mirror the mRNA changes. Preliminary results show increased T-type current, of which 70% was insensitive to 50μM $NiCl_2$. Because Cav3.1 is highly regulated in these conditions, the hypoxia/reoxygenation effect on the Cav3.1 gene promoter was confirmed by transfecting promoter-reporter constructs into HEK 293 cells and measuring luciferase activity under the same conditions. We show that T-type calcium channels are regulated in hypoxia/reoxygenation conditions, and we propose that T-type calcium channels contribute to intracellular calcium accumulation and the severity of injury in cardiomyocytes.

Biophysics of Ion Permeation

2203-Pos The Path Of Ions In Ompf Pore: An Analysis Using X-ray Crystallography

Suchismita Raychaudhury[1], Benoit Roux[2]

[1] *New York University, NewYork, NY, USA,*
[2] *University of Chicago, Chicago, IL, USA.*

Board B318

OmpF porins are proteins found in the outer membrane of Escherichia coli which form wide aqueous pores that are slightly cation-selective. They serve to facilitate the translocations of hydrophilic solutes with molecular mass up to 600 Da across the outer membrane. Because they are well-characterized, both structurally and functionally, OmpF and other porins represent ideal systems for addressing questions about the fundamental principles underlying ion flow in molecular pores. Previously, PD, BD and MD simulations hadshown that there exist two well-separated pathways for cations and anions inside OmpF. Thus, suggesting that the charge specificity of OmpF porin does not arise from a few local interactions in the constriction zone, but rather from a number of residue distributed over a large fraction of the aqueous pore.

In this study, we use x-ray crystallography to observe the path taken by rubidium ions inside OmpF. X-ray diffraction data on OmpF were collected from crystals equilibrated with solutions containing rubidium chloride and snap frozen (100 degrees K). Difference electron density maps [F(rubidium) - F(native)], at 3.0 Angstrom resolution as well as rubidium anomalous scattering maps suggest that the cations prefer to be located along the walls of the OmpF pore, following a screw axis path. Also observed are densities of water molecule not seen in previous structures. Taken together the xray structure and previous PD, BD and MD studies add to our understanding of the factors that determine the path taken by ions inside the porins.

2204-Pos Interaction Of Ampicillin With The OmpF Channel Studied By Site Directed Mutagenesis

Ekaterina M. Nestorovich[1], Mathias Winterhalter[2], John Pang[1], Sergey M. Bezrukov[1]

[1] *NICHD, Bethesda, MD, USA,*
[2] *Jacobs University, Bremen, Germany.*

Board B319

We use site directed mutagenesis to study molecular mechanism of β-lactam antibiotics translocation through the single trimers of outer membrane protein F (OmpF). Using high resolution conductance recordings and noise analysis, we investigate ampicillin interaction with the following mutants: *D113A, R82A*R132A, R168A, R82A, R42A, E29A, E117A*R132A, E29A*R168A, E117A, D113A*R42A, E62A*R82A*, and *E71A*R132A*. For all mutants we observed a measurable current noise increase in the presence of antibiotics in the membrane bathing solutions. However, we could distinguish at least two different modes of ampicillin-channel interaction. The first group of mutants binds ampicillin in a way similar to the wild type exhibiting clear 1/3 blockages of the current through the single trimeric pore. The second group demonstrates no 1/3 blockages but induces a pronounced high-frequency spectral component suggesting that the characteristic time of the interaction was too short to be resolved.

Comparing the results for the first and second groups of mutants we discuss the possible involvement of certain amino acid residues in the ampicillin transport through the channel. We examine two different scenarios of antibiotic interaction to the mutants of the second group. One of them is that we deal with extremely fast ampicillin translocation through the pore, while the other is that the high-frequency component of the spectra is caused by antibiotic binding somewhere close to the opening of the channel without translocation through the pore. To discriminate between these scenarios, we compare channel blockage parameters under conditions when antibiotic was asymmetrically added only to *cis* or only to *trans* side of the membrane.

2205-Pos

Board B320

WITHDRAWN

2206-Pos The Effects Of High Frequency Electromagnetic Field On The Behavior Of Single OmpF Channel In Planar Bilayer

Mehdi Mohammadzadeh[1], Hamid Mobasheri*[1], Farrokh Arazm[2]

[1] *Lab. Memb. Biophys. , Inst. Biochem. & Biophys. , University of Tehran, Tehran, Iran (Islamic Republic of),*
[2] *Faculty of Electrical and Computer Engineering, University of Tehran, Tehran,, Tehran, Iran (Islamic Republic of).*

Board B321

The Effects of high frequency electromagnetic waves, 800 MHz, on the single-channel activity of OmpF porin channel reconstituted in lipid bilayer membrane were studied here. Ion channels as one the major components of biological membranes involved in the appropriate function of cell can be considered of the major target sites for

electromagnetic radiation. Channel activities before, in the presence and following the exposure to HF-EMF were recorded by means of voltage clamp technique. The effects of the electromagnetic field properties including its strength, polarity, frequency, modulation and duration on various channel characteristics such as conductance, gating pattern, voltage sensitivity, mean open time, symmetrical/asymmetrical response and so on were investigated at single molecule level. The preliminary results indicate that the exposure of OmpF to electromagnetic field increased the conductance, voltage sensitivity and the frequency of channel gating to some extent. Furthermore, mean open time decreased and reversing the polarities of various membrane potentials didn't show significant effect on the channel activity.

The financial support of the University of Tehran offered as Project Type 6 is acknowledged.

Keywords

Biophysics, single channel, electromagnetic field, voltage clamp

2207-Pos Understanding antibiotic translocation through porins from *E. coli* and *E. aerogenes*

Kozhinjampara R. Mahendran[1], Andrey N. Bessonov[1], Helge Weingart[1], Alexandr Ivanov[1], Tivadar Mach[1], Chloe E. James[2], Jean-Marie Pages[2], Amit Kumar[3], Matteo Ceccarelli[3], Mathias Winterhalter[1]

[1] *Jacobs University Bremen, Bremen, Germany,*
[2] *Université de la Méditerranée, Marseille, France,*
[3] *University of Cagliari, Cagliari, Italy.*

Board B322

Porins are channel forming membrane proteins in the outer cell wall of Gram-negative bacteria and the main pathway for a number of antibiotics. Bacterial resistance against antibiotics such as beta-lactams or quinolones can be triggered by preventing their influx into the cell. Characterization of the interactions between porins and the antibiotic molecule might improve the design of novel drugs that efficiently reach their target sites. In this study we focus on the transport pathways of novel classes of antibiotics. *Escherichia coli* OmpF, OmpC and its homologue from *Enterobacter aerogenes* - Omp36 were incorporated into the bilayer and ion currents through the channels were analyzed in the presence of ertapenem, cefepime, ceftazidime and enrofloxacin. Noise analysis of ion currents through porin in the presence of antibiotics allows to reveal binding kinetics and transport parameters at the single molecule level. Experimental data was compared with results obtained from molecular dynamics simulations and biological assays.

