sites or domains required for either modulation. Robust oxidative modulation is also observed in receptors that enter the desensitization state after prolonged exposure to capsaicin in the presence of extracellular Ca2+. We show that this modulation can be fully recapitulated in the excised inside-out membrane patches under the divalent cation free condition, ruling out the involvement of major protein or lipid phosphorylation pathways. Pretreatment with the cysteine-reactive alkylating agent maleimide blocks the modulation, while cysteine oxidizing chemicals produce pronounced sensitization and occlude each other's effects. The effect of oxidation on channel activity can be reversed by application of strong reducing agents. By constructing receptor chimeras and point cysteine mutations, we identified multiple cysteines required for full modulation of TRPV1 by oxidative challenges. We conclude that the oxidative modulation is a robust mechanism to tune TRPV1 activity via covalent modification of conserved cysteine residues across different species and may play a role in pain sensing process during inflammation, infection or tissue injury.

2020-Plat

PKCßII-Specific Phosphorylation Counteracts Regulation Of Trpv6 By ATP And Points Towards A Functional Difference Between Its Polymorphic Alleles

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The calcium selective ion channel TRPV6 shows a highly unusual evolution of one of its polymorphic alleles which might have conferred a selective advantage during migration of humans out of Africa. Because the ancestral allele contains an additional PKC consensus site, we analyzed regulation by intracellular ATP and phosphorylation. We found that ATP prevents run-down of TRPV6-mediated currents and have mapped a relevant site for regulation by ATP to the finger loop between ankyrin repeat domains (ARD) 3 and 4. Stimulation of PKC preserves run-down even in the presence of ATP and uncovers a difference between the alleles. Using different inhibitors and isoforms of PKC, we show that regulation requires $PKC_{\beta II}$, which is able to phosphorylate the channel. Site-directed mutagenesis shows that phosphorylation sites within the ARD and also within the C-terminus are necessary to confer the effect. We propose a model where ATP stabilizes the channel by tethering the C-and N-termini of the subunits together. $PKC_{\beta II}$ disrupts this interaction and yields channels susceptible to inactivation and run-down.

2021-Plat

Ca^{2+} Activates TRPM2 Channels By Binding In Deep Crevices Near The Pore, But Intracellularly Of The Gate

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TRPM2 is a tetrameric Ca^{2+} permeable channel involved in immunocyte respiratory burst and postischaemic neuronal death. In whole cells TRPM2 activity requires intracellular ADP ribose (ADPR) and intra- or extracellular Ca^{2+}, but the mechanism and the binding site(s) for Ca^{2+} activation remain unknown. Here we study TRPM2 gating in inside-out patches while directly controlling intracellular ligand concentrations. Concentration jump experiments at various voltages, and Ca^{2+} dependence of steady-state single-channel gating kinetics, provide unprecedented insight into the molecular mechanism of Ca^{2+} activation. In patches excised from *Xenopus* oocytes expressing human TRPM2, co-application of intracellular ADPR and Ca^{2+} activated ~50-pS non-selective cation channels; $K_{1/2}$ for ADPR was ~1μM at saturating Ca^{2+}. Intracellular Ca^{2+} dependence of TRPM2 steady-state opening and closing rates (at saturating [ADPR] and low extracellular Ca^{2+}) reveals that Ca^{2+} activation is a consequence of tighter binding of Ca^{2+} in the open- than in the closed-channel conformation. Four Ca^{2+} ions activate TRPM2 with a Monod-Wymann-Changeux mechanism: each binding event increases the open-closed equilibrium constant ~33-fold, producing altogether 10^6-fold activation. Experiments in the presence of 1mM free Ca^{2+} on the extracellular side clearly show that closed channels do not sense extracellular Ca^{2+}, but once channels have opened Ca^{2+} entering passively through the pore slows channel closure by keeping the ''activating sites'' saturated, despite rapid continuous Ca^{2+}-free wash of the intracellular channel surface. This effect of extracellular Ca^{2+} on gating is gradually lost at progressively depolarized membrane potentials, where the driving force for Ca^{2+} influx is diminished. Thus, the activating sites lie intracellularly from the gate, but in a shielded crevice near the pore entrance. Our results suggest that in intact cells which contain micromolar ADPR even brief Ca^{2+} spikes likely trigger prolonged, self-sustained TRPM2 activity.

2022-Plat

X-ray Crystal Structure Of A Trpm Assembly Domain Reveals An Antiparallel Four-stranded Coiled-coil

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Transient receptor potential (TRP) channels comprise a large family of tetrameric cation-selective ion channels that respond to diverse forms of sensory input. Previous studies have shown that members of the TRPM subclass possess a self-assembling tetrameric C-terminal cytoplasmic coiled-coil domain that underlies channel assembly and trafficking. Here, we present the high-resolution crystal structure of the coiled-coil domain of the channel enzyme TRPM7. The crystal structure, together with biochemical experiments, reveals an unexpected four-stranded antiparallel coiled-coil architecture that bears unique features relative to other antiparallel coiled-coils. Structural analysis indicates that a limited set of interactions encode assembly specificity determinants and uncovers a previously unnoticed segregation of TRPM assembly domains into two families that correspond with the phylogenetic divisions seen for the complete subunits. Together, the data provide a framework for understanding the mechanism of the TRPM channel assembly and highlight the diversity of forms found in the coiled-coil fold.

