Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

GlyR PAMs. Here, we present a new fluorescence imaging assay that allows reliable HTS for GlyRs and can be easily adapted for drug discovery by individual research laboratories with affordable instrumentation. Several elements set this new assay apart from traditional fluorescence assays. First, we adopt a new yellow fluorescent protein halide sensor for monitoring GlyR response with improved sensitivity, fluorescence intensity, and halide selectivity to provide more reliable data. Second, we increase transparency and selection rigor in the quantity and quality of the cells contributing to the detection signals to ensure screening reliability and reproducibility. Unlike most commercial instruments that use the average of uncontrolled mixtures, our method takes into consideration signal analysis from only healthy cells meeting *a priori* criteria of normal response to a receptor's orthosteric agonist at EC_{100}. Third, we implement an interleaved data collection method that allows reliable measurements of steady-state fluorescence quenching due to GlyR channel function, but takes only a fraction of the time used in conventional data collection to achieve the same steady state levels. These innovative elements will benefit the development of new glycinergic analgesics, and can also be easily adapted to other screening assays for new therapeutics.

1175-Pos

Using protein semi-synthesis to identify proton-sensing residues in an acid-sensing ion channel

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Acid-sensing ion channels (ASICs) are widely expressed in central nervous system and their activation by protons contributes to synaptic plasticity. Recently, ASICs have gained interest as potential drug targets due to their role in fear and pain processing, ischemic strokes and cardiac pathologies. Detailed functional studies and cryo-EM structures have identified a cluster of protonatable residues in the extracellular domain of the channel, also known as the 'acidic pocket'. However, due to the limited chemical space accessible by conventional mutagenesis, it remains unknown which amino acids within the acidic pocket contribute to proton-mediated activation. In an effort to insert subtle Glu and Asp analogs into the acidic pocket we turned to split intein-mediated protein semi-synthesis. This approach allows insertion of synthetic peptides bearing one or more non-canonical amino acids (ncAAs) between two recombinantly expressed protein fragments through the use of flanking intein sequences. Here, we conduct a small-scale screen to evaluate the efficiency of the splicing reactions in the extracellular domain of ASIC1a with a goal to identify suitable splice sites for the insertion of peptides carrying non-canonical Glu and Asp analogs. Using two-electrode voltage clamp electrophysiology as a means to assess the levels of channel reconstitution, we identify two sites with high splicing yields in recombinant expression experiments. We anticipate this work to provide insights in the mechanism of protonation, as well as binding of other pharmacological modulators of ASICs, such as toxins or neuropeptides.

1176-Pos

Probing the activation mechanisms of a muscle-type acetylcholine receptor using a combination of structural and functional measurements

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Determining the activation mechanisms for the prototypic pentameric ligand-gated ion channel, the muscle-type nicotinic acetylcholine receptor (nAChR), has been a major quest in neurobiology for nearly a century. Energy gained from the binding of agonist to the extracellular domain (ECD) is used to drive the opening of the ion conducting pore in the transmembrane domain (TMD), but the pathway(s) connecting the closed and open states have remained unclear. Functional studies based on medium-resolution structures originally identified critical interactions at the ECD-TMD interface, although the lack of high-resolution structures in multiple conformations prevented direct visualization of the gating process. Recently solved cryo-EM structures of the *Torpedo* nAChR in closed and channel open conformations, however, shed new light on the gating mechanism. In particular, these structures highlight potential interactions between the β8-β9 loop and β10-M1 linker from the complementary δ/γ subunits and the M2-M3 loop from the principal α subunits in coupling agonist induced movements in the ECD to gating movements in the TMD. Using these new structures as a guide, we use mutagenesis and electrophysiology to probe the role of inter-subunit interactions in channel function. Our data suggest that a quartet of polar residues in the αM2-M3 loop interact with residues from both the complimentary β8-β9 loops and β10-M1 linker to preferentially stabilize the activated or resting states, respectively. Together, our data highlight new interactions at the ECD — TMD interface that play a previously unappreciated role in coupling agonist binding to channel gating.

1177-Pos

Functional and structural characterization of a novel pH-modulated open state of a pentameric ligand-gated ion channel

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Pentameric ligand-gated ion channels are critical mediators of electrochemical signal transduction in a variety of excitable cells. Biophysical and pharmacological development in this family relies heavily on high-quality structural data in multiple distinct functional states. Bacterial family members could provide valuable insight into conducting states, as they often desensitize to a lesser extent than eukaryotic channels. Recently, a new prokaryotic family member (DeCLIC) from a Desulfofustis deltaproteobacterium was identified, including a previously uncharacterized N-terminal domain. The channel was found to be nonconductive by electrophysiology in the presence of Ca^{2+}, while in the absence of Ca^{2+}, it conducted ions and crystallized with a dramatically expanded pore. The functional correlates of these distinctive structures, and their relevance to eukaryotic family members, remained unclear. Here, we report enhancement of DeCLIC currents at low pH, a common modulator of pentameric channels. Furthermore, we used cryo-electron microscopy to identify multiple conformations of the channel under acidic conditions. When Ca^{2+} was present, one class of particles was similar to the crystallographic closed state, while a second class represented an apparent open state notably divergent from the open X-ray structure. When Ca^{2+} was absent, the predominant class had a similar open pore, with evidence of dynamic rearrangements in the N-terminal domains. These data provide new insight into pH modulation and the under-characterized open state of pentameric channels, as well as a novel mechanism of dynamic ion-channel regulation via an N-terminal module.

