Mechanosensitive protein-protein interactions in nascent focal adhesions determined by FRET sensing using multiphoton fluorescence lifetime imaging

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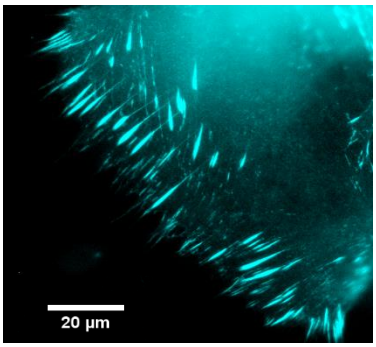

Figure 1: Vinculin-mTFP1 expressed in vinculin null MEFs.

Focal Adhesions (FAs) are large sub-cellular structures comprised of macromolecular assemblies (see figure 1) that anchor cells to the extracellular matrix and play a key role in force transduction and intracellular mechano-signalling pathways. Understanding how mechanical signalling influences specific molecular and cellular mechanisms is crucial in progressing our knowledge of how the external environment affects normal cell physiology.

In this project we used two tension sensitive biosensors to detect changes in force across two mechanosensitive FA proteins – Vinculin and Talin. These FA proteins both have a Tension Sensing Module[1,3] (TSM), which contains two fluorescent proteins joined by a short-coiled linker that extends when force is applied.

If the biosensors are not under tension, the two fluorescent proteins are in close enough proximity to undergo FRET (Förster Resonance Energy Transfer). By measuring FRET using multiphoton TCSPC (time-correlated single-photon counting) fluorescence lifetime imaging we observe the loss of FRET, compared to a control, as a direct consequence of an applied intracellular force across the biosensor[2].

These force-dependent conformational changes enable us to describe the role that tension plays within specific protein-protein interactions found in FAs. These interactions affect FA assembly, maturation and degradation through force dependent pathways and mechanisms. Ultimately, these pathways effect the adherence of cells to the extracellular matrix, which is of critical importance in understanding tumour growth and metastasis.

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