LCP, lamellar structures provide a more favorable setting in which GPCRs can oligomerize as a prelude to nucleation and crystal growth. These new findings lay the foundation for future studies of in meso crystallization mechanisms and for a rational approach to the generation of structure-quality crystals of membrane proteins.

2382-Pos Board B152

GPCRTooKit: A Computational Platform for Structural Comparison of GPCR Crystal Structures and Homology Model Refinement

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G-protein coupled receptors (GPCRs) constitute an important family of transmembrane receptors that regulate major life processes including sensory perception, cell proliferation and hormonal regulation. In lieu of the recent surge in GPCR crystal structures, structure based drug design methods are becoming more viable for GPCRs. However there is a huge disparity in the number of GPCR structures available and the number of GPCRs being studied in research. Thus homology models play an important role in a wide variety of GPCR research ranging from drug design to studying the functional mechanisms in these receptors. GPCR homology models do not capture the critical structural differences between the template and the GPCR being investigated.

We have developed GPCRToolKit that lays the computational framework for building GPCR based modeling tools. We have developed a robust and accurate homology model refinement method called LITiCon2.0, based on optimizing the helical translation, rotational orientation, tilt and gyration angles of the seven helices. The structural differences between two GPCRs stem mostly from the rigid body degrees of freedom. The algorithm is highly parallel to enhance the computational speed. We have tested and validated this method by refining the homology models of several class A GPCRs with known crystal structures, one of them being the chemokine CXCR4 using β2-adrenergic receptor as template. We have also analyzed the statistical distribution of translations, tilts and rotations of TM helices in all available GPCR crystal structures. We found that TM3 and TM7 showed relatively less variation in rigid body orientation compared to the other helices. Overall, TM4 and TM5 showed the highest diversity in spatial orientation among the TM domains. These results along with the description and validation of the LITiCon2.0 method will be presented.

2383-Pos Board B153

Rhodopsin Activation is Modulated by Non-Specific Membrane Lipid-Protein Interactions

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Rhodopsin activation proceeds through an ensemble of conformational substates: Meta I ↔ Meta II_a ↔ Meta II_b ↔ Meta II_bH^+ [1]. These substates are characterized by Schiff base deprotonation (Meta I), an outward tilt of helix H6 (Meta II_b), and protonation of Glu134 (Meta II_bH^+). Lipid bilayer composition has been shown to affect rhodopsin activation, providing an opportunity to systematically investigate membrane protein-lipid bilayer interactions on the mesoscale [2]. To quantify lipid acyl chain effects on GPCR activation, rhodopsin was reconstituted in lipids with symmetric unsaturated acyl chains (DOPC) or asymmetric lipids with *sn*-1 saturated and *sn*-2 unsaturated acyl groups (POPC). Proteolipid recombinant membranes were studied by UV-visible and FTIR spectroscopy. Symmetric membrane lipids (DOPC) stabilize Meta II_a and render the normally weakly populated substate accessible to study. The Meta II_a substate is characterized by an opening of the Schiff base ionic lock, and an activation switch in a conserved water-mediated, hydrogen-bonded network involving helices H1/H2/H7 that is sensed by Asp83. Replacement of an unsaturated acyl chain with a saturated chain (POPC) increases the pK_a value for the Meta I ↔ Meta II equilibrium, and consequently destabilizes the Meta II_a substate. Modulation of the bilayer curvature stress due to a negative monolayer spontaneous curvature (H_0) (DOPC) contributes a mechanical force that shifts the Meta I ↔ Meta II equilibrium towards Meta II, leading to rhodopsin activation. The flexible surface model (FSM) explains how chemically non-specific interactions between membrane proteins and the lipid bilayer contribute to GPCR activation [3,4].

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2384-Pos Board B154

Molecular Simulations Illuminate Rhodopsin Activation Based on New Crystal Structures

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The rhodopsin proteolipid system was modeled through all-atom molecular dynamics (MD) simulations on the microsecond timescale and compared to experimental ^2H NMR data. The post-isomerization behavior of the covalently bound ligand, retinal, was tested for two independent models: (i) the counterion-switch (neutral binding pocket) and (ii) complex-counterion (negative binding pocket) [1]. Each model leads to distinct geometrical rearrangements, with direct implications for interpretation of recent rhodopsin crystal structures [2,3]. The distinctive feature of the counterion-switch simulation entails fluctuations of the C11=C12-C13=C14 dihedral angle. These motions are correlated with changes in the C5-, C9-, and C13-methyl group orientations, and result in a long-axis flip of the polyene chain within the binding pocket of rhodopsin. In contrast, the complex-counterion simulation produced retinal fluctuations at the C7=C8-C9=C10 dihedral. This facilitates stabilization of the methyl group orientations ($\approx 60°$ with respect to the membrane normal), consistent with ^2H NMR results for the Meta I state [4]. Recently, a putative structure of the fully-activated Meta II state revealed a long-axis flip of the retinylidene chain relative to its orientation in the dark state [2]. Our simulations show that electrostatic changes to the rhodopsin binding pocket lead to alternate pathways of retinal conformational release. Agreement between simulation and spectroscopy indicates that the retinylidene flip may only occur in the Meta I ↔ Meta II transition. These simulations provide an essential framework for interpreting molecular snapshots (crystal structures) of membrane protein activation, illuminating how small-scale changes in a GPCR binding pocket can affect large-scale membrane protein-lipid bilayer dynamics.