1178-Pos

cryo-EM and small-angle scattering of a pentameric ligand-gated ion channel reveals a dynamic regulatory domain

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Pentameric ligand-gated ion channels (pLGICs) perform electrochemical signal transduction in organisms ranging from bacteria to humans. Among the prokaryotic pLGICs there is an architectural diversity involving N-terminal domains (NTDs) not found for the eukaryotic relatives, exemplified by the calcium-sensitive channel DeCLIC. Here, we characterized DeCLIC structure using cryogenic electron microscopy (cryo-EM), small-angle neutron scattering (SANS), and molecular dynamics (MD) simulations. In both the presence and absence of calcium, cryo-EM reconstructions were similar to a previously reported calcium-bound x-ray structure. The NTDs exhibited lower local resolution than the canonical unit, consistent with these domains being relatively mobile. The small-angle scattering profile revealed a feature not explained by the available structures, indicating that further conformational diversity is available to DeCLIC. MD simulations indicated that this profile is largely attributable to rigid-body motions of the NTDs relative to the protein core, including conformations similar to those in experimental structures, as well as more expanded and asymmetric conformations. Using these expanded conformations, it was possible to fit the previously unexplained SANS feature, indicating the presence of such conformations under solution conditions. This work reveals the range of motion available to the DeCLIC NTDs, expanding the conformational landscape of the pLGIC family; and demonstrates the power of combining low-resolution, high-resolution, and simulations data in the study of protein structure.

1179-Pos

Impact of SARS-CoV-2 spike protein on α7 nicotinic acetylcholine receptor in cells

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SARS-CoV-2 infection relies on its Spike glycoprotein (S) binding to ACE2 in the host cells. S may also interact with other receptors to cause cellular damage. The α7 nicotinic acetylcholine receptor (α7nAChR), a primary component in the cholinergic anti-inflammatory pathway and a modulator of coagulation, has been proposed as a target of S. However, experimental evidence for

full-length S binding to α7nAChR remains to be substantiated. Here we report our study of S/α7nAChR interactions using fluorescence imaging of cells. HEK293T/17 cells were transfected with a construct expressing α7nAChR and ZsGreen from the same plasmid, with or without co-transfection of a plasmid expressing soluble S (HexaPro, Addgene). Effects of S expression on functional α7nAChR were detected using fluorescent α-bungarotoxin (Invitrogen). We observed a dramatic reduction in bungarotoxin binding to α7nAChR in cells co-expressing S. These cells did not show significant change in the fluorescence of ZsGreen expressed from the same plasmid as α7nAChR, suggesting that plasmid competition was unlikely a mechanism to cause the reduction of α7nAChR for bungarotoxin binding. Whether the observed reduction of bungarotoxin-bound α7nAChR resulted from S interference in α7nAChR maturation to the cell surface and/or from competitive S binding to α7nAChR is still under investigation. These new findings may have significant implications for understanding of SARS-CoV-2 pathology.

1180-Pos

Glycine or D-serine activation of delta subtype of ionotropic glutamate receptor

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Delta receptors are members of the ionotropic glutamate receptor superfamily as they have a similar overall structure, arranged as dimer of dimers, with each subunit having extracellular amino terminal domain and agonist binding domain, transmembrane segments and intracellular domain. Unlike the other members of the family no ligand gated ion channel activity has been observed in the delta receptors. Delta receptors are hence thought to play a structural role by forming trans-synaptic connections through interactions with cerebellin-1 and pre-synaptic transmembrane protein neurexin-1β. They are also thought to mediate secondary messenger signaling upon binding agonist glycine and D-serine. We show that delta receptors can mediate ionotropic currents similar to the other members upon binding agonist glycine and D-serine. Such currents are only observed when the receptors are interacting with cerebellin-1 and neurexin-1β by cell to cell interactions. Using fluorescence lifetime imaging and chemical cross linkers we establish that this agonist mediated ionotropic currents are possible due to cerebellin-1 and neurexin-1β acting as biological cross-linkers stabilizing the extracellular domain in close contact, allowing for the agonist mediated conformational change to be transmitted to the channel segments.

