rotational movement of the transmembrane helix 1 (TM1), switching its interaction partner to a neighboring subunit. However, properties and underlying mechanisms underlying PAC channel desensitization remain to be largely unknown. In this study, we demonstrate that PAC undergoes pH-dependent desensitization upon prolonged acid exposure with patch-clamp electrophysiology. Additional studies with structure-guided mutagenesis identified several residues critical for PAC desensitization, including histidine (H) 98, glutamic acid (E) 94, and aspartic acid (D) 91 at the extracellular extension of the TM1, as well as E107, D109, and E250 at the interface of the extracellular domain (ECD) and transmembrane domain (TMD). Structural analysis and molecular dynamic simulations revealed extensive interactions between residues at the TM1 extension and those at the ECD-TMD interface. These interactions likely facilitate PAC desensitization by stabilizing the desensitized conformation of TM1. Our studies establish a new paradigm of channel desensitization in this ubiquitously expressed ion channel and pave the way for further investigation of its relevance in cellular physiology and disease.

2230-Plat

A LRRC8 chimera with native functional properties is a heptamer with a large lipid-blocked pore

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The volume-regulated anion channel VRAC mediates volume regulatory Cl- and organic solute efflux from vertebrate cells. VRACs are heteromeric assemblies of LRRC8A-E proteins with an undefined, variable, and experimentally uncontrollable stoichiometry. The heteromeric structure of VRACs greatly complicates structure-function analyses. We circumvented this serious limitation by developing homomeric LRRC8 chimeric channels that recapitulate the functional properties of native heteromeric VRACs. Cryo-EM structures of abnormally functioning LRRC8A and non-functional LRRC8D homomeric channels demonstrated that they have a narrow-pore hexameric structure. In striking contrast, the 8C-8A(IL125) chimera comprising LRRC8C and 25 amino acids unique to the first intracellular loop (IL1) of LRRC8A is a heptamer of asymmetrically arranged subunits. Five conformationally distinct structures from a single dataset exhibit diverse subunit arrangements and interactions, especially in the cytoplasmic LRR domains. The narrowest region of the 8C-8A(IL125) pore has a solvent-accessible radius of 4.7 Å. This limiting radius is similar to that estimated for native VRACs and is more than twice that of the limiting radii of homohexameric LRRC8A and LRRC8D channels. The 8C-8A(IL125) chimera is strongly inhibited by DCPIB, similar to that of native VRACs. In contrast, homohexameric LRRC8A channels exhibit grossly abnormal DCPIB pharmacology indicating a non-native pore structure. Within the 8C-8A(IL125) pore, we observe two layers of lipids running parallel to the membrane plane. Gaps between protomers are occupied by lipid molecules providing a potential route for lipids to move in and out of the channel pore. Like native VRACs, 8C-8A(IL125) chimeric channels are activated strongly by cell swelling and low intracellular ionic strength. We suggest that VRACs are heptamers similar to closely related pannexin channels and that movement of lipids into and out of the channel pore plays a central role in channel gating.

2231-Plat

Cotranslational association of mRNA transcripts encoding heteromeric GABA-A receptors

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Type A γ-aminobutyric receptors (GABAR) are pentameric ligand gated ion channels that mediate fast inhibition in the adult brain and are drug targets for barbiturates, benzodiazepines, intravenous anesthetics, and neurosteroids. Consistent with their essential role in regulating neuronal excitability, dysregulation of GABAR activity contributes to anxiety, autism, depression, epilepsy, substance abuse and schizophrenia. GABARs are heteropentamers that can be assembled from α(1-6), β(1-3), γ(1-3), δ, ε, θ and π subunits. The abundance, type, and function of GABARs at synaptic and extra-synaptic sites directly controls the strength of GABAergic transmission. Surprisingly, our understanding of how different GABAR subtypes, each comprising a unique complement of subunits, are assembled is rudimentary. Moreover, how the appropriate levels of these receptors are maintained to control excitatory/inhibitory balance remains unclear. In preliminary experiments, when expressed in HEK293 cells, we find that mRNA transcripts encoding the GABA-A receptor α1, β2 and γ2 subunits are physically associated and can be co-immunoprecipitated with nascent GABAR protein using an antibody against the GABAR α1 subunit suggesting that hetero-oligomeric assembly of GABARs is mediated by a complex comprising the mRNAs encoding each of the subunits. The transcript association occurred only when GABAR subunits were co-expressed and not when lysates from cells independently expressing different subunits were mixed. When the potassium channel, hERG, was co-expressed with GABARs, *hERG* transcript was not co-immunoprecipitated. The GABAR transcript association was also observed when GABARs were immunoprecipitated from mouse brain cortex. Our data suggests a new paradigm for heteromeric assembly of GABARs.

