2016-Pos Board B786
Computational Analysis of the Interactions Between Carbon Nanotubes and Cell Membranes
Mickael G. Lelimousin, Mark SP Sansom.
University of Oxford, Oxford, United Kingdom.
Carbon nanotubes (CNTs) have potential benefits in medicine, e.g. as drug delivery vehicles. However, CNTs and related nanoparticles might be significantly toxic. Although it is well established that cells ingest CNTs, we still have a limited understanding of the interactions at a molecular level between CNTs and cell membranes. Rational CNT derivatizations may allow targeting specific receptors as well as better penetrating cells, while pristine CNTs have shown strong antibacterial activity. In many cases, mechanistic details of such experimental results remain unknown. Consequently, recent computational and theoretical studies have tried to model possible internalization mechanisms of functionalized nanoparticles into cells. Here, we report coarse-grained molecular dynamics simulations of pristine CNTs in interaction with a dipalmitoylphosphatidylcholine (DPPC) lipid bilayer. Both single- and multi-wall CNTs, of different lengths (from 2 to 10 nm) and diameters (from 1.5 to 5 nm), are investigated. We characterize the insertion mechanism of pristine CNTs into the cell membrane model. Strong perturbations of the membrane are observed, as assessed by important phase transitions in the lipid bilayer. Based on our simulations of pristine CNTs, we finally suggest a mechanism for their antibacterial activity. The overall results shed light on the action of CNTs in cellular environment, which will contribute to guide both prevention of health risks and development of therapeutic applications.

2017-Pos Board B787
Protonation and the Matrix Effect of Oleate Vesicles, a Coarse Grained Molecular Dynamics Study
Bram van Hoof, Albert J. Markvoort, Rutger A. van Santen,
Peter A.J. Hilbers.
Technische Universiteit Eindhoven, Eindhoven, Netherlands.
Oleate vesicles provide an intriguing system to study amphiphile aggregation. Their behavior has been studied extensively in literature, revealing several interesting properties. Particularly interesting is the so called ''matrix effect''; when vesicles are formed de novo, the process is slow and the size distribution is broad, whereas in presence of ''seed vesicles'' with a narrow size distribution, the formation speeds up and the size of new vesicles correlates with the seed vesicles' size. To explain this matrix effect, a ''replication by division mechanism'' has been proposed, where division occurs due to asymmetric growth of the membrane caused by the fast insertion of fatty acids into the outer leaflet. The resulting area imbalance between the two leaflets can only partially be restored by flip-flop to the inner leaflet. The remaining imbalance is relaxed by deformation of the vesicle, followed by fission, competing with vesicle growth to determine the resulting vesicle size. In growing oleate vesicles, the generation of a transmembrane pH gradient is reported in the literature, explained by a net inward flip-flop upon oleate adsorption to the outer leaflet. In agreement with the replication by division mechanism, we hypothesize that in the matrix experiments a built-up proton gradient across the membrane during growth, counteracts inward flip-flop and, therefore, directly promotes the division of the vesicles. Here, the role of protonation at the molecular level at each stage of the replication by division process is investigated using molecular dynamics, using a coarse grained model developed specifically for this purpose, employing the recently developed CUMULUS coarse graining method.

2018-Pos Board B788
CaM Induced Gating Mechanism of AQP0
Daniel M. Clemens, Juan A. Freites, Karinne L. Németh-Cahalan,
Douglas J. Tobias, James E. Hall.
UC Irvine, Irvine, CA, USA.
Aquaporin 0 (AQP0) is a water channel protein necessary for lens transparency. AQP0 water permeability is lower than that of other aquaporins, and is regulated by calcium through the action of calmodulin (CaM). NMR studies indicate that one CaM monomer interacts with the C-terminal α-helices of two adjacent AQP0 monomers residing in an AQP0 tetramer. Our goal is to determine the mechanism by which CaM gates AQP0 water permeability. We utilize molecular dynamics simulations of a model of the AQP0-CaM complex. Trajectories of AQP0 and the AQP0-CaM complex embedded in a POPC lipid bilayer show differences between the water occupancy of the AQP0 pore with and without bound CaM. The motion of tyrosine 149 (Y149), a residue residing in the 'phenolic barrier' of AQP0, is reduced in the AQP0-CaM complex. Hypotheses concerning the gating mechanism of AQP0 are tested utilizing the *Xenopus* oocyte-swelling assay. Specifically, Y149 mutants are tested for calcium regulation. Analysis of the interface between AQP0 and CaM shows a chain of interacting residues connecting the interface and Tyr149. Analysis of our simulations indicates that Tyr149 serves as the gate in the regulation of AQP0 water permeability by CaM. Under conditions of low CaM binding, Tyr149 moves dynamically into and out of the AQP0 pore with a low open (out-of-pore) probability. Upon CaM binding, the motion of Tyr149 is decreased and the open probability is reduced. These results reveal a novel gating mechanism in which the binding of a protein to AQP0 reduces the dynamics of pore lining residues, thereby reducing the open-probability of the pore.

