Explicitly stating all potential states and their potential interactions is not feasible for very large networks. Modelers often deal with this problem by limiting the scope of the network considered, limiting the biochemical resolution considered, or imposing non-physiological reaction specifications that dramatically reduce the number of states. These simplifications are generally unsatisfactory. The cost of these common simplifications on predictive ability is not well understood. We have rather developed a rule-based approach for efficiently specifying and simulating reaction networks. This ''rule-based'' approach enables simulations of mechanistic models of cell signaling networks with resolution and scope far larger than traditional modeling methods. We have built a comprehensive model of IGF1R phosphorylation and SH2/PTB signaling that can account for over 10^{14} possible non-isomorphic complexes. We have also built a model of ErbB family signaling that spans from the four ErbB receptors through ERK and Akt activation and that can account for over 10^{150} possible non-isomorphic complexes. We have used these models to investigate how protein promiscuity may modulate signaling and also to investigate how feedback loops expand the range of signals a network may generate. Now that we have demonstrated the ability to develop and simulate such large models, we turn our attention to how oncogenic mutants disrupt these signaling networks.

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Investigating Structure and Sequence Dependence in the Dimerization of G Protein-Coupled Receptors

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Recent studies have reached mixed conclusions regarding the lifetime and fraction of G Protein-Coupled Receptor (GPCR) oligomers in living cells. Whilst a few, recently published, single-molecule imaging studies suggest transient association between GPCRs, fluorescence recovery after photobleaching (FRAP) has led to variable conclusions. Two closely related GPCRs, the β1-adrenergic receptor (B1AR) and β2- adrenergic receptor (B2AR), were proposed to form transient interactions and stable homomeric complexes, respectively. To obtain a rigorous mechanistic insight into the association of B1AR and B2AR in the cell membrane, at a level of molecular detail beyond that currently attainable by experimental techniques, we have calculated the free energy of association of these receptors using biased molecular dynamics simulations, in particular, a combination of umbrella sampling and metadynamics. Representing explicitly solvated (in a palmitoyl-oleoyl-phosphatidylcholine (POPC)/10 % cholesterol bilayer) B2AR and B1AR crystal structures using the MARTINI coarse-grained force field, we studied their homodimerization at symmetric interfaces formed by transmembrane (TM) helices that have been implicated in GPCR association (i.e., TM1, TM4, and TM4/5). Reconstruction of the free-energy surfaces as a function of the interprotomeric distance demonstrates different relative stability of the B1AR or B2AR dimers depending on the sequence and/or the different structural features at the interface. Specifically, we observe that: a) For both receptors, dimers interacting at interfaces defined by TM1 are more stable than TM4 or TM4/5 dimers; and b) Between the two receptors, the B2AR dimers appear to be generally - albeit not significantly - more stable than the B1AR dimers. Our calculations can be generally applied across family A GPCRs, and offer a novel insight into the mechanism of GPCR dimerization in the cell membrane.

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Interaction with the Membrane Uncovers Essential Differences Between Highly Homologous GPCRs

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The lipid membrane environment has been shown to play a significant role in the function and organization of G-protein coupled receptors (GPCRs) and other transmembrane proteins. We now show quantitatively how small sequence differences between otherwise highly homologous GPCRs can result in strikingly different membrane interaction characteristics. This is evidenced by comparing the membrane interactions of two pairs of functionally related family A GPCRs - (1) the beta1 and beta2 adrenergic receptors; and, (2) the kappa- and delta- opioid receptors, embedded in a lipid bilayer composed of a 16:0-18:1 PC (POPC) /10% Cholesterol mixture. We used the recently described 3D Continuum-Molecular dynamics (3D-CTMD) approach (Mondal et al., BJ (in press)) to quantify the membrane deformation profile and corresponding energy costs due to the protein/membrane hydrophobic mismatch. The novel computational method accounts for the irregular hydrophobic surface of the protein and the hydrophobic mismatch at particular TMs that is not alleviated by membrane deformations. A description of the irregular membrane-protein interface from MD simulations of protomeric receptors with the coarse-grained Martini force field provided the information on the membrane-protein boundary needed to quantify with 3D-CTMD the energetics of membrane deformation for each system. The specific residues involved in unfavorable polar-to-hydrophobic interactions not alleviated by membrane deformations at each TM were identified from solvent accessibilities in the MD trajectories. We found strikingly different energy costs of hydrophobic mismatch at TMs 4,5 between the beta1 and beta2 adrenergic receptors. In contrast, both kappa and delta opioid receptors exhibited a similar pattern of (small) energy cost around the protein with slightly more pronounced residual mismatch at TM4. These distinct patterns of energy differences indicate how small sequence differences in otherwise homologous GPCRs can affect the mechanisms driving their organization in the cell membrane.

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Free Energy Difference Calculations on Thermodynamic Model of Beta 2 Adrenergic Receptor Activation

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We have developed a thermodynamic model that describes the full agonist activation of the β1 and β2 adrenergic receptors. The activation mechanism of Rhodopsin is well understood, for other class A GPCRs the process is not as well resolved. Rhodopsin combines conformational change with proton uptake by two internal proton switches to achieve a stable active conformation. There is evidence to suggest that the β1 and β2 adrenergic receptors use a similar process. It has been demonstrated that the β2 adrenergic receptor activity increases at acidic pH, the result of Asp134 protonation. Molecular dynamics simulations revealed that deprotonation of Asp79 results in disruption of the ionic lock. Our hypothesis is that a proton transfer from Asp79 to an unknown proton acceptor facilitates the transition from inactive to the intermediate state in our model. This is followed by a conformational change that results in the protonation of Asp134, a transition that stabilizes active state. We tested our hypothesis by performing free energy difference (ΔΔG) calculations from a set of Molecular Dynamics simulations of the β1 and β2 receptors with these Aspartic acid residues occupying different ionization states. These calculations test whether transitions in ionization states of these residues provide favorable energy for activation.

2623-Pos Board B393

Functionally Important Structurally-Specific Homodimerization of the Glucagon Like Peptide 1 Receptor

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The glucagon-like peptide-1 (GLP-1) receptor is an important target for agonist drugs that may be useful in the treatment of type 2 diabetes mellitus. This receptor is a member of class B GPCRs, a group believed to associate with themselves and with each other to form oligomeric complexes. However, the way such complexes might affect the action of these drugs is not known. In the current work, we have studied the ability of GLP-1 receptors to oligomerize and have explored the influence of receptor oligomerization on the effects of both peptide and small molecule agonists that activate this receptor. Bioluminescence resonance energy transfer and bimolecular complementation were used to demonstrate that GLP-1 receptors constitutively form homodimers that were unaffected by occupation with any of these agonists. The lipid-exposed face of transmembrane segment 4 (TM4) was the critical determinant for complex formation, based on observations that competition with TM4 peptide could disrupt such receptor complexes and that TM4 mutants could interfere with the formation of these complexes. The affinities for binding and the potencies for agonist stimulation were lower for the monomeric state than for the dimeric state of this receptor. Treatment with GppNHp shifted the affinity of the dimeric receptor state of the receptor, but not its monomeric state. Negative cooperativity of natural ligand binding was observed only for the dimeric receptor state as well. We also characterized the influence of the dimeric state on signaling activities of various orthosteric and allosteric GLP-1 receptor agonists. The work provides novel insight into the importance of receptor oligomerization on the function of Family B GPCRs.