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Molecular Dynamics Simulations Reveal Mechanistic Details of Polymyxin Penetration into both Membranes of E. coli
Syma Khalid[1], Nils A. Berglund[1], Peter J. Bond[2], Thomas J. Piggot[3].
[1]School of Chemistry, University of Southampton, UK, Southampton, United Kingdom, [2]4 Bioinformatics Institute (A*STAR), Singapore, Singapore, [3]Detection Department, Defence Science and Technology Laboratory, Salisbury, United Kingdom.
Gram-negative bacteria such as E.coli are protected by a surprisingly complex cell envelope. The cell envelope is composed of two membranes that form a protective barrier around the cells, and control the influx and efflux of solutes via various routes. Lysing the cell by disrupting the membranes, or permeating across them to gain access to the cell interior are key properties for antimicrobial agents. Polymyxins are a class of antibiotics that have been shown to be highly active against Gram-negative bacteria. It is thought they enter bacterial cells through a self-uptake mechanism, although the molecular details of the mechanism are still unclear.
We present, to our knowledge, the first molecular dynamics simulation study of an antimicrobial peptide, with both membranes of E.coli. Our model of the outer membrane contains lipopolysaccharide molecules in the inner leaflet and a mixture of ohosphilipids in the inner leaflet. In contrast, the inner membrane is comprised only of phospholipids. Our simulations reveal the effects of Polymyxin B1 (PMB1) binding on the physical properties of each membrane. Thus they are able to identify potentially different mechanisms for membrane disruption by PMB1. Peptide aggregation and insertion of one peptide tail was observed in the outer membrane. In contrast, PMB1 peptides insert readily as monomers, accompanied by water penetration into the inner membrane. Our simulations demonstrate the importance of capturing relevant details of biological complexity, in molecular models of biological membrane systems.

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Thermodynamics Govern the Mechanism of Antimicrobial Lipopeptides: Insights from Coarse-Grained Molecular Dynamics Simulations
Dejun Lin, Alan Grossfield.
University of Rochester, Rochester, NY, USA.
Antimicrobial lipopeptides (AMLPs) are a series of acylated cationic peptides with broad-spectrum antimicrobial activity and low hemolytic activity. We used microsecond-scale coarse-grained molecular dynamics simulations with the MARTINI force field to understand AMLPs' modes of action. Using rigorous free energy calculations with a novel reaction coordinate, we quantified formation of lipopeptide micelles in solution, as well as the the affinity of those micelles for different membrane compositions. The results yield both kinetic and thermodynamics arguments for the lipopeptides selectivity for bacterial over mammalian membranes. Our results indicate that the acyl chain of C16-KGGK, one of the AMLPs, is mainly responsible for its affinity to membrane while the peptide portion determines the selectivity towards different membrane lipid compositions. The micelle results suggest both kinetic and thermodynamics arguments for the lipopeptides selectivity for bacterial over mammalian membranes. Our results provide biophysical insights into the mechanism of lipopeptides' antimicrobial action.

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The Nature of Daptomycin Aggregates
Ming-Tao Lee[1,2], Wei-Chin Hung[3], Yen-Fei Chen[4], Huey W. Huang[4].
[1]Life Science Group, National Synchrotron Radiation Reasearch Center, Hsinchu, Taiwan, [2]Department of Physics, National Central University, Jhongli, Taiwan, [3]Department of Physics, R. O. C. Military Academy, Fengshan, Taiwan, [4]Department of Physics & Astronomy, Rice University, Houston, TX, USA.
Daptomycin is the first FDA-approved member of a new structural class of antibiotics_the cyclic lipopeptides. It is an important drug against multidrug-resistant gram-positive pathogens. The peptide interacts with the lipid matrix of cell membranes, inducing membrane permeability to ions, but its molecular mechanism has been a puzzle. Unlike the ubiquitous membrane-acting host-defense antimicrobial peptides, daptomycin does not induce molecular-leaking pores in the cell membranes–no calcein leakage was detected from lipid vesicles. Thus how it affects the membrane permeability to ions is not clear. The antibacterial activity and induced ion leakage by daptomycin correlate with the target membrane's content of phosphatidylglycerol (PG) and occur only in the presence of Ca^{2+} ions. Fluorescence resonance energy transfer (FRET) experiments and the recently discovered lipid extracting effect have shown daptomycin aggregation in membranes, but the chemical structure of daptomycin gives no clue to the nature of daptomycin aggregates. Jung et al discovered an inversion of the CD spectrum of daptomycin that occurs only in the simultaneous presence of PG and Ca++. We have correlated the inversion of the CD with the lipid extracting effect of daptomycin. Here we make use of the CD spectral change with Ca^{2+} ion concentration and with PG concentration to analyze the nature of the daptomycin-Ca++-PG aggregates. The data shows a sharpe threshold (or micellization) effect on the Ca^{2+} ion concentration dependence, indicating that each aggregate contains 20 or more calcium ions. This result shows that the aggregates are large, inconsistent with the idea of daptomycin aggregates forming oligomeric pores, since such a pore must be made of a relatively small number of daptomycin. Once we realize the micellization effect, the stoichiometry of the daptomycin-Ca++-PG aggregates can be analyzed from the calcium and PG concentration dependence.

