(5) [^{3}H]epibatidine competition experiments indicate that ibogaine analogs interact with the agonist sites with very low affinities. Interaction of 18-MAC with the hα3β4 ion channel could be important for its anti-addictive property.

204-Pos Board B83
Noble Gas Anesthetics and Immobilizers Show Different Binding Distributions to KcsA Channel
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Neuronal ion channel is prominent candidate of molecular target anesthesia, but still not yet identified. Using KcsA potassium ion channel as model, anesthetic-protein interactions are investigated. We choose xenon, krypton and argon as anesthetics, which have simple structure. Neon and helium were also studied, which are structurally similar to anesthetics but do not have the anesthetic effects predicted by the Overton-Meyer rule (nonimmobilizers). Using computer simulation these binding sites of KcsA are searched. From the noble gas-KcsA complex structure we discuss binding characteristics of anesthetic and nonimmobilizer. **Methods:** 1k4c (PDB) was used as KcsA structure. Cavities in KcsA was searched with alpha-site finder (geometric search) in Molecular Operating Environment 2007.0902 (MOE, Chemical Computing Group, Canada), that is candidates of binding site of noble gas. Obtained dummy atom from alpha-site finder was used as initial position. Noble gas binding position was searched with energy minimization around initial position. MMFF94x was used for forcefield. **Results:** Binding energy of Xe, Kr, Ar were -8 to -4 kcal mol^{-1}, whereas Ne and He were -2 kcal mol^{-1}. Xe, Kr, Ar bound to gating region first, then they distributed to inter-helical space of transmembrane region. Ne bound to inter-helical space first, then to the gating region. Energy gaps of inter-helical sites were small, so noble gas was consider to be possible to transit from site to site with thermal energy. We considered that inter-helical binding have small position specificity (nospecific binding). Ne and He binding distributed inter-helical sites, the energy gaps were further small. They showed nonspecific binding. Anesthetics and nonimmobilizers of noble gases show different binding distribution to KcsA. We speculate that pharmacological difference of anesthetic and nonimmobilizer originates from the difference in binding distribution of these substances.

205-Pos Board B84
Ligand Induced Conformational Changes in GPCRs: Insight Into the Activation of Rhodopsin and β-adrenergic Receptors
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Signal transduction in GPCRs is initiated by ligand binding at the extracellular domain of the receptor. Recent experimental evidences indicate that structurally different ligands with varied efficacies stabilize distinct receptor conformations. Understanding the relationship between ligand structure and the stabilized receptor conformation is critical in designing GPCR drugs with functional selectivity for a particular signaling pathway. We recently developed a computational method (LITiCon) to study the ligand induced transmembrane conformational changes in GPCRs. Using this method, we have predicted the active conformation of bovine rhodopsin stabilized by the full agonist all-trans retinal. The major conformational changes upon activation are the straightening of the TM6 kink and tilting of the intracellular end of TM5 towards TM6. These predictions are in agreement with the recently published crystal structure of ligand-free opsin, which is believed to be in a partially active conformation. We then study the conformational changes in human β-adrenergic receptors induced by full and partial agonists as well as inverse agonists. In the predicted conformation of the β_2-adrenergic receptor stabilized by the full agonist norepinephrine, the three serines on TM5 come inside the binding pocket and the extracellular end of TM6 tilts towards TM3. These changes lead to shrinking of the norepinephrine binding pocket thus tightening the protein-ligand contacts. A new HB between N293 on TM6 and the β-OH of norepinephrine is formed in the norepinephrine stabilized conformation, which was not possible in the inactive conformation. Virtual ligand screening of the inactive receptor conformation shows higher selectivity for antagonists compared to agonists, whereas that of the norepinephrine stabilized conformation shows higher selectivity for agonists compared to antagonists. These results along with new insights into the ligand specificities between β_1 and β_2 receptor subtypes will be presented.

206-Pos Board B85
Structural Determinants Of Antibiotic And β-lactamase Diffusion Through Bacterial Porins
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General diffusion porins such as OmpF and OmpC, located in the outer membrane of bacteria, represent the main entry point for different classes of antibiotics. Bacteria can resist the action of antibiotics by underexpressing and/or mutating porins. Nowadays the problem of bacterial resistance calls for new antibiotics.
Another way bacteria exhibit resistance is by expressing enzymes that degrade antibiotics, such as β-lactamase that act on β-lactam antibiotics. Inhibitors of such enzymes are prescribed in combination with antibiotics to block β-lactamase and let antibiotics to reach their target. Again, β-lactamase inhibitors have to diffuse through porins in order to reach their target. Understanding how antibiotics and β-lactamase inhibitors diffuse through porins would help to design new molecules with improved permeation properties, solving this problem of resistance.
To investigate the diffusion process of molecules through bacterial porins we used classical MD simulations using OmpF in monomeric and trimeric form. Indeed, as showed experimentally, diffusion is controlled mainly by interaction at the molecular scale. However the high level of accuracy of MD represents also a limitation for simulations to reach the typical time scale of diffusion, from microsecond to millisecond. To overcome this problem we used an acceleration scheme, metadynamics, that allow extending simulations time to biological time scale.
From MD simulations we identified the structural determinants that play a key role in the diffusion process : (i) Flexibility of the molecule diffusing and porin (ii) particular localisation of charged residues (iii) presence of hydrophobic pockets. Further, we observed reciprocal influence of each monomer, in particular in the external loops and the constriction region. We compared diffusion of different antibiotics through various classes of porins, to understand better the problem of bacterial resistance to antibiotics.

