

a complex formation between activated G α and the chemoattractant-bound receptor. The complex formation caused the increase in the membrane resident time of individual active G α subunits and therefore the accumulation of G α at the membrane region that is stimulated by a focal chemoattractant. Such a mechanism positively retains the gradient signals from the receptor leading to the signal transmission into the downstream pathways.

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Association of Thromboxane A2 Receptor with Endoplasmic Reticulum-Membrane Spanning Proteins

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Human thromboxane A2 receptor (TP) density is significantly increased in atherosclerotic coronary arteries. The elevated TP protein levels in the diseased vasculature could be due to the increment of the TP protein synthesis/traffic. To reveal the mechanisms that regulate TP synthesis/traffic, we first analyzed its subproteome in TP-transfected HEK293T cells. TP immunoprecipitates were separated by SDS-PAGE and bands at 37, 65 and 110 kDa were excised for trypsin digestion and LC/MS/MS analysis. A group of endoplasmic reticulum (ER)-membrane spanning proteins were identified in complex with TP in transfected cells (n=3) but not in non-transfected cells (n=3). These proteins include phosphatidylinositol phosphatase SAC1 (SAC1), Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit STT3A (STT3A) and Dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 2 (RPN2). Six peptides of SAC1, 4 peptides of STT3A and 6 peptides of RPN2 were detected. Next, co-immunoprecipitation experiments showed that TP was able to pull down c-Myc tagged SAC1 (c-Myc-SAC1) in HEK293T cells coexpressing TP and c-Myc-SAC1 but not when TP or c-Myc-SAC1 were transfected alone (negative controls). The dynamic localization of SAC1 in the ER and Golgi apparatus regulates protein secretion from the Golgi apparatus in response to proliferating signals, while STT3A and RPN2 are components of the oligosaccharyltransferase complex, which catalyzes co-translational N-glycosylation and mediates protein translocation across the ER membrane. Thus, SAC1 likely aids TP secretion and trafficking to the plasma membrane of cells undergoing proliferation, and STT3A and RPN2 may be involved in TP Asn4- or Asn16-glycosylation which are required for TP expression. In summary, proteomic analysis identified ER membrane-spanning proteins that form macromolecular complexes with TP. Co-immunoprecipitation experiments confirmed TP and SAC1 association. Association of TP with ER-membrane spanning proteins may be involved in the regulation of TP synthesis, modification and trafficking in the proliferating vasculature. Supported by NIH.

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Structure and Dynamics of Helix-8 in GPCR-PDZ-Domain Interactions

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The interaction of GPCRs with PDZ-domain-containing proteins is essential for signaling. Helix-8 (H8) is a structurally conserved amphipathic helical motif in class-A GPCRs, adjacent to the C-terminal sequence that is responsible for PDZ-domain-recognition. To understand the role of H8 in molecular level mechanisms of GPCR/PDZ interactions we investigated the interaction of the PDZ-containing GIPC1 protein with the dopamine D2 receptor (D2R) in homology models of the systems based on the X-ray structures of very closely related analogs: the D3R, and the PDZ domain of GIPC2, respectively. The 5 C-terminal residues of D2 receptor's relatively short C-terminal stretch next to H8 was docked into the GIPC1-PDZ model using a simulated-annealing-based PDZ docking scheme. On the resulting GIPC1-D2R complex we carried out several regular MD simulations in sphingolipid/cholesterol membranes to determine the stability of the complex. The results show that the free carboxyl group of C-terminus of the D2R preferred to sample the space around the main-chain atoms of the conserved GFGL motif located on the carboxylate-binding loop of the GIPC1-PDZ. We investigated the relation between distance of H8 from the membrane and its secondary structure by metadynamics MD simulations and found that as the H8 moved away from the membrane, it became energetically easier for the helix to unravel into an extended conformation that is the canonical conformation for interaction with PDZ-domain. Because the C-termini of GPCRs are palmitoylated, we also investigated effect of palmitoylation on the structure and dynamics of H8 in the context of its role in repositioning H8 for the interaction with the PDZ domain. We find that in the palmitoylated C-terminus the H8 backbone penetrates deeper into the membrane, whereas de-palmitoylation renders it more accessible to the cytoplasm where it can interact with the PDZ domain-containing proteins.