References

Danelon C, Nestorovich EM, Winterhalter M,* Ceccarelli M, Bezrukov SM. Interaction of Zwitterionic Penicillins with the OmpF Channel Facilitates their Translocation. Biophys. J. ***90*** (2006) 1617–27.

2208-Pos Charge-based Selectivity Inversion In Ompf Porin With Salts Of Divalent Cations

Antonio Alcaraz[1], Elena García-Gimenez[1], María L. López[1], Sergey M. Bezrukov[2], Vicente M. Aguilella[1]

[1] *University Jaume I - Department of Physics, Castellon, Spain,*
[2] *NIH - Laboratory of Physical and Structural Biology, Bethesda, MD, USA.*

Board B323

From Reversal Potential measurements performed at neutral pH, we find that the moderate cationic selectivity of the general bacterial porin OmpF in sodium and potassium chloride solutions is reversed to the anionic selectivity in solutions of calcium and magnesium chloride. The interpretation of this phenomenon is discussed on the basis of:

(i) the role of liquid junction potentials and their contribution to the measured selectivity
(ii) the significant "anionic" diffusion potential for calcium and magnesium chloride bulk solutions and
(iii) the overcharging of the protein channel by divalent cations.

Wide channels, with a large number of charged residues mostly inaccessible to permeant counterions but contributing to ionic selectivity, challenge current physical theories of charge inversion in multivalent electrolyte solutions. Different mechanisms of counterion correlation near channel charged residues are analysed in the light of our selectivity measurements.

2209-Pos Atomistic Simulations of the Porin oprF in a Lipopolysaccharide Membrane

Thereza A. Soares, Tjerk P. Straatsma

Pacific Northwest National Laboratory, Richland, WA, USA.

Board B324

The outer protein F (oprF) is the major non-specific porin of *Pseudomonas aeruginosa,* a important causative agent of nosocomial infections in burn, immunocompromised, and cystic fibrosis patients. It was previously found that the N-terminal domain of oprF forms small, low conductance channels and folds into a β-sheet-rich structure. However, more recent findings indicate that only a conformation formed by the N- and C-terminal domains could form channels with well-defined conductance levels. In the present study, we have investigated the structural dynamics of the N-terminal domain conformation of oprF immersed in a lipopolysaccharide (LPS) membrane by means of atomistic molecular dynamics simulations. Because crystallographic or NMR spectroscopy-derived structures of oprF are unavailable, a structural model based on the X-ray structure of the homolog ompA was used.

The outer membrane is found to have a pronounced influence on the structure and dynamics of this protein. In presence of the LPS membrane, oprF exhibits very low flexibility, most of which is restricted to the periplasmic portion of the protein. The pore undergoes a small contraction to a diameter between 0.15–0.20 nm. The

sole exceptions are the regions corresponding to residues M1/Q2/E43 and H77 whose diameter is increased compared to the initial structure. These findings suggest that i. the eight beta-strand conformation of oprF exhibits a fairly tight channel to allow channel-forming activity, and ii. the unique properties of the LPS membrane, such as fluidity and charge density, will markedly affect the structural dynamics of the outer membrane proteins with implications for atomistic simulations of these class of proteins.

2210-Pos Antibiotics Translocation through Bacterial Porins : A Molecular Dynamics Study

Amit Kumar, Paolo Ruggerone, Matteo Ceccarelli

University of Cagliari, Monseratto, Italy.

Board B325

General diffusion porins like OmpF are the first pore forming proteins to be indentified in bacteria. Their ubiquitous presence among gram-negative bacteria and their extremely large abundance in the outer membrane make them an easily tractable protein to investigate. The shape of OmpF is of an hour glass with the central region rich in charged amino acids. The presence of three stacked arginines on one side and aspartic acid , glutamic acid on another produces a strong transverse electric field in the central region. This is believed to facilitate translocation of zwitterionic antibiotics like ampicllin shown by BLM experiments [1]and also by MD simulations[2,3] . Mutation on the key residues in the central region have been shown to modify the structural and functional properties of the pore for example channel conductance, voltage gating[4].

We apply molecular dynamic simulation to investigate the role of mutations on translocation of antibiotics. However the long time scales, hundreds of microsecond of the process does not permit the use of standard simulations. We use a recent algorithm METADYNAMICS [5] , that allows to overcome the timescale problem and at the same obtain quantification of free energy barrier for the translocation process.

References

[1]. Nestorovich et al . PNAS, *99* , 2002
[2]. M. Ceccarelli et al. Biophys . Journal *87* 2004
[3]. C. Danelon et al. Biophys. Journa *90* 2006
[4]. Bredin et al. J. Memb Biol *156* 1997, Vidal et al . J. Med Chem. 48 2005
[5]. Laio et al PNAS, *99* 2002

2211-Pos Free energy study of ion permeation through Gramicidin

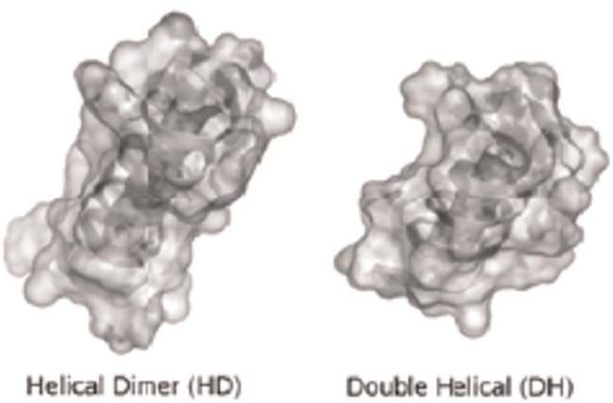
Shirley W. I. Siu, Rainer A. Böckmann

Theoretical & Computational Membrane Biology, Saarland University, Saarbrücken, Germany.

