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measure of oligomerization in contrast to the complex environment of cellular membranes.
We developed a miniaturized bioassay that allowed us to quantify GPCR oligomerization at the level of single proteoliposomes [4]. We employed confocal microscopy to monitor immobilized single proteoliposomes loaded with fluorescently labeled GPCRs, and measured the oligomerization efficiency as the Förster Resonance Energy Transfer (FRET) between donor and acceptor labeled receptors. This assay was used to probe the homo-oligomerization properties of two prototypical GPCRs, the cannabinoid receptor 1 (CB1) (5) and the photoreceptor opsin (6, 7). We anticipate our quantitative single proteoliposome assay will provide unique details on GPCR homo-oligomerization unobscured by ensemble averaging and cellular complexity.
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551-Pos Board B306
Use of Bioluminescence Resonance Energy Transfer (Bret) for Studies of Protein-Protein Interactions Between the GLUD2 Receptor and Intracellular Interaction Partners
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The GluD2 receptor is a member of the ionotropic glutamate receptor (iGluR) superfamily and is expressed in cerebellar Purkinje cells (PCs). GluD2 receptors recognize the neurotransmitters D-serine, but lack excitatory ligand-gated ion channel activity. Recently, D-serine activity mediated via GluD2 has been shown to modulate cerebellar long-term-depression (LTD). The intracellular C-terminal domain (CTD) of GluD2 is required for D-serine regulation. A working hypothesis for this regulation is that D-serine binding to the extracellular ligand-binding domain is translated to intracellular rearrangement of the CTD and hereby potentially affects protein-protein interactions (PPIs) with intracellular scaffolding proteins or signaling enzymes involved in expression of cerebellar LTD. Various interacting partners of the GluD2 CTD have been identified; including the PDZ-domain containing scaffolding protein S-SCAM and Delphilin and the tyrosine kinase PTPMEG. Furthermore, the CTD contains multiple phosphorylation sites, which may regulate PPIs.
In the present study, our aim is to study the potential regulation of GluD2 PPIs by phosphorylation and D-serine. For this purpose, we have established a bioluminescence resonance energy transfer (BRET) assay that enables measurement of GluD2 PPIs in intact cells by inserting the resonance energy donor Renilla luciferase 8 (Rluc8) at various positions in full-length GluD2 or the isolated CTD and acceptor molecule green fluorescence protein 2 (GFP2) to known GluD2 interaction partners.
For PSD95, Delphilin, AP-4 and S-SCAM, it was possible to detect robust BRET between GluD2 and GFP2-tagged full-length interacting partners as well as truncated versions containing the isolated PDZ domain. Hereby, an experimental platform has been created for studying GluD2 PPIs in intact cells. Results from ongoing characterizations of the influence of CTD phosphorylation and D-serine ligand-binding on PPIs will be presented.

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Multidimensional Solid-State NMR of Functional Chemotaxis Receptor Signaling Complexes
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Bacterial chemotaxis receptors serve as an ideal model system for elucidating molecular mechanisms of transmembrane signaling, due to the experimental accessibility and established biochemical characterization of these proteins. A gap in the structural understanding of bacterial chemotaxis is present between electron microscopy of the intact arrays of receptor complexes found in cells and high-resolution crystal structures of individual proteins and truncated complexes. To bridge this gap we have initiated multidimensional solid-state NMR spectroscopy studies of a kinase-active ternary complex composed of the soluble 30 kDa cytoplasmic fragment (CF) of the aspartate chemoreceptor, the 70 kDa histidine kinase CheA, and the 18 kDa adaptor protein CheW. We prepared uniformly ^{13}C & ^{15}N labeled CF and obtained both homonuclear and heteronuclear correlation spectra of frozen and unfrozen complexes.
The initial data indicates that such experiments are feasible on this functional multi-protein complex. A number of narrow resonances with sub-ppm linewidths are observed in spectra of both frozen and unfrozen samples, and both states exhibit sufficient signal-to-noise for acquiring multidimensional spectra. Overall, the spectra demonstrate the strongly overlapping resonances expected for an alpha-helical 30 kDa protein, which suggests selective labeling for future measurements of structural constraints. For these samples containing uniformly labeled receptor fragment, comparison with predicted chemical shift spectra are being used to test the current structural model. Comparisons of frozen and unfrozen spectra are also being used to assess the presence of static disorder or dynamics in the side chain and backbone resonances. The goal for uniformly labeled receptor samples is to compare spectral properties of both signaling states to gain insight into possible differences in structure and dynamics.
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Mechanisms Underlying CaMKII Regulation of Astrocytic Glutamate Transporters
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Excitotoxic calcium signaling, a hallmark of aberrant neuronal activity during cerebral ischemia and brain trauma, induces a loss of CaMKII activity that appears to sensitize neurons to glutamate-induced toxicity. Because CaMKII is present in astrocytes and other glia, we postulate that aberrant CaMKII signaling could compromise neuronal signaling and survival to excitotoxicity by disrupting astrocyte function. In support of this model, inhibition of CaMKII in cortical astrocytic cultures with a peptide (tat-CN21) as well as a small molecule (KN-93) inhibitor significantly reduces glutamate uptake compared to inactive controls, suggesting that glutamate transporters in astrocytes require CaMKII for their basal activity. The two primary excitatory amino acid transporters (EAAT) in astrocytes are EAAT 1 and EAAT2. Co-immunoprecipitation from rodent brain and immunocytochemical studies in cortical astrocytes reveal an association and co-localization respectively between EAAT2 and CaMKII. Although additional studies are required to determine if these interactions exist for EAAT1 and CaMKII in cells, in vitro studies using GST-fusion proteins and peptide tiling arrays indicate that purified δCaMKII binds to the N terminal and second intracellular loop of EAAT1, whereas, CaMKII preferentially binds to the N terminus and third intracellular loop of EAAT2. Using P32 incorporation as an index of CaMKII phosphorylation, phosphorylation of immunoprecipitated transporters as well as peptide tiling assays for EAAT2 reveal that astrocytic glutamate transporters are indeed CaMKII substrates. In total, our data support the hypothesis that astrocytic glutamate transporters are regulated by CaMKII binding and/or phosphorylation. Further studies are required to determine if a loss of CaMKII activity that is associated with pathological states, including epilepsy and ischemic stroke, compromises neuronal survival due to reduced glutamate transporter function previously associated with excitotoxicity.

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Optimized Zebrafish Apolipoprotein A-I Expression and Purification for Nabbs Assembly
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G protein-coupled receptors (GPCRs) are heptahelical transmembrane proteins that are highly susceptible to their lipid environment. Reconstitution of GPCRs into detergent micelles or liposomes allows researchers to control the lipid environment. Yet, micelle and liposome preparations are in homogeneous and protein incorporation is difficult to control. To address these issues, nanoscale apolipoprotein bound bilayers (NABBs), soluble phospholipid bilayers stabilized by the helical protein apolipoprotein A-I derived from zebra fish (zap1), have been previously devised. Yet, our approach has been limited by the poor expression of zap1 in E. coli. [1] To increase expression of zap1, we designed a synthetic gene (zapN1) with a codon-optimized sequence encoding the same amino acid sequence as zap1. We transformed BL21-Gold (DE3) cells with the zapN1 gene and performed a double-screen of protein expression from single transformed colonies. We show that zapN1 expresses in a shaking culture at higher levels than the native gene and the membrane scaffold protein MSP1D1 from Stephen Sligar's group. We carefully parameterized the induction and temperature values, finding that induction at an OD600 of 0.8 with