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Characterization of cellular forces using FRET-based sensors

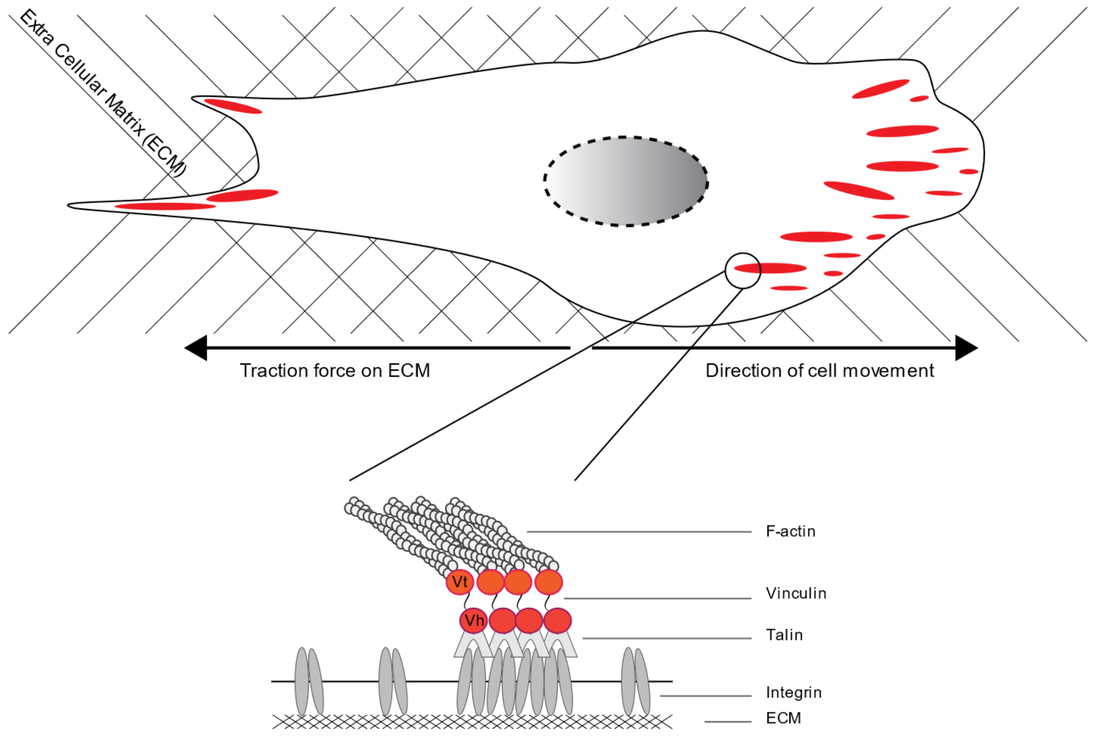
Quinten Coucke[a], Susana Rocha[a], Johan Hofkens[a]

[a]Laboratory for Photochemistry and Spectroscopy, Department of Chemistry, KU Leuven, Belgium

1. Why study cell-to-ECM forces?

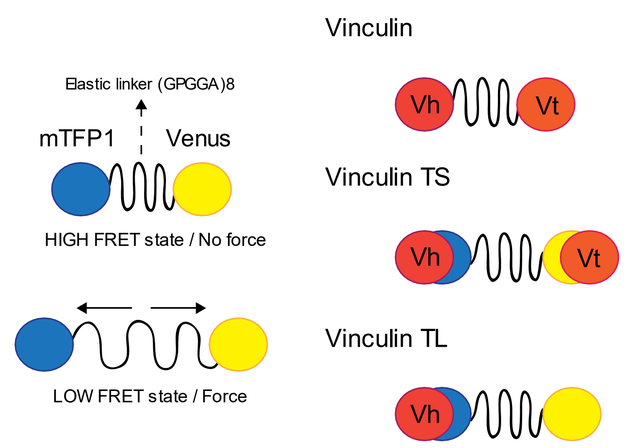
Mechanical forces play an undisputed elementary role in the interactions between cells and the surrounding extracellular matrix (**ECM**) [1]. Not only are these forces essential for the cells **migratory behavior**, they also influence **proliferation** (including tumor growth) and **differentiation** [2–4]. These forces are transferred across **focal adhesions** (FAs) which connect ECM and cell skeleton through patches of activated integrin proteins. Since the origin of exerted cellular forces lies in these FAs, they are the ideal starting point for characterization of **mechanotransduction pathways**.