Board B326

The pentadecapeptide gramicidin forms a cation-specific ion channel in membrane environment. Two conformations are known up-to-date: the head-to-head helical dimer (HD) and the intertwined double helical form (DH). These two conformations are favored depending on the specific conditions but the biologically active form is still a matter of debate.

In this study, the energetics of potassium ion permeation by means of the potential of mean force (PMF) of both gramicidin conformations are studied applying the GROMOS G53a6 force field. Comparing the energy profile of the two conformations, the DH has a significantly lower central barrier and broader binding sites at the pore entrances than the HD. The clearly identified external and internal binding sites in both conformations show the energetics and location of ion dehydration upon channel entry. The barrier to ion passage is found to be closely related to the channel flexibility. Multiple ion permeation appears significantly facilitated for the DH conformation due to its opposing pore water dipole moments at the pore entrances [1].



References

[1]. Shirley W.I. Siu and Rainer A. Böckmann (2006) Electric field effects on membranes: Gramicidin A as a test ground. J. Struct. Biol. *3*: 545–546.

2212-Pos CMAP Solves the Gramicidin Problem

Morad Alawneh, Douglas J. Henderson, David D. Busath

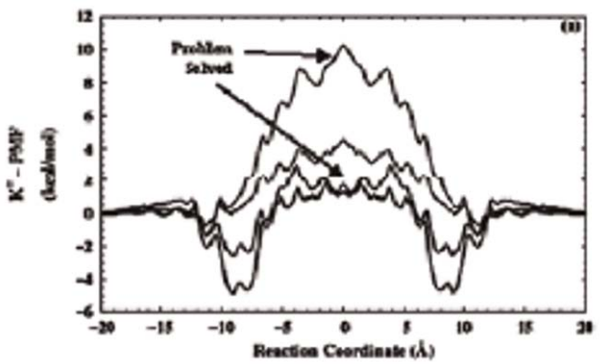
Brigham Young University, Provo, UT, USA.

Board B327

A revised CHARMM force field for Trp residues is studied as well as the effect of the new peptide dihedral correction algorithm (CMAP) using molecular dynamics simulations of gramicidin A (1JNO) embedded in a lipid (DMPC) bilayer with 1 m NaCl or 1 m KCl saline solution. The new Trp force field produces an ion-PMF_{Trp} that is consonant with the prediction from the experimental results, analyzed with rate theory by Durrant et al. (2006), but it is confounded by secondary effects that eliminate the net effect on the PMF. On the other hand, the CMAP for the peptide backbone is the key for solving a long-standing problem in the field, namely, the computed free energy barrier to ion translocation is too high compared with interpretations from the experimental observations. Unexpectedly, CMAP reduces the excessive translocation barrier by reducing the barrier through effects on the ion-PMF_{H2O} rather than

on ion-PMF_{gA}. This indicates a change, induced by CMAP, in the ion-H_2O interactions. The results have been confirmed to be robust using an alternative umbrella potential method.

Supported by NIH AI23007.



2213-Pos Calculating Free Energy Profiles from Nonequilibrium MD Simulations with Small Sample Sizes: Application to Pulling K+ Through a Gramicidin A Ion Channel

Christopher P. Calderon[1], Lorant Janosi[2], Ioan Kosztin[3]

[1] *Rice University, Houston, TX, USA,*
[2] *University of Houston, Houston, TX, USA,*
[3] *University of Missouri, Columbia, MO, USA.*

Board B328

Calculating free energy profiles, i.e., potentials of mean force (PMFs), along selected reaction coordinates in biomolecular systems is a challenging problems in computational molecular biophysics. Recently, there has been a great deal of interest in designing non-equilibrium PMF calculation methods, which compared to the standard equilibrium ones (e.g., the umbrella sampling method) may require less computational time and resources. Here we present two such non-equilibrium PMF calculation methods:

1. the "Forward/Reverse" (FR) method [1], and
2. the Surrogate Process Approximation (SPA) method [2].

These methods can not only provide reliable PMFs from a small number of forward (F) and reverse (R) non-equilibrium steered molecular dynamics (SMD) trajectories along given reaction coordinates, but can also estimate the corresponding underlying diffusion coefficients. The FR method utilizes the Crooks' Fluctuation Theorem and the PMF and diffusion coefficient are determined from the mean work along the F and R trajectories. The SPA method approximates the stochastic dynamics observed in the F and R SMD paths using maximum likelihood estimation techniques. The calibrated diffusion models are then used to augment the data set used to estimate the PMF. After the additional data is generated, any method (like the FR) can be used to compute the PMF. In order to explore their relative merits and limitations, we apply the FR and SPA methods to determine the PMF and the underlying diffusion coefficient of the potassium ion in the gramicidin A channel.

CPC was supported by NSF grants #DMS-0240058 and ACI-0325081. Computational resources we provided by the University of Missouri Bioinformatics Consortium.

References

[1]. Kosztin, I., Barz, B., and Janosi, L. J. Chem. Phys. *124*, 064106 (2006).
[2]. Calderon, C. J. Chem. Phys. *126*, 084106 (2007).

2214-Pos Mechanism and Energetic of Substrate Selectivity and Conduction in Ammonia Channel

Ugur Akgun, Shahram Khademi

University of Iowa, Iowa City, IA, USA.

Board B329

The transport of ammonia, which is fundamental to nitrogen metabolism in all domains of life, is provided by the Rh/Amt/MEP membrane protein superfamily. While bacteria and yeast use Amt/Mep to uptake ammonia as a nitrogen source, mammals uses Rh proteins to excrete ammonia which is toxic to the cell.

The first structure from this protein family, AmtB from *E. Coli*, has been determined at 1.35 Angstrom resolution using x-ray crystallography.

Here, we report Molecular Dynamics (MD) simulations and Brownian Dynamic (BD) simulations of trimeric *E. Coli* AmtB embedded into POPE bilayer for NH_3 and NH_4^+ conduction. Moreover since the Rh proteins are speculated to conduct CO2, we also report MD and BD simulations of the conduction of CO_2 through AmtB, which is a prokaryotic member of Rh/Amt/MEP superfamily.