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Molecular Dynamics Simulations of Transmembrane and Juxtamembrane Domain of EGFR and its Interaction with Membrane

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The juxtamembrane (JM) domain of the EGFR is crucial for receptor activation and also plays an important role in regulation of the kinase domain (Hubbard, 2004). The JM region starts immediately after the TM domain, at residue Arg645, referred to as JM-A (residues 645-663) and JM-B (residues 664-682). It is suggested that the JM-A region forms an antiparallel dimer and interacts with negatively charged lipids in the adjacent lipid bilayer. However its precise orientation and involvement in the activation mechanism is not clearly understood.

In this study, we employ coarse grained molecular dynamics (CG-MD) simulations to investigate the behaviour of the EGFR TM-JM domain dimer in various membrane environments. Our simulations reveal that the JM region interacts favourably with charged lipids, and in particular forms a high number of contacts between positively charged residues in the JM-A and anionic lipid headgroups. The resulting CG models were refined further using atomistic simulations. We have also extended this study to investigate the behavior of the juxtamembrane region in the presence of extracellular and kinase domain in order to gain more insights into the orientation of the JM region and its possible involvement in EGFR activation mechanism.

S.R. Hubbard, "Juxtamembrane autoinhibition in receptor tyrosine kinases."

Nature reviews. Molecular cell biology, vol. 5, Jun. 2004, pp. 464-71.

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Deciphering Metastable EphA1 Transmembrane Helices Association using PMF Calculations and Multiscale Molecular Dynamics Simulations

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The association of transmembrane helices plays a significant role in the signaling of receptor tyrosine kinases. Recently the structures of the transmembrane domain of EphA1, which is a receptor tyrosine kinase implicated in a variety of cancers, were obtained via NMR spectroscopy [1]. Recent studies present the existence of multiple possible interaction modes for EphA1 which has lead to the suggestion that this system is governed by the rotation-coupled activation mechanism, with packing via different interaction motifs corresponding to active and inactive states of the dimers.

To investigate further these results we performed potential of mean force (PMF) calculations for the association of the transmembrane helices of the receptor tyrosine kinases EphA1 using coarse-grained (CG) molecular dynamics calculations. The resulting profiles suggest two stable or metastable states for this system, consistent with the idea of a rotation-coupled activation mechanism. The most stable state for EphA1 involves a right-handed dimer interacting via an N-terminal glycine zipper motif, consistent with a recent structure from NMR spectroscopy. Interestingly the second state involves a left-handed dimer with interacting residues different from the glycine zipper. Analysis of unrestrained CG molecular dynamics based on the NMR structure of this dimer seems correlate these two states. The final analysis based on all atom simulations for the most representative structures of CG MDs give also valuable details for these interactions. So, multiscale molecular dynamics and PMF calculations shed a new light of the dynamical behaviour of such a system.

Reference:

1.Bocharov EV, Mayzel ML, Volynsky PE, Goncharuk MV, Ermolyuk YS, et al. (2008) Spatial structure and pH-dependent conformational diversity of dimeric transmembrane domain of the receptor tyrosine kinase EphA1. J Biol Chem 283: 29385-29395. doi:10.1074/jbc.M803089200.

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Structural Modeling of Human Growth Hormone Receptor using Computational Simulations and NMR Spectroscopy

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Growth hormone receptor (GHR) is a central component of growth hormone (GH) induced signaling pathway. It is engaged in various cell functions related to growth and metabolism. Despite its crucial role in these functions, knowledge of its structure remains elusive, especially the transmembrane (TM) domain that bridges extracellular signals to the cytosolic downstream recipients. We modeled the TM domain structures of the active and inactive receptors using temperature replica-exchange simulations and further tested their