2019-Pos Board B789
Pore Gating of K+ Channels Studied by Essential Dynamics Simulations using the Simplified Bacterial K+ Channel KcsA
Tobias Linder, Anna Stary-Weinzinger.
Institute of Pharmacology and Toxicology, University of Vienna, Vienna, Austria.
Voltage gated K+ channels open and close in response to voltage changes. While crystal structures capture a limited number of gating conformations (open, closed), the transition steps and mechanism are still unknown. A limited number of voltage gated potassium channels structures in either open or closed conformation have been crystallized. Since the bacterial K+ channel KcsA has been crystallized in open, intermediate (Cuello et al., 2010) and closed (Zhou et al., 2001) states, this channel offers an ideal model for pore gating simulations. Consequently, we applied essential dynamics simulations to study the gating pathway and energetics underlying pore gating. Essential dynamics simulations were performed at timescales ranging from 1 to 100 ns. Gating simulations were considered complete when the RMSD was below 1.5 Å to the target structure. This criterion was reached in most runs, however shorter simulation times usually resulted in lower RMSD values. In agreement with structural data (Cuello et al., 2010), all available KcsA transition structures (PDB identifier: 3fb5, 3fb6, 3f7y) were sampled with our essential dynamics protocol. Furthermore, molecular dynamics runs revealed that channel opening and closing sampled the same conformations along the transition pathway. Removal of pH-Sensor residues (H25, E120, R121, R122, H124) led to spontaneous channel openings on the nanosecond timescale. These unbiased opening simulations sampled the same conformations as our essential dynamics simulations, further supporting the validity of our method. Finally, umbrella sampling was used to estimate the energy profiles of the various gating states and the transition pathways of WT and mutant channels. As expected, the energy needed to open the channel is significantly decreased in the pH-Sensor deleted KcsA.

2020-Pos Board B790
Monte Carlo Loop Refinement of Trans-Membrane Domain of the Thyroid Stimulating Hormone Receptor
Rejwan Ali[1,2], Rauf Latif[1,3], Terry Davies[1,2], Mihaly Mezei[1].
[1]Mount Sinai School of Medicine, New York, NY, USA, [2]Thyroid Research Center, VA Hospital, Bronx, NY, USA, [3]Thyroid Research Center, VA, Bronx, NY, USA.
While the ectodomain structure of the Thyroid Stimulating Hormone Receptor (TSH-R) a GPCR, has been recently reported (Sanders, Chirgadze et al. 2007), for the transmembrane domain (TMD) only model structures exist. However, for virtual screen of small molecules targeting the TMD domain region reliable structure is a prerequisite. In this work we have used an ab initio method, that previously had reported to reproduce crystal structures within 2 Å RMSD, to model 3 extra-cellular and 3 intra-cellular TSHR-TMD loops (Cui, Mezei et al. 2008). The method employs Modeller (Sali and Blundell 1993) to generate an initial loop structure. This is followed by a Metropolis Monte Carlo run in the torsion angle space of the of loop regions at high temperature for extensive sampling of the conformation space. Selected conformations are subjected to simulated annealing and the final structure with the lowest energy is chosen as the loop structure.
The Charmm molecular mechanics force field is used for the VdW interactions between the protein atoms and for the torsion angles; water is represented by a sigmoidal distance dependent dielectric function and the Autodock desolvation term (Cui, Mezei et al. 2008). In addition, explicit hydrogen-bonding terms are used. We will present the computational and structural details of the methods used.

