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► To cite this version:

Audrey Ntadambanya, Julien Pernier, Violaine David, Kimihiro Susumu, Igor L Medintz, et al.. Quantum-dot based nanosensors to study focal adhesion assembly and mechanosensing. IUTAM 2024 Theoretical and numerical developments in cellular mechanobiology, Jun 2024, Sevilla (Spain), Spain. hal-04671395

HAL Id: hal-04671395

<https://hal.science/hal-04671395v1>

Submitted on 14 Aug 2024

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QUANTUM-DOT BASED NANOSENSORS TO STUDY FOCAL ADHESION ASSEMBLY AND MECHANOSENSING

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INTRODUCTION

Transmission of force between the cell and its environment is essential for the cell to accomplish adhesion and migration processes required for tissue building and repair. Transmission of force across the cell membrane is facilitated by focal adhesions (FAs) [1]. The mechanosensors that make up FAs are proteins sensing cytoskeletal tension. Talin is largely responsible for the mechanosensing of FA [2]. Filamentous actin binds to Talin and with myosin exerts a pulling force. However, the mechanism of mechanosensing still requires in-depth observation and due to lack of tools, how cells sense the cytoskeleton force remains unclear. To improve our comprehension of how Talin interacts with the membrane and its stretching, we have developed intra and inter molecular biosensors. This FRET (Förster Resonance Energy Transfer) nanosensor is made of Quantum Dot (QD) donor, able to measure distances greater than those from conventional FRET, and a dye acceptor.

In this study, we could deduce how Talin assemble according to the membrane and measure separating distances thanks to FRET efficiencies obtained using fluorescence spectroscopy and microscopy. Changes of Talin conformation with cytoskeleton tension were observed.

MATERIALS AND METHODS

Three Talin variants produced in E.Coli bacteria strain were used: AB-Ta (actin binding Talin) variant, composed of F₂-F₃-R₁-R₂-R₃-R₁₃, ΔABD-Ta (delta actin-binding domain Talin) composed of F₂-F₃-R₁-R₂-R₃-R₄-R₅-R₆ R₇-R₈ and mini ΔABD-Ta (mini delta actin-binding domain Talin) composed of F₂-F₃-R₁-R₂-R₃. CdSe/CdS/CdZnS/ZnS core/multishell QDs were prepared and coated with CL4 capping ligands [6]. FRET nanoprobe were prepared by mixing QD (λ_{em}=600nm) at 20 nM and Talin in a 1:2 concentration ratio in KCl 100mM/HEPES 20 mM buffer with 0.06% BSA for 1h at RT. Talin-QD conjugate was added on GUVs. Later, MemBright640 acceptor (λ_{em}=664nm) diluted at 400 nM was added for integration in GUV lipidic bilayer. GUVs made by PVA gel-assisted vesicle formation method. They are made of egg PC with 20% mole cholesterol, 10% mole PIP₂ and 3.5 % mole DSPE-(PEG2000)-biotin; in chloroform.

Spectroscopy experiments were conducted on FluoroMax-4 where emission spectra of donor alone (I_D) and donor in the presence of the acceptor (I_{DA}) were acquired using 625V gain, 2 nm slit range, and excitation 405 nm. For the microscopy experiments, we used Spinning-Disc microscopy to register I_D and I_{DA} . FRET efficiencies (η) were calculated as following: $\eta = 1 - \frac{I_{DA}}{I_D}$ (Eq. 1), since η varies proportionally with the inverse sixth power of the distance r between the donor and acceptor molecules, it was calculated as: $r = \left(\frac{1}{\eta} - 1\right)^{1/6} \times R_0$ (Eq. 2), with R_0 being the Förster distance.

