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

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The Biophysics Of Antibiotics Translocation Through OmpF Revealed By Computer Simulations

Eric Hajjar[1], Amit Kumar[1], Enrico Spiga[1], Jean-Marie Pages[2], Kozhinjampara Mahendran[3], Tivadar Mach[3], Andrey Bessonov[3], Mathias Winterhalter[3], Paolo Ruggerone[1], Matteo Ceccarelli[1].
[1]University of Cagliari, Cagliari, Italy, [2]2Université de la Méditerranée, Marseille, France, [3]Jacobs University Bremen, Bremen, Germany.

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.

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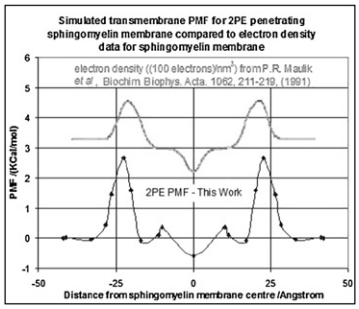
Simulation Studies Of Trace Amines Passing Through Neuronal Membranes

Bruno L. Tomberli, Jarrod Nickel, Mark Berry.
Brandon University, Brandon, MB, Canada.

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.



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

William J. Drasler, Jonathan N. Sachs.
University of Minnesota - Twin Cities, Minneapolis, MN, USA.

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.

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Electroporation Sensitivity of Oxidized Phospholipid Bilayers

Zachary A. Levine[1,2], Yu-Hsuan Wu[1], Matthew J. Ziegler[1,2], D. Peter Tieleman[3], P. Thomas Vernier[1,2].
[1]University of Southern California, Los Angeles, CA, USA, [2]MOSIS, Information Sciences Institute, Marina Del Rey, CA, USA, [3]University of Calgary, Calgary, AB, Canada.

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.

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

Sunhwan Jo[1], Jeffery B. Klauda[2], Wonpil Im[1].
[1]The University of Kansas, Lawrence, KS, USA, [2]The University of Maryland, College Park, MD, USA.

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.