2021-Pos Board B791
Structure of the Antimicrobial Peptide HHC-36 and its Interaction with Model Cell Membranes
Mostafa NateghołEslam[1], Shaghayegh Vafaei[1], Matthew Nichols[2], Miljan Kuljanin[2], Masoud Jelokhani-Niaraki[2], Bruno Tomberli[3], Chris G. Gray[1].
[1]University of Guelph, Guelph, ON, Canada, [2]Wilfrid Laurier University, Waterloo, ON, Canada, [3]Brandon University, Brandon, MB, Canada.
HHC-36 is an antimicrobial peptide, designed through neural network algorithms. It has been tested in vivo and in vitro, and has proved to be strongly

effective against strains of multidrug-resistant P. aeruginosa, methicillin-resistant Staphylococcus aureus , and a few other 'superbugs' (Cherkasov et al., ACS Chem. Biol., 2009, 4 (1), pp 65-74). The peptide has also been observed through in vivo tests to be greatly pathogen-specific, hence proving to be a great candidate for developing future antibiotics.
To understand the mechanism of activity of this peptide against bacterial membranes, we have performed a number of all-atom simulations, together with a series of circular dichroism spectroscopy (CD) and isothermal titration calorimetry (ITC) experiments. The small size (9 amino acids) and great charge density of HHC-36 make it problematic (if not unreliable) to find the structure of HHC-36 through conventional spectroscopy and/or crystallography methods. We have thus performed microsecond-scale molecular dynamics simulations, starting from an unfolded structure of the peptide, to find its folded structure. An amphipathic turn structure has been obtained, which was observed to be very stable over few hundred nanosecond timescales of simulation. This result has been compared to circular dichroism spectroscopy results, and the presence of the turn structure has been verified. To assess the stability of the observed structure, we have also performed temperature-dependent simulations and CD measurements, which have shown the stability of the turn structure at close-to-physiological temperatures.
The obtained structure is then used in peptide-membrane simulations with a few different membrane compositions mimicking both bacterial and animal cell membranes. Profiles of the potential of mean force have been obtained, and the relevant binding parameters extracted from these simulations are then compared with the binding free energies obtained from ITC experiments.

2022-Pos Board B792
All-Atom Molecular Dynamics Simulations of Bacterial Porins Phoe from *E. Coli* And VCA1008 from *V. Cholerae*: Revealing the Molecular Origin of Ion Selectivity
João L. Vianez-Junior, Pedro A. Lapido-Loureiro, Carolina L. Goulart, **Paulo M. Bisch**.
Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
Outer membrane pore-forming proteins play an essential role in survival, adaptation and response to environment of Gram-negative bacteria, and contribute for invasion of host organisms and even for drug resistance of such pathogens. These membrane proteins form large and almost unspecific ion channels through the membrane and a lot of effort was made to determine and to justify the ion selectivity of these pores (Alcaraz et al. 2009). Altough there is some sequence and structural similarities between porins PhoE from *E. coli* and VCA1008 from *V. cholerae* (Goulart et al. 2009) their selectivities for different inorganic ions seem to be quite different. On the other hand, molecular dynamics simulation of large systems are now reliable and should give detailed atomic information about ion dynamics though the channel, charge distribution and specific interacting site. Molecular dynamics simulations of the related porins OmpC and OmpF from *E. coli*, were recently presented and some of these aspects were explored (Biró et al. 2010). We have built up two systems, one for PhoE (PDB ID: 1PHO) and another one for VCA1008 (constructed by comparative modeling, Goulart et al. 2009), consisting of the channel trimer embedded in a DPPC lipid bilayer. A constant electrical field will be applied for different ionic concentrations of LiCl, NaCl, KCl and $CaCl_2$. The conductance, charge distribution and the identification of binding sites for each condition should be compared with experimental results. The elucidation of the charge-controlled mechanism inside the channels could emerge from our approach.
[1] Alcaraz et al., Biophysical J. **96** (2009) 56-66.
[2] Biró et al., Biophysical J. **98** (2010) 1-10.
[3] Goulart et al., FEMS Microbiol Lett **298** (2009) 241-248.