2232-Plat

Elucidating the structure-function of the ionotropic delta receptor in the presence of trans-synaptic partners

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The ionotropic glutamate receptors (iGluRs) are glutamate-gated cationic channels expressed in the brain. They are known for their involvement in learning and memory, behavior, and motor coordination. The delta receptor is a member of the iGluR family due to its structural and sequence similarity. This receptor has been demonstrated to play a role in synaptic long-term depression, learning and memory, and implicated in neurological disorders like autism and schizophrenia. Although CryoEM structures show the formation of a pore-like structure like other iGluRs, the delta receptor does not demonstrate isolated ligand-gated currents like its family members, making it difficult to study their function. Our lab recently demonstrated the delta receptor is capable of glycine-gated activity when the extracellular domains come into closer proximity by using chemical linkers at the amino-terminal domain. Using FRET/FLIM, we further demonstrated that the delta receptor trans-synaptic partners, presynaptic Neurexin1β and soluble cerebellin, are capable of bringing the extracellular domains into closer proximity. Based on these findings, I developed a Neurexin1β dimer complex using two different coiled tags at the carboxyl-terminal domain and tagged them with separate purification tags for co-immunoprecipitation. Using this Neurexin1β dimer complex in conjunction with cerebellin, we are capable of inducing glycine-gated activity in single channel patch recordings. Furthermore, using this Neurexin1β dimer complex, I have started investigating the conformational effects of Neurexin1β on the delta receptor using single molecule FRET, and have begun to uncover the conformational landscape of the extracellular domains when in the presence and absence of glycine and the Neurexin1β/cerebellin dimer complex. The findings obtained from these studies will further elucidate the required structure and conformational changes for delta receptor activity, and reduce the challenges of targeting the ionotropic activity of the delta receptor.

2233-Plat

Identification of residues involved in homotrimeric assembly of the human P2X4 receptor by simulations and site-directed mutagenesis

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P2X receptors (P2XRs) are a family of ATP-gated cation channels with seven members, P2X1-P2X7. P2XRs are involved in numerous physiological and pathophysiological functions and are therefore interesting drug targets. Functional P2XRs are obligate homotrimers or heterotrimers of three identical or homologous subunits, respectively, with each subunit having a large ectodomain flanked by two membrane-spanning helices, TM1 and TM2, and N- and C-terminal intracellular domains.

Although much is known about the structure and biochemical function of P2XR channels, little is known about the stabilization of the trimeric complex and its influence on function. Here, we used molecular dynamics simulations to identify residues involved in the homotrimeric stabilization of the human P2X4R receptor (hP2X4R) and tested the predictions following expression in *Xenopus laevis* oocytes using biochemical techniques (native polyacrylamide gel electrophoresis) and two-electrode voltage clamp electrophysiology. By contact analysis, we identified five groups of multiple interacting residues that

contribute to the overall stability of homotrimeric hP2X4R. Among all contacts evaluated, three predominant couplings become apparent: (1) salt bridges between acidic and basic residues, (2) coupling with aromatic amino acids, (3) coupling of polar amino acids via hydrogen bonds. For several contact sites identified by our simulations, we were able to biochemically and electrophysiologically demonstrate significant structure-stabilizing or functional effects. However, for some of the structure-stabilizing residues, we did not find effects on hP2X4R channel function.

By simulating closed and open structures, we aim to find out whether the contact sites are static or dynamic in nature. These results may help to identify new druggable sites.

2234-Plat

Structural basis for ligand modulation and partial agonism of the 5-HT$_3$R

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Type-3 serotonin receptors (5-HT$_3$Rs) are pentameric ligand gated ion channels that mediate fast synaptic signaling in response to binding of the neurotransmitter serotonin. 5-HT$_3$Rs play a major role in regulating gut motility, secretion, and visceral perception. Hyperactivity of 5-HT$_3$Rs underlies pathologies such as chemotherapy-induced nausea and vomiting, irritable bowel syndrome, depression, anxiety, bipolar disorder, and excessive visceral pain, making it an important drug target. Previous research has resulted in the structures of the 5-HT$_{3A}$R in complex with the agonist serotonin and setrons, 5-HT$_3$R antagonists. Recently, 5-HT$_{3A}$R partial agonists have been proposed to treat 5-HT$_3$R pathologies with less severe side effects than full antagonists. However, the molecular mechanisms of partial agonism by orthosteric ligands of the 5-HT$_3$R are still poorly understood. Here we present structures of the 5-HT$_{3A}$R in complex with two novel orthosteric partial agonists generated from cryo-electron microscopy imaging. Structural stability of the ligand binding poses was assessed by molecular dynamic simulations, and ligand function was assessed by two-electrode voltage clamp in wild type and mutant receptors. Using our ligand-bound models of the 5-HT$_{3A}$R, we propose structure-based mechanisms for the functional differences between orthosteric partial agonists, full agonist, and antagonists.

