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SINGLE CHANNEL MEASUREMENTS OF N-ACETYLNEURAMINIC ACID-INDUCIBLE CHANNEL (NANC) IN E. COLI

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Abstract

Escherichia coli can use *N*-acetylneuraminic acid (Neu5Ac) as its sole carbon source even if the general outer membrane proteins OmpF and OmpC are not expressed: NanC - a monomeric outer membrane channel - allows Neu5Ac to move into the bacterial periplasm. Recently, a high resolution structure of NanC in two different crystal forms was reported by Wirth et al., J.Mol.Biol., (2009) 394:718 (PDB codes: 2WJQ and 2WJR). Our goal is to determine appropriate 'baseline' ionic conditions to study the transport of Neu5Ac through NanC using single channels in lipid bilayers. Measurements of single channel currents showed that NanC has two modes of time dependent behavior ('gating'). In the many situations we have tested, the modes are not induced or changed by surrounding ionic conditions or voltage. Single channels of NanC at pH 7.0 have: (1) a large conductance (around 100 pS to 800 pS in 100 mM KCl to 3M KCl) that varies with the polarity of the applied voltage; (2) anion over cation selectivity (Vreversal around +16 mV in 250 mM KCl || 1 M KCl); (3) voltage-dependent gating (channel closures above ±200 mV). Single channel conductance of NanC decreases about 50% when HEPES concentration is increased from 100 µM to 100 mM in 250 mM KCl at pH 7.4, consistent with the two HEPES binding sites observed in the crystal structure (PDB code: 2WJR). Studying alternative buffers, we found that phosphate interferes with the channel conductance, whereas TRIS could not be used because it reacts with Ag/AgCl electrodes producing artifacts even in the presence of Agar-KCl bridges. Our further studies of NanC will use no pH buffers, but low concentration (250 mM) salt solutions adjusted to neutral pH 7.0.

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SIALIC ACID TRANSPORT IN E. COLI: ROLE OF OUTER MEMBRANE PORIN NANC

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Abstract

Sialic acid is a nutrient of bacteria important in host-pathogen interactions. The mechanism of transport of sialic acid from outer membrane to periplasmic space of *Escherichia coli* is not known. N-acetylneuraminic acid (Neu5Ac) - the most abundant form of sialic acid - induces a specific porin NanC (N-acetylneuraminic acid Channel) in the outer membrane of *E. coli*. Recently, a high resolution structure of NanC (Wirth et al., J.Mol.Biol., (2009) 394:718) revealed unique structural features that support Neu5Ac transport. However, patch-clamp experiments seemed to show that NanC conductance is unaffected by sialic acid (Condemine et al., J.Bacteriol., (2005) 187:1959). We report single channel current measurements of NanC in bilayers in the presence of Neu5Ac. Neu5Ac changes gating and considerably increases the ionic conductance of NanC in 250 mM KCl, pH 7.0. (See our other NanC poster.) The unitary current through NanC increases when 7-12 mM of Neu5Ac is added to the grounded side of the bilayer. A distinct steady voltage dependent current (sub-level) is observed that seems to add to the unitary current. The single channel slope conductance of NanC increases by 51% in the presence of 7 mM Neu5Ac and by 74% in 55 mM. The effect of Neu5Ac on the unitary current through NanC seems to saturate at higher Neu5Ac concentrations. The unit conductance of NanC also increases when 20 mM Neu5Ac is added to both sides of the bilayer. It is likely that some of the current is carried by Neu5Ac. Interestingly, Neu5Ac reduces the ionic conductance of trimeric OmpF (Outer membrane porin F) under the same conditions: frequent, long closures are seen. Thus, we provide evidence that sialic acid translocation is specifically facilitated by NanC, and not by the general porin OmpF.