2023-Pos Board B793
Window Exchange Umbrella Sampling Molecular Dynamics Simulations for Transmembrane Helix Assembly
Soohyung Park, Taehoon Kim, Wonpil Im.
University of Kansas, Lawrence, KS, USA.
Membrane proteins whose structures and functions are defined by the orientation of each transmembrane (TM) helix play crucial roles in many cellular processes. Various polytopic membrane protein structures have been determined by crystallography/spectroscopy. However, it is still challenging to obtain the structural information of membrane proteins with small numbers of TM helices. Computational studies can play a vital role by providing structural models of such TM assembly. Yet, TM assembly modeling itself is intensive even in finding interfacial (contact) residues of a bitopic TM dimer because of multiple degrees of freedom (DOF), such as helix-helix distance, crossing angle, and rotation angles along each TM. Moreover, it is usually assumed that the interface of mutated TM helices resembles that of the wild type in most molecular dynamics (MD) simulations. Such an assumption may be problematic because of incomplete sampling due to strong interactions between interfacial residues. The replica exchange method may overcome such difficulties, but the potential of mean force (PMF) along the reaction coordinate(s) is not generally available and its applicability to all-atom explicit lipid bilayers is limited. We address these issues by the method of window exchange umbrella sampling MD (WEUSMD), in which windows along a certain helix DOF are exchanged. To illustrate its efficacy and advantage, we apply WEUSMD to a dimer of pVNVV TM model, an analog of GCN4 leucine zipper, whose initial configurations are away from the optimal TM dimer structure. In addition, we apply WEUSMD to a glycophorin A TM dimer to obtain the PMF with respect to helix-helix distance and crossing angle to investigate the energetics and mechanism of the association.

2024-Pos Board B794
Molecular Simulations Investigations of the Role of the M2 Influenza Protein in Viral Budding
Eduardo Mendez-Villuendas, D Peter Tieleman.
University of Calgary, Department of Biological Sciences and Institute for Biocomplexity and Informatics, Calgary, AB, Canada.
The budding of enveloped viruses is a complex multi-step process requiring alterations in membrane curvature and scission at the neck of the budding virion. M2 is a pH dependent matrix protein from influenza virus widely known for its role in virus uncoating and used as a target of the amadantine flu-drug that prevents ion transport. An additional and lesser known role played by M2 relies on collective effects where M2 clusters have been hypothesized to induce local membrane curvature resulting in a reduced energetic cost associated with the bending of the membrane, and where the budding of virus particles takes place on a cholesterol dependent manner(1).
We carried out molecular dynamics simulations to study the local composition and structural properties of curved membranes with lipid composition ratios A:B:C=4:1:x, x={0,1/2,1,2} (A=DOPC, B=DOPE, C=Cholesterol) and decorated with M2 protein channels arranged at various geometries. Further, the induced curvature effects produced by the M2 proteins is investigated and compared to equivalent M2-free membranes via the evaluation of both, the structural stress and the energetics of the model systems. This approach is used as an initial step towards the formulation of phenomenological models that quantify the thermodynamics of virus budding mechanism mediated by M2.
(1) J.S. Rossman, X. Jing, G. P. Lesser, R. A. Lamb. (2010) Cell 142:902-913.

2025-Pos Board B795
Packing of Amantadine-Like Compounds in the Central Cavity of Influenza a M2
Curtis B. Evans, David Busath.
Brigham Young University, Provo, UT, USA.
Over 291 variants of amantadine, mw 113.2 to 437.5, have been historically used as therapeutic agents for influenza A. These amantadine-like molecules have been thought to function as intrachannel blockers in the M2 channel of the influenza A virus. We explored whether these drugs fit in the central cavity of the channel using simple simulation methods with the CHARMM force field, the atomic coordinates of the solid state NMR structure for the M2 channel (PDB ID 2L0J, model 1), and a full set of amantadine-like molecules, with variations and titration states, gleaned from the literature. First, we examined the optimal water packing in the central cavity of the rigid channel using randomized, annealed water insertion, which indicates that optimally it holds 882 to 1116 Daltons of water. Drugs were inserted into water-filled channels with waters deleted based on various oxygen radius parameters. A radius of 2.2 Å was found to give optimal water packing. Sterically, the amantadine-like molecules from the literature all fit readily within the central cavity, judging from drug energies inside the channel compared to those of the drugs relaxed *in vacuo*. Future work will focus on the relative energetics of drug binding and configuration and the solvation of amantadine-like molecules in explicit water within the central cavity. Support is from NIH AI23007 and a Mentoring Grant from BYU.

2026-Pos Board B796
Mechanistic Insights into αIIbβ3 Integrin Signaling from Long-Timescale Molecular Dynamics Simulations
Davide Provasi[1], Ana Negri[1], Barry Coller[2], Marta Filizola[1].
[1]Mount Sinai School of Medicine, New York, NY, USA, [2]Rockefeller University, New York, NY, USA.
Integrin αIIbβ3 is a heterodimeric cell adhesion receptor composed of α and β subunits, each of which features an extracellular domain (ECD), a single transmembrane (TM) α-helix, and an intracellular cytoplasmic tail (CT).