This report shows that the extracellular vestibule is responsible for selectivity of AmtB for NH_4^+ vs NH_3, and CO_2. The vestibule facilitates the uptake of NH_4^+ and directs it toward the hydrophobic lumen of the channel via oxygens of backbone carbonyls in AmtB. As NH_4^+ enters the narrow hydrophobic pore, it releases its proton to the bulk of water through a chain of hydrogen bonds. Our results show that Asp 160 is not involved in deprotonation of NH_4^+. We also observed an energy barrier for NH_4^+ at the exit of the intracellular vestibule. This suggests that the NH_4^+ cannot enter to the channel through cytoplasmic vestibule.

Our results also show that the AmtB periplasmic vestibule applies selectivity against CO_2 through the oxygen atoms of the backbone carbonyl groups.

2215-Pos K+ Channel Selectivity Depends On Kinetic As Well As Thermodynamic Factors

Michael Grabe[1,2], Delphine Bichet[3,2], Lily Y. Jan[2]

[1] *University of Pittsburgh, Pittsburgh, PA, USA,*
[2] *UCSF, San Francisco, CA, USA,*
[3] *CNRS, Valbonne, France.*

Board B330

Potassium channels are necessary for a number of essential biological tasks such as the generation of action potentials and setting the resting membrane potential in cells, both of which require that these channels selectively permit the passage of potassium ions while suppressing the flow of other ions. Generally, selectivity is attributed to a narrow stretch of the channel known as the selectivity filter. Over this stretch ions are dehydrated, and the backbone oxygen atoms of the protein mimic the ion's loss of coordination by water. However, channels are long pores with spatially distinct ion binding sites that must all be traversed during ion permeation. I will discuss experiments where we show that selectivity of mutant Kir3.2 (GIRK2) channels can be substantially amplified by introducing acidic residues into the cavity, a binding site below the selectivity filter [1]. I will go on to discuss electrostatic calculations that we carried out on homology models to quantify the degree of stabilization that these mutations have on ions in the cavity [2]. I then present a multi-ion model of ion permeation to calculate the channel's permeability to potassium relative to sodium. This kinetic model uses rates derived from the electrostatic calculations, and it demonstrates that non-selective electrostatic stabilization of cations in the cavity can amplify channel selectivity independently of the selectivity filter. This non-intuitive result highlights the dependence of channel properties on the entire channel architecture, and it suggests that selectivity may not be fully understood by focusing on thermodynamic considerations of ion dehydration and the energetics of the selectivity filter solely.

References

[1]. Bichet, D., M. Grabe, Y.N. Jan, and L.Y. Jan (2006) PNAS, *103*:14355–14360

[2]. Grabe, M., D. Bichet, X. Qian, Y.N. Jan, and L.Y. Jan (2006) PNAS, *103*:14361–14366

2216-Pos A Novel Transition Path Ensemble Study of Electrolyte Solution Structure at Physiological Conditions Suggests an Extended Multi-Well Pore Design for Selective Ion Filtering

Ilja V. Khavrutskii[1], Joachim Dzubiella[2], Mikolai Fajer[3], Xiaolin Cheng[1], J. Andrew McCammon[1]

[1] *Howard Hughes Medical Institute and University of California San Diego, La Jolla, CA, USA,*
[2] *Technical University Munich, Garching, Germany,*
[3] *University of California San Diego, La Jolla, CA, USA.*

Board B331

We present a generalized gradient-augmented Harmonic Fourier Beads (ggaHFB) method for finding minimum free-energy transition path ensembles and computing accurate free energy profiles. The ggaHFB method is histogram, Jacobian, metric tensor and reaction coordinate free and is therefore applicable to complex multidimensional systems. The high accuracy of the ggaHFB free-energy profiles is demonstrated by gauging against two independent, conventional ion-ion potential of mean force (PMF) benchmarks. The ggaHFB method is then applied to study concentration and size dependence of the ion-ion PMFs of monovalent electrolyte solutions in the physiological concentration range. We find that ion activities remain constant in the concentration range between 0.1 and 0.3 M NaCl, which might be of biological significance. Furthermore, we observe that for the like-charged ion pairs in mixed NaCl:KCl solutions the increase in size of the ions from Na-Na to Na-K and K-K switches the short-range interaction from repulsive into attractive mode. Based on this finding we propose a linked ion-pair incommensurate passage model and design a hypothetical K selectivity filter that selects ions of larger size over those of smaller size. Our filter is compared to that of the KcsA ion channel. We then investigate the conformational transition of the four alpha-strands of the KcsA selectivity filter in response to a change in the ion concentration. Finally, before studying the ion permeation through the KcsA selectivity filter, we compute the free-energy profile for the permeation of water through a carbon nanotube channel of sub-nanometer diameter. Here we identify the driving force behind water entering the hydrophobic pore and provide a simple explanation for its pulsating behavior.

2217-Pos A Stochastic Approach to Transport In Potassium Ion Channels

Enrique Abad[1,2], Philip Fowler[1], Mark Sansom[1]

[1] *University of Oxford, Oxford, United Kingdom,*
[2] *Université Libre de Bruxelles, Brussels, Belgium.*

Board B332

We present a discrete Markovian approach which allows one to compute theoretical conductance curves for a family of potassium ion channels. The method relies on a fundamental matrix approach for the dwelling times of potassium ions at different binding sites. A closely related model has been used in a previous reference to obtain reasonable estimates for ion residence times in the selectivity filter of the KcsA K+ channel (E. Abad and J.J. Kozak Physica A *380*, 172–190(2007)). In the present model, switching between different states of the underlying Markov chain is governed by Arrhenius-like transition probabilities. The latter are constructed from free energy profiles provided by detailed molecular dynamics simulations based on structural data at the atomistic level. The ultimate goal of our stochastic model is to bridge the gap between the atomistic level of description and a more mesoscopic approach by providing transport equations able to describe basic conduction properties of ion channels in terms of a small number of coarse-grained observables characterizing the relevant interactions.

2218-Pos Why Can't Potassium Ion Channels Conduct Sodium? - A Critical Comparison Of The Competing Hypotheses Of Ion Selectivity

Philip W. Fowler, Kaihsu Tai, Mark S. P. Sansom

University of Oxford, Oxford, United Kingdom.