Platform AR: Membrane Protein Function

2023-Plat

Simultaneous Monitoring The Two Rotary Motors Of A Single F_OF_1-ATP Synthase

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Synthesis of ATP from ADP and phosphate is performed by a stepwise internal rotation of subunits of the enzyme F_oF_1-ATP synthase. The bacterial enzyme also catalyzes ATP hydrolysis. The opposite direction of rotation during ATP synthesis and hydrolysis was confirmed by single-molecule fluorescence resonance energy transfer, FRET, using specific labeling of the rotary subunits γ or ϵ in the F_1 motor and the stator subunits [1-3]. The step size in the F_1 motor was 120°. In contrast the step size during proton-driven rotation of the c subunits in the F_o motor was 36° using single-molecule FRET. FRET artifacts could be minimized by 'duty cycle optimized alternating laser excitation'. As the two coupled motors of F_oF_1-ATP synthase showed apparently different step sizes, this mismatch has to be unraveled by mapping the contributions of rotor and stator subunits for transient energy storage. We present the simultaneous observations of F_1 and F_o motor rotations using a single-molecule triple FRET approach, which indicate elastic deformations of the rotor between ϵ and c subunits during ATP hydrolysis as well as synthesis.

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2024-Plat

Tracking Single Protein Translocation Complexes In The Membranes Of Living Bacteria

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The Twin Arginine Translocation (Tat) system transports fully folded en sometimes even oligomeric proteins across the inner membrane of bacteria. Its mechanism is largely unknown. Remarkably, a stable translocation complex has not been observed. Instead, the three components of the system, i.e., TatA, TatB and TatC, are isolated from the membrane of Escherichia coli in various complexes of different sizes, which suggests that a complete and active Tat complex is formed only transiently. We have used single particle tracking in living bacteria to gain more insight into the dynamics of the Tat proteins. TatA has been genetically fused tot enhanced Green Fluorescent Protein (eGFP). Living bacteria expressing low levels of TatA-eGFP have been immobilized on glass slides and imaged with a sensitive wide-field fluorescence

microscope. Mobile fluorescent spots are observed, and their intensity and location have been tracked by fitting a 2D Gaussian function to successive frames. Analysis of the data shows that diffusion of TatA-eGFP is heterogeneous, and that the average diffusion coefficient of fluorescent TatA particles decreases when excess substrate is expressed. The latter suggests that TatA forms larger complexes upon substrate binding.

2025-Plat

Opening the SecYEG Protein Translocon

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The Sec61/SecYEG translocon is the central component of the major biosynthetic pathway for membrane and secreted proteins. To incorporate membrane proteins into the endoplasmic reticulum membrane of eukaryotes (Sec61), or into the plasma membrane of prokaryotes (SecYEG), the translocon relies on the opening of a lateral helical gate formed by transmembrane (TM) helices TM2 and TM7. The crystallographic structure of the closed state of the translocon (B. van der Berg et al, Nature 427, 36-44, 2003) solved in the absence of bound signal peptide provides valuable information on possible scenarios of membrane protein insertion. Nevertheless, the sequence of structural rearrangements that leads to the opening of the translocon and the geometry of the translocon in its open state remain unknown. To derive information on the structure and dynamics of possible open conformations of the SecYEG translocon, we performed prolonged molecular dynamics simulations of the SecYEG translocon in which we mutated residues determined to participate in stabilizing interactions of the closed state. We have also performed a simulation of the SecYEG translocon with a bound signal peptide. The computations provide insights into interactions essential for keeping the lateral gate closed and how perturbations of these interactions cause rearrangements of the gate.

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2026-Plat

Real Time ^{31}P and Steady State ^{19}F/^{13}C Solid State NMR on Integral Membrane Protein *E.coli* Diacylglycerol Kinase

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The integral membrane protein Diacylglycerol Kinase (DGK) from *E.coli* can be used as model system for prokaryotic kinases, playing an important role in microbial physiology. In its active conformation, DGK, consisting of three transmembrane helices, forms a homotrimer with one putative active site per monomer. DGK, as the smallest known kinase, catalyses the phosphorylation of diacylglycerol to phosphatidyl acid by utilizing MgATP at the interface membrane-cytoplasm. These features make DGK an attractive model protein for research on structure of membrane proteins in lipid bilayers, as well as on its function as a lipid regulator.

Solid-state NMR is a unique tool for the investigation of membrane proteins in their native environment and for probing enzymatic reactions regardless of their compartmentalization. For such experiments, amount of protein and quality of the sample preparation are crucial. Expression, purification, reconstitution and sample preparation were optimised, so that a sample of DGK in high quality became available in amounts necessary for ssNMR experiments, while maintaining its specific activity.

