Membrane Receptors and Signal Transduction I

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Activation and Drug Design of a Muscarinic G-Protein Coupled Receptor

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The M2 muscarinic acetylcholine receptor is a key G-protein coupled receptor (GPCR) that regulates the human heart rate and contractile forces of cardiomyocytes, and is thus targeted for treating many heart diseases. Here, we have performed long-timescale accelerated molecular dynamics (aMD) simulations and captured activation of the M2 muscarinic receptor at an atomistic level(1). The receptor activation is characterized by large-scale structural rearrangements of the transmembrane helices and conformational changes in the inter-helical salt bridge and hydrogen bond interactions(1, 2). Furthermore, using the aMD simulation-derived structural ensembles that account for the receptor flexibility, we have mapped the receptor surface for druggable allosteric sites(3) and then targeted the extracellular vestibule for designing allosteric modulators. Retrospective docking of known ligands is first carried out to validate the simulation receptor ensembles, followed by prospective docking to predict new allosteric modulators from the National Cancer Institute (NCI) compound library. The computationally selected compounds will be tested using experimental binding and functional assays, which may allow us to discover selective allosteric drugs of the M2 muscarinic receptor.

References

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Allosteric Effects of G-Protein Coupled Receptor Heteromerization: Relevance to Psychosis

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A broad symptom spectrum has been evidenced in psychoses, while nearly 80 years of research has not produced adequate anti-psychotic drugs (APDs). G-protein coupled receptors (GPCRs) in three neurotransmitter pathways are targeted by APDs. Each pathway contains an integral GPCR: dopamine 2 receptor (D2R), serotonin 2A receptor (5HT2AR), and metabotropic glutamate 2 receptor (mGluR2). While each GPCR forms homomers, heteromers of 5HT2AR have been shown to form with D2R or mGluR2. Two-Electrode Voltage Clamp in Xenopus oocytes (Hatcher-Solis, et al., 2014 - submitted) has allowed us to show that lateral allosterism (heteromerization with another receptor) of mGluR2-5HT2AR enhances Gi activity of mGluR2 in response to glutamate, while decreasing Gq activity of 5HT2AR in response to 5HT. However, addition of the second endogenous ligand does not cross-signal to the other receptor. Binding of drugs that changed conformation at one receptor in mGluR2-5HT2AR revealed inverse coupling and cross-signaling. Thus APDs, inverse agonists of 5HT2AR or agonists of mGluR2, restore high Gi over Gq signaling (Fribourg et al., 2011). In contrast to mGluR2-5HT2AR, lateral allosterism of D2R-5HT2AR revealed an increase in both Gi activity of the D2R in response to dopamine and Gq activity of the 5HT2AR in response to 5HT. Furthermore, the endogenous ligands of D2R-5HT2AR cross-signaled, increasing G-protein activity of the other receptor. Like the mGluR2-5HT2AR, agonists cross-signaled, decreasing G-protein activity of the other receptor. We also discovered the above allosterism only resulted from specific amounts and ratios of injected cRNA, presumably reflecting different stoichiometries of each receptor in the heteromer and/or changes in trafficking to the cell surface. These results lead us to hypothesize that the combined allosteric effects evidenced upon GPCR heteromerization are unique for each heteromer and that RNA and protein amounts are critical for signaling through heteromers.

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Microtubules Shape GPCR Spatiotemporal Membrane Organization and Function by Scaffolding Cortical Signaling Hubs

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G-protein coupled receptors (GPCR) activity is precisely regulated in space and time, however the exact molecular mechanisms regulating GPCR localization within the plasma membrane are still poorly defined. Combining single receptor tracking and a diffusion state classification method together with imaging of early downstream signaling events, we explored the activity of two GPCR subtypes, the prostanoid EP2 and EP4 receptors. Although both receptors trigger the Gαs-cAMP pathway, we found that EP4-mediated cAMP production is stronger but transient, whereas EP2 induces shallow but continuous cAMP levels. This is structured by the dynamic localization of slowly diffusing EP4, but not EP2, in elongated areas of the cell membrane that are templated by the underlying cortical microtubule network and allow EP4 to interact with its downstream interactors. These results reveal a new paradigm of dynamic plasma membrane organization based on sub-membraneous microtubules that scaffold hubs for GPCR signaling initiation and attenuation.

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Localization and Dynamics of Beta-Adrenergic Receptor Mediated EGFR Transactivation on Micro-Patterned Surfaces

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Recent evidence suggests that activation of G protein-coupled receptors (GPCR), e.g. the β-adrenergic receptor (βAR), leads to transactivation of adjacent membrane receptors such as the epidermal growth factor receptor (EGFR) [1]. The process of GPCR-mediated receptor tyrosine kinase (RTK) transactivation is highly complex and appears to depend on a number of factors including the cell line, the type and class of the GPCR and the cellular environment. Although a number of molecules have been found to play a role in the mechanisms underlying this process, in-depth knowledge of complete machinery is still lacking.

We used micro-patterned surfaces in combination with Total Internal Reflection Fluorescence (TIRF) microscopy [2,3] to study the localization and dynamics of key molecules involved in β2AR-EGFR transactivation in living cells. The time-resolved co-recruitment of receptors and important second messengers into pre-clustered microdomains in the live cell membrane was visualized. In addition, the mobility of the β2AR, EGFR and downstream molecules was monitored by fluorescence recovery after photobleaching (FRAP) experiments to unravel the underlying molecular dynamics.

GPCR-mediated EGFR transactivation is likely to be an important contributor to the molecular complexity of specific diseases associated with GPCR and EGFR dysregulation such as cancer and cardiovascular diseases. This assay could serve as a platform to identify novel molecular candidates that are involved in this process.

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Membrane Curvature Regulates the Localization of G Protein Coupled Receptors and Ras Isoforms

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The shape of biological membranes (membrane curvature) has been recognized to regulate the binding and thus the spatial localization of soluble proteins containing BAR domains, amphipathic helices or lipid anchors1-3. However, with few exceptions4,5, the overwhelming majority of the experiments that have validated quantitatively sensing of membrane curvature have been performed with model reconstituted systems in vitro6-10, and thus strictly speaking remain inconclusive regarding the biological relevance of these measurements. Here I will discuss unpublished data on two new assays that allow quantitative characterization of membrane curvature in live cells. We exploited these assays to investigate the influence of membrane shape on the localization of transmembrane G protein coupled receptors and the three major isoforms of Ras (K-, N-, H-).

Selected References

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