207-Pos Board B86
Conformational Transitions and Proton Conduction in the Multidrug Efflux Pump AcrB
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The increasing problem of multi-drug resistance (MDR) in cancer therapy or bacterial infections is to a large degree caused by multi-drug efflux pumps in pathogenic cells. In Escherichia coli, a major resistance mechanism against antibiotics is based on a tripartite multi-drug export complex comprising the inner membrane translocase AcrB, the membrane-fusion protein AcrA and the outer-membrane channel TolC. AcrB functions as the engine of this complex, using proton motive force to expel a wide variety of unrelated toxic compounds such as antibiotics, disinfectants or detergents. The molecular mechanisms of how proton conduction through AcrB is coupled to drug expulsion are not fully understood yet. Here we report a combination of normal mode analysis (NMA) and molecular dynamics (MD) simulation to investigate conformational transitions occurring in the AcrB reaction cycle and to identify residues crucial for proton conduction. In the crystallographic structure of AcrB each monomer is trapped in a different conformation, representing consecutive states in the transport mechanism. Applying the elastic network NMA variant of minimum action pathway (Kim et al. 2002), we computed transitions between these states. The resulting c-alpha trajectories were then converted back to all atom in an approach of steered energy minimization. We also performed multi-copy MD simulations of AcrB embedded in a phospholipid/water environment using the GroMACS simulation package. Mapping the proton conduction pathway was done on the basis of protein-internal water dynamics and monitoring their frequency of forming hydrogen bonds to adjacent residues.
References:
Kim, M. K., R. L. Jernigan, and G. S. Chirikjian. 2002. Biophysical Journal. 83: 1620-1630.

208-Pos Board B87
Dynamics Of Water Molecules In Bacteriorhodopsin Mutants
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Water molecules are essential for the functioning of proton-pumping proteins. Bacteriorhodopsin is a light-driven proton pump whose reaction cycle is accompanied by changes in the interactions between the protein and the retinal chromophore with water molecules. Of particular importance is the formation of a chain of water molecules that mediate the reprotonation of the retinal Schiff base from the Asp96 residue. Asp96 is replaced by histidine in channelrhodopsin-1 (G. Nagel et al, Science 296, 2395-2398, 2002), and by glutamate in *Neurospora* rhodopsin (Y. Fan, L. Shi & L. S. Brown, FEBS 581, 2557-2561, 2007). Significant effects of mutating Asp96 on the proton-pumping kinetics of bacteriorhodopsin, and effects of mutating the corresponding residues in other retinal proteins, have been documented. To understand how replacement of

Asp96 affects the dynamics of water molecules in bacteriorhodopsin, we perform molecular dynamics simulations of bacteriorhodopsin wild type and Asp96 mutants.

209-Pos Board B88

The Biophysics Of Antibiotics Translocation Through OmpF Revealed By Computer Simulations

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In Gram-negative bacteria, the outer membrane porin F (OmpF) constitute the preferred entry point of antibiotics. Since bacteria can resist antibiotics by altering the expression or structures of OmpF, it is of fundamental importance to investigate on the permeation mechanisms at a molecular level. A key feature in the structure of OmpF is the presence of a constriction region, characterized by both a spatial (with dimensions as low as 7x11Å) and an electrostatic (a transversal field formed by negative and positive residues facing each others) restriction.

To study the translocation process at a molecular scale, we performed molecular dynamic simulations combined with the metadynamic algorithm. This recently designed algorithm overcomes the time scale problem by accelerating properly defined reaction coordinates. We compared the following modeling methodologies: (i) OmpF as monomers or trimers, (ii) membranes as surrounding detergent molecules or lipid bilayers, (iii) antibiotics of different structural and chemical properties (penicillins, fluorokinolones, cephalosporines).

We evaluated how site mutations on OmpF alter electrostatic or spatial restriction at the constriction region and affect antibiotics binding and transport. We reconstructed the free energy surface of each antibiotic translocation and compared their preferred path, orientation, affinity sites. We find that translocation is governed by specific (polar, hydrophobic) interactions. This leads us to discuss the applicability of analytical models in this transport. Our results, such as energy barriers for translocations, compared well with the translocation rates obtained by experimental collaborators using electrophysiology and MIC measurements. Furthermore, our methodology suggested new measurements, such as testing novel OmpF variants, low-temperature measurements and liposome swelling assays.

This study demonstrates how theory and experiments combined can reveal the mechanism and the molecular basis of OmpF permeation. This work will benefits to the design of antibiotics with improved transport properties.

