cryo-transmission electron microscopy. We visualised the intermediate initial bilayer contacts and stalk formation, followed by pore development, pore evolution into 2D hexagonally packed lattices, and finally creation of 3D bicontinuous cubic structures (3).
In a biological context, the experimental corroboration of transitional lipid self-assembly structures furthers the understanding of organelle morphogenesis and maturation.
The ability to manipulate intermediate structures in nanoparticulate dispersions of self-assembled structures may provide a unique system for encapsulation and controlled release of bioactives. The capability to control intermediate transformations may also permit the development of flexible growth media for applications such as in-cubo integral membrane protein crystallization or liquid crystal templating of nanostructured materials.
1.Drummond, C.J.; et al., Current Opinion in Colloid & Interface Science 1999, 4, (6), 449-456.
2. Conn, C.E.; et al., Physical Review Letters, 2006, 96, (10), 108102
3. Mulet X., et al., ACS Nano, 2009, 3 (9), 2789-2797

1499-Pos
Native Pulmonary Surfactant Membranes in Mice Show Coexistence of Two Different Phases in Bilayers and Monolayers: When the Lipid Composition can Predict the Structural Phase Segregations
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Pulmonary surfactant is a surface active material composed both of lipids (aprox. 90% by weight) and proteins (aprox. 10%) produced by type II pneumocyte cells in the alveoli. This tension-active material forms a unique air-liquid interface at the alveolar cell surface that reduces surface tension close to 0 mN/m and maintains lung volumes and alveolar homeostasis at the end of the expiration. There are four pulmonary surfactant proteins (SP-A, -B, -C and -D). SP-A and -D have an important role in the immunological response against pathogens. The particular lipid composition of the lung surfactant suggests that surfactant mono- and bi-layer-based structures could exhibit lateral phase segregation at physiological temperatures. This work shows that in native pulmonary surfactant membranes a close lipid compositional study is crucial to understand the structure and biophysical function of these complex mixtures. Observing Giant Unilamellar Vesicles under conventional and two-photon excitation microscopy allow us to characterize and quantify the coexistence of two fluid-like phases in the wild-type (wt) native pulmonary surfactant membranes and a gel/fluid-like segregation pattern in the Knocked-out protein D (KOD) membranes. The atomic force microscopy studies of supported Langmuir-Blodgett bilayers and monolayers at different surface pressure show the same phase pattern before the collapse surface pressure of native pulmonary surfactant (~40mN/m). Above this surface pressure different protruded structures can be observed arising from the more fluid phases. A closer look at the lipid composition reveals a higher content of saturated phospholipid species in the KOD native pulmonary surfactant membranes. This last finding explain the coexistence phenomena observed and allow us to conclude that the pulmonary surfactant segregation pattern could be predicted by an accurate lipid compositional study.

Membrane Receptors & Signal Transduction I

1500-Pos

WITHDRAWN.

1501-Pos
Quantitative GPCR Assay Using Time-Resolved Fret
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Quantitative study of membrane proteins presents considerable technical challenges. Here, we report a time-resolved fluorescence resonance energy transfer (TR-FRET) assay to quantify functional Chemokine (C-C motif) Receptor 5 (CCR5). CCR5 is a seven-transmembrane helical G protein-coupled receptor (GPCR) expressed primarily on immune cells. It is of significant interest due to its role as the co-receptor of R5-tropic HIV-1. The TR-FRET assay exploits energy transfer between a long-lived europium cryptate donor fluorophore and an appropriate acceptor. We have developed a homogeneous sandwich-type immunoassay with labeled antibodies against a conformationally-sensitive epitope on the extracellular domain of CCR5 and an engineered C-terminal 1D4-mAb epitope. The assay yields a quantitative FRET signal corresponding to the total amount receptor. To quantitate ''functional'' receptor, a labeled anti-HA (hemagglutinin) antibody against an engineered N-terminal HA epitope is used in conjunction with labeled MIP-1a, a chemokine ligand for CCR5. We are also interested in quantifying the degree of chemical labeling of unnatural amino acids incorporated into expressed GPCRs by amber codon suppression technology. The Staudinger-Bertozzi ligation links a commercially-available FLAG-phosphine reagent to p-L-azidophenylalanine. A europium-labeled anti-FLAG antibody can then be used in another version of the sandwich assay. The resulting FRET signals are directly proportional to amount of total, functional, and labeled receptor, but must be calibrated precisely to extract an absolute concentration. Calibration is accomplished by measuring binding of a fluorescent derivative of a small-molecule CCR5 antagonist. The assay is highly specific due to the long lifetime of the europium donor, and nanomolar concentrations of receptor are detectable. This GPCR assay technology can be used to optimize CCR5 reconstitution conditions and can be readily extended to other members of the chemokine receptor family.

