Additionally, crystallographic studies could not explain the binding of the F3 subdomain to the membrane, which is well-characterized in mutational studies. Utilizing the highly-mobile membrane mimetics, we were able to capture large-scale conformational changes in talin F2F3 which promote the interaction of the F3 subdomain with the membrane via several residues in the β-β loop. This study represents one of the first examples of membrane-induced large-scale conformational changes observed using MD simulations. Using the membrane-bound talin F2F3 subdomain, we then studied how the integrin β3 will be affected by membrane binding of talin. These simulations revealed alterations in the conformation of the F3 subdomain with the membrane via several residues in the membrane-proximal hydrophobic pocket. Additionally, talin F2F3 induces the bending of integrin β3, changing the angle between the cytoplasmic and transmembrane domains by approximately 50°. This buries the conserved β-Asp within the headgroup region, possibly giving a mechanism as to how the z-β salt bridge is broken and consequently how integrin is activated.

Lipopolysaccharide Recognition via Toll-Like Receptor Signaling Complexes: A Large-Scale Simulation Study

Teresa Páramo, Thomas J. Piggot, Peter J. Bond

Toll-like receptors (TLRs) are type I transmembrane glycoproteins that have emerged as key pathogen sensors in the mammalian innate immune response. TLR4 is of particular biomedical interest, because it mediates the response to bacterial lipopolysaccharide (LPS), a potent early indicator of microbial infection and the primary inducer of fatal septic shock syndrome. Unfortunately, subtle alterations in the structure of LPS derivatives can profoundly alter the resultant immunological response, hampering the rational design of TLR4 immunomodulation. Here we show that the sequence-specific structure-activity relationships we have performed long-timescale, all-atom molecular dynamics simulations of the signaling-active receptor complex. The system, which consists of two TLR4 molecules bound to two MD-2 lipid recognition domains, constitutes approximately half a million atoms in solvent. The conformational dynamics of the hetero-oligomer, and essential conserved loops, are highly responsive over timescales of hundreds of nanoseconds in a manner dependent upon the presence of a variety of bound "activating" or "inactivating" ligands, including e.g., hexa-acylated lipid A, the main inducer of the immunological response to LPS; its tetra-acylated precursor lipid IVa, which acts as an agonist in mice but as an antagonist in humans; and Ertrorlan, a strong synthetic antagonist currently undergoing clinical trials. This helps to rationalize how specificity is achieved in the activation of TLR4 signaling pathways. In addition, a novel, spontaneously "collapsed" conformation of the MD-2 fold has been identified in the lipid-free complex, consistent with previously hypothesized "clamshell-like" gating motions in homologous mite allergen proteins. Supported by further simulations of the isolated MD-2 domain, this provides a possible mechanism for ligand gating and the prevention of TLR4 pathway stimulation in the absence of pathogenic signals.

The PKa of Retinal Depends on the Conformation of the Beta-Ionone Ring

Shengshuang Zhu, Scott E. Feller

While much attention has been placed on how methyl substituent groups, charge distribution, and protein environment influence the loss of the proton from the Schiff base in retinal during the phototransduction process, the influence of the conformation of the β-ionone ring has not been explored. Here, we examine the effect of β-ionone ring conformation, defined by the value of the C6-C7 torsion, on the retinal phototransduction process, specifically how the ring conformation influences the pKa value. We have carried out quantum chemical calculations, including a detailed examination of the effect of different basis sets and the inclusion of correlation, to predict the pKa as a function of the C6-C7 torsion angle. The results of the calculations suggest that the pKa value shifts around 2 units as the C6-C7 dihedral changes with the minimum pKa value arising from nonplanar conformations. Our high level MP2 quantum mechanical results also reveal very different torsional potential energy surfaces for the protonated and deprotonated forms of retinal, an observation with important implications for empirical force fields employed in molecular simulations of rhodopsin.

