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1645-Pos
Drug binding alters pathways of information flow in ERK2
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ERK2 is a kinase in the MAPK/ERK pathway, which is involved in several cellular functions such as cell survival, proliferation, and differentiation. Disruption of this pathway is known to lead to the development of cancer. We performed molecular dynamics simulations of structures of unphosphorylated (inactive) ERK2 with and without the inhibitory drug FR180204, as well as simulations of phosphorylated (active) ERK2 with and without the inhibitory drug Vertex-11e. We found that both inhibitory drugs had the same effects on the driving behavior of the N-terminal region, activation lip, and part of the L16 region. Some regions were not significantly affected by either drug, while the drugs had opposite effects on the driving behavior of the ATP binding site and other areas of the kinase. These observations may indicate that Vertex-11e and FR180204 inhibit ERK2 via different mechanisms, consistent with recent experimental results that different ERK2 inhibitors binding in the same site can have opposite effects on the conformation of the kinase. More research must be conducted to explain the mechanisms of communication and discover if there are patterns of information flow that are consistently observed in active and inactive ERK2 bound with various inhibitors.

1646-Pos
Calmodulin binds conserved motifs in fibroblast growth factor homologous factors
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Voltage-gated sodium channels (Na_vs) are tightly regulated by multiple proteins that bind the intracellular C-terminal domain. These include calmodulin (CaM), a calcium-signaling protein that binds the Na_v IQ motif, and fibroblast growth factor homologous factors (FGFs) that bind the Na_v EF-hand-like domain (EFL) proximal to the plasma membrane. We discovered that two sequences in the N-terminal domain (NTD) of A-type FGFs (FGF11A, FGF12A, FGF13A, and FGF14A) bind both domains of calcium-saturated CaM (Mahling et al (2021) J. Biol. Chem. 296:100458). These FGF sites (LTP, long-term inactivation particle, and CaMBD, CaM-binding domain) possess a high degree of evolutionary conservation in eukaryotes. Computational analysis and modeling predicts these sites adopt helical secondary structures within a larger intrinsically disordered region. We hypothesize that CaM binding to the CaMBD, the higher affinity site, causes a conformational change that facilitates the movement of LTP towards the pore, resulting in long-term inactivation. To visualize possible states of this 3-protein complex, we are using structural and hydrodynamic studies (NMR, crystallography, DLS, and analytical chromatography). To understand calcium-induced switching in this allosteric network, we are conducting titrations monitored by steady-state fluorescence spectroscopy to determine differences in effects of A-type FGFs on CaM bound at the IQ motif of $Na_v1.2$, and CaM bound to the FGF NTD. These titrations suggest that A-type FGFs can compete with Na_v IQ motifs for Ca^{2+}-saturated CaM. During spikes in the cytosolic Ca^{2+} concentration that accompany an action potential, CaM may translocate from the Na_v IQ motif to the FGF NTD or the A-type FGF NTD may recruit a second molecule of CaM to the channel.
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Posters: Membrane Protein Structures I

1647-Pos
Assembly of serotonin receptor ion channel 5-HT3$_A$
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The 5-HT3$_A$ receptor (5-HT3$_A$R) is a serotonin-activated pentameric ligand-gated ion channel (pLGIC) that mediates fast synaptic transmission throughout the nervous system. The channel modulates multiple behavioural functions and controls both gut motility and emesis. 5-HT3$_A$R is known to form functional homopentamers composed of five pseudo-symmetrically arranged subunits surrounding a central ion-conducting pore. Previously, blue native PAGE analysis and various thermostability assays on 5-HT3$_A$R suggested that upon structurally destabilizing events the channel goes through different oligomeric states (i.e. tetramer, trimer, dimer and monomer). However, very few is known about how pLGICs assemble at the level of the plasma membrane. Many cryo-EM structures of the pentameric 5-HT3$_A$R solubilized with detergent or reconstituted in Salipro have been reported. However, none of those studies contributed to elucidate the oligomerization modality of the channel. Recently, cryo-EM structures of another pLGIC, the Glycin receptor, in different assembly states (i.e. alfa-tetramers and -trimers) have been released, allowing the authors to speculate on the eventual assembly modality of this channel. Here we show for the first time near atomic resolution cryo-EM structures of 5-HT3$_A$R in a tetrameric intermediate assembly state. The cryo-EM map of the tetramer revealed an asymmetrical organization of both the extracellular domain (ECD) and intracellular domain (ICD), while the transmembrane domain (TMD) exhibits a packed four-fold symmetry. We speculate that the tetramer could be a physiologically relevant precursor of the final pentameric functional channel. Moreover, it could also correspond to a pathological state of the channel.We propose a model that sheds light on 5-HT3$_A$R genesis and activity modulation at neuronal level.

