Substrates for Study of 2-D lipid Bilayer Diffusion using Two Foci Fluorescence Lifetime Correlation Spectroscopy

Tia E. Keyes

National Biophotonics and Imaging Platform Ireland, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland

Abstract

Supported lipid bilayers are widely applied as models of the cell membrane for study of key issues at the cell interface such as protein or lipid diffusion, lipid-protein interaction and transmembrane protein-protein interaction. Although the preparation of lipid bilayer on glass is straight forward, this system suffers from several key drawbacks. Most notably, non-specific interactions between the substrate and the bilayer components (lipid and/or protein) can lead to strong frictional drag which can modify the lateral diffusion, aggregation of lipid or protein, as well as the activity of an embedded protein. In this contribution we overview some of our strategies for building lipid bilayer films for study of both lipid diffusion and embedded protein diffusion using FLCS fluorescence lifetime correlation spectroscopy and FRAP (fluorescence recovery after photobleaching).

The fabrication of a range of substrates; aminosilanized glass, oxidized hydrophilic polymer and metal an polymer micropore arrays is described along with assembly of lipid bilayer films onto these substrates. The diffusion rates of the assembled lipid are studied using a 2D diffusion model to assess the diffusion coefficient. We compare the lipid diffusion rates across the substrates with giant unilamelar vesicles using fluorescently labeled lipid and FLCS. To improve precision 2- foci FLCS can be implemented. We demonstrate that using this approach it is possible to estimate the coefficient of diffusion with better accuracy and with high reproducibility. The latter feature is particularly important because it then allows direct comparison between different platforms. Finally the use of the 2- foci FLCS technique to study the influence of PEG polymer on lipid lateral diffusion in a supported lipid bilayer is described.