AuPS/ASB Meeting - Adelaide 2010

Symposium: Computational studies on biological and synthetic nanotubes

Wednesday 1st December 2010 - The Gallery - 11:30

Chair: Shin-Ho Chung

Selective ion binding and its role in potassium channel selectivity

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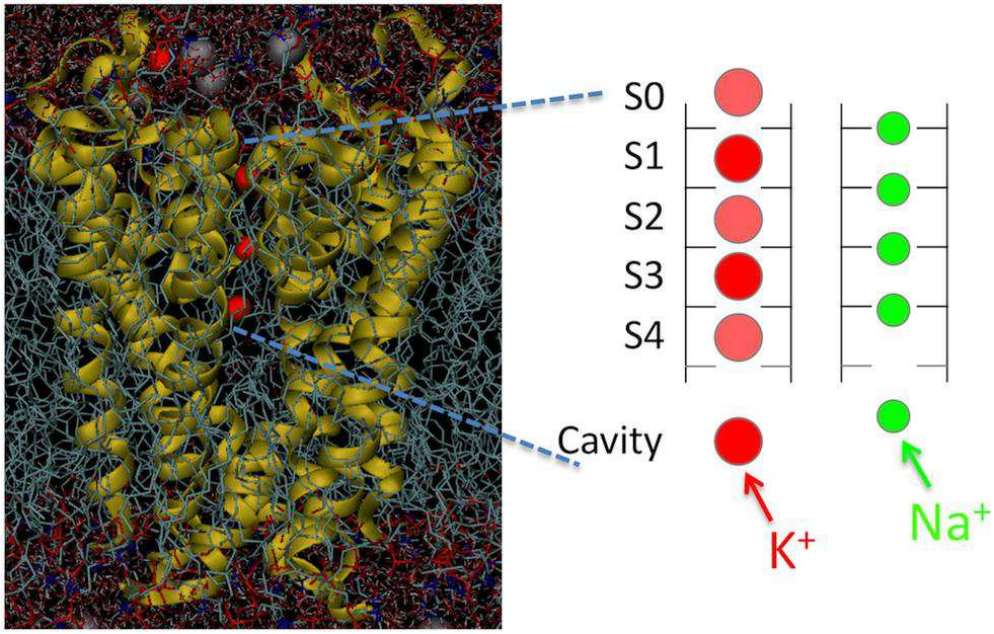
Ion channels catalyse rapid and selective ion movement across cell membranes to control electrical and chemical activity in the body. K^+ channels have the remarkable ability to pass K^+ ions at near diffusion-limited rates, while sensitively excluding Na^+ ions; a characteristic essential for membrane repolarization during action potentials. Since it was suggested, over 20 years ago (Neyton & Miller, 1988), that K^+ channel pores consisted of multiple K^+-selective binding sites, and high resolution structures (Doyle *et al.*, 1998; Morais-Cabral *et al.*, 2001) subsequently revealed these sites, the prevailing view has been that K^+ channels select for K^+ ions *via* a mechanism of selective binding.

Recent molecular dynamics simulations (Figure, left), x-ray crystallography and patch clamping (Thompson *et al.*, 2009) have unveiled a putative Na^+ binding site within the K^+ channel selectivity filter, and new calculations (Kim & Allen, 2010) have demonstrated the existence of multiple such sites, leading us to question the hypothesis of selective permeation *via* selective binding. Each K^+ binding site (S0-S4, red balls in the Figure) consists of a cage of 8 carbonyl (or threonine OH for S4) oxygen ligands, made from two planar rings formed by the KcsA tetramer (only two subunits depicted as lines). Each of these sites is adjacent to one or two Na^+ binding sites, consisting of planar rings of 4 carbonyl oxygen atoms, as shown in the Figure (green balls). Free energy calculations indicate that these planar sites select for Na^+ over K^+, and that the net selectivity for K^+ over Na^+ in the selectivity filter is much lower than previously calculated. These results suggest the need for a broader view of selectivity mechanisms, including possible kinetic entry barriers for Na^+ ions (Bezanilla & Armstrong, 1972), with fully-atomistic molecular dynamics simulations helping to reveal those barriers within the multiple-ion permeation process.



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Kim I & Allen TW. (2010). On the selective ion binding hypothesis for potassium channels. *In preparation*

Morais-Cabral JH, Zhou Y & MacKinnon R. (2001). Energetic optimization of ion conduction rate by the K^+ selectivity filter. *Nature* **414,** 37-42.