210-Pos Board B89

Simulation Studies Of Trace Amines Passing Through Neuronal Membranes

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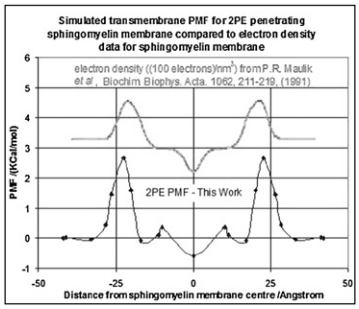
The trace amine phenylethylamine (2PE) has been the focus of a number of recent studies attempting to ascertain its physiological role (M.D. Berry, *J. Neurochem*, **90**, 257-271, (2004)). An important unknown is the role of passive diffusion in allowing 2PE to cross the synaptic cleft. Although molecular dynamics (MD) can be be used to determine the diffusion rate, a key difficulty is evaluating the penetration energy or Potential of Mean Force (PMF) inside the membrane. Penetration energies have been determined by other workers for small anesthetic molecules like NO and butanol (A. Pohorille, M.A. Wilson, M.H. New and C. Chipot, *Toxicology Letters,* **100-101**, 421-430,(1998)) but little work has been done on penetration energies for larger molecules. Using specially developed free energy simulation techniques, approximately twenty several nanosecond MD trajectories have been generated and analyzed to determine the mean force exerted on the trace amine at distances ranging from 20 angstrom right to the middle of a symmetric sphingomyelin membrane. From this data, the PMF and diffusion rate for 2PE through the membrane will be calculated. The techniques developed may be extended to study the binding of antimicrobial peptides to phospholipid membranes.



211-Pos Board B90

All-atom Molecular Dynamics Simulations of a Membrane Protein Stabilizing Polymer

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Amphipols are amphipathic, polyacrylate-based polymers that have shown great promise in stabilizing membrane proteins for structural analysis. We have used all-atom molecular dynamics simulations in order to probe details of the behavior of the amphipols. First, we have reproduced experimental SANS measurements on pure amphipol particles. Analysis of these simulations has focused on how varying the chemical ordering of polymer side-chains affects the self-organization and dynamic behavior of the particle. In particular, we describe the manner in which hydrophobic and hydrophilic side-chains arrange inside each particle, as well as differences in water permeability. A second set of simulations of amphiol and a membrane protein, namely OmpX, probes how the amphipol is able to stabilize the protein in its native conformation, and further illustrates the impact of chain order and chemistry on this stabilization.

212-Pos Board B91

Electroporation Sensitivity of Oxidized Phospholipid Bilayers

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Molecular dynamics (MD) studies showing that oxidized lipids increase the frequency of water defects in phospholipid bilayers suggest that the presence of oxidized lipids in a bilayer will also increase the sensitivity of the bilayer to electropermeabilization. To investigate this possibility we applied external electric fields during MD simulations of PLPC (1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine) bilayers containing varying concentrations of oxidized PLPC species - the peroxidized linoleic acid derivatives 12-oxo-9-dodecenoic acid (12-al), which contains an aldehyde group, and 13-trans, cis-hydroperoxide linoleic acid (13-tc), which contains a hydroperoxide group. Systems with higher concentrations of oxidized lipids form hydrophilic electropores in significantly shorter times than do systems with lower oxidized lipid concentrations, and at lower electric fields. Furthermore, bilayers containing 12-al electroporate more quickly than bilayers containing 13-tc, possibly a result of the decreased thickness of membranes containing 12-al. Sites of water defect formation and subsequent electroporation appear to coincide with local clustering of oxidized lipids in the bilayer. In large-area simulations containing localized high oxidized lipid concentrations, pores formed preferentially in these oxidized regions. The tendency of the oxidized lipids to bend their sn-2 tail toward the aqueous interface, which may result in membrane thinning and a decrease in the lipid areal density, was not noticeably enhanced by the application of an external electric field, but the presence of the aldehyde and hydroperoxy oxygens on the otherwise nonpolar lipid tails appears to facilitate the penetration of water into the bilayer interior. Simulation results were verified by experimental observations of enhanced permeabilization of oxidized membranes in living cells.

213-Pos Board B92

CHARMM-GUI Membrane Builder for Mixed Bilayers and Its Application to Yeast Membranes

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Most biological membranes are composed of many different kinds of lipid, and can be characterized by the composition of the lipids. Although more and more researchers have shown their interests in molecular dynamics simulation of lipid bilayer or protein-membrane complex system, the setup of such system remains quite challenging for even relatively experienced researchers. In the previous work [1], we have shown that the setup of molecular dynamics simulation for protein-membrane complex can be dramatically simplified by automating the process and providing intuitive and straightforward user interface. In this work, we have further elaborated the process to include 25 different kinds of lipid, which makes it possible to build more biologically relevant lipid bilayers, and we also added the facility to make a lipid bilayer system alone. The efficacy of the web interface at the CHARMM-GUI website [2] has been tested by building and simulating lipid bilayer systems that resemble yeast membrane, which is composed of cholesterol, DPPC, DOPC, POPE, POPA, and POPS. In this work, we will present the usages of the mixed bilayer generation in Membrane Builder and the simulation results of the yeast membrane systems.