groups. Membrane thickness was measured by using energy transfer between the surface fluorescent probe 1-anilinonaphthalene-8-sulfonic acid(ANS) and hydrophobic fluorescent probe Py-3-Py. Lidocaine·HCl increased bulk lateral and rotational mobilities, and had a greater fluidizing effect on the inner than outer monolayer of liposome. Thickness of SPMVTL, SPMVPL lipid bilayer have been decreased by lidocaine·HCl, which means that membranes have been expanded. The sensitivities to increasing effect of lateral and rotational mobilities of liposomal lipid bilayer by local anesthetic differed depending on the native and model membranes in the descending order of SPMV, SPMVPL and SPMVTL. These effects are not only due to the influence of local anesthetic on lipids, but they are magnified by the interaction between lipids, proteins and water.

429-Pos

Drug Delivery Systems Featuring Withdrawn Fluoroquinolones

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With increasing menace of bacterial resistance, constant development of new drugs and strategies to increase their efficacy is of great importance. Quinolones are a very well know class of antibacterial agents, as well as one of the most prescribed drugs in medicine for treatment of various bacterial infections. This wide use seems to be the main cause for bacterial resistance and this class of antibacterial agents grew significantly in the past. Fluoroquinolones, which include newer generation quinolones, were developed by implementing structural changes to the basic drug structure. Although highly prescribed, these antibacterial drugs are known for their various side effects and toxicity, and some of the agents have been withdrawn or not approved for use.

Drug delivery systems have been the target, for the past few years, of intense research since they aim to achieve a greater efficacy in the site of action as well as to improve aspects such as pharmacokinetics and/or minimizing side effects, contributing to the development of these systems.

From the existing controlled drug delivery systems, liposomes are frequently used due to their high versatility and biocompatibility. Lipid vesicles are considered for drug delivery when therapeutic agents are toxic, have high potency and low blood circulation times. Encapsulation of drugs, such as antifungal agents, has been reported and even commercialized, but research, regarding quinolones and liposomes, consists, mainly, in membrane permeability and physicochemical studies.

Different lipid formulations for drug delivery of similar fluoroquinolones, withdrawn or not approved for use (due to side effects) were prepared, studied and optimized. Physicochemical characterization of the antibacterial drugs (free and encapsulated) and lipid interaction is also a target of this work.

Acknowledgments: I.S thanks ''Fundação para a Ciência e a Tecnologia'', FCT Lisboa, for a PhD grant (SFRH/BD/47486/2008).

Membrane Active Peptides I

430-Pos

Multiscale Simulations of RNase E From E.coli: A Membrane Binding Protein

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RNase E is an essential endoribonuclease involved in RNA processing and mRNA degradation. The N-terminal half of the protein encompasses the catalytic domain; the C-terminal half is the scaffold for the assembly of the multienzyme RNA degradosome. Here we describe multiscale MD simulations of 'segment-A', an element in the beginning of the non-catalytic region of RNase E that is required for membrane binding. It has previously been demonstrated *in vitro*, that an oligopeptide corresponding to segment-A has the propensity to form an amphipathic α-helix and that it avidly binds to protein-free phospholipid vesicles. Disruption or mutation of segment-A *in vitro* and *in vivo* in full-length RNase E abolishes membrane binding.

We present a thorough multiscale MD simulation characterization of the behavior of RNase E in model membranes:

We have performed atomistic simulations of the wildtype segment-A and mutants in phospholipid bilayers to uncover the molecular-level details of membrane-binding. Furthermore, we have performed coarse-grained simulations of the same peptides in phospholipid vesicles of various sizes and lipid compositions to investigate the effect of membrane curvature, and lipid type on the membrane-binding, dynamics and potential aggregation of RNase E. The lipid compositions are designed to provide a realistic mimic of the *E.coli* inner membrane.

Not only are our simulations in good agreement with experimental work, but in addition, they provide molecular-level interpretations of the experimentally observed phenomena.

431-Pos

Poration of Lipid Vesicles By Antimicrobial Peptides: Simulation Studies With a Polarizable Coarse-Grain Model

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Antimicrobial peptides are a large family of peptides that include small cationic peptides that can permeabilize lipid membranes by disrupting the bilayer structure. Previous atomistic simulations of two specific antimicrobial peptides, magainin and melittin, show that they act by forming toroidal transmembrane pores in model bilayers. However, only systems of limited size and length scales have been studied and direct comparisons to experimental observations could not be made. Here, we study the poration propensity of these peptides with lipid vesicles using a coarse-grain description. A new version of the MARTINI force-field has been used which accounts for the polarizability of water. The explicit screening of the new MARTINI force-field provides for a more realistic description of membrane poration by antimicrobial peptides.