Board B333

It is not yet understood how potassium ion channels can conduct potassium, but not sodium, ions. A number of different hypotheses have recently been suggested; these can be summarised as the snug-fit, carbonyl-repulsion and topological-constraint hypotheses. All the theoretical studies necessarily used simplified models of a single ion binding site and did not (usually) attempt to explain the expected differences in selectivity between the four ion binding sites. A further constraint on these hypotheses is that they must also be able to explain why NaK conducts sodium ions but KcsA does not, despite the two ion channels having similar structures.

We use nanosecond classical molecular dynamics simulations to (i) test each of these hypotheses by analysing the behaviour of ions in the selectivity filters of NaK and KcsA, (ii) investigate what structural/dynamical differences in the behaviour of the selectivity filters could lead to the observed variation in selectivity along the pore and (iii) compare the dynamics of the selectivity filters at physiological temperatures.

2219-Pos Classical and Quantum Chemical Studies of Rb^+ & Cs^+ ions to Understand Mechanism in K-channels

Sameer Varma, Dubravko Sabo, Susan B. Rempe

Sandia National Labs, Albuquerque, NM, USA.

Board B334

Cs^+ interferes with the permeation of K^+ ions through potassium (K−) channels and serves as a channel blocker. Presumably, the difference between its binding free energy to the selectivity filter and its hydration free energy must be more negative than that of K^+, and even the other permeable ion, Rb^+. Nonetheless, what still remains unclear regarding the mechanism by which it interacts with K-channels includes the following. In our previous quantum chemical studies, we found that highly selective K-channels maintain a special local environment around their binding sites devoid of competing hydrogen bond donor groups, which enables spontaneous transfer of K^+ from states of low coordinations in water into states of over-coordination by 8 carbonyl ligands in the channel filter. This over-coordination is physiologically important to achieve K^+ over Na^+ selectivity. Does the binding of Rb^+ or Cs^+ to the 8-fold sites in the channel also require the presence of this special local phase? In addition to the properties of the solvation phase beyond the individual binding sites, are there any structural differences between the inner coordination shells of the Cs^+, Rb^+ and K^+ ions that designate the former as a blocker and the latter two as permeable ions? To resolve these issues, we carry out a series of classical and quantum chemical simulations and probe the effects of such determinants as coordination number, ligand chemistry and local phase on the structural and thermodynamic solvation properties of Rb^+ and Cs^+ ions. We then compare these results to our previous results on Na^+ and K^+ ions to understand why K-channels appear to be selective for ion size.

2220-Pos Energetics of Ion Selectivity in a Calcium Channel: The Ryanodine Receptor Case Study

Dirk Gillespie

Rush University Medical Center, Chicago, IL, USA.

Board B335

A model of the ryanodine receptor (RyR) calcium channel is used to study the energetics of ion binding selectivity. RyR is a calcium-selective channel with a DDDD locus in the selectivity filter, similar to the EEEE locus of the L-type calcium channel. While the affinity of RyR for Ca2+ is in the millimolar range (as opposed to the micromolar range of the L-type channel), the ease of single-channel measurements compared to L-type and its similar selectivity filter make RyR an excellent candidate for studying calcium selectivity. A Poisson-Nernst-Planck/Density Functional Theory model of RyR is used to calculate the energetics of selectivity. In RyR, ion selectivity is driven by the charge/space competition mechanism in which selectivity arises from a balance of electrostatics and the excluded volume of ions in the crowded selectivity filter. While electrostatic terms dominate Ca2+ selectivity, the much smaller excluded-volume term also plays a substantial role. In the D4899N and D4938N mutations of RyR that are analyzed, substantial changes in specific components of the chemical potential profiles are found far from the mutation site. These changes result in the significant reduction of Ca2+ selectivity found in both theory and experiments.

2221-Pos A Common Mechanism For The Anomalous Mole Fraction Effect In Biological Calcium Channels And In Abiotic Pores

Dirk Gillespie[1], Dezso Boda[1], Yan He[2], Zuzanna Siwy[2]

[1] *Rush University Medical Center, Chicago, IL, USA,*
[2] *University of California, Irvine, CA, USA.*

Board B336

A theory of the anomalous mole fraction effect (AMFE) in biological calcium channels is presented that does not assume single-filing of multiple ions in the pore. In a mole fraction experiment, a mixture of two ion species is at a fixed total concentration and the channel conductance is measured while the relative concentrations are changed. In some cases, the conductance of the mixture is less than that of the conductances of either species individually (an AMFE). The textbook explanation of the AMFE is the coordinated movement of multiple ions moving through a single-file channel. The theory that is presented takes a different approach and builds on the ideas of Nonner and Eisenberg that depletion zones of ions in the permeation pathway correspond to high resistance elements in series. Experimental verification that the AMFE can exist in wide pores without correlated ion motion is shown in a micrometer-long pore in plastic with a negative surface charge of density -1e/nm2. The diameter of the plastic pores ranges from 20 to 50 Angstroms.

The same theory with a reduced model of this abiotic pore reproduces the experimental normalized currents.

2222-Pos Competition of Steric repulsion and Electrostatic Attraction in the Selectivity Filter of Model Calcium Channels

Dezso Boda[1], Wolfgang Nonner[2], Monika Valisko[3], Douglas Henderson[4], Bob Eisenberg[1], Dirk Gillespie[1]

[1] *Rush University Medical Center, Chicago, IL, USA,*
[2] *Miller School of Medicine University of Miami, Miami, FL, USA,*
[3] *University of Pannonia, Veszprém, Hungary,*
[4] *Brigham Young University, Provo, UT, USA.*

Board B337

Calcium channels conduct Na ions in the absence of Ca, but they selectively conduct Ca ions when Ca ions are present at physiological concentrations. In the anomalous mole fraction effect, even a micromolar amount of Ca ions effectively blocks Na current . Many attempts have been made to explain the mechanism behind these phenomena. In our model of the selectivity filter of Ca channels, the endgroups of the side chains of amino acids–four glutamates–in the selectivity filter are represented as mobile ions that are restricted so they move inside the filter. These structural ions form a liquid-like self-adjusting environment for the passing ions so that the system assumes minimum free energy. They also fill part of the pore so the counterions have to compete for space in the crowded selectivity filter. In this picture electrostatic attraction and repulsive entropic excluded volume effects compete with each other to determine which ions can enter the selectivity filter. We argue that this competition is crucial in explaining the selectivity mechanism of Ca channels. We show Monte Carlo simulation results for competition between ions of different valence and diameter. We predict that Ca-selectivity depends on the background concentration of NaCl. We show that our model can explain the micromolar Ca-selectivity observed in the L-type Ca channel. We also show results for an alternative model of Ca channels developed by Corry *et al.* In this model, the structural ions are placed in fixed positions behind the protein wall. We show that this rigid model cannot reproduce the micromolar selectivity of the L-type Ca channel.

