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Emulsification of Cholesterol in Bile Salt Micelles:relevance For Cholesterol Absorption

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A high level of cholesterol in the blood is associated with predisposition for cardiac diseases and is one of the major human health problems[1]. Some cholesterol is synthesized in the liver and a significant amount is also absorbed from dietary cholesterol at the small intestinal brush border membranes. Dietary cholesterol is first emulsified in mixed micelles of bile salts (BS) and fatty acids (FA) and the currently accepted mechanism suggests that absorption involves the interaction between the mixed micelles and the apical membrane of brush border cells where passive mechanisms play a significant role[2]. However, the detailed mechanism and the dependence on the dietary mixture of lipids are far from being completely understood. In most studies, mixtures of BS and FA have been used and the distribution of cholesterol between different phases and/or the in vitro cholesterol intake has been measured[3]. The complexity of the systems studied precludes the interpretation of the effect of each component in the process. Here we present the study of the solubilization of a cholesterol analogue, Deydroergosterol, in micelles of glycocholic acid and glycochenodeoxycholic acid (most abundant BS in the upper intestine[3]) followed by fluorescence. We develop a kinetic model to describe the rate of sterol emulsification and its maximum solubility in the BS micelles. The study was also performed with cholesterol labeled with C13 in carbon 4 in the sterol ring and followed by C13 NMR Spectroscopy. From preliminary results we can identify and quantify the emulsified cholesterol in the BSM at a chemical shift of 41.6 ppm that is well separated from the C13 NMR resonances of BS[4]. From the data obtained we obtain the cholesterol saturation index and the kinetic profile for the solubilization of cholesterol in the BS micelles.

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Partition of Amphiphilic Molecules To Lipid Bilayers By ITC

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The partition of the amphiphile Sodium Dodecyl Sulfate between an aqueous solution and a POPC bilayer was followed by ITC as a function of the total concentration of SDS. It was found that the obtained partition coefficient is strongly affected by the ligand concentration, even after correction for the charge imposed by SDS to the bilayer due to its partition. The partition coefficient decreased as the concentration of SDS increased and this was accompanied by an increase in the molar enthalpy. This behavior is due to saturation of the lipid bilayer leading to non-ideal behavior. Some rules are proposed to enable the retrieval of the parameters that describe the interaction of ligands with unperturbed lipid bilayers.

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Cholesterol Orientation and Tilt Modulus in DMPC Bilayers

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We performed molecular dynamics (MD) simulations of hydrated bilayers containing mixtures of dimyristoylphosphatidylcholine (DMPC) and Cholesterol at various ratios, to study the effect of cholesterol composition on its orientation, and to study the link between cholesterol tilt and overall phospholipid membrane organization. The simulations show a substantial probability for cholesterol molecules to transiently orient perpendicular to the bilayer normal. Our results further suggest that cholesterol tilt may be an important factor capable of inducing membrane ordering. In particular, we find that, as cholesterol concentration increases, the average cholesterol orientation changes in a manner strongly (anti)-correlated with the variation in membrane thickness. This correlation persists within the broad range of lipid/cholesterol ratios (1%-40% cholesterol) that we have studied. However, cholesterol orientation is found to be strongly determined by the aligning force induced by other cholesterol molecules.

To discuss this aligning field quantitatively, we analyzed cholesterol orientation using, to our knowledge, the first estimates from MD simulations of the cholesterol tilt modulus. Our calculations suggest that the tilt modulus (hence the aligning field) is indeed strongly dependent on sterol composition. Beyond providing valuable energetic insights pertaining to cholesterol orientation in phospholipid membranes, this empirical parameter should become a useful quantitative measure to describing cholesterol interaction with lipid bilayer, particularly in various coarse-grained force fields. The results discussed in this work should aid in understanding how cholesterol may induce a ''nematic'' aligning field on membrane proteins and thus shift their preferred conformational state.