Consequently, studying how the properties of the ECM affect the cellular forces is key in understanding how cells connect to their environment and alter their behavior appropriately. While the scientific field of cellular mechanosensation has been studied for years, recent developments in imaging techniques and force sensor development enable us to dig deeper.



2. How to visualize forces?

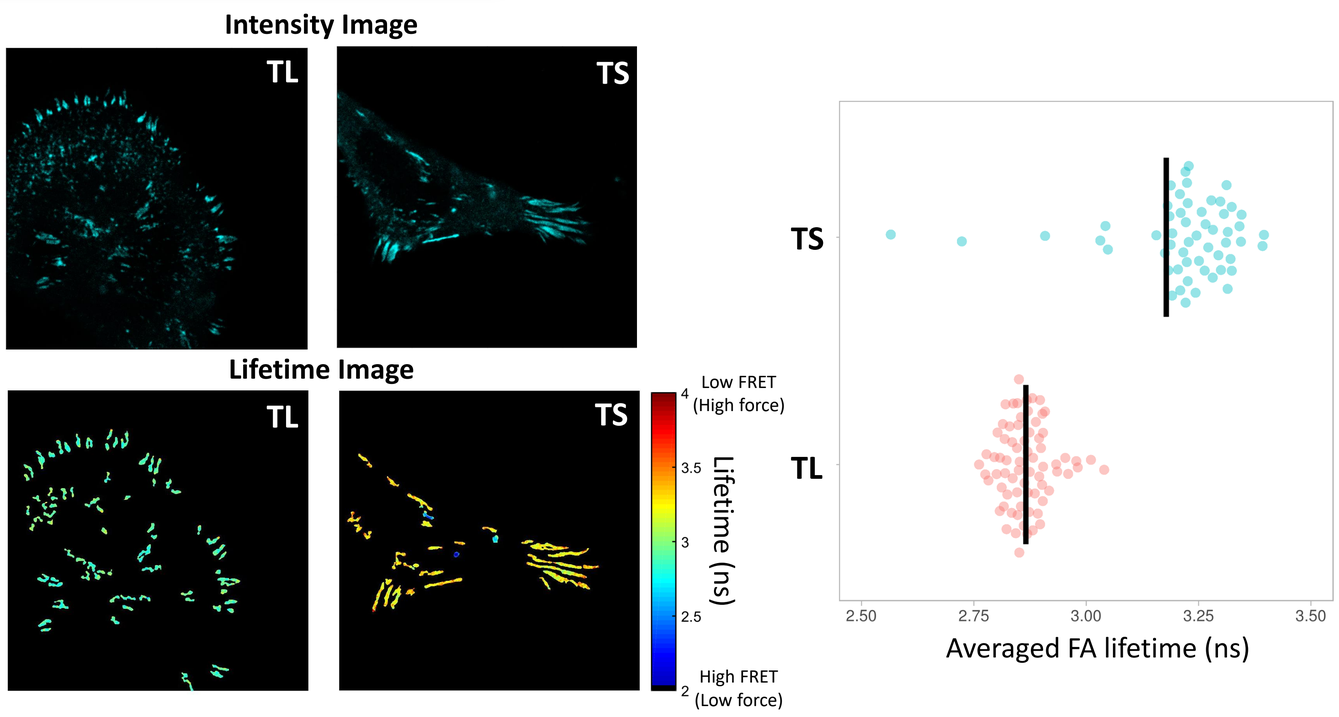
To investigate the forces applied in FAs, the well established **TSMod** force sensor is used. This sensor consists of two fluorescent proteins separated by an elastic linker. This elastic linker keeps both chromophores close to establish a high **Förster Resonance Energy Transfer (FRET)** state. This sensor is incorporated in the **Vinculin protein**, sandwiched by its head (Vh) and tail (Vt) domain. Only when a force is applies to the FA complex, the fluorescent proteins separate resulting in a lower FRET signal. Two types of sensor were used, the tension sensitive (**TS**) and a tail-less mutant (**TL**, positive control). FRET efficiencies are calculated by measuring accurate donor **lifetimes**, which lowers when FRET towards acceptor (Venus) quenches the donor (mTFP1) [1].



