Title
Membrane-protein dynamics: Spatio-temporal interactions by fluorescence correlation spectroscopy (FCS)

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Abstract
Giant unilamellar vesicles (GUVs) are useful as model membrane systems to study protein-membrane (P-M) interactions. Processing sequential GUVs fluorescence images only detects slower dynamics of P-M interactions. We have developed a new FCS technique, termed scanning FCS (SFCS) that performs multiple FCS measurements simultaneously by rapidly directing the excitation laser beam in a uniform (circular) scan across the bilayer of the GUVs in a repetitive fashion. SFCS measurements on GUVs allow for detection of spatial-temporal interactions between the P-M based on the diffusion rate of the protein. In this study, GUVs were assembled from rat kidney brushborder membrane fragments, which included the integral membrane proteins. To test membrane proteins incorporation into the GUVs, antibodies against NaPi II cotransporter were labeled with Alexa 488. Fluorescence images of the GUVs in the presence of the antibody showed no apparent binding. With SFCS, the binding of the antibody to the GUVs was detected directly from the analysis of diffusion rates of the fluorescent antibody. The diffusion coefficient of the antibody bound to NaPi II in the GUVs was 200 fold smaller than that in solution. SFCS provides a simple, quantitative yet highly sensitive method to study P-M interactions.