1502-Pos
Effects of Sensory Rhodopsin II Complexation with its Cognate Transducer HtrII on the Local Environment of Internal Water Molecules
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Recent studies indicate that internal water molecules play a critical role in membrane protein function. Here we report evidence that the local environment of one or more internal water molecules in sensory rhodopsin II (SRII) is altered by interaction with its cognate transducer HtrII. The SRII-HtrII complex mediates blue-light repellent phototaxis in halophilic archaea, using a signaling pathway similar to that in bacterial chemotaxis. We studied the photocycle of SRII and a SRII-HtrII fusion complex from *Natronobacterium pharaonis* using low-temperature static and room temperature time-resolved Fourier transform infrared (FTIR) difference spectroscopy. When cooled to 80 K, and illuminated the protein is trapped in its K state. A shift of 2 cm^{-1} between SRII (3626 cm^{-1}) and SRII-HtrII (3628 cm^{-1}) is found for a negative OH stretching band assigned to an internal water molecule, most likely located near the active Schiff base. In contrast, the OH stretching band for this water in the K state appears at the same frequency (3619 cm^{-1}) for both the free and complexed receptor. Similar shifts are observed upon hydration with H_2O^{18} shifted to a lower frequency confirming these bands arise from the OH stretching mode of water. Data are also presented on the effects of lipid environment on structural changes of internal water molecules and the receptor-transducer interactions. This work was supported by National Institutes of Health Grants
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1503-Pos
Exploring the Mechanics and Energetics of Epidermal Growth Factor Receptor Activation
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The activation of epidermal growth factor receptor (EGFR) is a complex molecular process. To date, because of technical limitations in dealing with a full-length receptor construct, our understanding of this process comes from structural and biochemical studies of isolated fragments of the receptor. Here we seek detailed molecular insight into the activation process of EGFR in the context a full-length receptor construct. To be able to handle computationally such a large protein (~1000 amino acids) we have developed ELNEDIN: a coarse-grained modeling approach that can describe reliably the dynamics and interactions of proteins. We have built full-length models of human EGFR in the active (extended) and inactive (tethered) states. The models include explicit representations of the lipid membrane and aqueous environments. The conformational space of the tethered and extended state at equilibrium was sampled extensively in the microsecond time scale using a combination of classical molecular dynamics and enhanced sampling techniques. Using these enhanced sampling techniques we have also generated paths that describe the transition from the tethered to the extended state, i.e. the activation of the receptor. These paths have yielded a clear molecular picture of the sequence of conformational changes that lead to activation of EGFR. This picture is remarkably consistent with that derived from experimental approaches, but it also provides new insights into the activation process. Notably, it shows how the conformational changes that occur on the extracellular side of the membrane affect the structure dynamics of the intracellular components of the receptor. Finally, the free-energy surface associated the activation was obtained

and a putative model for the transition state is proposed. This information can now be used to rationalize the energetic and conformational effects of oncogenic mutations and the binding of antibodies.