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2385-Pos Board B155

Sampling of GPCR Second Extracellular Loops using Geometric Constraints

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Second extracellular loops (ECL2) of G protein-coupled receptors (GPCR) are known to play important roles by accommodating various GPCR ligands and providing ligand specificity. Despite the structural similarity among GPCR proteins, ECL2 structure is particularly hard to predict because of the relatively large size and ill-conserved sequence. In this study, we developed an efficient sampling algorithm for GPCR ECL2 that utilizes geometric constraints specific for GPCR. Two applications of the triaxial loop closure algorithm were employed to sample geometrically plausible ECL2 conformations that form a well-conserved disulfide bond with a particular transmembrane helix. Scores based on geometric constraints that effectively describe ECL2 environment were introduced to facilitate filtering of implausible ECL2 structures. All of these components are purely geometric, hence sampling and filtering can be performed with extremely low computational cost. A benchmark test was performed on seven unique GPCRs for which all-atom structures have been revealed. The result shows that the best model out of 50 sampled structures is of acceptable accuracy with the median loop RMSD less than 5 Å. Combined with energy-guided global optimization, further refined ECL2 structures could be obtained. New ideas introduced in this study may be useful for developing methodologies for further GPCR modeling and docking studies.

2386-Pos Board B156

Prediction of the Monomer-Monomer Interface Region of the ß2Ar Homodimer Receptor via Docking Experiments

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Increasing body of evidence indicate that G protein-coupled receptors (GPCRs) exist as dimers and oligomers in the cell membrane. The goal of this study is to estimate the homodimeric form of beta-2 adrenergic ($ß_2AR$) receptor which is a member of the GPCR family, via multiple docking experiments.

The transmembrane domain VI (TMVI) of the receptor is suggested to be a significant part of the interface both experimentally and theoretically [1,2]. The

amino acid sequence motif, [75]**L**IXX**G**VXX**G**[83]VXXT, which is proposed to be essential for dimerization in glycophorin A is similarly observed on TMVI of ß$_2$AR as in **L**KTL**G**[276]IMM**G**[280]TFT**L**[284]. A peptide is derived from TMVI consisting of residues from 276 to 296, GIIMGTFTLCWLPFFIVNIVH and blindly docked to one conformer of the monomeric structure using a rigid body approach, via AutoDock v4.0 software tool [3]. Bound conformations were then reevaluated with a knowledge-based scoring function called DSX^{online} v0.88 [4]. Docking results shows that the peptide has the highest binding affinity for TMVI which supports the possible role of TMVI at the interface of a dimeric structure. Also, a residue interface propensity data derived from a set of 32 nonhomologous homodimers [5] is able to identify TMVI as the domain which contains the highest number of residues with the highest interface propensity values.

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2387-Pos Board B157

Characterizing the Motion of W6.48 in the Active State of a GPCR

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Early biochemical and biophysical studies of GPCRs (G-Protein Coupled Receptors) have suggested that there are a series of switches which trigger the conformational changes associated with activation[1]. These switches include i) the rupture of the cytosolic ‘‘ionic-lock’’ between the E/DRY motif on TMH3 and the intracellular end of TMH6, with a subsequent outward displacement of TMH6, as well as ii) a rotameric transition of W6.48, a modulator of the geometry of TMH6 about the conserved P6.50. This ‘‘toggle switch’’ mechanism effectively couples ligand binding to intracellular conformational changes necessary for activation. Recently several activated state crystal structures have been released, of particular significance is the agonist bound β2AR in complex with the Gs heterotrimer[2]. This state is noteworthy as it is the first structure of an activated GPCR in complex with it’s cognate G-protein. Although these structures display the expected outward movement of TMH6, none indicate a rotameric transition of W6.48, implying that a rotameric change is not required for activation. Alternately, it has been suggested that the transition is transitory[3]. Given that the β2AR displays constitutive activity[4], it seems plausible that the energy required to transition from the inactive to the active state is thermally accessible. In order to test the hypothesis that W6.48 makes transient rotameric changes we have undertaken microsecond long molecular dynamics simulations embedding the full β2AR complex, derived from the crystal structure, into an all atom phospholipid environment. The thermal motion/fluctuations available to the complex and the propensity of W6.48 to undergo rotameric changes will be discussed in light of recent structural studies.

