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Functionalized lipids and surfactants for specific applications☆

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ABSTRACT

Synthetic lipids and surfactants that do not exist in biological systems have been used for the last few decades in both basic and applied science. The most notable applications for synthetic lipids and surfactants are drug delivery, gene transfection, as reporting molecules, and as support for structural lipid biology. In this review, we describe the potential of the synergistic combination of computational and experimental methodologies to study the behavior of synthetic lipids and surfactants embedded in lipid membranes and liposomes. We focused on select cases in which molecular dynamics simulations were used to complement experimental studies aiming to understand the structure and properties of new compounds at the atomistic level. We also describe cases in which molecular dynamics simulations were used to design new synthetic lipids and surfactants, as well as emerging fields for the application of these compounds. This article is part of a Special Issue entitled: Biosimulations edited by Ilpo Vattulainen and Tomasz Róg.

1. Introduction

According to the biochemistry handbook, "lipids are biological molecules insoluble in water and well soluble in organic solvents such as chloroform" [1]. Thus, lipids are a large and diverse class of naturally occurring compounds, such as triglycerides, phospholipids, and sterols, among others. Recent studies of lipidomics have revealed the existence of thousands of lipid species. Lipid profiles differ between organisms, cells types, cellular organelles, and among healthy and pathological cases [2]. For example, exosomes have been shown to be enriched in the long tails of sphingomyelin (SPM) and phosphatidylserine compared to the cell from which they were derived [3]. In addition, adipocytes from obese patients have been shown to be enriched in ethanolamine plasmalogens that contain arachidonic acid compared to the patients' non-obese identical twins [4]. Taking into account the large number and diversity of lipids, one could question why the addition of synthetic lipids is necessary, but synthetic lipids have countless applications in both applied and basic science.

The largest field of synthetic lipid application is pharmacology. Lipid analogs may be used directly as drugs, such as lipase inhibitors or analogs of bacterial lipid A to stimulate the immune system. Currently, one of the most important research areas in pharmacology is drug delivery. Various lipid assemblies, such as liposomes, micelles, bicelles, and nanodiscs, are used as carriers for drug molecules. These carriers have to pass rather high technical requirements, such as having an optimal lifetime, low permeability for drugs, and the ability to prolong storage, among others. Not surprisingly, numerous designed lipids have been synthesized and tested for this purpose (for a recent review, see [5]). The most important type of synthetic lipid is likely lipids functionalized with poly(ethylene glycol) (PEG), known as PEGylated lipids. Molecular dynamics (MD) simulations have been used extensively to characterize the physicochemical properties of these lipids (e.g., [6]).

Non-viral gene transfection, a method with scientific, medical, and technological importance, largely relies on synthetic lipids [7]. Synthetic lipids are used to form aggregates with DNA called *genosomes* or, more commonly, *lipoplexes*. Because nucleic acids carry a large negative charge, lipids need to be positively charged. As cationic lipids do not exist in nature, only synthetic lipids can be used for this purpose.

Basic research is another large field of synthetic lipid application. Most straightforward and commonly used synthetic lipids are lipid-based reporting molecules. Specifically, deuterated phospholipids are used in NMR studies of lipids to measure the order and dynamics of specific acyl tail segments [8]. Spin-labeled lipids are used in electron paramagnetic resonance (EPR) measurements to describe bilayer structures and dynamics [9], as well as oxygen transport thorough membranes [10] and bilayer hydrophobic profiles [11]. Lipids with fluorescent labels are used in fluorescent spectroscopy to determine both structural and dynamics parameters and in microscopy to visualize various lipid compartments [12]. Finally, clickable lipids, which have reactive groups that form covalent bonds with their nearest neighbors as a result of external

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stimuli such as light, are used to recognize the lipid nearest neighbors [13]. Attachment of labels or clickable groups is expected to affect the properties of native molecules. MD simulations may be used to evaluate such effects and a large number of studies have provided insight into, for example, the effects of labels on the native molecule location, orientation, and structure, providing background for the interpretation of experimental results (e.g., [14]). In the case of spin labels, simulations may be used to calculate the EPR spectrum of the molecule, providing direct validation of simulation methodologies [15].