RESULTS

To quantify intermolecular distances, spectroscopy was employed to study the interaction between Talin and the membrane. This interaction results in the quenching of the donor's emission (Fig 1 A, blue curve). For three Talin variants: AB-Ta, ΔABD-Ta and mini ΔABD-T, corresponding η values of 0.65, 0.38, and 0.51, were found from Eq.1. Eq. 2 was used to determine the distance between the QD donor and acceptor for AB-Ta, ΔABD-Ta and mini ΔABD-Ta as 6.3 ± 0.8 , 7.3 ± 0.2 and 6.7 ± 0.2 nm respectively. This distance reflects the distance separating Talin from the membrane.

Single GUV measurements were made in microscopy with images in "donor channel. Mean I_D and I_{DA} were extracted before and after MemBright640 addition (Fig 1 B₁, B₂). We can see a difference in intensity between I_D and I_{DA} images. In fact, the intermolecular distance shortening makes the donor quenching more visible. The calculated η were of 0.35, 0.17 and 0.32 and the deduced intermolecular distances were $r_{AB-Ta}=7.3 \pm 1$ nm, $r_{\Delta ABD-Ta}=8.6 \pm 1$ nm and $r_{mini\Delta ABD-Ta}=7.5 \pm 1$ nm, respectively for AB-Ta, $\Delta ABD-Ta$ and mini $\Delta ABD-Ta$.

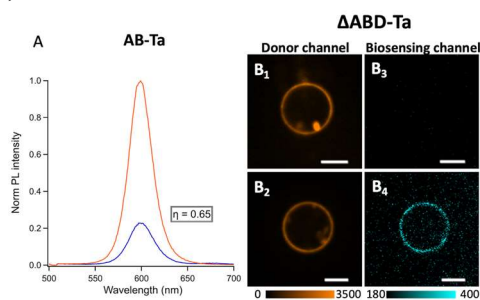


Figure 1. A) Talin-membrane interaction revealed by fluorescence spectroscopy. Normalized emission spectra of AB-Ta. B) Talin-membrane interaction revealed by fluorescence microscopy ($\Delta ABD-Ta$ images). In donor channel PL intensity of QD donor before (B₁) and after acceptor addition (B₂) and in biosensing channel for donor alone (B₃) and donor+acceptor (B₄) Scale bars: 5 μm .

To mimic the tensile force of cytoskeleton, Actin and Myosin II (Two major microfilament components) have been used on the AB-Ta. The mechanosensing process happens when actin filaments contract and Talin stretches upon Myosin addition. As a result, Talin changes conformation. A slight variation of η is observed. Here, for the first time to our knowledge, Talin mechanosensing mechanism, as a consequence of actin-myosin activity, has been revealed and quantified at the nanometric scale.

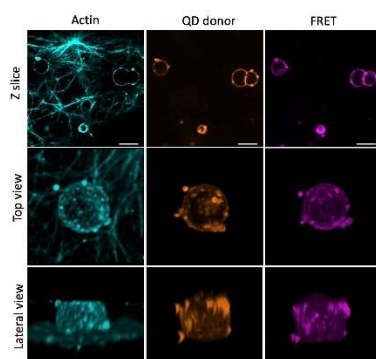


Figure 2. Talin mechanosensing. Talin AB-Ta stretched by actin-myosin: z slice of an area of few GUVs (top); 3D Visualization of top view (middle) and lateral view (bottom) on one GUV in Actin; QD nanosensor and FRET channels.

DISCUSSION AND CONCLUSIONS

Results of η for the Talin intermolecular distance explain the quenching decrease. Our findings were compared with theoretical models in Alphafold where we observed the orientation of domains interacting with the membrane relative to Talin. Talin interacts with various partners regulating the mechanical response of the adhesion. However, these interactions are complex and require further investigation. In our study, we could observe a distance shortening with actin-myosin. In conclusion, our QD-based FRET nanosensors have yielded quantitative insights into talin stretching dynamics. We will be able to elucidate emerging models for Talin assembly with different partners.

ACKNOWLEDGMENTS

This work was partially funded by the Interdisciplinary Object "OI Bioprobe". Authors thank the Light Microscopy facility of Imagerie-Gif and Integrative Bioinformatics platform (BIOI2) @I2BC.

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