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2388-Pos Board B158

Molecular Determinants for a Mutant Mu-Opioid Receptor in which Naloxone Acts as a Partial Agonist

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The Law group has identified a mutant μ-opioid receptor (MOR), S4.54A, at which traditional opioid alkaloid antagonists become agonists (Yang et al., 2003). Subsequent targeted gene therapy studies using the S4.54A mutant receptor have indicated that naloxone can act as an antinociceptive agent *in vivo* (Chen et al., 2007). The goal of the work presented here was to understand at the molecular level, the origins of this unusual phenotype. Compounding the unusual nature of this mutant is the fact that S4.54 is a lipid facing residue. Ballesteros and colleagues have reported that the hydrogen bonding capacity of lipid facing Ser/Thr residues in α-helices can be satisfied by an intrahelical hydrogen bond interaction, in either the *g-* or *g+* conformation, between the O-γ atom and the *i-3* or *i-4* carbonyl oxygen (Ballesteros et al., 2000). Ser/Thr residues in the *g-* conformation can induce a bend in an α-helix, and we have found that changes in wobble angle and face shift can also occur (Ballesteros et al., 2000). To explore the effect of the S4.54A mutation on the conformation of TMH4, we used the Monte Carlo/simulated annealing technique, Conformational Memories (CM) (Konvicka et al., 1998; Whitnell et al., 2008). CM results showed that the S4.54A mutation alters the conformation of TMH4, such that the top of TMH4 bends into the bundle towards TMH3. When the output S4.54A mutant TMH4 was incorporated into our inactive state MOR model, packing of the TMHs in the TMH3/TMH4 region was altered such that the opioid antagonist, naloxone could no longer stabilize the toggle switch residue, W6.48 in its inactive state conformation. [Support: DA023905, P-Y L; DA021358, PHR]

2389-Pos Board B159

TMBB-Explorer: A Webserver to Predict the Structure, Oligomerization State, Ppi Interface, and Thermodynamic Properties of the Transmembrane Domains of Outer Membrane Proteins

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Beta-barrel membrane proteins are found in the outer membrane of gram-negative bacteria, mitochondria, and chloroplasts. They are important for pore formation, membrane anchoring, enzyme activity, and are often responsible for bacterial virulence. Due to difficulties in experimental structure determination, they are sparsely represented in the protein structure databank. We have developed a webserver (TMBB-Explorer) capable of predicting the three-dimensional structure, oligomerization state, protein-protein interaction interface, and thermodynamic properties such as heat capacity and relative melting temperature of the transmembrane domains of beta-barrel membrane proteins. Our method is based on a physical interaction model, a simplified conformational space for efficient enumeration, and an empirical potential function from a detailed combinatorial analysis [1]. It also accounts for loop entropy and an affinity for right-handedness of the natural beta-barrels. The webserver also reports the hydrogen bonds formed between the beta-strands in the predicted structure. The webserver can provide predictions using sequence information alone or by incorporating secondary structure information.

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2390-Pos Board B160

Memprotmd: Adding the Grease to Membrane Protein Structures

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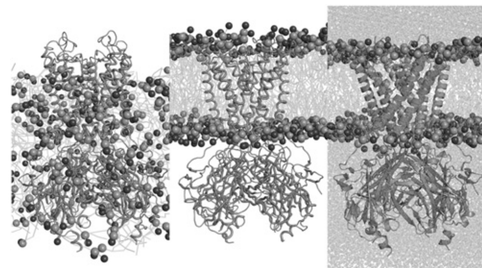
Membrane protein structural biology is one of the key biochemical challenges. With continuous improvements to the methods used by structural biologists there is a predicted exponential growth in the number of membrane proteins structures. Nevertheless, these biological assemblies are usually resolved in the absence of the native lipid environment. Coarse-Grained molecular dynamics (CGMD) simulations provide a means for assessing the assembly and interactions of molecular complexes at a reduced level of representation. This method has been shown to accurately predict the position and orientation of proteins within a cell membrane. The results of these predictions are available in a database (http://sbcb.bioch.ox.ac.uk/cgdb). We are in the process of pipelining the procedure, so that new membrane protein structures are automatically inserted into a DPPC lipid bilayer on release from the Protein Data Bank (PDB). The CG simulations are then assessed for protein-lipid interactions, bilayer deformation, lipid diffusion and protein tilt. The resulting models are then refined to include more physiologically relevant lipid mixtures and subsequently converted to an atomistic resolution [1] to enable more detailed simulations of lipid protein interactions [2].



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2391-Pos Board B161

Identification and Characterization of Transmembrane Segments

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Membrane proteins comprise 30-40% of all known proteins; however, they remain the most uncharacterized. The protein data bank contains very few detailed 3D structures of the transmembrane segments of transmembrane