2223-Pos Model Study Of Temperature Dependent Functional Motions Of N-type Ca^{2+} Voltage-sensitive Channel

Yun Xu, Larisa Adamian, Jie Liang

University of Illinois at Chicago, Chicago, IL, USA.

Board B338

N-type Ca^{2+} voltage-sensitive channels are expressed in neurons and play a role in the transmission of nociceptive stimuli such as noxious heat. To understand the mechanism of thermal-induced nociception, we developed a 3D model and studied the large-scale functional motions at different temperatures of N-type calcium channel. Using protein threading and homology modeling based on X-ray structure of mammalian K^+ shaker channel as a template (2A79), we have constructed a model of N-type Ca^{2+} channel. Despite of the low sequence identity (~10%), we were able to reliably align both proteins using published experimental data on the functional and conserved amino acid residues in voltage-sensitive ion channels. We first used MODELLER suite of programs to calculate a set of candidate homologous structures and then applied the LIPS algorithm to select a model with the best orientation of transmembrane helices. We have compared the template K^+ channel structure with the target Ca^{2+} channel structure and found that the ion filter radius in the model is smaller. This is in agreement with the fact that Ca^{2+} has a smaller atomic radius in comparison with K^+ (0.99Å *vs*. 1.38 Å, respectively). We then calculate dynamics of a N-type Ca^{2+} using anisotropic network model. We focus on large-scale motions of N-type channel at different temperatures which are required for the ion channel gating and Ca^{2+} influx. Our result of large-scale motions indicated that temperature increase significantly affects the functional motions of N-type channel as well as results in the increase of the amplitude motion of individual amino acid residues, an increase of the diameters of the ion filter and gate. This temperature dependent may facilitate the inward flow of Ca^{2+}, which, in turn may trigger the nociceptive response.

2224-Pos A New Reaction Field Method for Brownian Dynamics Simulations of Ion Channel Permeation

Bernhard Egwolf, Benoît Roux

Institute for Molecular Pediatric Sciences, The University of Chicago, Chicago, IL, USA.

Board B339

A new reaction field method has been developed for the Grand Canonical Monte Carlo/Brownian Dynamics (GCMC/BD) algorithm, which allows to simulate the permeation of ions through ion channels of arbitrary shape. In order to propagate the stochastic ion trajectories, GCMC/BD requires the effective microscopic forces acting on the ions. These forces are given by the gradient of the multi-ion potential of mean force (PMF), which corresponds to the free energy to insert the ions into a given simulation system. A crucial contribution to this PMF is the reaction field induced by the ions in their heterogeneous dielectric environment, which consists of a high dielectric solvent continuum representing the water and regions with low dielectrics describing the polarizability of the protein and the membrane interior. The new method quantifies the reaction field by a sum over the self reaction field energies of the ions and pairwise ion-ion interaction terms depending on the self reaction field energies, effective ion-protein distances, and the ion-ion distances. Inspired by methods for calculating generalized Born radii, an improved integration formula for the self reaction field energies of the ions has been derived and optimized for single ions in channels. The effective ion-protein distances are inspired by the image charge method and yield accurate values for the pairwise ion-ion interaction terms. It is possible to compute these reaction field parameters for ions located at the points of a regular grid before a

simulation is launched. This look-up grid allows a very efficient calculation of the reaction field energies and forces during a simulation.

2225-Pos Modelling and Simulation of Ion Channels

Kattrin Arning

Radon Institute for Computational and Applied Mathematics, Austrian Academy of Sciences, Linz, Austria.

Board B340

Ion channels play a vital role in controlling many important biological functions such as conducting electrical signals down nerve fibres and initiating muscle contraction. They provide a gateway for different kinds of ions enabling them to pass through otherwise impermeable cell membranes. One of their main features is their selectivity, allowing only specific types of ions to enter or leave the cell. This selectivity is determined by the structure of the channel protein, in particular by the channel geometry and fixed charges ("permanent charge") from confined species located inside the filter region.

Our goal is to use a mathematical model describing the transport process to identify individual structural features of the channel based on measurements of current-voltage curves.

There are both continuum and atomistic approaches for modelling the transport of ions through ion channels. One continuum approach is based on the Poisson-Nernst-Planck theory which leads to a standard model for electro-diffusion of charged species. The excess chemical potentials are determined using density functional theory originated from Rosenfeld. Since the full continuum model leads to a system of coupled partial differential equations (PDEs) that requires high computational effort, we derive a reduced surrogate model for characterizing the current-voltage relationship. This surrogate model will then be used to identify some basic channel properties like the amount of fixed charge inside the filter from voltage-current mesaurements. Such expedient computer models can provide helpful insights for determining the structure and function of biological membrane channels and for designing artificial channels that show a desired behaviour, e.g. with respect to selectivity towards a certain species.

2226-Pos An Ionic Model for Action Potentials and Fast Ca^{2+} concentration Dynamics in Pancreatic Beta-cells

Leonid E. Fridlyand, Louis H. Philipson

University of Chicago, Chicago, IL, USA.