Structural Dynamics of a Signalsome: The Receptor-G protein Complex

Thomas Huhaier, Parag Mukhopadhyay, Thomas Huber, Parag Mukhopadhyay, Thomas Huber

Heptahelical G-protein-coupled receptors (GPCRs) allosterically couple extracellular ligand binding to guanine-nucleotide exchange on intracellular G-proteins. Using the vertebrate visual system, we combined relevant existing structural data to assemble an "signalsome" complex model comprising "active" opsin, GDP-bound transducin and all post-translational modifications solvated in a membrane. In sub-microsecond molecular dynamics simulations, we observe reversible opening of the GDP-binding pocket, which is coupled to conformational changes in the receptor. In particular, transmembrane helix 6 distorts and the rotamer of conserved Trp265 toggles to a new orientation, opening a channel from the hydrocarbon core of the bilayer to the ligand-binding pocket of the receptor. Thermodynamic analysis of the retinal-binding kinetics in rhodopsin-F212A mutant demonstrates its role in the toggle switch. The simulations reveal the mechanism of allosteric coupling of agonist- and nucleotide-binding pockets in a GPCR signalsome complex, and imply supramolecular organization that reconcile existing atomic force and cryoelectron microscopy data.

Metadynamics-Based Mechanistic Interpretation of Functional Crosstalk Between Serotonin 2A and Metabotropic Glutamate 2 receptors

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Serotonin 2A (2AR) and metabotropic glutamate 2 (mGlur2) receptors have been shown to form a functional and specific heterocomplex in mammalian brain and in tissue culture preparations with possible implications in the psychotic symptoms of schizophrenia. Unlike non-antipsychotic drugs, clinically effective antipsychotics (e.g., risperidone) that bind to 2AR increase the glutamate-mediated Gi signaling through the 2AR/mGlur2 heterodimer. The molecular mechanisms underlying these allosteric effects and functional crosstalk are unknown. Using molecular dynamics (MD) simulations enhanced with metadynamics, we investigated at the molecular level the conformational changes induced by atypical antipsychotic or non-antipsychotic 2AR ligands in atomistic representations of interacting 2AR and mGlur2 embedded in an explicit lipid-water environment. First, we sampled the conformational transitions from inactive to active (opsin-like) models of the ligand-free transmembrane regions of 2AR or mGlur2 with adiabatic biased MD simulations. We then reconstructed the free-energy landscape of the 2AR/mGlur2 heterodimer along the pre-determined transition trajectories in the presence of ligands, using a path collective variable approach based on metadynamics. The CHARMM force-field with the CMAP backbone energy correction was used to describe the full systems. All calculations were performed using NAMD enhanced with the Plumed plug-in. Our results suggest that the conformational transitions of 2AR and mGlur2 are populated by different inactive and active metastable states of the receptors, which are differentially stabilized by antipsychotic and non-antipsychotic ligands.

Investigating the Relative Stability of Opioid Receptor Homo- and Heterodimers Using Biased Molecular Dynamics Methods

Jennifer M. Johnston, Davide Provasti, Marta Filizola.

The crucial role of opiates in the clinical management of pain places opioid receptors (ORs) among the most pharmacologically important members of the family A G protein-coupled receptors (GPCRs). A body of evidence, gathered over the last decade, supports the ability of all of the major subtypes of OR, the MOR, DOR and KOR, to form oligomers of homogeneous or heterogeneous composition at the plasma membrane. The discovery of unique functionality for some of these arrangements has shifted the focus of research towards the study of dimers/oligomers of ORs to aid understanding of the fundamental molecular factors governing opiate binding and selective activation of ORs. Using biased molecular dynamics (MD) methods, we aim to determine thermodynamic and kinetic details of the molecular mechanisms underlying homo- and heteromerization of ORs. We recently performed coarse-grained umbrella sampling MD simulations of DOR from Mus musculus in an explicit palmitoyl-oleoyl-phosphatidyl-choline (POPC):10% cholesterol-water environment, in order to estimate the free-energy difference between isolated and interacting protomers, from which, we were able to predict the lifetime of DOR homodimers. Our results indicated that this lifetime was of the order of a few seconds, in rough agreement with recent observations from single molecule fluorescence experiments on other rhodopsin-like GPCRs. Here, we extend this investigation to an experimentally supported, physiologically relevant heterodimer of DOR and KOR, which exhibits unique antinociceptive signaling properties that differ from those of the individual protomers. We investigated the relative stability of DOR/KOR dimers utilizing a combination of umbrella sampling and metadynamics methods, and compared the results with those obtained for homodimers. Our results suggest that the dimer stability varies depending on the protein sequence at the interface, and offer a route to testable prediction of dimerization-disrupting mutations.