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Lipid Selectivity of Fungicidal Lipopeptides
Sebastian Fiedler, Quang Huynh, Hiren Patel, Heiko Heerklotz.
Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.
Cyclic lipopeptides act against a variety of pathogens and, thus, constitute highly efficient crop-protection agents. For example, commercially available fungicides contain mixtures of different Bacillus subtilis lipopeptide families, such as surfactins (SF), iturins (IT), and fengycins (FE). Besides other effects, the fungicidal activity of these peptides is mainly mediated by permeabilizing the membrane. Accordingly, surfactins behave like extremely powerful surfactants and induce graded membrane leakage, iturins synergize with surfactins, and fengycins follow an all-or-none leakage mechanism. However, little is known about how the lipid composition of the membrane affects the capability of these lipopeptides to induce membrane leakage. To shed more light on their lipid selectivity, we performed fluorescence-lifetime-based leakage assays on various types of lipid-bilayer compositions. To this end, we compare leakage efficiencies and mode of actions of SF, IT, FE, and mixtures of them in lipid vesicles composed of phosphatidylcholine/phosphatidylethanolamine (PC/PE), PE/phosphatidylglycerol (PE/PG), and analyze the effect of phosphatidylserine and cardiolipin. Furthermore, we assessed the influence of ergosterol as a main component of fungal cell membranes. Our results aid in the understanding of the mechanism of lipopeptide-induced fungicidal activity and demonstrate that B. subtilis tailors these lipopeptide mixtures to specifically attack fungal membranes.

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Structure and Membrane Topology of the Pore-Forming Peptide Maculatin 1.1
Marc-Antoine Sani[1], Terry P. Lybrand[2], Frances Separovic[1].
[1]School of Chemistry Bio21 institute, Univeristy of Melbourne, parkville, Australia, [2]Center for Structural Biology, Vanderbilt University, Nashville, TN, USA.
Antimicrobial peptides (AMP) that target membranes are an attractive alternative to classic antibiotics, since they do not require internalization nor target a specific stereo-structure, thus limiting development of bacterial resistance. Their mode of action involves the disruption of lipid membranes; however, the relationship between lipid membrane structure and peptide potency remains unclear. We present the structural investigation of the AMP maculatin 1.1 (Mac1) in DPC micelles and DHPC/DMPC isotropic (q= 0.5) bicelles. Using solution and solid-state NMR with paramagnetic relaxation enhancement agents (PRE) and molecular dynamics (MD), we demonstrate the important role of the membrane structure in modulating the structure and location of Mac1. HSQC of specifically 15N labeled Mac1 in buffer displayed a narrow chemical shift dispersion that is typical of random coil structures. Introduction of micelles and bicelles produced chemical shift dispersions characteristic of helical structures, with differences suggesting that Mac1 adopts a different degree of helicity dependent on the curvature. 3D TROSY-NOESY allowed assignment of the sequential 15N labeled residues, and determination of a 3D helical structure in phospholipid micelles and bicelles, the latter producing the greatest helical stretch. Titration of the PRE agent Gd3+-(DTPA) showed that the central core of Mac1 is protected in bicelles while in micelles only the N-term is exposed to the PRE effect. MD simulations in DPC micelles revealed N-term exposure to the solvent, and they also suggested that Mac1 bent to adapt the curved micelle structure. Experiments will be repeated with 4 and 8 peptides per micelles.

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Structure of Transmembrane Pores Stabilized by Antimicrobial Peptides Magainin and PGLa
Almudena Pino Angeles, John M. Leveritt III,, Themis Lazaridis.
Chemistry, City College of New York, New York, NY, USA.
Antimicrobial peptides are found in many organisms as part of their defense system against bacterial infections. They have similar structural and functional features: most consist of amphiphilic helices that bind to a membrane and disrupt it by diverse methods. Among these peptides, magainin 2 and PGLa are found in the frog skin and exhibit synergistic effects in lipid bilayer disruption by the