1504-Pos

Oligomerization of Membrane Receptors: FRET Analysis Using Coiled-Coil Tag-Probe Labeling and Spectral Imaging

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Oligomerization of membrane receptors in living cell membranes plays an important role in regulation of receptor activity and trafficking. Förster resonance energy transfer (FRET) techniques are often employed to detect receptor oligomerization using receptors fused with fluorescent/luminescent proteins. However, the large size of fluorescent proteins often interferes localization and function of target receptors. Furthermore, controlling the labeling ratio (donor/acceptor), which is important for analysis of FRET, is usually difficult when two fusion receptors with different colors are co-expressed. Posttranslational labeling methods using a short tag peptide and a fluorescent probe that specifically binds to the tag enable a smaller size of label and easy control of labeling ratio in multicolor labeling [1,2]. We recently developed a quick (< 1 min) and surface-specific tag-probe method using a high-affinity heterodimeric coiled-coil formation between the E3 tag (EIAALEK)3 attached to the target receptor and the Kn probes (KIAALKE)n (n = 3 or 4) labeled with a fluorophore [3]. Here we examine oligomerization of the metabotropic glutamate receptor (mGluR) using this novel technique. The receptors were labeled with Rhodamine Green (donor) or Tetramethylrhodamine (acceptor) fluorophores. A constant FRET signal was observed for mGluRs transiently expressed in CHO cells, indicating constitutive oligomer formation. Spectral imaging and demixing of emission spectra abolish crosstalk between channels that is inevitable in conventional filter detection therefore simplify quantification of FRET efficiency from sensitized acceptor emission, allowing analysis of stoichiometry of the oligomerization.

[1] Murel et al. Nat. Mathods (2008) 5, 561-567.
[2] Meyer et al. PNAS (2006) 103, 2138-2143.
[3] Yano et al. ACS Chem. Biol. (2008) 3, 341-345

1505-Pos

How does the State of Aggregation of Rhodopsin in Retinal Discs Influence the Variability of its Activated Life Time?

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Single photon responses (SPRs) in the retinal rod are less variable than expected assuming that deactivation of the receptor rhodopsin (R*) occurs in a single memoryless step. It has been suggested that SPRs reproducibility can be explained by a sequential increase of affinity of R* to the protein involved in its deactivation arrestin (Arr) which leads to a reduction in the life-time variability of R*. This increase in affinity is promoted by a serial phosphorylation of rhodopsin catalyzed by rhodopsin- kinase (RK).

This deactivation mechanism has been tested successfully by means of stochastic simulation assuming rapid diffusion of all signaling molecules. However, evidence suggests that, in native rod discs, rhodopsin is found forming dimers organized in paracrystalline arrays covering about half of the membrane surface.

In this work, we test the hypothesis that packing induced crowding effects, in conjunction with the competitive interactions between R* an the other proteins involved in the signaling cascade (G protein (G), RK, and Arr) will influence the variability of the half-time of R*. In particular, we explore whether the local decrease of inactivated G around R* (as it becomes activated by R*) facilitates interactions of the receptor with Arr and RK, increasing the probability of R* deactivation. This would then lead to a reduction in its trial to trial variation.

In order to explore these issues, we implement a mesoscopic Monte Carlo simulation in a two-dimensional grid representing the membrane, and follow the stochastic encounters and reactions between the species involved in the signal cascade. We perform the simulations and present data on the variability of the half life of R* under two scenarios: rapid diffusion of all proteins, and immobile paracrystalline arrays of rhodopsin.