Board B341

We formulated a Hodgkin-Huxley-type ionic model for the action potential in pancreatic beta-cells producing insulin based on the voltage- and current-clamp results from our laboratory and published experimental data. The model contains an inward L- type Ca^{2+} current (I_{VCa}), a "rapid" delayed rectifier K^+ current (I_{Kr}), a small slowly activated K^+ current (I_{Ks}), a small conductance Ca^{2+}-activated K^+ current, an ATP-sensitive K^+ current, a plasma membrane calcium pump current and a Na^+ background current. This model is coupled to an equation describing Ca^{2+} homeostasis in cytoplasm. The model replicates the glucose-induced spontaneous spikes in beta-cells, and the effects of blocking K^+ currents by tetraethylammonium ions, stromatoxin and tolbutamide, as well as specific spike behavior in beta cells lacking the Kv2.1 K^+ channel. In this model I_{VCa} generates the spikes, whereas I_{Kr}, I_{Ks}, or Ca^{2+}-activated K^+ currents contribute to repolarization. However, all currents contribute to the regulation of spontaneous spike activity and can be responsible for burst behavior. Original features of this model include new equations for the I_{VCa}, assessment of the role of the I_{Kr} and demonstration of the possible influence of Ca^{2+} pump and background currents on the action potential and bursts. This model provides acceptable fits to voltage-clamp, action potential and Ca^{2+} concentration change dynamics and can be used to seek biophysically based explanations of the electrophysiological activity and Ca^{2+} flux in the pancreatic beta-cells under a variety of physiological conditions.

2227-Pos Electrodiffusion Through A Protegrin Pore - Molecular Dynamics And Continuum Theory

Dan S. Bolintineanu, Allison A. Langham, Abdallah Sayeed Ahmad, Yiannis N. Kaznessis

University of Minnesota, Minneapolis, MN, USA.

Board B342

Protegrins are small, cationic antimicrobial peptides that are believed to act against bacterial infections by compromising the integrity of the bacterial cell membrane. We have used the results of extensive, fully atomistic molecular dynamics (MD) simulations of a protegrin pore in a lipid bilayer as input to a continuum electrodiffusion analysis of ion permeation (commonly referred to as Poisson-Nernst-Planck theory). The coupled steady-state Poisson-Nernst-Planck equations are solved numerically, using the geometry, charge density, and ion diffusivity profile obtained from the MD simulations. This multiscale approach allows for a quantitative analysis of ion transport through a protegrin pore in the biologically relevant non-equilibrium state (in particular, ion transport in the presence of an applied field, or in the presence of a concentration gradient). Results from this approach are shown to be in reasonable agreement with previous experimental measurements of similar systems, in particular conductance and ion selectivity measurements. The relatively low computational cost of this approach allows us to explore numerous aspects of ion transport through a protegrin pore, including current-voltage relationships, the relative importance of geometry and charge distribution, ion types and diffusivities, and external conditions. We discuss the biological implications of these results, in particular with regards to the ability of protegrins to disrupt the transmembrane potential, which is likely an important mechanism in the action of these and other antimicrobial peptides.

This work was supported by a grant from NIH (GM 070989). Computational support from the Minnesota Supercomputing Insti-

tute is gratefully acknowledged. This work was also partially supported by National Computational Science Alliance.

2228-Pos Ion Flux Through Membrane Channels - an Enhanced Algorithm for the Poisson-Nernst-Planck Model

Witold Dyrka[1], Andy Augousti[2], Malgorzata Kotulska[1]

[1] *Wroclaw University of Technology, Wroclaw, Poland,*
[2] *Kingston University, London, United Kingdom.*

Board B343

The Poisson-Nernst-Planck (PNP) electrodiffusion theory provides a basis for computing charge fluxes through narrow pores, especially biological ion channels. A novel algorithmic scheme for numerical solution of the 3D Poisson-Nernst-Planck model is proposed. The algorithmic improvements are universal and independent of the detailed physical model. They include three major steps: an adjustable gradient-based step value, an adjustable relaxation coefficient, and an optimised segmentation of the modelled space. The enhanced algorithm very significantly accelerates the speed of computation and reduces the computational demands without loss of the accuracy. All the methods are effective also when applied alone. The adaptive gradient-based optimisation technique is analogous to an extension of the over-relaxation technique, and we term it as a super-relaxation technique. It appears to exhibit greater stability than the standard over-relaxation technique with a choice of the weighting parameter significantly less than -1, probably because our technique is based on a mean of several iteratively-derived values, rather than a simple projection from a single value. The adjustable relaxation coefficient technique has also proven to be more efficient that the basic algorithm, although less efficient than the super-relaxation technique, probably because it is more susceptible to high-frequency fluctuations in the iteration profile. The combination of these two techniques provides a powerful improvement in iteration efficiency, typically reducing the number of iteration steps by approximately 80%. The space segmentation technique is similarly effective, reducing the number of iterations between 50–75% approximately, with good accuracy in achieving the final values. The accuracy typically differed by less than 0.5% from values obtained from the basic unsegmented algorithm.

2229-Pos Pore Formation In Bipolar Lipid Membranes: A Coarse Grained Molecular Dynamics Model

Alain Gauthier, Bela Joos

University of Ottawa, Ottawa, ON, Canada.

Board B344

Archaeal bacteria thrive in extreme environments. In order to survive such harsh conditions as low PH, high temperatures, and near-saturated salt brines, some of these organisms have evolved specialized membranes composed of bipolar lipids. Vesicles composed of bipolar lipids from these organisms have been shown to have very low permeability to protons, ions and small molecules when compared with vesicles composed of polar lipids typically found in prokaryote and eukaryote cells. The lower permeability has usually been explained by the tighter packing of bipolar lipids. A higher packing density increases the energy barrier to diffusive permeability and reduces the probability of formation of water wires and hydrophilic pores. To understand the effects of the monolayer nature of bipolar lipid membranes, we performed coarse grained molecular dynamic simulations of both a monolayer, composed of single chained bipolar lipids with a head group at either end, and a bilayer system, composed of the same lipids cut in half, in other words, single chain lipids, half the tail length of the bipolar lipids, with one head group. We compare the structural properties, elasticity, and line tension of the two membranes. We will present a detailed analysis of the nucleation of pores, its edge structure and line tension, as a function of pore radius. In brief, the monolayer system is found to be more rigid, ordered, and tightly packed than the bilayer system. It is also found to have a greater resistance to pore formation, in agreement with recent experimental observations.

2230-Pos Ionic transport in nanopores

Arthur Evans[1], Johan Lagerqvist[1], Michael Zwolak[2], Massimiliano Di Ventra[1]

[1] *University of California San Diego, San Diego, CA, USA,*
[2] *California Institute of Technology, Pasadena, CA, USA.*

Board B345

We study ionic transport in nanopores from the perspective of the microscopic electrostatics. In the confining geometry of a nanopore, the breaking of hydration layers that surround an ion in bulk water creates an energetic barrier to transport that depends on the number of broken layers. As a result, we predict the existence of step-like structures in the ionic conductance as a function of the nanopore radius. The transport barrier can also be modified by the external bias and by a strong ionic concentration gradient. In this talk, we discuss various properties of the hydration layers and the parameter range necessary to experimentally observe set-like ionic conductance through nanopores. We will also explore the possible impact of this work to nanoscopic DNA sequencing methods.