Neyton J & Miller C. (1988). Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high-conductance Ca^{2+} -activated K^+ channel. *Journal of General Physiology* **92,** 569-586.

Thompson AN, Kim I, Panosian TD, Iverson TM, Allen TW & Nimigean CM. (2009). Mechanism of potassium-channel selectivity revealed by Na^+ and Li^+ binding sites within the KcsA pore. *Nature Structural and Molecular Biology* **16,** 1317-1324.

The induction and stabilization of transmembrane pores by peptides

A.E. Mark, School of Chemistry and Molecular Bioscience, The University of Queensland, St Lucia, QLD 4072, Australia.

Atomistic molecular dynamics simulation techniques have been used to examine the interaction of a range of pore-forming and cell-penetrating peptides with lipid membranes. Such systems are archetypical examples of self-organizing molecular systems leading to functional complexes. In general it had been assumed that the structured formed were highly regular. However, simulations of the spontaneous induction of transmembrane pores by the antimicrobial peptides Magainin and Melletin suggest that the pores are at least initially highly disordered casting doubt on the validity of current models (Leontiadou, Mark & Marrink, 2006; Sengupta *et al.*, 2008). In contrast in the case of the cell-penetrating peptides Penetrin and the TAT-peptide no spontaneous formation of transmembrane pores was observed. Instead, the simulations suggest that the peptides may enter the cell by micropinocytosis, whereby the peptides induce curvature in the membrane, ultimately leading to the formation of small vesicles within the cell that encapsulate the peptides (Yesylevskyy, Marrink & Mark, 2009).

Leontiadou, H, Mark, AE & Marrink, SJ, (2006) Antimicrobial peptides in action. *Journal of the American Chemical Society* **128,** 12156-12161.

Sengupta, D, Leontiadou, H, Mark, AE & Marrink, SJ.(2008) Toroidal pores formed by antimicrobial peptides show significant disorder. *Biochimica et Biophysica Acta - Biomembranes* **1778,** 2308-2317.

Yesylevskyy, S, Marrink, SJ & Mark, AE. (2009) Alternative mechanisms for the interaction of the cell-penetrating peptides Penetratin and the TAT peptide with lipid bilayers. *Biophysical Journal* **97,** 40–49.

Monitoring the conformational changes involved in MscL channel gating using FRET microscopy and simulation

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Large scale conformational changes often play an essential role in the functioning of proteins, yet they can be hard to probe with experimental methods and take place over timescales that are difficult to simulate. We are using a combination of low resolution experimental techniques in combination with a variety of computational methods to try to understand the large structural rearrangements taking place in the gating of the mechanosensitive channel MscL. These bacterial channels open large pores in response to membrane tension in order to rescue the cell under osmotic shock. In this case we gain structural data on the protein in a natural environment using patch-clamp studies and confocal FRET microscopy. Combining this with existing EPR data as restraints in molecular and coarse grain simulations has allowed us to determine the likely structure of the open state of the pore. While there are many challenges to be overcome in this methodology, including careful approaches to labelling; control of the protein state; careful analysis of the orientation, geometry and number of fluorescent probes; and rigorous sampling of the conformational space; we believe that it provides a useful tool for studying the structures of a range of membrane proteins in natural environments.

Free energy simulations of Asp/Glu transporter GltPh

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Glutamate is the dominant excitatory neurotransmitter in the brain. Its extracellular concentration is kept at the nanomolar level by glutamate transporters – membrane proteins that continuously pump glutamate back to the neurons using the existing ionic gradients. Because small changes in glutamate concentration have major effects in signaling in the brain, glutamate transporters are key targets for treatment of various neurological conditions. Here we use the recently determined bacterial transporter structure GltPh in MD simulations to delineate the basic steps involved in glutamate transport and provide a structural basis for the transport mechanism. We have confirmed the binding sites Na1, Na2 and Asp, suggested by the experimental structure, and found a third Na ion binding site (called Na3), which is critical for the functioning of the transporter. This also strengthens the case for constructing homology models for human glutamate transporters (EAAT1-5) from the GltPh structure. Our proposed binding site for Na3 is completely consistent with the structural data, which is not the case for other proposed sites.