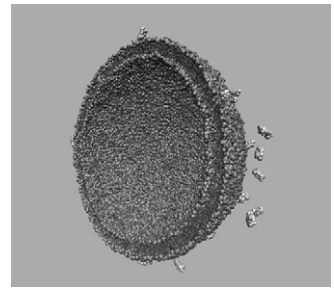

Figure: A snapshot of the starting structure of simulations of magainin-H2, an antimicrobial peptide, ''attacking'' a DPPC lipid vesicle. The vesicle is cut through to reveal its cross section. The head group beads are shown in purple and pink and the tails in gray. The antimicrobial peptides are shown in green (backbone beads) and yellow (side-chain beads). The water beads are not shown for clarity.

432-Pos

Binding of Antimicrobial Lipopeptides To Lipid Bilayers Characterized By Microsecond Molecular Dynamics Simulations

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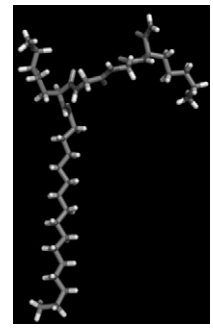

The emergence of antibiotic resistant pathogens is one of the major medical problems of the 21st century, prompting renewed interest in the development of novel antimicrobial compounds. Here we use microsecond-scale all-atom molecular dynamics simulations to characterize the structure, dynamics, and membrane-binding mechanism of a synthetic antimicrobial lipopeptide, C16-KGGK. The results of the simulations are validated by comparison with solid state NMR experiments, and yield new insights into the molecules' mechanism of action.

433-Pos

On the Roles of Anionic Lipids in Protein Localization and Permeability of Membranes

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Anionic lipids, such as phosphatidylglycerol and phosphatidylserine, play important structural and functional roles in cell membranes. In particular, they appear to provide strong interactions with positively charged protein side chains to promote membrane localization of lipid binding domains and antimicrobial peptides, as well as to modulate the function of many membrane proteins. All-atom molecular dynamics simulations were used to explore the strength of these interactions and the impact they have on the ability of charged protein residues to penetrate into membranes. Using an analog of arginine and bilayers of pure phosphatidylcholine or mixtures with phosphatidylglycerol, we have computed the thermodynamics of charged side chain translocation, as well as the binding affinity of each lipid within the membrane. We found that arginine deforms the bilayer in a similar fashion, regardless of composition, and that the free energy profile for translocation is relatively unaffected by anionic lipids: the ''neutralization'' of the protein side chain does not reduce the large ~20 kcal/mol barrier significantly. We decomposed these free energies to explain why anionic lipids do not play a significant role, with implications for the actions of many charged peptides and ion permeability. We also find

that arginine binding to phosphatidylglycerol is more favorable by only ~1 kcal/mol, suggesting that lipid binding domains and antimicrobial peptides likely require many charged side chains acting together to promote membrane localization.

434-Pos

Physical Modeling of Membrane-Lytic Antimicrobial Peptides: Toward Optimizing Their Membrane Disrupting Activity

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Antimicrobial Peptides (AMPs) are fast microbe-killing molecules found in virtually-all living organisms. Membrane-active AMPs are of particular interest, since they do not easily induce bacterial resistance. Accordingly, these peptides offer promising design principles for developing potent peptide antibiotics, especially for fighting conventional antibiotic-resistant bacteria. Here, we present a physical basis for optimizing the selective membrane-disrupting activity of cationic AMPs. Our approach explains the vital feats of the peptide, shedding quantitative insights into their design principles: Threshold peptide coverage on the membrane surface required for disruption can easily be reached for microbes, but not for the host cell - large peptide charge (> 4) is shown to be the key ingredient for determining the optimal activity-selectivity of AMPs (in an ambient-salt dependent way). Our results also illustrate how reduced fluidity of the host cell membrane by cholesterol enhances the selectivity.

435-Pos

The Role of Hydrophobicity in Peptide-Membrane Interactions: Insights Through Coarse-Grained Molecular Dynamics Simulations

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Peptide-membrane interactions are complex and diverse phenomena, highly important for various biological processes, such as antimicrobial defence mechanisms, viral translocation, membrane fusion and different functions of membrane proteins. Despite the extensive theoretical and experimental on-going research in the area of these interactions, the underlying mechanisms remain unclear. Here, we will present the latest results of our study on simulations of peptide-membrane interactions, based on the coarse-grained MARTINI force field [1, 2]. We will discuss about the possibility of classifying α-helical peptides into groups according to their hydrophobicity and predicting their interaction with cell membranes [3, 4]. Structural and dynamical properties related to various interaction patterns will be presented. Moreover, results of the potential of mean force (PMF) for peptide translocation across the lipid bilayer calculated for each class of peptides will be presented and compared. Finally, we will examine the possibility of simulating stages of the endocytic pathway and discuss about the reliability as well as wider implications of these results.