Platform: Exocytosis and Endocytosis

2235-Plat

Poly-ubiquitin catalyzes endocytosis by promoting liquid-like assembly of early endocytic proteins

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Clathrin-mediated endocytosis is an essential cellular pathway that enables cell signaling and receptor recycling. During endocytosis, dozens of cytosolic proteins come together at the plasma membrane, assembling into a highly interconnected network that drives trafficking vesicle assembly. Recently it has been reported that early endocytic proteins, Eps15 and Fcho1/2, form liquid-like droplets through weak multivalent interactions, which provide a dynamic, catalytic scaffold for assembly of endocytic vesicles. How do cells modulate the stability of this network? Many receptors and endocytic proteins are ubiquitinated, and early endocytic proteins, such as Eps15, contain ubiquitin-interacting motifs (UIMs). Therefore, we examined the influence of poly-ubiquitin on the stability of the early endocytic protein network. In vitro, we found that recruitment of ubiquitin to Eps15 condensates dramatically increased the critical temperature for assembly of Eps15 networks, suggesting that ubiquitination may play an important role in initiating endocytosis. In live cell imaging experiments, we added a deubiquitinase (DUB) domain at the C terminus of Eps15 (Eps15-DUB) to remove ubiquitin from Eps15 and its interaction partners. Expression of Eps15-DUB drove a sharp increase in the frequency of non-productive endocytic events that failed to form vesicles. Interestingly, the fraction of non-productive events in cells expressing Eps15-DUB exceeded that for Eps15 knockout cells, demonstrating the importance of ubiquitination in endocytosis. Collectively, these results suggest that poly-ubiquitination of the endocytic machinery and its target receptors plays an essential role in stabilizing the flexible protein network responsible for catalyzing endocytic events.

2236-Plat

A conformational switch in clathrin light chain regulates lattice structure and endocytosis at the plasma membrane of mammalian cells

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The nanoscale localizations, interactions, and conformations of endocytic proteins are key regulators of clathrin-mediated endocytosis. Although super-resolution microscopy has revealed the detailed organization of endocytic proteins, the resolution of super-resolution imaging cannot assess molecular interaction and conformational changes. Fluorescence resonance energy transfer (FRET) occurs when dyes are separated by less than 10 nm for most fluorescent proteins pairs. Thus, it can be used to map close-range molecular complexes and dynamics. Yet, to understand how endocytosis works, measurements from FRET must be correlated to the distinct stages of endocytosis. To accomplish this, we developed a new correlative lifetime-based FRET (FLIM-FRET) and platinum replica transmission electron microscopy (PREM) method, named FRET-CLEM. Here, FRET-based atomic distances can be mapped directly to individual cellular structures visualized in EM at the plasma membrane. We used this method to measure the conformational changes in clathrin light chain (CLC), a component of the clathrin triskelion and assembled clathrin lattice. CLC conformational changes have been proposed to regulate the assembly of clathrin in solution. However, CLC conformational changes and their effects on clathrin lattice growth, curvature, and endocytosis at the membrane of living cells are unknown. Using FRET-CLEM, we discovered that CLC undergoes a conformational switch as clathrin lattices curve. Preventing this conformational switch with acute chemical tools increased clathrin lattice sizes and inhibited endocytosis. Therefore, a specific conformational switch in CLC regulates lattice curvature and endocytosis in mammalian cells. These new correlative light and EM data will help develop a complete mechanistic model of endocytosis. More generally, FRET-CLEM can map molecular interactions and conformational changes at targeted membrane-associated proteins at identified cellular compartments including exocytic sites and neuronal synapses.

2237-Plat

Shear stress shifts clathrin-coated vesicle formation to favor the flat-to-curved model in endothelial cells

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Endothelial cells (ECs) experience a variety of highly dynamic mechanical stresses. Among others, cyclic stretch and increased plasma membrane tension inhibit clathrin-mediated endocytosis (CME) in non-ECs cells. How ECs overcome such unfavorable conditions and maintain CME remains elusive. Previously, we have used simultaneous two-wavelength axial ratiometry (STAR) microscopy to resolve how clathrin-coated vesicles (CCVs) from in fibroblast-like cells (Cos-7) and human umbilical vein endothelial cells (HUVECs). This revealed multiple productive ways of CCV formation, supporting the flexible model of CME. We next asked whether biophysical stresses generated by blood flow could favor one mechanism of CCV formation to overcome unfavorable environment present in vasculature. Addressing this question required development of an automated MATLAB-based accelerated STAR data processing pipeline (DrSTAR) to enable the processing of multiple experimental conditions and biological replicates in a robust and reproducible environment. Moreover, DrSTAR employs a dynamic local referencing algorithm, which resolves the curvature of long-lasting CCSs. We used this new platform to examine if clathrin dynamics are altered in HUVECs grown under sheer stress. We found sheer stress led to an increase in clathrin dynamics. Surprisingly, we found this was due to an increase of flat clathrin accumulations in flow-stimulated cells, while the number of curved events remained consistent between groups. The curvature-positive events had significantly delayed curvature initiation in flow-stimulated cells, highlighting a shift toward flat-to-curved clathrin transitions in vesicle formation. Moreover, CME was more resistant to increased osmotic pressure in HUVECs grown under shear stress. Overall, our findings indicate that clathrin dynamics and CCV formation can be modulated by the local environment and represents an important regulatory mechanism.

2238-Plat

A dynamic mechanically stabilized membrane reservoir mediates fast endocytosis

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