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A NEW POISSON-NERNST-PLANCK EQUATION (PNP-FS-IF) FOR CHARGE INVERSION NEAR WALLS

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Abstract

The plasmas of biology are interacting mixtures of ions — often charged spheres — that do not behave like the ideal solutions of textbooks. Interactions are always present because of strong electrical forces. Flows are usually present. Life without flow is death. We analyze ionic solutions as complex fluids with an approach that has successfully analyzed complex systems like liquid crystals that are dominated by interactions between composite components. The finite size of ions is particularly important in biology in crowded environments like channels, active sites of enzymes, or charged surfaces. We here deal with surfaces and try to capture the essential features of charge inversion (layering) near a charged wall. Charge inversion (layering) near walls is a characteristic phenomenon resulting from the electrostatic interactions in systems with charged walls. The mathematical model is derived by the energy variational approach (EnVarA) — J.Chem.Phys. (2010) 133:104104 — that combines the action of conservative (Hamiltonian) systems and the dissipation of Onsager and Rayleigh. Both are written in the same laboratory coordinates after variational derivatives of variables are taken. The generalized energy and dissipation include entropic and electrostatic components, and repulsion between spheres. An interfacial electroneutrality constraint between bulk and charged wall captures some essential features of charge inversion. Taking variational derivatives yields a field theory of partial differential equations and boundary conditions that are appropriate for life's solutions — that interact and flow — as well as thermodynamic equilibrium. The new equations, PNP-FS-IF, include (1) a nonlocal contribution of finite size (FS) and (2) an interfacial constraint (IF) of electroneutrality. PNP-FS-IF produces charge inversion near walls. We compare the charge inversion seen with PNP-FS-IF and Monte-Carlo simulations.

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ACTIVE SITES OF ENZYMES ARE CROWDED WITH CHARGE

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Abstract

The chemistry of enzymes occurs at active sites that concentrate biological function into functional pockets. Functional pockets mix catalytic amino acids and substrate in tiny volumes. Here, we look for biological properties of that small space. We imagine that electric charge plays important roles, because even one charge in a small space produces large electric fields. To estimate densities of fixed charge, we measure the volume of functional pockets and count 'charged residues' in it. We collect locations of functional pockets from enzymes of known structure that catalyze the main six enzymatic reactions. Functional amino acids are identfied by their participation in catalysis. We measure the volume of pockets using both solvent-accessible and molecular-surface models. 'Charged residues' are R, K and H (positive); E and D (negative).Charge density is extraordinarily large (~20 Molar on average, often larger).Mobile counterions for thefixed charge are presumably nearby in high density. Active sites do not resemble the infinitely dilute ideal solutions of classical enzyme kinetics. Their enormous charge density is comparable to the charge density of solid NaCl. Different types of enzymes have different charge densities. Hydrolases show the largest values of charge density. Some enzymes have extraordinarily large charge density—phosphoglycerate mutase (PDB = 1o98,density of charge 104 Molar, Molecular Surface), or sulfurtransferase (PDB =1e0c, 109 Molar, Molecular Surface). Crowding of charged side-chains and ions produces enormous steric and electrostatic forces in these tiny active sites. The balance of these forces seems likely to be of great importance to enzyme function. Many charged pockets are also found away from active sites. Charged pockets are likely to be involved in many surface interactions. They may be reservoirs of electromechanical energy that can drive conformational changes.

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ELECTRODIFFUSION AND OSMOTIC WATER FLOW AND ITS VARIATIONAL STRUCTURE

