological molecules. Magnetic tweezers (MT), optical tweezers, and atomic force microscopy (AFM) are specific examples of high-resolution tools that allow users to investigate how changes in the biomechanical function of a single protein, for example, can lead to larger effects in the complete organism. We have chosen to develop and implement an MT apparatus due to its unique potential to expand our current capabilities by allowing many single-molecule measurements in parallel. In this system, molecules are tethered to a glass surface and to magnetic beads, and a magnetic field is applied to pull on the beads and thus the molecules. We then used a bead-tracking algorithm to derive quantitative force measurements. We hope that multiplexing will allow for a more efficient study of how mechanical forces regulate molecular structure and function. Ultimately, this knowledge can be applied to both engineering innovative materials and designing therapeutic interventions. For example, we are using our MT to investigate how mutations in a blood clotting protein called von Willebrand factor (VWF) can lead to bleeding disorders. We intend to accomplish this by measuring how VWF's sensitivity to forces comparable to those in the blood affect its roles in platelet binding. VWF also plays a major role in stroke and myocardial infarction, which we hope to study and quantify using our MT system.