1181-Pos

Lsm12, an NAADP receptor, is also a PI(3,5)P_2-competitive antagonist of human two pore channels

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Human two pore channels (TPCs) are endolysomal cation channels that regulate endomembrane dynamics and are involved in NAADP signaling and many pathological processes including virus infection and fat liver. TPCs are activated by two endogenous signaling molecules, NAADP and PI(3,5)P_2. We recently identified Lsm12 as an NAADP receptor and a TPC regulatory protein required for NAADP-induced TPC activation and calcium mobilization from acidic organelles. In contrast to its NAADP-dependent activating effect on TPCs, we here report that Lsm12 is a PI(3,5)P_2-dependent antagonist of TPCs. With inside-out patch-clamp recording of TPC2 and TPC1 mutant channels, we observed inhibition of PI(3,5)P_2-induced TPC2 and TPC1 currents upon perfusion of purified Lsm12 protein on the intracellular side. Preapplication of Lsm12 before application of PI(3,5)P_2 alone also led to reduced efficacy of PI(3,5)P_2 in TPC2 activation, suggesting that Lsm12 acts on TPC2 rather than on PI(3,5)P_2. Lsm12 inhibited PI(3,5)P_2-induced TPC2 currents in a protein and PI(3,5)P_2-concentration dependent manner. Schild regression analysis showed that Lsm12 is PI(3,5)P2-competitive antagonist of TPC2 channel. We further tested the effects of Lsm12 on TPC activated by other agonists. Interestingly, the Lsm12 inhibition occurred similarly with TPC2 activation by TPC2-A1-P; however, Lsm12 had no inhibitory effect on the TPC2 currents when the channels were activated by either the constitutively-open mutation or TPC2-A1-N. Thus, the inhibitory effect of Lsm12 is specific to channel activation by PI(3,5)P_2 or TPC2-A1-P. Furthermore, with cell-attached patch-clamp recording, we found that Lsm12 expressed at endogenous level was able to produce significant inhibitory effect on TPC2 when activated by TPC2-A1-P. We concluded that Lsm12 is a PI(3,5)P_2-competitive antagonist of TPC2 channels. Overall, Lsm12 plays a dual role in TPC channel regulation by modulating the efficacy of the channels' two endogenous ligands in opposite directions.

1182-Pos

Atomic models from low resolution maps with density-guided MD simulations

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Ion channels, transporters, and receptors are some of the most important pharmaceutical targets in our body. Their whose physiological role is tightly coupled to their ability to cycle through various functional states with high fidelity. Though membrane proteins have historically been recalcitrant to structural studies, the 'resolution revolution' of cryoEM has opened the floodgates, leading to a rapid influx of structures for previously unseen targets. These structures have revealed the architecture of many exemplar channels, transporters, and receptors. However, the characterization of the full conformational cycle is often incomplete, and only the most stable states are resolved to high- enough resolution for atomic modeling (<3.5 angstroms). Improvements in single-particle analysis have increased our capabilities to extract electron density maps for multiple conformations from a single dataset, but often the density maps for lesser populated states remain too low for manual atomic modeling (4-6 angstroms). Here, we assess the potential of a new density-guided molecular dynamics (MD) simulation approach to generate atomic models from these low-resolution maps in cases when an atomic model is already available in a different conformational state. Using maps filtered or decimated to ∼5 angstrom resolution, we show that density-guided simulations are often capable of rescuing the high-resolution features that were removed. Thus, density-guided MD is a promising approach to extract atomistic detail for conformational states that do not yield atomic resolution maps in cryoEM. The resulting atomic models can be used for further studies with MD simulations or to guide mutagenic studies to define the role of such states in the overall functional cycle.

1183-Pos

Concanavalin A biding to homomeric GluK2 receptor leads to closer proximity of the extracellular domains

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Kainate receptors belong to the family of ionotropic glutamate receptor family and are important in excitatory neuronal transmission within the central nervous system. Glutamate binding to the agonist biding domain of kainate receptors leads to a fast-activating cation-specific transmembrane channel, however, the stress caused by glutamate biding is relieved quickly, leading to fast-desensitization. Concanavalin A (Con-A), a lectin, is known to reduce the extent of desensitization and, therefore, increase the active open-channel state in kainate receptors. However, the specific mechanisms at which Con-A modulates kainate receptors remained unknown. Using single-molecule fluorescence resonance energy transfer (smFRET), we investigated the conformational changes occurring in kainate receptors in the presence of Con-A. Our findings show that Con-A binding leads to closer proximity of the amino-terminal domain dimer-dimer interface and the agonist-binding domain dimer interface. Furthermore, the modulation of Con-A on kainate receptor is independent of Na^+, which is known to bind and stabilize the dimer interface of the agonist-biding domain. These results lead us to conclude that Con-A causes a tighter packing of the extracellular domains of kainate receptor, therefore, driving the conformation to active-open state.

1184-Pos

Allosteric control of NMDA receptor calcium permeability by extracellular protons

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Excitatory neurotransmission in the central nervous system is primarily mediated through the activation of ionotropic glutamate receptors (iGluRs). Among iGluRs, N-methyl-D-aspartate receptors (NMDARs) are unique in their ability to pass large Ca^{2+} currents, which are essential to the normal physiology and development of excitatory synapses. Classically, the content of Ca^{2+} current passed by NMDARs has been viewed as a static property of the receptor. However, recent evidence shows that extracellular protons, which are strong negative allosteric modulators of NMDA receptors, are capable of also modulating