LOW EXPRESSION PALM-LIKE OBSERVATION OF DUALLY TAGGED TALIN MOLECULES

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In order to gain a more thorough insight of the stretch-release process of Talin in focal adhesions during spreading we resorted to optical observation of tags at the ends of the molecule due to its relative low-damaging nature to the functionality of the adhesions.

Talin was mutated to carry a fluorescent protein of a different absorption/emission peak at each end. The design of the experiment was that if the expression could be regulated low enough, or the fluorescent proteins can be activated selectively enough, the location of each fluorophore tag can be localized by means of centroid methods and hence deliver an accuracy of the stretching state in the range of 20nm.

While the photoactivation proved to be feasible and working as predicted, recording a two color PALM image required a prohibitively long time given the dynamics of the underlying processes. Also, as the activation is purely stochastic, the fluorescent tags on a single Talin molecule are unlikely to be activated in the same frame of observation.

At sufficiently low expression rates, high activation level and relatively low bleaching rates, a good localization as well as the evolution of the stretch over several seconds was observed in a large fraction of the tagged molecules.

We present the experimental difficulties we encountered during the design of the experiment and some of the findings from the described observations.