1648-Pos
Molecular determinants of desensitization of mGluR3 by β-arrestins
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G protein-coupled receptors (GPCRs) interact with a variety of intracellular transducers that can initiate signaling events, post-translationally modify the receptor, and desensitize receptor signaling. For many GPCRs, desensitization is mediated by sequential phosphorylation of the GPCR C-terminal domain (CTD) and recruitment of β-arrestins to the modified CTD. β-Arrestin binding can lead to a variety of outcomes including clathrin-mediated endocytosis of the GPCR or additional intracellular signaling events. β-arrestin binding to phosphorylated GPCR CTDs has been studied primarily using a small set of monomeric family A GPCRs. Metabotropic glutamate receptors (mGluRs) are dimeric family C GPCRs that are important for synaptic modulation and plasticity. Recently, a study of the eight metabotropic glutamate receptor (mGluR) isoforms found that mGluR3, but not the closely-related mGluR2, is robustly internalized in a β-arrestin-dependent manner. Like all other known GPCR-β-arrestin interactions, the internalization of mGluR3 was found to be mediated by a Ser/Thr-rich region in its intrinsically disordered CTD. To better understand the regulation of mGluR3 by both β-arrestin-1 and -2, we have employed an array of biophysical, biochemical, and cell biology approaches to assess the driving forces of this interaction. We investigate the role of the phosphorylatable residues in the mGluR3 CTD in β-arrestin-1 and -2 recruitment, the contribution of transmembrane core interactions and assess the stoichiometry of the mGluR3-β-arrestin complex using single-molecule fluorescence methods. This work elucidates the biophysical underpinnings of β-arrestin interactions with a dimeric family C GPCR and establishes new experimental methodologies to dissect GPCR-transducer coupling.

1649-Pos
Intricacies of the SARS-CoV-2 spike transmembrane trimer organization
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The spike glycoprotein (SGP) of SARS-CoV-2 effectuates membrane fusion and virus entry into target cells. A transmembrane domain (TMD) represents a homotrimer of alpha helices anchoring SGP in the viral envelope. Although SGP models available to date include TMD, its precise configuration was given brief consideration. Understanding viral fusion entails realistic TMD models, while no reliable approaches towards predicting the 3D structure of transmembrane homotrimers exist. Here, we adopted diverse computational tools to model the SGP TMD based solely on its primary structure. We performed amino acid sequence pattern matching and compared molecular hydrophobicity potential (MHP) distribution on the helix surface against transmembrane homotrimers with known 3D structures. Eventually, the TMD of TNFR-1 was selected for template-based modelling. Adjusting so-called ‘‘dynamic MHP portraits’’, we iteratively built an all-atom homotrimer model of the SGP TMD, whereof each helix possessed two overlapping interfaces interacting with either of the remaining helices. The interfaces included conservative residues like I1216, F1220 and I1227. Stability of this model was tested in an all-atom molecular dynamics (MD) simulation in a POPC bilayer mimicking the viral envelope and compared to several alternative configurations, including a recent NMR structure of a trimerised peptide identical in sequence to the SGP TMD bar four point mutations. Unlike other configurations, our model trimer remained tightly packed over a microsecond-range MD and retained its stability when palmitoyl chains were added at cysteine residues located downstream and shown to be palmitoylated experimentally. We additionally probed our model's stability introducing mutations present in the aforesaid NMR structure; MD simulations revealed no impact of the mutations on the

TMD stability. This model will be used to explore the complex machinery of membrane fusion from a broader perspective beyond the TMD. This work was supported by RSF grant 18-14-00375.

