2473-Symp
Real-Time Shape Determination and 5-D Fingerprinting of Single Proteins
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This talk describes the use of electrolyte-filled nanopores to determine, simultaneously and in real time, the shape, volume, charge, rotational diffusion coefficient, and dipole moment of individual proteins. It introduces the main concepts for a quantitative understanding and analysis of modulations in ionic current that arise from rotational dynamics of single proteins as they move through the electric field inside a nanopore. The resulting multi-parametric information raises the possibility to characterize, identify, and quantify individual proteins and protein complexes in a mixture. This approach interrogates single proteins and determines parameters such as the shape and dipole moment, which are excellent protein descriptors and cannot be obtained otherwise from single protein molecules in solution. Therefore, this five-dimensional characterization of proteins at the single particle level has the potential for instantaneous protein identification, quantification, and possibly sorting with implications for structural biology, proteomics, biomarker detection, and routine protein analysis.

2474-Symp
Pores with Undulating Diameter for Multipronged Characterization of Single Particles and Cells in Resistive-Pulse Technique
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Single pores in resistive-pulse technique have been successfully used for the detection of cells, viruses, particles, and even molecules such as DNA and proteins. We have investigated application of pores with undulating opening diameter for the detection of particles and characterization of their biophysical and mechanical properties including size, shape and squishiness. The resistive pulses generated by polymer spheres passing through these pores had a repeatable pattern of large variations corresponding to these diameter changes. We showed that this pattern of variations enabled the unambiguous resolution of multiple particles simultaneously in the pore, that it could detect transient sticking of particles within the pore, and that it could confirm whether any individual particle completely translocated the pore. These results have practical importance for increasing the speed of resistive-pulse sensing, optimizing the detection of specific analytes, and identifying particle shapes. We also showed pores with undulating opening diameter developed local pressure drops, which were sufficiently large to probe mechanical properties of passing objects. Application to hydrogels as well as biological cells will be discussed.

Methods to measure diffusion coefficient and electrophoretic velocity of individual particles will be discussed in context of performing detection from diluted solutions of an analyte. Balancing all forces acting on particles allowed us to observe random walk of individual particles in a pore and estimate their diffusion coefficient from the variance of diffusion velocities of a particle. Trapping individual particles will be discussed in context of performing detection from diluted solutions of an analyte. Balancing all forces acting on particles allowed us to observe random walk of individual particles in a pore and estimate their diffusion coefficient from the variance of diffusion velocities of a particle. Trapping individual proteins and protein complexes in a mixture. This approach interrogates single proteins and determines parameters such as the shape and dipole moment, which are excellent protein descriptors and cannot be obtained otherwise from single protein molecules in solution. Therefore, this five-dimensional characterization of proteins at the single particle level has the potential for instantaneous protein identification, quantification, and possibly sorting with implications for structural biology, proteomics, biomarker detection, and routine protein analysis.

Platform: Voltage-gated Na and Ca Channels

2475-Plat
Sodium Ion Coordination in the Selectivity Filter of a Voltage-Gated Sodium Channel
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Voltage-gated sodium channels are essential for electrical signalling across eukaryotic cell membranes. They exhibit strong selectivity for sodium over other cations, thus enabling the finely-tuned cascade of events associated with action potentials. A new high resolution crystal structure of the prokaryotic sodium channel pore NavMs from Magnetococcus marinus provides the first view of the locations of sodium ions within the selectivity filter of a sodium channel. The structure reveals three sodium ions are bound within the selectivity filter. Unlike potassium ions in potassium channels, the sodium ions in these channels appear to be hydrated and make no direct contact with the polypeptide backbone, instead there are interactions with conserved mainchain glutamate and serine residues, as well as backbone carbonyl atoms, all of which are likely mediated by a disordered hydration shell. Electrostatic calculations on the structure are compatible with relative cation selectivities of Na⁺ ≈ Li⁺ ≈ K⁺, Ca²⁺ which correspond with the ion permeability ratios measured for these channels. Mutation of the conserved glutamate 178 to aspartate results in reduced sodium ion conductance through the pore. Interestingly, our structure of the E178D mutant reveals that it lacks the sodium ion nearest the extracellular vestibule and most closely associated with E178 in the native structure, explaining the reduced conductance. These results provide insight into the biophysical determinants of sodium-selectivity, which initiates the opening of other ion channels to shape the action potential waveform.

2476-Plat
Conduction and Selectivity in Na⁺ Channels Analyzed by Bias-Exchange Metadynamics Simulations
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Bacterial Na⁺ channels have been the subject of numerous computational studies since the first experimental structure of a Na⁺ selective channel was solved in 2011. Molecular Dynamics simulations revealed the presence of 2 binding sites for Na⁺ ions, respectively at the intracellular and extracellular entrance of the selectivity filter, separated by low energy barriers. While there is a general agreement about these features, there are also important differences among various published studies. In particular, ion conduction has been described both as a 2-ions or a 3-ions process, and this difference has been correlated to the direction of conduction, or to the state of the intracellular gate. A current limit of the computational strategies usually adopted to estimate the energy profiles for permeation events, is that the number of permeating ions has to be defined in advance. As consequence, it is difficult to compare energetically conduction mechanisms characterized by different number of ions, and this could explain the lack of congruence in the literature. In order to overcome this limit, we tested a novel approach for the analysis of ion conduction based on bias-exchange metadynamics simulations. In bias-exchange, several replicas of the system are simulated in parallel. A metadynamics simulation is performed for each replica, along one or a few collective variable, and at fixed time intervals swaps of configurations between replicas are attempted. Using this approach it was possible to analyze by a single set of simulations the five energy for permeation events with different number of ions. The analysis revealed that several conduction mechanisms are indeed possible for Na⁺ channels. This computational strategy could find wide applications for the study of ion channels, in particular to characterize conduction of ion-mixtures, or channels that exhibit heterogeneous conduction events.

2477-Plat
Inactivation Voltage Sensor S4 in Domain IV of Nav1.2 Controls Imobilization of S4 in Domain III as Shown by Omega Currents
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The role of S4DIV for inactivation of skeletal muscle Na channel Nav1.4 was recently described after deciphering the channelopathia Paramyotonia congenita. We showed with point mutations in the rat brain sodium channel Nav1.2 the central role of S4DIV for inactivation (Kühn and Greeff, 1999). The single mutation R4H in S4DIV slowed the recovery from inactivation about 20 times in parallel for tonic current and immobilized gating charge. Immobilization concerns amount of 50 % of total gating charge returning slowly to the resting state during recovery while the other half of gating charge returns very quickly. Clearly, the amount of immobilized charge is more than just the one from S4DIV. So we speculated that S4DIV would control S4R in other domains. Now, we are able to monitor the return of S4 into the resting position for each domain separately by recording the peak current of resting-state omega pore mutants (this Meeting). We find that S4DIV with the omega mutation RR12QQ shows a fast onset of omega peak current for channels at rest; however, after an inactivating prepulse, the peak current grows with the time course of recovery as expected, since this voltage sensor controls the recovery and returns into resting position accordingly. Checking the return of S4 in the other domains, we find a fast return in DI and DII while in DIII the return follows DIV. Combining these mutations with R4H in DIV, the return in both domains III and IV is about 20 times more slowly. This suggests that immobilization of gating charge across the domains is most likely achieved by the cytoplasmic loop between DIII and DIV which under control of S4DIV closes the alpha-pore and immobilizes S4DIV.