

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

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ABSTRACT:

We present the results of an ongoing 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 hence a robust collaborative process [1].

We were able to achieve a stable 16% labeling ratios of vinculins 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 dSTORM channel. Each recruitment process translates into a bright flare that enables timing with better than 15ms jitter.

FIGURES AND REFERENCES

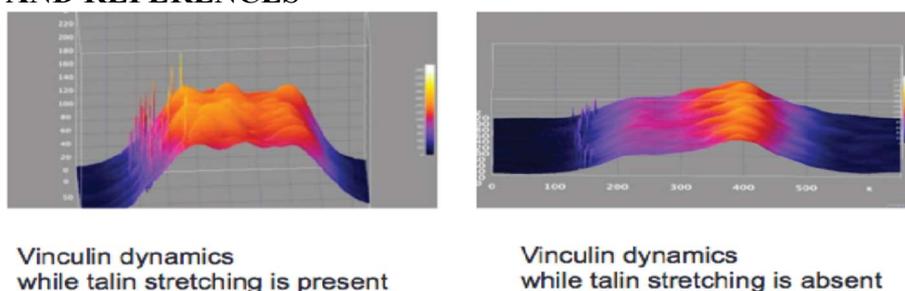


Figure 1: Talin in leading edge adhesions is generally highly stretched both between the integrin layer and the actin [4]. We illuminate the necessity of the cyclic stretch for the efficient vinculin binding.

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