2473-Symp
Real-Time Shape Determination and 5-D Fingerprinting of Single Proteins
Michael J. Mayer
Biomed. Eng. and Chem. Eng., University of Michigan, Ann Arbor,
MI, USA.
This talk describes the use of electrolyte-filled nanopenes to determine, simul-
taneously and in real time, the shape, volume, charge, rotational diffusion
coefficient, and dipole moment of individual proteins. It introduces the main
corcepts 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 nanopene. The resulting multi-parametric in-
formation 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 charac-
terization 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 Multichannel Characterization of
Single Particles and Cells in Resistive-Pulse Technique
Zuzanna S. Siwy1, Laura Innes1, Matthew Schiel1, Ivan Vlassiouk1,
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, 4University of
California, Santa Barbara, Santa Barbara, CA, USA.
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 open-
ing diameter for the detection of particles and characterization of their phys-
tical 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 re-
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 prop-
erties of passing objects. Application to hydrogels as well as biological cells
will be discussed.

Methods to measure diffusion coefficient and electokinetic velocity of individ-
ual 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 Bagnères2, Paul G. DeCaen2, 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 prokary-
totic sodium channel pore NavMs from Magnetococcus marinus provides the
first view of the locations of sodium ions within the selectivity filter of a sodi-
um 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,
of 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. Inter-
estingly, 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 re-

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 Domene2.
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 various computational 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 energet-
ically the 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 rep-
licas of the system are simulated in parallel. A metadynamics simulation is per-
fomed 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 re-
vealed that several conduction mechanisms are indeed possible for Na+ chan-
nels. This computational strategy could find wide applications for the study
of ion channels, in particular to characterize conduction of ion-mixtures, or chan-
nels that exhibit heterogeneous conduction events.

2477-Plat
Inactivation Voltage Sensor S4 in Domain IV of Nav1.2 Controls Immobi-
lization 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 of skeletal muscle Na channel Nav1.4 was
recently discovered 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 DIV slowed the recovery from inactivation about 20 times in parallel
for ionic current and immobilized gating charge. Immobilization concerns
about 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 S4s in other domains. Now, we are
able to monitor the return of S4 in the resting position for each domain sepa-
rately by recording the leak current of resting-state omega pore mutants (this
Meeting). We find that S4DIV with the omega mutation RR12QQ shows a
fast onset of omega leak current for channels at rest; however, after an inacti-

vating prepulse, the leak current grows with the time course of recovery as ex-
pected, 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 slower than in DI and DII. 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.