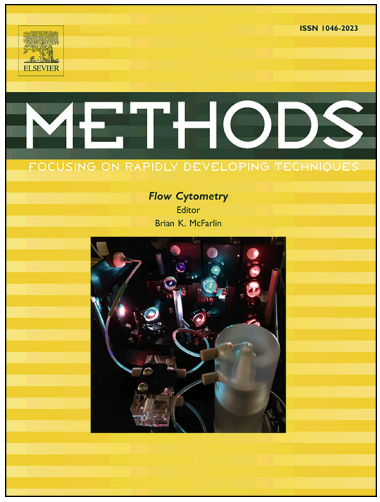

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Single molecule fluorescence for membrane proteins

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Abstract
The cell membrane is a complex milieu of lipids and proteins. In order to understand the behaviour of individual molecules is it often desirable to examine them as purified components in *in vitro* systems. Here, we detail the creation and use of droplet interface bilayers (DIBs) which, when coupled to TIRF microscopy, can reveal spatiotemporal and kinetic information for individual membrane proteins. A number of steps are required including modification of the protein sequence to enable the incorporation of appropriate fluorescent labels, expression and purification of the membrane protein and subsequent labelling. Following creation of DIBs, proteins are spontaneously incorporated into the membrane where they can be imaged via conventional single molecule TIRF approaches. Using this strategy, in conjunction with step-wise photobleaching, FRET and / or single particle tracking, a host of parameters can be determined such as oligomerisation state and dynamic information. We discuss advantages and limitations of this system and offer guidance for successful implementation of these approaches.

Keywords

Membrane protein, single molecule fluorescence, droplet interface bilayer, FRET, photobleaching, TIRF.

Introduction (~600 words)

The cell membrane can be envisaged as a crowded lipid bilayer in which protein-protein and protein-lipid interactions are responsible for regulating membrane function including maintenance of cell structure, transport of molecules in and out of the cell and signal transduction. Historically, most biological analyses have been performed on populations using ensemble averages. However, cells represent heterogeneous environments where fluctuations in protein activity may be inherent to biological function. This is especially true in the membrane where the "solvent", i.e. the lipids in the membrane, is itself heterogeneous and the presence of localised populations of proteins and/or microdomains could have significant impacts on protein function [1].

Single molecule techniques provide an incredibly powerful approach with which to probe membrane protein localisation, interactions and dynamics [2]. The strength of these approaches lies in the visualisation and analysis of individual molecules which can reveal information not accessible via ensemble techniques [3]. For example, single molecule analysis removes ensemble averaging and can therefore reveal, and be used to analyse, subpopulations of molecules. In addition, kinetic analysis can be performed on unsynchronised populations, enabling insight into the nature of dynamic equilibria. The ability to analyse heterogeneous populations can provide insights that would be masked in ensemble techniques [4], affording the opportunity to reveal spatio-temporal dynamics, rare-states, transient intermediates and population stoichiometries.

Single molecule fluorescence techniques have been applied to a range of both soluble and membrane proteins. In order to undertake these approaches, a number of technical challenges must be overcome. Firstly, unless a direct activity can be measured e,g, ion flux imaging the membrane protein of interest