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Combining FRET tension sensor and optical tweezer for mechanical studies on living cells

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Introduction: Focal adhesions (FAs) are the anchoring point which link the actin cytoskeleton to the extracellular matrix and though which cells sense their environment. Several proteins are involved in FAs assembly, stabilization and disassembly but their roles are still not fully understood. The vinculin protein is force-dependently recruited and is suspected to be responsible for FAs stabilization. FRET-based tension sensors are a remarkable tool used to study the forces exerted on FAs proteins.
Förster resonance energy transfer (FRET) is a unique way to measure nanometer-scale distances between two fluorophores, a donor and an acceptor, without the need for high resolution imaging. When the donor and acceptor pair are connected with an elastic linker subjected to tension, the distance measurement can be translated into a force measurement, with pN sensitivity. These FRET tension sensors have been inserted inside proteins involved in cell attachment.

Method: Direct measurement of FRET efficiency with fluorescence lifetime measurements (FLIM), typically require a costly scanning microscopy setup with a short-pulse laser and a detector able to measure nanosecond lifetimes. Sensitized FRET measurements, based on fluorescence intensities, can be done on simpler setups, providing images over a large field of view without scanning. The originality of our setup lies in the simultaneous detection of the fluorescence of the donor and the acceptor, and the sequential excitation with two LEDs, avoiding any moving parts in the system. Our setup combines fluorescence excitation and an optical tweezer to simultaneously monitor the force applied on the cell membrane in vivo and the FRET efficiency of the vinculin tension sensor near the point of application of the force. The optical tweezer is based on a laser at 1069nm and a detection by reflection, allowing a rapid and precise measurement of the force exerted by the optical tweezer.

Results: Our measurements have been performed at FAs sites of CHO cells, on a tension sensor inserted in vinculin (VinTS), and compared with a force insensitive tail-less control (VinTL), lacking the actin-binding domain of vinculin. Small changes in FRET efficiency of VinTS can be translated into force variations (ranging from 1 to 10 pN). Variation of these forces depending on the surface treatment on which the cells are plated has been observed.
Our simple optical setup has proven capable of measuring FRET efficiencies in live cells with adequate sensitivity to resolve small changes in FRET efficiency. Microbeads coated with fibronectin are brought in contact with cell membrane to create FAs. FAs formation is stimulated by constraining the bead with the optical tweezer. Vinculin recruitment around the bead is monitored by fluorescence, then FRET gives access to forces exerted on vinculin. Simultaneously, force exerted by the cell on the beads is known by tracking the bead position relative to the optical trap.

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