Using Induced Aggregation of PA-mCherry to Monitor Changes in Local Protein Concentration

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Photo-activatable and photo-switchable proteins used in Photoactivated Localization Microscopy (PALM) can show a propensity to aggregate. Also, some photo-activatable proteins that are monomeric by themselves can aggregate when fused to a protein that also has weak self-aggregation properties [1]. We have used this property of PA-mCherry to probe changes in the local concentration of a crucial signaling protein involved in T cell activation.

We used PALM imaging and bivariate pair correlation statistics to investigate the organization of proteins found in T cell signaling microclusters [2]. PA-mCherry fused to the transmembrane protein, Linker for Activation in T cells (LAT) segregated from LAT conjugated to Dronpa. This segregation required the self-association of PA-mCherry as LAT-PA-GFP and LAT-Dronpa followed a random model of mixing. We then sought to use this induced aggregation to obtain information about changes in the local concentration of LAT and membrane domain structure during T cell activation [3].

The segregation of LAT-PA-mCherry from LAT-Dronpa develops after several minutes of activation, suggesting that the local concentration of LAT increased during activation perhaps due to confinement in ordered membrane domains. We determined that the segregation of LAT-PA-mCherry from Dronpa-LAT was impaired by treatments that increase membrane fluidity such as Lidocaine and elevated temperature. We believe that this shows that LAT is concentrated into ordered lipid domains during the spreading of T cells, increasing the local concentration of LAT to the point where weak interactions of LAT combined with weak interactions from PA-mCherry become sufficient to drive aggregation of the fusion protein. Thus, the tendency of PA-mCherry to interact with itself can be used to provide information about the local protein concentration of its fusion partner.

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