1650-Pos
Ensemble-based, glycan-dependent epitope analysis of SARS-CoV-2 spike protein

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The global pandemic caused by the SARS-CoV-2 virus has impacted all aspects of human life. The spike protein, which is key to targeting and entering host cells by the virus, provides a major target for protective antibodies against the virus. Various neutralizing antibodies against the spike have been identified. Furthermore, the spike protein is the main therapeutic objective in development of vaccines. To explore potential B-cell epitopes on the spike protein, we have constructed a full-length, membrane-embedded, palmitoylated and fully glycosylated spike model and performed a 5-μs equilibrium simulation. A control simulation was also performed in the absence of glycans. We first examined the shielding effect by the glycans on the spike surface and did network analysis between the occupied regions of glycans at different positions. A residue-wise epitope score for the whole spike was developed using a metric based on the sequence- or structure-dependent scores and the solvent accessible surface area of each residue. Then, knowing that the function of the epitopes can be modulated by the nearby glycans, we calculated the root-mean-square deviation of the Cα atoms in the two trajectories. The presence of the glycans is found to correlate with the flexibility of some epitopes, especially the N2 and N3 loops in the N-terminal domain. Furthermore, as the number of spike variants continues to grow and more effective antibodies may be needed, in a first attempt in this direction, we have also simulated and analyzed the Delta variant of spike and characterized its differences with wild type spike.

1651-Pos
Detoxification of bee venom melittin to create a selective pore-forming peptide by substitution of key amino acids

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Melittin, the archetypal 26 residue α-helical peptide toxin is the main component of European Honeybee venom. With its ability to partition into lipid bilayer membranes and form an amphipathic helix, melittin permeabilizes many different types of membranes indiscriminately and has a wide toxicity against mammalian cells, bacteria, fungi, insects and more. MelP5 and M159 are two melittin variants discovered by high-throughput screening. They share similar amino acid sequences while having distinct characteristics. Notably, MelP5, like its parent melittin, is highly toxic to a variety of cells, while M159 forms macromolecule-sized pores in PC vesicles but has essentially no cell toxicity. In this study, we investigate the sequence-structure-function relationships by comparing the biophysical and functional features of three MelP5 or M159 variants in which potentially key amino acids are substituted. The results indicate that substitution of the acidic amino acids at the 4th or 8th site of M159 increased both the cell toxicity and the disruption of synthetic bilayers containing cholesterol implying that these residues contribute to the detoxification of melittin. Substitution of leucine by glutamine at the 17th position of MelP5 decreased MelP5 toxicity, indicating how the toxicity of MelP5 is alleviated in the non-toxic M159 and related peptides. Lastly, we propose a theoretical model describing the relationship between the peptide-lipid pore structure and peptide sequence, which provides insights to enable the design or screening of even more useful melittin variants in the future.

