2473-Symp
Real-Time Shape Determination and 5-D Fingerprinting of Single Proteins
Michael Mayer
Biomed. Eng. and Chem. Eng., University of Michigan, Ann Arbor, MI, USA.
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
Zuzanna S. Siwy1, Laura Innes1, Matthew Schiel1, Ivan Vlassiouk1, Kenneth J. Shea1, Luke Theogarajan1.
1Dept. of Physics and Astronomy, University of California, Irvine, Irvine, CA, USA.
2Oak Ridge National Laboratory, Oak Ridge, TN, USA.
3Dept. of Chemistry, University of California, Irvine, Irvine, CA, USA.
Abstract: 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 by combining several technical 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 electrokinetic 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 of particles/cells for a controllable amount of time between few milliseconds and a few minutes will be presented as well.

Platform: Voltage-gated Na and Ca Channels

2475-Plat
Sodium Ion Coordination in the Selectivity Filter of a Voltage-Gated Sodium Channel
Claire E. Naylor1, Claire Bagners1, Paul G. DeCaen1, David E. Clapham1, B.A. Wallace1.
1Institute of Structural and Molecular Biology, Birkbeck College, University of London, London, United Kingdom.
2Department of Cardiology, Howard Hughes Medical Institute, Children’s Hospital Boston, Boston, MA, USA.
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 from Magnetoococcus 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
Simone Furini1, Paolo Barbini1, Carmen Domenè1,
1Department of Medical Biotechnologies, University of Siena, Siena, Italy.
2Department of Chemistry, King’s College London, London, United Kingdom.
Bacterial Na+ channels have been the subjects 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 the numerous 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 a consequence, it is difficult to compare energetically relevant 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 free 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 Immobilization of S4 in Domain III as Shown by Omega Currents
Nikolaus G. Greeff, Claudia Lehmann, Hansjakob Heldstab.
Biophysics Institute Greeff, Uetikon am See, Switzerland.
The role of S4DIV for inactivation (Kuhn and Greeff, 1999): The single mutation S4DIII.
1, Laura Innes1, Matthew Schiel1, Ivan Vlassiouk2, Nikolaus G. Greeff2.
2Biophysics Institute Greeff, Uetikon am See, Switzerland.
Nikolaus G. Greeff1, Laura Innes1, Matthew Schiel1, Ivan Vlassiouk2, Nikolaus G. Greeff2.
1Department of Medical Biotechnologies, University of Siena, Siena, Italy.
2Department of Chemistry, King’s College London, London, United Kingdom.
Bacterial Na+ channels have been the subjects 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 the numerous 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 a consequence, it is difficult to compare energetically relevant 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 free 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.

2478-Plat
Inactivation Voltage Sensor S4 in Domain IV of Nav1.2 Controls Immobilization of S4 in Domain III as Shown by Omega Currents
Nikolaus G. Greeff, Claudia Lehmann, Hansjakob Heldstab.
Biophysics Institute Greeff, Uetikon am See, Switzerland.
The role of S4DIV for inactivation (Kuhn and Greeff, 1999): The single mutation S4DIII.
1, Laura Innes1, Matthew Schiel1, Ivan Vlassiouk2, Nikolaus G. Greeff2.
2Biophysics Institute Greeff, Uetikon am See, Switzerland.
Nikolaus G. Greeff1, Laura Innes1, Matthew Schiel1, Ivan Vlassiouk2, Nikolaus G. Greeff2.
1Department of Medical Biotechnologies, University of Siena, Siena, Italy.
2Department of Chemistry, King’s College London, London, United Kingdom.
Bacterial Na+ channels have been the subjects 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 the numerous 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 a consequence, it is difficult to compare energetically relevant 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 free 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.