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Opinion Article

Molecular Tracking and Localization of Bio Orthogonally Labeled Plasma Membrane Proteins in Live Cells

Logan Brennan*

Department of Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, USA

Introduction

Genetic Code Expansion (GCE) in combination with orthogonal labelling provides an elegant method for labelling proteins in live mammalian cells with organic fluorescent dyes (Fl-dyes). A Non-Canonical Amino Acid (ncAA) with a functional group is incorporated into the protein sequence in response to an in-frame amber stop codon (TAG) via an orthogonal tRNA/tRNA-synthetase pair (reviewed in Labeling is then carried out by a rapid and specific bio orthogonal reaction between the functional group and a Fl-dye in GCE-based labelling. As a result, the protein of interest is fluorescently labelled on a specific residue, eliminating the requirement for protein tag fusions (i.e. fluorescent proteins or self-labelling proteins) in live cell applications.

For quantitative, high-end live cell imaging, direct labelling of proteins in live cells with Fl-dyes has various advantages. First, because fluorescent dyes are brighter and more photostable than fluorescent proteins, cells can be observed for longer periods of time and with lower laser intensity. Second, the label itself is tiny (Fl-dye, 0.5 nm; GFP, 4.2 nm; antibodies >10 nm; quantum dots 2–60 nm), allowing for greater precision in localization studies and a better depiction of the protein's physiological features. Third, because labelling does not require amplification, the quantity of molecules can be counted. Fourth, the Fl-dye is applied at the end of the reaction, allowing for greater freedom in tailoring the Fl-dye to specific applications.

Multiple dyes are used to label protein populations at the same time. Indeed, live cell imaging and the super resolution techniques Structured Illumination Microscopy (SIM), Stimulated Emission Depletion (STED), and Single Molecule Localization Microscopy have all proven GCE paired with bio orthogonal labelling in recent years.

We wanted to look at the dynamics and geographical distribution of plasma membrane (PM) proteins that have been labelled with GCE using bio orthogonal chemistry. To do this, we used extracellular bio orthogonal labelling to calibrate settings for performing live-cell single-molecule applications like as Single Particle Tracking (SPT) and live-SMLM.

The diffusion coefficients of bio orthogonally tagged epidermal growth factor receptor (EGFR) and the archetypal Shaker B voltage dependent potassium (Kv) channel were then measured using our optimized SPT assay. When compared to EGFR-GFP, bio orthogonally tagged EGFR yielded much more tracks and longer trajectories. Furthermore, while SPT was successful on bio orthogonally labelled Shaker B, no tracks were created with mCherry-Shaker B. In the presence of the actin polymerization inhibitor Latrunculin A, EGFR diffusion became more limited upon ligand activation, while Shaker B diffusion became less confined (LatA). As a result, GCE and bio orthogonal chemical labelling of proteins is consistent with, and outperforms, Fl-protein labelling for particle tracking.

Using our calibrated technique, SMLM investigations with bio orthogonally labelled EGFR and Shaker B were successfully performed in fixed and living cells, allowing quantification of the dynamic distribution of PM proteins throughout time. Finally, we performed live-SMLM and SPT in a single cell employing simultaneous labelling with two distinct Fl-dyes and assessed the density and dynamics of EGFR in subcellular areas at Nano scale precision. We concluded that GCE-based bio orthogonal protein labelling is a better, more versatile technique for quantitative live cell single molecule applications based on these findings.

Plasma Membrane

Heterogeneities in the distributions of its constituents have been documented since technology enabled the characterization of eukaryotic plasma membranes. Various models defining plasma membrane architecture, such as lipid shells, picket-and-fences, lipid rafts, or protein islands, have been proposed over the years, as discussed in several publications and reviews. Rather of stressing one model, we present a quick summary of current models in this study, emphasizing how current experimental findings in one way or another does not support the existence of a single overarching paradigm. Instead, we emphasis the wide range of membrane features and components, as well as their influences and consequences. We believe that highlighting such controversial discoveries will stimulate unbiased research on

***Corresponding Author:** Brennan L, Department of Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, USA, E-mail: Loganbrennan@case.edu

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plasma membrane organization and functionality, leading to a better understanding of this essential cellular structure.

Membranes are an important part of cell life. Aside from compartmentalizing and safeguarding cells, their importance as organizing centers for functions like metabolism and signaling is becoming increasingly apparent. In reality, membranes are involved in the bulk of biological operations. Membranes serve as useful docks for proteins' proper localization, which is critical for their function. Importantly, in humans, mislocalization of membrane proteins causes loss of function and, in some cases, illnesses. However, the mere presence of proteins at a membrane is frequently insufficient for their function. The effectiveness of biological processes is frequently influenced by the nanoscopic localization, oligomerization, and/or clustering of membrane proteins. The nanoscale architecture and function of these molecules are influenced by membranes, the lipid environment, and membrane characteristics in general. It is, therefore, important to understand molecular details of membrane structure and mechanisms responsible for its dynamics organization.

With a special focus on the plasma membrane of higher eukaryotes, we cover membrane characteristics, models of membrane organization, and relevant tools for studies of membrane organization and dynamics (mammals). Our specific goal is to re-emphasize and discuss the relevance of currently overlooked or underappreciated biophysical concepts in dynamic membrane formation. We strive to present a complete account of membrane complexity as well as recommendations for interpreting membrane-related occurrences outside of the confines of a particular theory. We feel there is no general model of plasma membrane dynamic lateral organization, as the reader will see. The last section will go through these more general difficulties. Let's start with the fundamental structure of membranes.