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Combined Use of Steady-State Fluorescence Emission and Anisotropy of Merocyanine 540 To Distinguish Crystalline, Gel, Ripple, and Liquid Crystalline Phases in Dipalmitoylphosphatidylcholine Bilayers

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This study investigated the effects of lipid phase on monomer and dimer fluorescence of merocyanine 540. Emission and anisotropy spectra were assessed at multiple temperatures covering all four lamellar phases of pure dipalmitoylphosphatidylcholine. The probe segregates in the bilayer into two populations: monomers (emission maximum ~585 nm) and dimers (emission maximum ~621 nm). Induction of the crystalline (L_C) phase by extended pre-incubation at 4 °C produced a strong wavelength dependence of anisotropy values (0.33 at 580 nm, 0.14 at 625 nm). Wavelength dependence was strong at 15 and 25 °C, weak at 38 °C and absent above the main phase transition (>41.4 °C) or after returning the temperature from 46 to 25 °C. Average anisotropy values across the complete temperature range revealed both the sub-and main phase transitions. The temperature dependence of total fluorescence intensity likewise displayed both transitions. In contrast, changes in the shape of the emission spectrum were sensitive to the sub- and pre-transitions but not the main transition. These changes were quantified by calculating the ratio of intensities at the two peaks in the emission spectrum (585 and 621 nm). These results indicate that dimer fluorescence mostly vanishes at the pre-transition because the spectrum shape was unchanged above 35 °C. Thus, the absence of wavelength dependence of anisotropy values at higher temperatures was largely due to loss of fluorescence from probe dimers. These observations are consistent with a model in which merocyanine dimers are localized to the region between membrane leaflets where their motion is greater than that of the monomers, which reside among the packed lipid head groups. Moreover, dimer fluorescence intensity is enhanced by constraints on its movement imposed by highly-ordered lipids.

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Characterizing Vesicle Leakage By Fluorescence Lifetime Measurements

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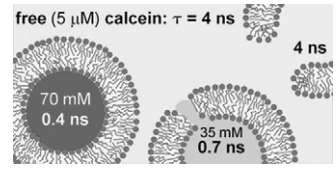
The membrane leakage assay based on vesicles loaded with self quenching dyes has been widely used for quantifying the activity of antibiotic peptides and other compounds that induce membrane pores or leaks. Here we show that this assay can be substantially improved if it is based on time-resolved decay curves rather than steady-state intensities only. A bi- or triexponential fit of calcein fluorescence decays allows for a parallel quantification of the free and (one or two) entrapped dye fractions and their effective local concentrations (governing the lifetime). The advantages of this technique are that it (i) allows the distinguishing of all-or-none from graded leakage for each sample, (ii) reveals the heterogeneity of graded leakage, (iii) truly quantifies the released dye rather than providing an empiric de-quenching value, and (iv) is independent of many errors that may affect the intensity. In addition to the example C12EO8 shown in Patel et al. (2009) Soft Matter 5:2849, we will present data obtained for a series of detergents and peptides, comprising all-or-none as well as graded leakage.



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The Effect of Lidocaine·HCL on the Physical Properties of Liposomes of Total Lipid and Phospholipids Extracted From Neuronal Membranes

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Fluorescent probe techniques were used to evaluate effect of lidocaine·HCl on physical properties (asymmetric lateral and rotational mobilities, membrane thickness) of liposomes of total lipid(SPMVTL) and phospholipids(SPMVPL) extracted from synaptosomal plasma membrane vesicles(SPMV). An experimental procedure was used based on selective quenching of 1,3-di(1-pyrenyl)-propane(Py-3-Py), 1,6-diphenyl-1,3,5-hexatriene(DPH) by trinitrophenyl

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.

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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.

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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.

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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.

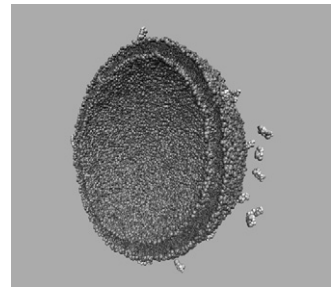

Figure: A snapshot of the starting structure of simulations of magainin-H2, an antimicrobial peptide, ''attacking'' a DPPC lipid vesicle. The vesicle is cut through to reveal its cross section. The head group beads are shown in purple and pink and the tails in gray. The antimicrobial peptides are shown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

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

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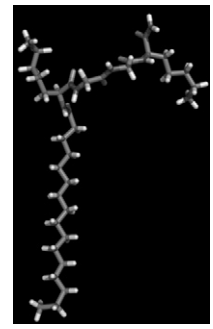

The emergence of antibiotic resistant pathogens is one of the major medical problems of the 21st century, prompting renewed interest in the development of novel antimicrobial compounds. Here we use microsecond-scale all-atom molecular dynamics simulations to characterize the structure, dynamics, and 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.

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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