3. Preliminary results:

Using a custom built PIE-FLIM setup, HeLa cells transfected with VinTS and VinTL were imaged. By using the phasor method, accurate lifetimes were obtained from the image photon data without fitting bias [5].

A clear distinction has been found upon lifetime analysis of FAs containing either the TS or TL sensor. As expected, **the lifetime** from FAs in the VinTL (control) expressing cells (high FRET, high donor quenching) is clearly **lower** than in the tension sensitive VinTS, where force separates the chromophores to reduce FRET (low FRET, low donor quenching). **This proves system and sensor performance.**

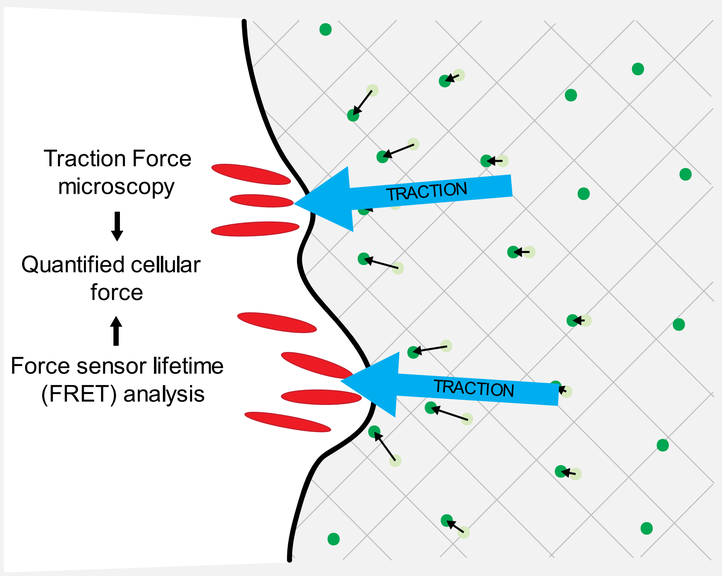


4. Future perspectives:

1. We aim to combine **traction force microscopy (TFM)** with **FRET-FLIM** to establish a link between the forces applied by FAs and matrix deformation. By comparing our biosensor derived force values to the established TFM values, we can continue research on more complex samples such as cancer cells, i.e. **Cancer Associated Fibroblasts (CAFs)**.

2. By using biomimetic-engineered Poly Ethylene Glycol hydrogel (**PEG**) with tunable stiffness (different degree of crosslinking), we can **simulate the effect of stiffer ECM** environments and study the cellular mechanical response.

3. Cancer cells have been shown to alter **vesicle trafficking** upon changed matrix rigidity. Hence, unraveling the link between cancer metastasis, cancer cell mechanical properties and their **deregulated clathrin-mediated endocytosis** is a future research field [4, 6]



References:

[1] C. Grashoff *et al.*, "Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics," *Nature*, vol. 466, no. 7303, pp. 263–266, Jul. 2010.
[2] F. Li *et al.*, "Vinculin Force Sensor Detects Tumor-Osteocyte Interactions," *Sci. Rep.*, no. September 2018, pp. 1–11, 2019.
[3] A. J. Engler, S. Sen, H. L. Sweeney, and D. E. Discher, "Matrix Elasticity Directs Stem Cell Lineage Specification," pp. 677–689, 2006.
[4] Y. Wang *et al.*, "Deregulation of AP-1 Proteins in Collagen Gel-induced Epithelial Cell Apoptosis Mediated by Low Substratum Rigidity * □," vol. 282, no. 1, pp. 752–763, 2007.
[5] M. A. Digman, V. R. Caiolfa, M. Zamai, and E. Gratton, "The phasor approach to fluorescence lifetime imaging analysis," *Biophys. J.*, vol. 94, no. 2, pp. L14–L16, 2008.
[6] J. L. Leight, M. A. Wozniak, S. Chen, M. L. Lynch, C. S. Chen, and Y. Wang, "Matrix rigidity regulates a switch between TGF- β 1 – induced apoptosis and epithelial – mesenchymal transition," vol. 23, 2012.

For additional information or questions please contact quinten.coucke@kuleuven.be

Lab website: