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3053-Pos Board B100

Changes in the Secondary and Tertiary Structures of Secreted Phospholipase A_2 upon Activation

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Activation of human pancreatic phospholipase A_2 (PLA_2) in the presence of DPPC/DPPG (7:3) vesicles was induced by a temperature shift from 4 to 38 ^oC. PLA_2 activity was monitored by changes in fluorescence of bis-Pyrene-PC (2.5 mol % in the membranes), while simultaneous far- and near-UV circular dichroism (CD) spectra identified changes in the secondary and tertiary structures of the protein in real time. The 4-to-38 ^oC temperature shift caused dramatic changes in both bis-Pyrene-PC fluorescence and the protein CD spectra. The monomer fluorescence signal of bis-Pyrene-PC rapidly increased and the excimer signal decreased, demonstrating PLA_2 activation. Drastic weakening in the α-helical CD signal of the protein, i.e., a 20% decrease in the n-π* transition intensity at 222 nm, was detected upon enzyme activation. The α-helical signal exhibited a significantly smaller change upon a similar temperature shift under non-catalytic conditions (1 mM EGTA), while little changes were detected in the absence of lipid. Strong changes in the tertiary structure during PLA_2 activation were also identified. Initially, at 4 ^oC, the near-UV CD spectra showed a weak negative band around 280 nm. Upon a shift to 38 ^oC, strong positive CD bands rapidly developed around 250 and 280 nm, implying significant changes in the conformation and/or the microenvironment of Tyr and Trp side chains of PLA_2, possibly accompanied with a global tertiary structure perturbation associated with deformation of the abundant disulfide bonds in the protein. These experiments provide new information on the structure-function relationship of PLA_2 by near-simultaneous measurements of PLA_2 activity and its secondary and tertiary structures.

3054-Pos Board B101

Global Fitting and Kinetic Modeling of the Drug Transport Cycle of Human P-glycoprotein

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Human P-glycoprotein (ABCB1) is important in pharmacokinetics, tissue distribution, oral bioavailability and disposition of therapeutic compounds. Overexpression of P-glycoprotein can lead to adverse clinical effects such as the failure of cancer chemotherapy by the induction of multidrug resistance. Thus a detailed understanding of P-glycoprotein's drug transport cycle is a prerequisite to modulating its various activities. Here we present a detailed kinetic and mechanistic model of drug transport by P-glycoprotein. This was achieved by global fitting of time-based progress curves of the transport of spin-labeled verapamil as a function of ATP concentrations and measurements of the force-flux relationships observed during drug transport. By measuring the ATPase activity simultaneously with the transport velocity in proteoliposomes containing purified P-glycoprotein we established a limiting stoichiometry of one ATP hydrolyzed per spin-labeled verapamil molecule transported. Next, using standard chemical kinetic rate laws, we compared different proposed models of drug transport by trying to globally fit available experimental data numerically to coupled differential equations generated by each competing model. Our original seed values and constraints were generated from measurements of K_M, *Vmax*, and K_i values for all reacting ligands during the steady state transport cycle together with knowledge of the overall thermodynamics of the transport cycle. We achieved a unique global fit of the progress curves of spin-labeled verapamil transport as a function of ATP concentration. A single internally consistent set of rate constants was shown to account for the data. Additionally, these same rate constants also accounted for the experimentally observed force-flux relationships when utilized in our ''reaction power stroke'' model. In contrast we were not able to fit the data as uniquely and as effectively with other competing drug transport models. Supported by NIH grant GM52502.

3055-Pos Board B102

Agonist and Antagonist Interactions in Beta Adrenergic Receptors

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G-protein coupled receptors (GPCRs) are a large family of integral membrane proteins involved in signal transduction pathways, making them appealing drug targets for a wide spectrum of diseases. The recently solved X-ray structures of beta1 (B1AR) and beta2 (B2AR) adrenergic receptors bound to inverse agonists/antagonists open up a large field of potential investigations to understand the binding modes and mechanisms of activation of GPCRs.
To investigate their structural and dynamic properties under pseudo in vivo conditions, we performed extensive molecular dynamics simulations (in an explicit membrane) of adrenergic receptors in complex with partial inverse agonists and agonists as well as in their apoform. To this end, we applied MD-based enhanced sampling techniques (steered MD, metadynamics, …) to describe ligand binding and to elucidate the process of ligand entrance and release.
In this contribution, we rationalize the differences in binding mode between B1AR and B2AR for both agonists and antagonists (focusing on a limited set of key residues surrounding the binding pocket that are different between B1AR and B2AR). We also discuss main structural changes upon agonist/antagonist binding also in comparison with the most thoroughly studied GPCR, rhodopsin.

3056-Pos Board B103

Expression and functional characterization of Metabotropic Glutamate Receptor Type 6 (mGluR6) in detergent micelles

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Metabotropic glutamate receptors (mGluRs) are G protein coupled receptors which are implicated in different brain functions and dysfunctions including learning, memory, pain perception, neurodegeneration, schizophrenia and addiction. Metabotropic glutamate receptor type 6 (mGluR6) is a subtype of mGluRs which is exclusively expressed in ON-bipolar cells and is involved in night vision. Recent genome-wide association studies have discovered involvement of mGluR6 in heroin addiction. mGluR6 is emerging as a new drug target but structure-function relationships of this receptor and in general of mGluRs are poorly understood. These receptors have very low expression levels in their native cells and are only active in a membrane environment. The major problem in studying these receptors with biophysical approaches is in obtaining sufficient quantities of functional protein. To overcome this, we have constructed a tetracycline inducible mammalian stable cell line expressing full length human mGluR6. We optimized the detergent and buffer conditions required for mGluR6 purification. We are developing a reliable, quantitative *in vitro* assay for verifying the function of purified mGluR6 in different detergents. We have been successful in developing a medium scale expression and purification system of mGluR6. Preliminary data suggests that this receptor responds to a number of ligands relevant to its implicated role in vision and addiction with changes in activity and structure as evidenced by G protein binding and cysteine accessibility measurements, respectively.

3057-Pos Board B104

Calcium Enhances The Proteolytic Activity Of BACE: An In Vitro Biophysical And Biochemical Characterization Of The BACE-Calcium Interaction

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BACE is a novel type I transmembrane aspartyl protease that has been implicated in the pathogenesis of Alzheimer's disease. Cleavage of the amyloid precursor protein by the beta-secretase, BACE, is the first step in the production of the amyloid-beta peptide and is a prime target for therapeutic intervention. Using circular dichroism, we provide evidence that show differences in stability between active (pH 4.8) and inactive (pH 7.2) BACE. Active BACE (T_m ~51°C) is comparably much less stable than the inactive form (T_m ~84°C). In this study we have also examined Ca^{2+} binding to BACE, the effect of this binding on the secondary and tertiary structural characteristics of BACE, and the influence of this binding on the specific activity of the purified protein. Initially, we used isothermal titration calorimetry to characterize the Ca^{2+}-BACE interaction. Our results suggest that there is a high affinity of binding ($K = 2.0 \times 10^5\ M^{-1}$) between Ca^{2+} and BACE and that the binding process was exothermic (-3.5 kcal/mol). Circular dichroism and endogenous tryptophan fluorescence measurements demonstrated that the secondary and tertiary structure, respectively, is sensitive to increasing concentrations of Ca^{2+}. We also could demonstrate that low concentrations of Ca^{2+} (μM) significantly increased the proteolytic activity of BACE. Collectively, these results define a role for Ca^{2+} in both modulating the structure and proteolytic activity of BACE and suggest

that under physiological conditions, the function(s) of BACE is influenced by Ca^{2+}.

3058-Pos Board B105

Investigation Of The Multidrug ABC-transporter LmrA By Multinuclear MAS-NMR And EPR

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The multidrug ABC-transporter LmrA from *Lactococcus lactis* is a structural and functional homologue of human P-glycoprotein. Just like its famous relative, LmrA extrudes hydrophobic drugs from the membrane and can thus confer resistance to its host cell. The energy for this process is provided by ATP hydrolysis in the two nucleotide binding domains (NBDs). During a hydrolysis-transport cycle, the NBDs communicate a structural change to the transmembrane domains (TMDs), where the substrate is recognized and extruded. However, it is not understood for any ABC-transporter at which point during those cycles the two domains interact and how ATP hydrolysis and substrate recognition and extrusion are coupled. In order to some shed light on these phenomena, we have trapped the transporter during the hydrolysis cycle with fluorinated phosphor analogues (BeFx, AlFx) and investigated the electronic environment within the NBD with a complementary $^{19}F/^{31}P$ MAS ssNMR approach. Additionally, we have labelled the TMDs with ^{19}F- and EPR spinlabels for ssNMR and EPR in order to probe the relationship between drug recognition, transport and coupling to the hydrolysis cycle. Activity of the transporter under MAS conditions has been verified by an ATPase Assay with 31P NMR that can also be employed to probe substrate phosphorylation within membranes.