A ^{31}P Real Time MAS experiment was designed and implemented, to investigate for the first time, simultaneously, ATP hydrolysis and phosphorylation of a substrate analog by a membrane protein, inside the lipid bilayer. From these data, the rate constants of enzymatic activity were determined. Furthermore, inhibition experiments with orthovanadate, $(BeF_2)_x$ and AlF_3 were carried out. The inhibiting species was identified by ^{19}F MAS NMR. This method provides the opportunity to investigate the enzymatic mechanism in real time, with atomic resolution.

A first fingerprint of structural details were obtained with a 2D ^{13}C-^{13}C MAS ssNMR PDSD type experiment on a uniformly ^{13}C/^{15}N labelled sample of a thermostable mutant- and WT-DGK. Further insight into structural details will be obtained by selective labelling.

2027-Plat

BetP - Structure and Function of an Osmosensor and Transporter

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The Na+ coupled betaine uptake system BetP of Corynebacterium glutamicum comprises three functions of stimulus (osmostress) sensing, activity regulation and solute transport. Its 2D structure was recently solved by electron and its 3D structure by X-ray crystallography. Within a trimeric structure, each BetP monomer harbours both an N- and a C-terminal domain involved in stimulus sensing and intramolecular signal transduction. Factors contributing to the sensory and regulatory function of BetP are (i) the two terminal domains, (ii) K+ ions as an osmostress related stimulus, and (iii) interaction with the surrounding membrane.

We used several techniques to analyze the contribution of the terminal sensory domains to BetP function. By scanning mutagenesis we identified the significance of single amino acids and parts of the C-terminal domain of BetP. EPR spectroscopy was applied to determine the mobility or the C-terminal domain under different functional conditions and to measure intra- and intermolecular distances in BetP. The 3D structure of BetP finally revealed a putative crosstalk between the three monomers of BetP via their C-terminal domain. On the basis of these results we suggest a novel functional model of the terminal domains of BetP during its sensory and regulatory function.

2028-Plat

Atomic Force Spectroscopy Measures Light Activation And Transducer Binding Induced Structural Changes In The Sensory Rhodopsin II

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Rhodopsins are a family of seven-helical transmembrane proteins responding to light. Sensory rhodopsin II (SRII) triggers two very different responses depending on the presence or absence of its transducer: Whereas light activation of the SRII-transducer-complex triggers a signalling cascade initiating the photophobic response of the bacterium, SRII alone acts as a proton-pump.

Using single molecule force spectroscopy we analysed the stability of SRII in dark and after light activation as well as in presence and absence of the transducer, which revealed a distinct pattern of changes in the protein stability. By improving the force spectroscopic data analysis we were able to predict the localisation of occuring forces within the protein chain with a resolution of about six amino acids.

Different regions showed up, where secondary structure elements of SRII are selectively stabilised or weakened by either light activation or transducer binding or both. Independent of the presence of the transducer light activation has a destabilizing effect in the middle of α-helix G. This suggests a loss in interactions between helices G and F, which would allow an outward tilt of α-helix F as previously observed. Additionally, the unfolding curves show an increased number of rupture events in the region of helix F upon transducer binding, which is most likely due to the formation of several interactions between α-helix F and TM2 of the transducer. Most interestingly, we found a loss of some of these interactions upon light activation, which might explain transducer activation and help to answer remaining questions concerning the precise molecular mechanism. Finally, in the absence of the transducer, destabilizing effects are observed at the cytoplasmic half of helix G, which might indicate its importance for the proton-pumping properties of SRII.

2029-Plat

Conformational Dynamics of PorB, a Helical Outer Membrane Protein from C. glutamicum: a Multi-scale MD Simulation Study

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PorB is one of the major porins found in the cell wall of C. glutamicum. Somewhat surprisingly, for an outer membrane protein, the recently determined X-ray structures of PorB monomers revealed a helical topology. A model for the pentameric protein was proposed base upon (1) the required polar interior and nonpolar exterior of the porin, (2) a recurring crystal packing contact around a 2-fold axis, (3) the assumption of a simple Cn symmetry, (4) the experimentally established electric conductivity and anion selectivity and (5) the generally observed shape of porin channels. The presence of divalent cations was thought to be required to balance the charged protein residues.

We have employed MD simulations to study the conformational dynamics of the putative PorB model. We have performed multiple, atomistic simulations of the protein in the presence and absence of divalent cations. Our simulations have allowed us to study specific protein-ion interactions and thus to speculate on the role of these ions. The structural role of the interdigitation of the N-terminal and C-terminal extensions has also been studied in detail. In addition, we have studied the conformational dynamics of the PorB pore and compared its flexibility to similar (in size) pores of barrel-shaped outer membrane proteins. We have employed coarse-grained MD simulations to determine the preferred location and orientation of PorB in phospholipid bilayers.