We have calculated the binding free energies for Na ions and Asp in various configurations and thus determined the order of binding as Na3, Na1, Asp, Na2 (following the notation used in the crystal structure). To understand the selectivity of GltPh for Asp, we have performed free energy perturbation calculations for the transformation Asp to Glu and found a selectivity free energy barrier of 4-5 kcal/mol consistent with the experimental observations. This is basically caused by steric interactions - the larger sidechain of Glu does not quite fit in the binding site. Once we understand the operation of the bacterial transporter, we will create a homology model for the mammalian glutamate transporter EAAT1 and investigate similarities and differences in the transport mechanism with the bacterial one, following steps similar to above.

Mimicking biological ion channels using nanotubes

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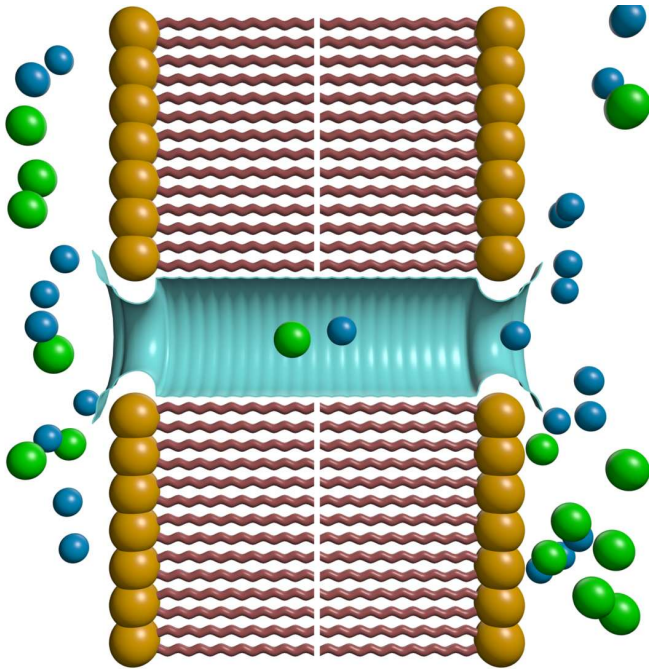
Biological ion channels are selectively permeable to specific ionic species, and regulate the flow of ions across the cell membrane. They maintain the resting membrane potential, generate propagated action potentials in nerves and control a wide variety of cell functions. We report that hollow nanotubes constructed from carbon atoms, and with hydrogen, carbonyl or carboxylic acid terminated ends, have the ability to mirror some of the important functions of various biological ion channels. In particular, these carbon nanotubes (CNTs), embedded in a lipid bilayer (illustrated in Figure), are selectively permeable to cations or anions, depending on their terminated ends and diameter. They broadly mimic some of the permeation characteristics of the antibiotic gramicidin, chloride channels, and the mutant glycine receptor.



Using a combination of molecular and stochastic dynamics simulations (see Gordon *et al.*, 2009 for details), we characterize certain properties of these engineered nanotubes, such as the free energy profiles encountered by charged particles, the current-voltage-concentration profiles and the overall conduction mechanism. We demonstrate that CNTs can be designed such that they are selective to either cations or anions by modifying their surface chemistry. In particular, we discuss three CNTs with a length of approximately 36 Å and varying surface chemistry, namely (i) with a radius of 4.53 Å and terminated with carbonyl groups (see Hilder *et al.*, 2010 for details); (ii) with a radius of 4.53 Å and terminated with hydrogen and with two regions near the entrance and exit of the nanotube exohydrogenated (outside surface hydrogenation); and (iii) with a radius of 5.08 Å and terminated with carboxylic acid. These CNTs are shown to broadly mimic the permeation characteristics of (i) the ClC-1 chloride channel and $GABA_A$ but with conduction rates 4 times and 2 times larger, respectively (see Hilder *et al.*, 2010 for details); (ii) the antibiotic gramicidin but with a potassium current 6 times larger; and (iii) the mutant glycine receptor in which anions chaperone sodium across the channel (illustrated in Figure) but with a sodium conductance 7 times larger. These synthetic nanotubes may lead to a host of pharmaceutical products to assist in treatments such as antibacterial, cancer and cystic fibrosis in addition to potential applications as sensitive biosensors.

Hilder, T.A., Gordon, D. and Chung, S.H. (2010) *Biophysical Journal*, **99**: *in press.*
Gordon, D., Krishnamurthy, V. and Chung, S.H. (2009) *Journal of Chemical Physics*, **131**: 134102.