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Abstract

We propose a system of partial differential equations (PDE) that describe electrodiffusion and osmotic water flow. From a physical standpoint, this is a far-reaching generalization of the standard treatment of osmosis and electrodiffusion in irreversible thermodynamics to spatially extended systems. As far as we know, this is the first mechanically and thermodynamically consistent model of osmotic water flow and electrodiffusion in systems with deformable cells and membranes with capacitance and conductance. We use an energetic variational approach to enforce consistency and derive a field theory describing the flow, diffusion, and migration of ions, water, and the solution itself. The variational approach is particularly useful because it treats interactions automatically and consistently with a minimal number of arbitrary parameters. Electrodiffusion and osmotic water flow are involved in a wide range of biological functions of organs, tissues, cells, and organelles, including the homeostasis of ions in the brain, fluid secretion by epithelial systems, electrolyte regulation in the kidney, fluid circulation in ocular systems, gastric protection, water uptake by plants, etc. The field equations can be written with boundary conditions and parameters appropriate for the anatomy of each system. The field equations then form a physically and anatomically consistent model of biological function in the variational framework of modern field theory. The variational approach deals naturally with the many ionic solutions (containing a multitude of interacting components in a wide range of concentrations) and the wide range of conditions and forces used in experiments. Solving the PDEs will help suggest and interpret new experiments to understand the interaction of components, conditions, structure, and forces. In the view of classical physiology and biophysics, these interactions are the essence of biological function.

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A CONTINUUM VARIATIONAL APPROACH TO VESICLE MEMBRANE MODELING

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Abstract

Biological membranes remodel in lipid pore formation, fusion, endocytosis and other processes. Traditionally, continuum membrane mechanics has been used to describe the physics of these remodelings. Membrane mechanics is a conservative, equilibrium theory and so cannot, a priori, describe the time course, flows and dissipations of a real system. Over the past few decades, physical scientists and mathematicians have developed global multi-physics field equations that describe the time course of processes for condensed matter in a thermodynamically consistent way. We use these equations to describe the membrane during lipid bilayer membrane remodelings. We analyze the vesicle membrane and its lipid layers as a bulk continuum variable in a Hamiltonian. The Hamiltonian includes the surface tension and curvature effects of the classical Helfrich model. The representations are, however, more flexible and can readily account for multicomponent systems, inhomogeneities, and changes in topology. Coupling the Hamiltonian to the motion of the aqueous medium with Rayleigh dissipation leads to a complicated, self-consistent system of partial differential equations that is solved numerically. Numerical schemes, designed specifically for this field theory, provide the position, velocity and forces of the fluid--vesicle system at each point in space and time. Classical models assume a specific shape for the vesicle (e.g., a sphere). The assumed shape will occur in the real world, however, only if it is a self-consistent solution of the equations. Our calculations yield values of all key variables and energies over time-the shape is an output. Movies that precisely illustrate the time evolution of the membrane configuration are generated. Changes over time are appreciated visually without reference to the equations--or even to the physics--of the remodeling processes.

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A novel Brownian-Dynamics algorithm for the simulation of ion conduction through membrane pores

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Abstract

Brownian-Dynamics (BD) is a powerful approach for the simulation of ion conduction through membrane pores. BD simulations are much less computational demanding than molecular dynamics simulations, thus allowing the analysis on the microsecond time-scale. Furthermore, compared to other simplified approaches like Poisson-Nernst-Planck that use point-charge ions, BD preserves the discrete nature of the ionic particles, which is particularly important in narrow pores. For these reasons, BD simulations have been widely used to analyze conduction in membrane proteins or carbon nanotubes, obtaining good agreement with experimental data.

Published implementations of BD suffer from severe shortcomings, both in terms of accuracy and efficiency. Electrostatic forces due to source and induced (polarization) charges are usually computed in advance, and then tabulated for fast recovery during the numerical integration of the BD equations. Simulation accuracy requires dense grids and this results in low efficiency. In order to improve the state of the art in this field, we have implemented BD code using the Induced Charge Computation (ICC) algorithm to solve the Poisson equation in discrete-charges systems. The accuracy and speed of ICC allows run-time solution of the Poisson equation during simulation. It does not need lookup tables. We compared our new implementation with a standard algorithm, based on tabulation of the electrostatic potential, using as a benchmark a toy-model of a pore with cylindrical profile. Our algorithm provides a considerable increase of efficiency at given accuracy, and a significant increase of accuracy at given computation time. We expect further improvements, in both accuracy and performance, when simulating pores with more irregular profiles, as required for the analysis of real membrane proteins.

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