

Optical Measurement of Forces between biological molecules

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Background:

Cadherin are vital proteins within the human body and in cell biology. Cadherins are primarily binding agents responsible for growth, wound repair and immune response. Observation of the motion during the binding of tissue surrounding a wound has engendered questions about the mechanisms and properties which distinguish Cadherins from simple adhesives. The proposed study requires development of fluorescence sensing techniques, such as a FRET (fluorescent resonance energy transfer) based technique (undergoing development at IMB) to find mechanisms activated during adhesion. Gain-of-function experimentation has found that Src has negative effects on cadherin function. However, in loss-of-function testing it has also been found that Src has positive effects on cadherin function. The complex nature of Src interaction and its implications in pathology for metastatic cancers and epithelial-mesenchymal transformation (EMT) make it a novel and worthwhile investigation. Using chromophores the distribution of Src will be monitored during cadherin controlled transduction events. The creation of an experiment to probe both the transductive force mediated by cadherin during cell-cell adhesion is highly non-trivial due to the complex nature of signaling and the integration of force sensing equipment. The planned experiments will start with a simple optical tweezers setup which will allow control and measurement of forces present in single sets of interacting Cadherins. Optical Tweezers can detect forces to an accuracy of a few pN/nm making them well suited to the study of protein-level interactions. Cadherins are well known to form homophilic ligands with neighbors so much of the effect of other cell adhesives such as integrins are eliminated by using a bead coated in cadherin. The initial optical tweezers apparatus will be extended to include fluorescence microscopy in an investigation of the interactions between a cadherin coated bead and a cell.

Outcomes:

- Fluorescence microscope with optical micromanipulation functions to measure forces and torques while simultaneously monitoring activation of cellular transmitters.
- Greater understanding of cadherin binding at both a single molecule level and its signalling role in cell transduction.

Progress to date:

- Mr Stilgoe has recently joined the project as a PhD student and will apply laser micromanipulation techniques previously developed for force and torque measurement to this new application. Dr Yap has an ongoing program on cadherins which will be enhanced by this project.

Funding is sought to:

No funding is sought at this time.

Timeline:

The project will extend over the next three years.