1506-Pos

Comparative Interaction of Tricyclic Antidepressants and Mecamlyamine with the Human α4β2 Nicotinic Acetylcholine Receptor

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We compared the interaction of tricyclic antidepressants (TCAs) with that for the noncompetitive antagonist mecamylamine with the human (h) α4β2 nicotinic acetylcholine receptor (AChR) in different conformational states, by using functional and structural methods. The results established that: **(a)** TCAsinhibit (±)-epibatidine-induced Ca^{2+} influx in HEK293-hα4β2 cells with potencies that are in the same concentration range (IC_{50} = 2.2-6.8 μM) as that for mecamylamine ($IC_{50} = 3.0 \pm 0.7$ μM), **(b)** [^{3}H]imipramine binds to a single binding site located in the hα4β2 AChR ion channel with relatively high affinity ($K_d = 0.83 \pm 0.08$ μM), **(c)** TCAsinhibit [^{3}H]imipramine binding to hα4β2 AChRs with affinities (K_i = 1.0-2.1 μM) higher than that for mecamylamine ($K_i = 143 \pm 31$ μM), **(d)** imipramine and mecamylamine do not differentiate between desensitized and resting AChRs, **(e)** imipramine interacts with the desensitized AChR mainly by an entropy-driven process, whereas the interactions with the resting AChR are mediated by a combination of enthalpic and entropic components, and **(f)** neutral imipramine and mecamylamine interact with a domain formed between the leucine (position 9') and valine (position 13') rings by van der Waals contacts, whereas protonated mecamylamine interacts electrostatically with the outer ring (position 20'). Our data indicate that although TCAs interact with a binding domain located between the leucine and valine rings, and mecamylamine predominantly acts at the outer ring and by intercalating between two M2 segments, both drugs may efficiently inhibit the ion channel.

1507-Pos

Common Dynamic Behavior of Inactive G-Protein Coupled Receptor Structures for Diffusible Ligands

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The crystal structures of three different family A G-protein coupled receptors (GPCRs) for diffusible ligands, i.e., engineered forms of human β2-adrenergic (B2AR) and adenosine A2A receptors (A2AR), as well as a turkey β1-adrenergic receptor (B1AR) mutant, have recently become available in the literature. Although the overall helical-bundle topology is conserved among these three inactive GPCR structures, several differences emerge from their comparison, particularly at TM1, the extracellular region, the cytoplasmic side of helices TM5-TM7, the ligand-binding pocket, and the long loop regions. Although one cannot exclude the possibility that crystallographic artifacts may be causing some of these structural differences, it remains to be addressed whether these different GPCR structures would share a common dynamic behavior during molecular dynamics simulations in an explicit lipid-cholesterol-water environment. The results of nanosecond-scale simulations of ligand-free inactive crystallographic forms of B2AR, B1AR, and A2AR were analyzed in terms of inter-residue contact variability over time. Contacts that remained in place during most of the simulations were recognized as stable contacts. Among them, the most stable contacts were found to be common among the three GPCR structures, and to involve residues that are conserved among family A GPCRs. We propose that these stable contacts define a common dynamic behavior of inactive GPCR structures for diffusible ligands, and are therefore important for keeping the receptors in an inactive state.

1508-Pos

Protonation Switches in GPCR Activation: Physiologically Significant Rhodopsin Photointermediates

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Rhodopsin is a paradigm for GPCRs, yet unlike other class A members, its bound chromophore's UV/vis absorbance provides excellent time-resolved information about GPCR activation steps. At least three species equilibrate on the millisecond time scale after rhodopsin photoexcitation in a membrane lipid environment. The first equilibrium is pH-independent and involves Meta I_{480}, the visible absorbing, protonated Schiff base (PSB) species, and Meta IIa, the UV absorbing, deprotonated SB species. The second equilibrium, involving spectrally silent proton uptake by Meta IIa to produce Meta IIb, accounts for the fact that low pH causes anomalous disappearance of the protonated SB species, Meta I_{480}. The equilibria affect production of the G protein-activating species R* and are of great interest. However they must be studied promptly because inactivation steps follow, and long illumination increases secondary photolysis of photoproducts. We used time-resolved absorbance measurements of bovine rhodopsin on the microsecond-to-hundred millisecond