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436-Pos

A Comparative Study on the Effect of Hydrophobicity and Net Positive Charge on the Antibacterial and Anti- Endotoxin Activities of Antimicrobial Peptides

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The bacterial outer membrane, lipopolysaccharide (LPS), can serve as a barrier that protects bacteria from antimicrobial peptides (AMPs). However, LPS can also activate immune cells that in sever cases may cause death. This activity can also be neutralized by several AMPs. However, it is difficult to determine common denominators required for antimicrobial and LPS neutralizing activities. To this end, we synthesized and investigated a series of 12-mer D,L-amino acid peptides and their fatty acid-conjugated analogs composed of Leu and Lys with increasing number of positive charges and decreasing hydrophobicities, and with preserved positions for the D-amino acids. The overall altered helical structure in the membrane is similar for all of them as determined by FTIR spectroscopy. All the peptides were tested for their antibacterial and hemolytic activity, their ability to permeate LPS vesicles, to neutralize LPS activation of macrophages, as well as their effect on LPS morphology, determined by negative staining electron microscopy. The data reveal that whereas antimicrobial activity increases linearly with the increase in the peptides' hydrophobicity, peptides with different hydrophobicities are endowed with similar LPS neutralizing activities. Besides its importance to the understanding of antimicrobial and LPS neutralizing activities, this study suggests the use of such diastereomers as potential templates for the development of simple molecules that carry out both types of functions.

437-Pos

Cationic Antimicrobial Peptides: A Physical Basis For Their Selective Membrane-Disrupting Activity

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Antimicrobial peptides (AMPs) are not only fast microbe-killing molecules deployed in the host defense of living organisms but also offer valuable lessons for developing new therapeutic agents. While the mode of action of AMPs is not clearly understood, membrane perturbation has been recognized as a crucial step in the microbial killing mechanism of many AMPs. Here, we present a physical basis for the selective membrane-disrupting activity of cationic AMPs. In particular, we calculate the surface coverage of peptides embedded in the lipid headgroup-tail interface and the resulting membrane-area change, in terms of peptide and membrane parameters (e.g., peptide charge and the fraction of anionic lipids). We find threshold peptide coverage on the membrane surface required for disruption can easily be reached for microbes, but not for the host cell - large peptide charge > 4) is shown to be the key ingredient for the optimal activity-selectivity of AMPs (in an ambient-salt dependent way). Intriguingly, we find that in a higher-salt environment, larger charge is required for optimal activity. Our results also illustrate how reduced fluidity of the host cell membrane by cholesterol is implicated in the selectivity.

438-Pos

Energy Barriers and Helix Plasticity in the Membrane Insertion of pHLIP

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The pH (low) insertion peptide (pHLIP) is a 36-aa monomeric peptide which is both soluble in water and able to insert as a transmembrane helix in lipid membranes at low pH. Thus, pHLIP has three major states with characteristic secondary structure: it is unstructured in solution (state I) and when bound to the surface of lipid membranes at neutral pH (state II). However, it forms a transmembrane helix in membranes at acid pH (state III), with a pKa of insertion between states II and III of 6.0. The lipid insertion of pHLIP is mediated by the protonation of at least two Asp residues. Thus, pHLIP has to deal with the translocation of acidic residues through the membrane to insert.
Here, we designed several mutant peptides where the number of aspartic residues in the hydrophobic region of pHLIP was modified. Some mutations altered peptide behavior in solution and their interaction with lipid. At the same time, we observed that there was an apparent linear relationship between the number of Asp and both the observed pKa and the cooperativity of the insertion and/or folding in the membrane.
In order to study the role of transmembrane helix formation in the lipid insertion of pHLIP, we designed a mutant peptide where the key residue Pro20 had been mutated to Gly. This peptide retained the overall properties of pHLIP, however both the interfacial (II) and the transmembrane (III) states had a higher helical content than wt pHLIP, and the pKa of insertion was also higher.
Our data suggest that i) the number of Asp and their location at the water-lipid interface affect the pKa and/or cooperativity of the transition and ii) the formation of the membrane interfacial helix promotes peptide insertion into the membrane.

439-Pos

Lipid Membrane Destabilisation By Arginine Peptides Is Chain Length Dependent

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The intracellular delivery of proteins and other bioactive molecules using membrane-permeable carrier peptide vectors is a way to elucidate and control cell functions with therapeutic potentials. One of the most typical peptide vector is a short arginine-rich peptide segment derived from the human immunodeficiency virus (HIV)-1Tat protein as well as various arginine-rich oligopeptides. These peptides seems to translocate with their cargo into eukaryotic cells through a physical mechanism which is neither receptor-mediated and does not implicate endocytosis. Other studies have however implicated an endocytic pathway involving macropinocytosis. We provide here evidence that arginine peptides induce membrane destabilisation in DMPC and DMPG liposomes which is dependent of the arginine peptides length. Evolution of the CH_2- vibration of lipids was monitored by ATR-IR (Attenuated Total Reflection