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Elucidating GPCR Functional Dependence on Plasma Membrane Composition using Giant Unilamellar Protein-Vesicles

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Using an agarose hydration technique for protein incorporation into vesicular bilayers, we elucidate the effects of membrane composition and ordering on G Protein Coupled Receptors (GPRCs). We successfully incorporate GPCRs into model membranes in the form of giant unilamellar protein-vesicles (GUPs). Using this completely *in vitro* platform we observe that the functional rate of the human serotonin receptor, GPCR 5-HT_{1A}, and the A_2a Adenosine GPCR is dependent on membrane composition and ordering. We use BODIPY-GTPγS as our fluorescent marker to track the irreversible exchange between GDP and GTP on G proteins over time in GUPs composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), brain sphingomyelin (BSM), and cholesterol (Chol) as well as synthetic lamellar phase diblock copolymer. Furthermore, using this approach we demonstrate that the incorporated receptors display a biased orientation with the N-terminus located on the exterior (extracellular) and the C-terminus on the interior (cytosolic).

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Controlling GPCR Rhodopsin Function by Small, Physiologically Relevant Changes in Bilayer Hydrophobic Thickness

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It was established previously that the G protein-coupled membrane receptor rhodopsin has transmembrane helices which match a hydrophobic bilayer thickness of 27 ± 1 Å. Here we demonstrate that small changes of bilayer thickness of ± 2 Å about that match point translate in the considerable changes of rhodopsin activation measured as the metarhodopsin I (MI)/metarhodopsin II (MII) equilibrium. We observed a biphasic behavior of the MI/MII equilibrium, with a sharp decline towards MI from 25-27 Å followed by a rapid increase of MII from 27-29 Å. Results are qualitatively identical for thickness changes induced by mixing of 16:0-16:1 PC and 18:0-18:1 PC, or 16:1-16:1 PC and 18:1-18:1 PC, or addition of 0-30 mol% cholesterol to 16:0-16:1 PC. The biphasic behavior was observed regardless of lipids used to alter bilayer hydrophobic thickness suggesting a relationship between small changes in hydrophobic thickness and rhodopsin function. It strongly favors an explanation based on a change of elastic stresses in lipid bilayers upon the transition from negative curvature in lipid monolayers near the protein below 27 Å hydrophobic thickness to positive monolayer curvature above the match point. A continuum elastic model of the membrane, including the effect of lipid monolayer curvature near the protein, predicts membrane mediated clustering of rhodopsin and stabilization of the MI photointermediate at the matching point. Small, physiologically relevant changes in cholesterol content of bilayers with a thickness in the physiologically relevant range do drastically down- or up regulate the amount of MII which is the state that activates G protein.

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Characterization of CEACAM1 and Lipid Raft Nanoclustering, Association and Structure by dSTORM and homo-FRET Imaging

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The advent of super-resolution microscopy has revealed that most membrane proteins cluster at nanoscale lengths. Clustering and compartmentalization of membrane proteins is partially caused by lipid rafts that exist at small spatial (10-100 nm) and temporal scales, that are not perceptible using classic fluorescence microscopy (1,2). Carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs) are cell surface glycoproteins involved in homo- and hetero-philic intercellular interactions that control cellular growth, differentiation, tumourigenesis, inflammation and infection. Here, we investigate the association of lipid rafts with CEACAMs using direct stochastic optical reconstruction microscopy (dSTORM). We have previously demonstrated using live cell TIRF-homoFRET-dSTORM that CEACAM1 can exist as monomers and oligomers at the cell membrane, with the monomers being predominantly organized in clustered regions and the dimers in more diffuse regions. We super-resolved CEACAM1 and lipid rafts using an anti-GFP labeled nanobody and a labeled Cholera Toxin subunit B, respectively. This approach allows us to obtain super-resolved images from which coordinates with a localization precision of 20 nm can be derived. Subsequent clustering analyses allowed us to quantify the degree of association between lipid rafts and CEACAM1. Moreover, correlating homo-FRET CEACAM data with these super-resolved maps allowed us to elucidate CEACAM monomer/oligomer distribution and association with lipid rafts. Our results show that both CEACAM1 and lipid rafts exhibit nano and micro-sized clusters of various shapes and partial spatial colocalization. This reinforces the body of work suggesting that lipid rafts are necessary to support cell adhesion, pathogen binding and intercellular signalling.

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Structural Determinants of Raft Partitioning for Single-Pass Transmembrane Proteins

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Transmembrane proteins (TMPs) comprise ~30% of the mammalian proteome and mediate nearly all functions of the cell. The subcellular localization, trafficking, signalling, and enzymatic activity of many TMPs is regulated by their association with lipid-driven lateral membrane domains known as lipid rafts. However, the structural determinants of TMP partitioning to raft domains remain almost unknown. We hypothesized that structural features of the transmembrane domain (TMD) of single-pass TMPs would guide raft partitioning and raft-dependent sub-cellular trafficking. To explore TMD-dependent raft partitioning, we isolated plasma membranes as Giant Plasma Membrane Vesicles (GPMVs), a model system that allows direct observation of protein partitioning between raft/non-raft phases by fluorescence microscopy. We generated 96 different fluorescent mutants of a model TMP and quantified their partitioning as a function of TMD features, namely hydrophobic length and accessible surface area. Raft partitioning was related to TMD length, with longer TMDs imparting greater raft association, confirming previously untested predictions. Investigation of 74 mutants with constant TMD length revealed that raft partitioning was also inversely related to the TMD accessible surface area, with larger surface areas preferring the non-raft phase. To explain these results, we present a simple physical model wherein raft partitioning is driven by differences in interfacial energy between the TMD and its surrounding lipid matrix in raft and non-raft phases. This model is in quantitative agreement with experimental observations and provides first predictions of protein-lipid surface tensions differences between coexisting membrane phases. The model could successfully connect predicted raft partitioning with subcellular localization of single-pass TMPs in *Eukaryota*. These results point the way to a general rule for raft partitioning of TMPs and underline the central role of membrane domains in cellular traffic.