Work supported in part by NSF and NIH.

2231-Pos Microwave Probing Of Artificial Ion Channels And Pores

Robert H. Blick, Sujatha Ramachandran, Hyun-Seok Kim, Hua Qin, Hyun-Cheol Shin, Eric Stava, Daniel W. van der Weide

University of Wisconsin-Madison, Madison, WI, USA.

Board B346

We present direct microwave spectroscopy measurements in the high-frequency end of 1 GHz on on artificial ion channels and pores. The measurements are performed making use of microstrip lines and micro-coaxes integrated in a on-chip patch clamp setup. This

allows us to monitor channel formation of alamethicin peptides and a-HL. From the microwave tranmission signature we can correlate the peptides' insertion and reveal radiation rectification. The results offer a new tool for high-frequency spectroscopy on single channels.

2232-Pos Ion Exclusion by Sub-2 nm Carbon Nanotubes: a Simplified Model for Ion Channels

Francesco Fornasiero[1], Hyung Gyu Park[1], Jason K. Holt[1], Micheal Stadermann[1], Costas P. Grigoropoulos[2], Aleksandr Noy[1], Olgica Bakajin[1]

[1] *LLNL, Livermore, CA, USA,*
[2] *UC Berkeley, Berkeley, CA, USA.*

Board B347

Narrow and relatively hydrophobic pore regions are often encountered in biological channels, including aquaporins, proton, and ion channels. Moreover, charged residues are frequently found in the selectivity filter of membrane ion channels. Understanding the mechanism of ion transport and ion selectivilty in biological pores is very difficult due to their inherent complexity.

Carbon nanotubes are considered simplified models of membrane channels because of their hydrophobicity, narrow diameter, and fast flow of water, comparable to membrane aquaporins. Thus, to avoid the complexity of biochannels while retaining their basic features, we investigate ion transport through carbon nanotube model pores. The nanofluidic platform used in this study consists of membranes made of aligned double-walled carbon nanotubes with sub-2 nm diameter. Negatively charged groups are introduced at the opening of the carbon nanotubes by oxygen plasma treatment. Pressure-driven filtration experiments coupled with capillary electrophoresis analysis of permeate and feed are used to understand the mechanism of ion transport for both large and small ions. Ion exclusion and selectivity is investigated as a function of solution ionic strength, pH, and ion valence. Observed trends show similarities with biological ion channels.

This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

Ion Channels, Other

2233-Pos Identification of the Pore-Lining Residues of the BM2 Ion Channel of Influenza B Virus

Chunlong Ma[1], Cinque Soto[2], Yuki Ohigashi[1], Albert Taylor[1], Vasilios Bournas[1], Brett Glawe[1], Maria K. Udo[3], William F. DeGrado[2], Robert A. Lamb[1,4], Lawrence H. Pinto[1]

[1] *Northwestern University, Evanston, IL, USA,*
[2] *University of Pennsylvania, Philadelphia, PA, USA,*
[3] *Loyola University, Chicago, IL, USA,*
[4] *Howard Hughes Medical Institute, Evanston, IL, USA.*

Board B348

The influenza virus BM2 proton-selective ion channel is essential for virus replication. The BM2 channel conducts proton into the interior of the virus particle, which results in viral matrix protein-RNPs dissociation. The BM2 protein is similar to the A/M2 protein of influenza A virus in that it is a single span proton channel and it contains a $H_{19}XXXW_{23}$ motif. Unlike the A/M2 protein, the BM2 protein is not inhibited by the antiviral drug amantadine. The identification of the pore-lining residues of the BM2 proton channel is essential to understand the mechanism of ion transport and to reveal the drug action target. In this study we used cysteine scanning mutagenesis in combination with the substituted cysteine accessibility measurement to ascertain the pore-lining residues of this ion channel. The specific activity (relative to wild-type), reversal voltage, and susceptibility to modification by MTSEA and NEM of the mutant proteins were measured in oocytes. We found that substitutions of cysteine at positions of Ser9, Ser12, Phe13, Ser16, His19, and Trp23 ion channels were most disruptive for ion channel function and/or most susceptible to MTSEA and NEM modification. Based on experimental data, a first BM2 transmembrane domain model is proposed. The presence of polar residues in the pore is a probable explanation for the amatadine-insensitivity of the BM2 protein and suggests that related, but more polar, compounds might serve as useful inhibitors of the protein.

This project was supported by NIH grant RO1 AI-57363 to LHP.

2234-Pos Channel Properties Of The Translocator Hmw1b Of The Two Partner Secretion System Of *Hemophilus Influenzae*

Guillaume Duret, Michal Szymanski, Hye-Jeong Yeo, Anne H. Delcour

University of Houston, Houston, TX, USA.

Board B349

H. influenzae is a pathogenic Gram-negative bacterium that colonizes the nasopharynx. A major virulence factor is the adhesin HMW1 which provides attachment of the bacterium to the host epithelium. The secretion of HMW1 requires the outer membrane translocator HMW1B. HMW1B belongs to the Omp85-Tps superfamily, which also includes proteins involved in the membrane biogenesis of mitochondria and chloroplasts. It is believed that HMW1B contains an N-terminal surface-exposed domain, followed by a large periplasmic domain possibly involved in HMW1 adhesin recognition and a C-terminal beta-barrel domain which may act as a pore for the translocation of the adhesin. Here, the whole HMW1B and the C-terminal domain CTD (HMW1B 234-545) were purified, and the pore-forming nature of the proteins was assessed by electrophysiology using planar lipid bilayers. CTD was a somewhat more unstable protein and required prior reconstitution into liposomes before insertion into planar membranes. Both proteins show similar channel activity. The proteins exhibit opening transitions to two distinct levels of conductance of 343 and 1417 pS in 1 M KCl. Transitions of small conductance tend to be frequent and extremely flickery. The large conductance level is accessed less frequently and