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Activation Dependent Organization of T Cell Membranes MARTIN FORSTNER, Department of Physics, Syracuse University, BJOERN LILLEMEIER, MARK DAVIS, Howard Hughes Medical Institute and Department of Microbiology and Immunology Stanford University School of Medicine, JAY GROVES, Howard Hughes Medical Institute and Department of Chemistry University of California — We investigate the role of lipid anchor motifs in the micro-organization of T-cell plasma membranes. To that end we generated a combinatorial library of protein constructs by fusing different lipid-modification sites of lipid anchored proteins with one of two fluorescent proteins (EGFP and Cherry). Two of these constructs that encode either for myristoylation, palmitoylation, geranylation or glycosylphosphatidylinositol (GPI) elaboration were co-expressed and dual color fluorescence cross-correlation spectroscopy (FCCS) was used to exploit comovement as a signature of co-localization. We find that in living T cells most anchors only co-localize with themselves, while different anchors move independently from each other. This suggests that a variety of domains with different chemical compositions exist in the plasma membrane and that the lipid anchor structure plays a key role in domain-specific recruitment of proteins. Furthermore, the degree of aggregation is found to depend on the activation state of the T cells. Cholesterol depletion and actin-drug experiments indicate that both are involved in the dynamic organization of the T cell plasma membrane.

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