

Measuring mechanical strains in live cells with force nanosensors

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Scientific context

Normal and pathological cells grow and function while being tightly associated with the diverse connective tissue components that form the extracellular matrix (ECM). In addition to sensing and reacting to chemical cues from ECM ligands, cells also react to internally generated or externally applied forces and transduce the stimuli into different appropriate mechanical and biochemical responses. This process of force transmission across cell membrane is widely known as mechanotransduction and is essential for cells to properly accomplish the adhesion and migration processes required for tissue building, remodeling and repair. The transmission of force is mediated by focal adhesions, which are highly organized, self-assembling nanostructures composed of plasma membrane receptors (integrins), the cytoskeleton, and various signalling adaptors. **The challenge of deciphering the molecular machinery of mechanotransduction is to develop devices that can measure the forces associated with specific receptors on the cell membrane** (i.e. integrins). Indeed, alterations of strength adhesion and cell adhesion mechanics play a role in the development of different pathologies. For example, it is established that cells crossing the endothelial barrier into blood vessels and form metastases in other tissues, reorganize their focal adhesion to adapt the speed of migration. It has therefore been proposed that adhesion strength may serve as a general marker of metastatic cells.^[1]

Objectives

The objective of this project is to propose a novel force nanosensor that will enable the mapping of the mechanical strain dynamics exerted by living cells in 2D environments. This nanosensor is based on two fluorescent emitters bound together with a molecular spring-like linker, that can be under tension when contractile force is applied by a cell. Force measurement will be achieved when molecular linker is stretched, and this length modification can be precisely assessed according to Förster Resonance Energy Transfer (FRET) between both emitters. FRET is now a standard technique, widely used in biophysics as a nanoscale ruler, for biosensing and binding measurements. We have previously demonstrated that it is possible to measure distance changes with ≈ 1 nm accuracy with FRET^[2,3]. FRET relies on a nonradiative energy transfer from excited donor molecules to acceptor molecules in their ground state. Due to their high photostability, we propose to use quantum dots (QDs) as donors for long time observations. Acceptors will be common organic dye molecules. The basic idea of the force nanosensor is then depicted in Fig. 1. First, due to the close proximity of donor and acceptor, high FRET signal is observable when the linker spring molecule is not under tension. Next, when the acceptor-labeled peptide ligand (e.g. RGD) is recognized by mechanotransduction associated membrane receptor (e.g. integrins), the linker is stretched,

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distancing donor from acceptor. In consequence, FRET gradually disappears owing to the intracellular traction force generated by actin cytoskeleton. Thus, FRET measurements can offer a true opportunity to realize, in real time, a map of forces generated by cells on their environment, and reveal mechanotransduction processes in cell biology or in biophysics.

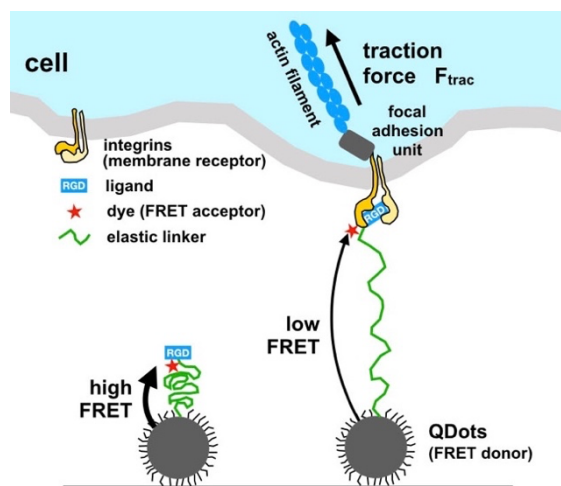


Figure 1: Schematic principal of force nanosensors. FRET occurs between quantum dot (QD) and dye molecule (left). When RGD tri-peptide is bound to integrin membrane receptor, traction force can take place in focal adhesion unit (right). This intracellular force can stretch the molecular spring linker. Consequently, FRET decreases.

In order to measure forces, nanosensors will first be calibrated using single molecule spectroscopy with optical tweezers and microfluidic device (6 to 12 months, RJ lab). Second, forces will be measured using biomimetic in vitro systems based on supported lipid bilayers (SLB). SLB offers a well-controlled biomimetic platform that allows for the reconstitution of focal adhesion and actin contractile machinery. The force exerted by polymerized actin and contractile myosin on integrin will be quantified using force nanosensors (1 year, MCDS lab). Finally, cytoskeleton contractile forces will be measured on live cells. Force nanosensors will be applied on 2D cells in culture to study the spatial-temporal dynamics of adhesion and contractility of migrating fibroblasts (1 year, MCDS lab).

Complementarity between supervisors and interdisciplinarity

MCDS is a cell biologist, while RF is a biophysicist. Both partners work in the field of biophysics and have an interdisciplinary background mixing physics, biology, chemistry and biochemistry. They had previously worked together to develop a nano-imaging technique called variable-angle TIRF, allowing to quantify cell binding energy. This new collaborative project is in line with the condition of interdisciplinarity ("3i"). In fact, both partners are associated with two different doctoral schools.

Resources

The project is already supported by an ANR funding (ANR-23-CE42-0009-01). QD-based force sensors are already available. The microscopy techniques such as single molecule fluorescence imaging, FRAP, FRET, TIRF and optical tweezers are already implemented in partner's labs. Both labs have as well access to cell culture unit. Protein purification will be implemented in MCDS lab.

Supervision experience

Both partners have extensive experience in doctoral supervision and hold Habilitation to Direct Research (HDR) degrees. RJ has supervised 11 doctoral theses. 8 have been defended and 3 are in progress. The MCDS supervised 4 PhDs, 2 of which were completed and 2 of which continue to progress. The partners have indicated their willingness to undertake the supervision of this project, with an allocation of 50% and 30% full-time equivalent (FTE) for MCDS and RJ, respectively.

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