3059-Pos Board B106

A Voltage Sensitive Phosphatase from Xenopus Laevis Testis

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Voltage sensitive phosphatases (VSPs) are transmembrane proteins comprised of a voltage sensor domain characteristic of an ion channel, coupled to a phosphatidylinositol phosphatase; their phosphatase activity is activated by membrane depolarization (Murata and Okamura, 2007, *J. Physiol.* 583:875-889). Because VSPs are expressed predominantly in sperm, it has been proposed that they might function in the voltage-dependent regulation of sperm-egg membrane fusion, which in many species provides a fast block to polyspermy. To characterize the properties of a VSP from a species amenable to transgenesis and fertilization studies, we identified a VSP homolog from *Xenopus laevis* testis and expressed it in *Xenopus* oocytes, together with the fluorescent PIP_2 sensor PLCδPH-GFP. Using a photodiode to measure PLCδPH-GFP fluorescence from the pigmented surface of voltage clamped oocytes, we showed that XlVSP enzymatic activity is regulated over a range of membrane potentials (50% activation at ~ +10 mV) similar to those that regulate sperm-egg fusion. In agreement with previous studies of ascidian and zebrafish VSPs (Hossain et al., 2008, *J. Biol. Chem*. 283: 18248-59), an R152Q point mutation shifted the XlVSP activation curve towards more hyperpolarized membrane potentials (50% activation at ~ −12mV). Future studies of the voltage dependence of fertilization using sperm from transgenic frogs expressing $XlVSP^{R152Q}$ should allow investigation of the functional significance of voltage sensitive phosphatases in sperm-egg fusion.

3060-Pos Board B107

The Substrate Translocation Pathway and Transport Mechanism in the Dopamine Transporter

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The crystal structure of a prokaryotic homolog of the dopamine transporter (DAT), LeuT provided essential structural insights about the family of sodium symport transporters, but only limited clues regarding the molecular mechanism of substrate transport. Utilizing this structure as a template we constructed an experimentally-validated homology model for DAT, and used SMD simulations to explore the substrate translocation pathways. The substrate was first pulled from its primary binding site (S1) toward the extracellular side to evaluate the presence of a secondary binding site (S2) in DAT analogous to the one identified in LeuT (Shi et al, Mol Cell 2008). With such a site identified, a doubly occupied (substrate in S1 and S2) model was constructed and equilibrated. During this MD equilibration, the S1,S2-DAT was found to open towards the intracellular side allowing water to enter. This is accompanied by specific changes in local conformations, e.g., the rotamer of residue F1.42, and a downwards move of the dopamine in S1, which primes it for translocation. Further pulling this substrate towards the cytoplasmic side causes further inward opening, and conformational changes of specific residues that correlate with SCAM data. Thus, residues lining the transport pathway and key interaction-networks stabilizing either inward-facing or outward-facing conformations (e.g., involving Y3.35-E8.66) are revealed. Large-scale helix rearrangements involved in transition between different states are identified, such as (TM1-TM4-TM5 and TM8-TM9) moving as one group, and (TM2-TM6-TM7 and TM10-TM11) moving as another. Hinge regions involved in these movements contain conserved residues such as G/P/S/T/C that could disrupt helix rigid-body motions and are known to be necessary for maintaining dopamine uptake or efflux. Together, our findings delineate both the mechanism and the pathway for substrate translocation at a level of detail that is directly amenable to experimental validation.

3061-Pos Board B108

Purification and Characterization of an Activated Rhodopsin/Transducin Complex

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Light-induced conformational changes of the dim-light photoreceptor rhodopsin promote efficient binding and activation of the intracellular guanine nucleotide-binding protein, transducin. This activation event initiates GDP/GTP exchange and subsequent dissociation of transducin, from the activated photoreceptor. In this work we have developed a method to assemble and purify an activated rhodopsin/transducin complex in both detergent micelles and lipid bilayers. Activated rhodopsin was immobilized on an affinity resin, allowed to bind to transducin, and extensively washed to remove nonbinding material prior to elution. Evaluation of the eluate by SDS-PAGE and UV-visible absorption spectoscopy confirm the presence of rhodopsin and transducin. The incubation of inactive rhodopsin with transducin in a control experiment resulted in the elution of rhodopsin but no transducin. Activity of the rhodopsin/transducin complex was measured by rhodopsin-dependent GDP release, the uptake of GTPγS, and the nucleotide-dependent release of transducin.