1652-Pos
Peptide tags and domains for expression and detection of mammalian membrane proteins at the cell surface

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Transport proteins mediating solute transfer across the plasma membrane constitute an important group of cell-surface proteins. There are several diseases resulting from mutations in these proteins that interfere with their transport function or trafficking, depending on the impact of the mutations on protein folding and structure. Recent advances in successful treatment of some of these diseases with small molecules which correct the mutations-induced folding and structural changes underline the need for detailed structural and biophysical characterization of membrane proteins. This requires methods to express and purify these proteins using heterologous expression systems. We described experimental strategies for this approach using the solute carrier transporter NaCT (Na^+-coupled citrate transporter). Loss-of-function mutations in NaCT cause a severe neurologic disease known as early infantile epileptic encephalopathy-25 (EIEE-25). EIEE-25 leads to epilepsy, impaired speech, limited motor skills, developmental delay, and tooth defects in children. NaCT was modified with various peptide tags, including a RGS-His10, a Twin-Strep, the SUMOstar domain, and an enhanced green fluorescent protein (EGFP), each alone or in various combinations. When transiently expressed in HEK293 cells, recombinant NaCT proteins underwent complex glycosylation, compartmentalized with the plasma membrane, and exhibited citrate transport activity similar to the non-tagged protein. Surface NaCT expression was enhanced by the presence of SUMOstar on the N-terminus. The dual-purpose peptide epitopes RGS-His10 and Twin-Strep facilitated detection of NaCT by immunohistochemistry and western blot and may serve as useful tags for affinity purification. This approach sets the stage for future analyses of mutant NaCT proteins that may alter protein folding and trafficking. It also demonstrates the capability of a transient mammalian cell expression system to produce human NaCT of sufficient quality and quantity to augment future biophysical and structural studies and drug discovery efforts.

1653-Pos
Antibody complexation with small helical-MPER epitope for structural studies of small membrane proteins

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Structural studies of small membrane proteins are challenging. The use of soluble binding proteins such as antibody fragments is a common approach to increase the size of membrane proteins for cryo-EM, or to aid in crystal formation for x-ray crystallography. However, several complications arise from the generation of stable antibodies or the discovery of specific binders. To establish a new approach for the structural determination of small membrane proteins, we identified a short and helical epitope known as the membrane-proximal external region (MPER) from HIV envelope glycoprotein gp41. As a helical extension of the first transmembrane helix, this epitope provides a rigid binding site for well-characterized antibody fragments. Using this method, we solved an x-ray crystal structure of a 30-kDa fluoride channel protein in complex with antibody fragments and collected a small single particle cryo-EM dataset. Hence, antibodies complexation with the MPER epitope serve as a promising avenue for the structural determination of small membrane proteins.

1654-Pos
Magnetically aligned peptoid-based macrodiscs for structure determination of membrane proteins by solid-state NMR

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We report on highly alignable, novel lipid mimetic for structural and dynamical studies of membrane proteins by oriented-sample (OS) solid-state NMR which can be used to determine structures of membrane proteins under their native-like conditions without the use of crystallization and cryogenic temperatures. As an alternative to the recently reported discoidal mimetics based on lipoproteins and styrene-maleic acid lipid particles, we have developed magnetically orientable discs of ca. 20-30 nm in diameter based on 15-mer peptoid constructs. Peptoids consist of poly-*N*-substituted Glycines, where the amino-acid side chains are bound to the backbone nitrogen instead of the α-carbons. Peptoid constructs were synthesized by alternating carboxylate and aromatic side chains at the 1:2 molar ratio exhibiting a high degree of alignment in the magnetic field over a wide temperature range (30-45°C). Due to their higher flexibility than for the belt peptides, peptoids are expected to wrap around the hydrophobic lipid interior more uniformly, thus forming more stable and homogenous soluble macrodiscs. ^{31}P and ^{15}N NMR spectra measured for a sample of membrane-reconstituted Pf1 coat protein yield superior order parameters to the magnetically aligned bicelles and peptide-based macrodiscs even at a moderate NMR magnetic field of 11T. Moreover, the dipolar linewidths in the two-dimensional separated local field spectra are ca. 50% narrower, thus indicating more uniform alignment than any previous magnetically aligned membrane mimics. Rotational dynamics of Pf1 coat protein embedded in the peptoid-based macrosdiscs has also been investigated by $T_{1\rho}$ measurement to assess the rotation of the