

Quantitative multiscale imaging and analysis of membrane protein and actin cortex co-organization

Huong-Tra Ngo¹, Aparajita Dasgupta¹, Deryl Tschoerner¹, Nicolas Touret², Bruno da Rocha-Azevedo¹, and Khuloud Jaqaman¹

Short Abstract — Membrane protein (MP) organization and signaling depends on the actin cortex (AC). Evidence for this has been largely obtained through actin perturbation experiments, which preclude the quantification of AC dynamics. To derive explicit relationships between the dynamic organization of MPs and the AC in live unperturbed cells, we developed a novel imaging and computational analysis framework that integrates single-molecule imaging and fluorescent speckle microscopy. Using machine learning approaches, we identified a novel biphasic relationship between MP mobility and AC density. Our framework will aid with elucidating the general principles of how the AC regulates the dynamic organization of MPs and with quantitatively predicting MP behavior based on AC activities.

Keywords — single-molecule analysis, quantitative fluorescent speckle microscopy, membrane organization, actin cortex.

I. BACKGROUND

CELL surface membrane protein (MP) organization and signaling are influenced greatly by the actin cortex (AC), a subset of the cytoskeleton very close to the plasma membrane [1-3]. While the AC is a dynamic structure, most studies to date investigating how the AC influences membrane protein organization have not been able to access cortical actin dynamics, because the AC is too dense to resolve with conventional light microscopy. Thus, most studies have perturbed the actin cytoskeleton to infer its role on MP organization, which precludes any quantification of actin dynamics in the absence of perturbation and raises concerns of cytotoxicity [4, 5].

II. METHOD

To overcome these challenges, we developed a novel multicolor imaging and computational analysis framework to simultaneously observe MP and AC dynamic organization in live single cells. Specifically, we visualized MPs and extracted their dynamic organization using single molecule

imaging (SMI) and single particle tracking [6]. At the same time, we captured the AC organization and dynamics using quantitative fluorescent speckle microscopy (FSM) [7]. SMI and FSM are two distinct imaging modalities that we optimized to follow MP and AC dynamics. We then built a computational pipeline to put temporally and spatially relevant information obtained from SMI and FSM together. Then, we derived quantitative relationships between the MP behavior and the AC dynamics using machine learning.

III. RESULTS

Application of this pipeline to the cell surface receptor CD36, and chimeric control transmembrane proteins with different abilities to bind the AC, revealed a novel biphasic relationship between MP diffusion and AC density. At lower actin densities, actin promoted CD36 movement and had little effect on CD36 clustering. At higher actin densities, actin inhibited CD36 movement and promoted CD36 clustering. While both freely diffusing and confined MPs exhibited a dependence on AC dynamics, confined MPs showed a stronger dependence. Our results highlight the unique information that can be learned through direct simultaneous imaging and analysis of the dynamics of both the AC and cell surface MPs. This work provides a general framework that can be used to quantitatively study the relationship between the dynamic organization of MPs and the AC.

REFERENCES

- [1] Kalappurakkal et al. (2019) Integrin Mechano-chemical Signaling Generates Plasma Membrane Nanodomains that Promote Cell Spreading. *Cell*.
- [2] Githaka et al. (2016) Ligand-induced growth and compaction of CD36 nanoclusters enriched in Fyn induces Fyn signaling. *J Cell Sci*.
- [3] Ketchum C, Miller H, Song W, and Upadhyaya A. (2014) Ligand mobility regulates B cell receptor clustering and signaling activation. *Biophys J*.
- [4] Calebiro D, Rieken F, Wagner J, and Lohse MJ (2012) Single-molecule analysis of fluorescently labeled G-protein-coupled receptors reveals complexes with distinct dynamics and organization. *PNAS*.
- [5] Jaqaman et al. (2011) Cytoskeletal control of CD36 diffusion promotes its receptor and signaling function. *Cell*.
- [6] Jaqaman et al. (2008) Robust single-particle tracking in live-cell time-lapse sequences. *Nat Methods*.
- [7] Ponti A et al. (2003) Computational analysis of F-actin turnover in cortical actin meshworks using fluorescent speckle microscopy. *Biophys J*.

¹ Department of Biophysics, The University of Texas Southwestern Medical Center, Dallas, TX, USA.

² Department of Biochemistry, University of Alberta, Edmonton, AB, Canada.