HIGH SPATIAL AND TEMPORAL RESOLUTION OBSERVATION OF VINCULIN RECRUITMENT ON TALIN DIMERS

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ABSTRACT: We present the results from a large survey of fundamental mechano-sensing and mechano-transducing molecules. The microscopy achievements of our work center on determining the precise timing when the activation protein vinculin is recruited onto talin during its periodic stretch cycle. Together they form an independent and robust force sensor and transducer in focal adhesions with the cycle providing a regulation mechanism with a time constant. Our work demonstrated that while the recruitment is at its core a stochastic process, the probability of recruitment is highly regulated and in consequence a collaborative process [1] that is fairly robust.

We were able achieve stable labeling ratios of vinculins (1 out of 6) available in the system without disrupting its functionality and were thereby able to determine sensible lower bounds for the frequency with which vinculins bound to single talin dimers. Two channel single molecule tracking was deployed to monitor the overall talin stretch cycle[2] and a small dye (Atto655) linked with a new covalently bound TMP tag that deactivates the dye while not yet bound [3] allowed for a near background-free observation of the vinculin recruitment processes in a far red dSTROM channel. Each recruitment process translates into a bright flare that enables timing with 15ms accuracy as well as conventional localization microscopy accuracy.

FIGURES AND REFERENCES

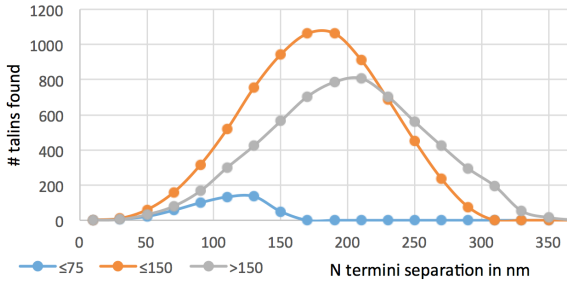

Figure 1: Talin in leading edge adhesions is generally highly stretched both between the integrin layer and the actin filament as well within the integrin layer. The former was well known[4], the latter is novel to this project. Here the integrin termini separation density we observed for 3 ranges of talin elongation (≤75nm,>75nm & ≤150nm,>150nm).

[1] X. Hu et al, "Cooperative Vinculin Binding to Talin Mapped by Time-Resolved Super Resolution Microscopy", Nano Letters 4062-4068 (2016)
[2]F. Margadant et al, "Mechanotransduction In Vivo by Repeated Talin Stretch-Relaxation Events Depends upon Vinculin", *PLOS Biology* 12 (2011)
[3] Z. Chen et al, "Second-generation covalent TMP-tag for live cell imaging", *J Am Chem Soc,* 13692–13699 (2012)
[4]P. Kanchanawong et al, "Nanoscale Architecutre of Integrin-based Adhesions", *Nature* 580-584 (2010)