2832-Plat
Identification of a Tetrameric Assembly Domain in the C-Terminus of Heat-Activated TRPV Channels
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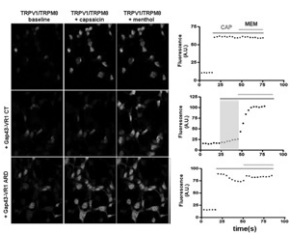
Transient receptor potential channels (TRPs) as cellular sensors are thought to function as tetramers. Yet, the molecular determinants governing homotetramerization of heat-activated TRPV1-4 remain largely elusive. In this study, we identified a segment comprising 20 amino acids after the known TRP-like domain in the channel C-terminus that functions as a tetrameric assembly domain (TAD). Purified recombinant C-terminal proteins of TRPV1-4, but not the N-terminus, mediated the protein-protein interaction in *in vitro* pull-down assay. Western blot analysis combined with confocal calcium imaging further demonstrated that the TAD exerted robust dominant-negative effect on wild-type TRPV1. When fused with membrane-tethered peptide Gap43, the TAD blocked the formation of stable homomultimers, and removal of the TAD from the full length TRPV1 resulted in nonfunctional channels. Calcium imaging and current recording showed that deletion of the TAD in a poreless TRPV1 mutant subunit suppressed its dominant-negative phenotype, confirming the involvement of the TAD in assembly of functional channels. Our findings suggest that the C-terminal TAD in heat-activated TRPV1-4 channels functions as a conserved domain that mediates a direct subunit-subunit interaction for tetrameric assembly.



2833-Plat
Complex Regulation of TRPV1 by Phosphoinositides
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TRPV1 is a nonselective calcium permeable cation channel present in polymodal nociceptors that plays a crucial role in the development of inflammatory pain and thermal hypersensitivity. Plasmamembrane phosphoinositides are recognized as important regulators of TRPV1; the precise nature of their effect is, however, controversial. Recent studies indicate that phosphatidyl inositol (4,5) bisphosphate [PI(4,5)P_2] and other phosphoinositides activate TRPV1 in excised patches. In concert with previous studies, we have shown that calcium influx-mediated activation of PLC results in a robust depletion of both phosphatidyl inositol (4) phosphate [PI(4)P] and PI(4,5)P_2 and is one of the mechanisms of TRPV1 desensitization. It is difficult however to reconcile these observations with preceding data indicating that PI(4,5)P_2 inhibits TRPV1, and relief from this inhibition by GPCR activation-induced PLC activity may contribute to TRPV1 potentiation during inflammation. In the present work we utilize several independent approaches to demonstrate that under specific circumstances TRPV1 currents are inhibited by PI(4,5)P_2. We show that selective dephosphorylation of PI(4,5)P_2 in the plasma membrane activates, while an increase in PI(4,5)P_2 levels inhibits TRPV1 currents elicited by low to moderate, but not high agonist concentrations. We also show that potentiation of whole-cell TRPV1 currents by bradykinin is partially impaired by dialyzing diC8-PI(4,5)P_2 as well as the PKC pseudosubstrate inhibitory peptide (19-31) through the pipette. Our data indicate that in addition to the well documented role of PKC-mediated phosphorylation in TRPV1 channel sensitization, PI(4,5)P_2 may also be involved in this phenomenon, implying a TRPV1 agonist concentration-dependent dual regulatory effect of PI(4,5)P_2. Such dual effects of PI(4,5)P_2 have previously been described for voltage-gated calcium channels as well as other TRP channels. Our findings support distinct roles for both activation and inhibition of TRPV1 by PI(4,5)P_2 and raise important questions as to the molecular background of the differential effects.

Platform BB: Micro and Nanotechnology; Nanopores

2834-Plat
DNA Translocation Through Graphene Nanopores
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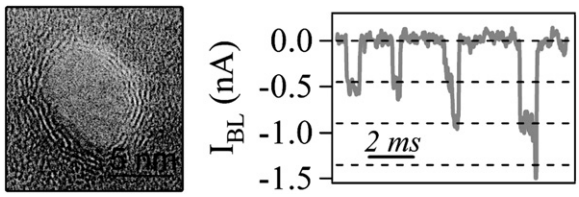
We report on DNA translocations through nanopores created in graphene membranes. Devices consist of nanometer-thick graphene membranes with electron-beam sculpted nanopores. Due to the thin nature of the graphene membranes, we observe larger blocked currents than for traditional solid-state nanopores. Unlike traditional solid-state nanopore materials that are insulating, graphene is an excellent electrical conductor. Use of graphene as a membrane material opens the door to a new class of nanopore devices in which electronic sensing and control are performed directly at the pore.



2835-Plat
Using Measurements of the ion Current Through a Synthetic Nanopore to Discriminate Nucleotides in a Single *DNA* Molecule
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Sequencing DNA with a nanopore relies on a distinctive electrical signal associated with each nucleobase that develops when a single DNA molecule, immersed in electrolyte, translocates across a membrane through the pore. We show that it is possible to discriminate nucleobases in DNA through measurements of the electrolytic current through a synthetic nanopore. We use a pore smaller in diameter than the double helix, in a silicon nitride membrane 15 nm thick. When an electric field is applied, biotinylated DNA homopolymers bound to streptavidin are impelled into and through the pore, but prevented from full translocation by the large streptavidin molecule at one end. Subsequent long time measurements of the blockade current through the pore reveal distinctive electrical signatures for poly (dA), poly (dC), and poly (dT) on a scale >100pA at high voltage (>100mV); differences much larger than the ~pA-level currents used to discriminate nucleotides in proteinaceous pores like α-hemolysin.

2836-Plat
In Vitro Measurements of Single-Molecule Transport Across an Individual Biomimetic Nuclear Pore Complex
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Nuclear pore complexes (NPCs) regulate the exchange of RNA and proteins between the nucleus and the cytoplasm in eukaryotic cells. We present a new method to investigate NPC transport at the single molecule level. We construct a biomimetic NPC by covalently bonding natively unfolded Phe-Gly rich (FG) domains of human nucleoporins (Nups) to a solid-state nanopore. Trans-pore ionic current measurements provide a probe to monitor single molecules traversing the pore. Importantly, we find that translocation events are indeed observed for transport receptors (Impβ) whereas the passage of non-specific proteins (BSA) is inhibited. A single type of Nups is thus sufficient to form a transport barrier that exhibits the selectivity found in NPCs. We find a translocation time for transporter molecule Impβ of 3 ms for both Nup153 and Nup98, which is 20-fold slower than the passage time through a bare pore, but comparable to in vivo measurements on NPCs. By reproducing key features of the NPC, our biomimetic approach opens the way to study a wide variety of nucleocytoplasmic transport processes at the single-molecule level in vitro.*
*Kowalczyk et al, Nature Nanotechnology, under review

2837-Plat
Integrated Microfluidic Platform for Studies on Membrane Proteins and Drug Screening Assays
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The combination of microfluidics with BLM (bilayer lipid membrane) experimentation provides a promising route towards high-throughput screening platforms for studies and drug screening on membrane proteins. The microfluidic format is ideal for multiplexed and automated assays, the miniaturization of the device goes together with enhanced electrical capabilities and more stable bilayers, and dual optical and electrical measurements are possible with a horizontal configuration of the membrane.
In that context, we have developed a sandwich device consisting of two glass substrates separated by a Teflon membrane. The glass substrates contain two independent and orthogonal microfluidic channels being the two fluidic