An understanding of the importance of lipid structure and the role of their various functional groups can be achieved in a comparison of naturally occurring lipids. Existing lipid diversity provides a large number of lipid variants, allowing for such a comparison, but in some cases synthetic lipids are the only possible analogs. For example, the role of chirality can only be studied with synthetic lipids because most natural lipids have only one stereoisomer (e.g., glycerol moiety in all glycerol-based lipids adopts the *R* configuration of phosphatidylglycerol, and sphingolipids always have a D-erythro enantiomeric configuration - 2S, 3R). Modifications in lipid functional groups can be used to study the bulk properties of lipid bilayers or to better understand lipid interactions with proteins in macromolecular assemblies [16]. Modifications in the functional groups of a lipid molecule could be seen as corresponding to site-directed mutagenesis, one of the major tools in structural biology [17]. Synthetic surfactants also have applications in membrane protein extractions, biochemical characterization, and crystallization. For example, a cholesterol (Chol) analog, cholesteryl succinate, is commonly used in G-coupled protein receptor studies [18]. Another example is linolein, a surfactant used in protein crystallization [19].

MD simulation is a method that provides information at the atomistic level in a time scale of picoseconds to milliseconds (for a more extensive review see [20]). In recent years, the quality of lipid models has greatly improved due to the development of force-field parameters specific to lipid molecules [21–25]. These parameters have been shown to reproduce experimental data with better accuracy [26]. MD simulations seem to be particularly useful methods for studying the properties of synthetic lipids; they are an inexpensive method and provide an understanding of the molecular structure at the atomistic level that is useful in the further design of lipid species with desired properties. In the past, MD simulations have been shown to have the ability to accurately predict the effects of lipid modification [27]. In this article, we provide examples of synthetic lipids and surfactants in all of the above-described applications. In particular, we discuss studies in which MD simulations were applied and concentrate on cases in which MD simulation is a suitable method for providing a background for the future development of synthetic lipids and their applications.

2. Pharmacological applications

2.1. Drug delivery

Drug delivery is challenging nanotechnology in which synthetic lipids are used intensively. Kohli et al. [5] pointed to three key steps in drug delivery that must be optimized, and synthetic lipids may be an option. The first step after the injection of liposomes into a blood vessel is its circulation. Liposomes composed of naturally occurring lipids are quickly removed from the circulation by the immune system with a half-life in the bloodstream of roughly 1 h. An extended circulation time increases drug accumulation in tumors and, thus, is highly desired. As liposomes have to deliver their cargo, stability and low permeability are important. The second step is the targeting of liposomes to specific cell types. The final step is the release of the drug, preferably in a controlled manner. MD simulations are applicable for studying all of these steps and are able to provide novel insight into the structure and properties of delivery systems.

The most common method for extending liposome circulation time is the use of so-called stealth liposomes. Stealth liposomes are shielded from the immune system by a layer of hydrophilic polymer covalently attached to lipid headgroups. The most commonly used polymer is PEG attached to a phosphatidylethanolamine headgroup. PEGylated liposomes have been studied extensively (for review see [28]). MD simulations provide a few novel observations. First, the PEG corona was shown to bind Na^+ cations; thus, PEGylated liposomes carry a small positive charge [29]. The binding of K^+ to PEG is much weaker and Ca^{2+} cations do not interact with PEG [30]. PEG chains penetrate the membrane hydrocarbon core when a membrane is in the liquid state but not when the membrane is in the gel state [29].

Another interesting result obtained in MD simulations concerns the location of a hydrophobic drug in the PEGylated bilayer, in this case porphyrin. Simulations have shown that porphyrin has two possible locations, in the bilayer hydrocarbon core below the carbonyl oxygens or in the PEG layer (Fig. 1) [31]. This result was validated by a quenching experiment that showed the existence of two subpopulations of porphyrin, one that is accessible to the quencher and one that is not accessible to the quencher. This observation is also in agreement with previous studies showing a higher binding constant of porphyrin to PEGylated liposomes than to conventional liposomes [32,33]. Taken together, these results indicate that PEGylation increases the drug load efficiency of liposomes.

Chol is a key molecule regulating almost all properties of lipid bilayers (for review see [34,35]). From the point of view of drug delivery, the most important effect of Chol on the structure of a lipid bilayer is an increase in the tail order of the lipid, which results in increased stability and lower permeability of liposomes. Thus, Chol is a common component of liposomes used in drug delivery [36]. However, Chol is a

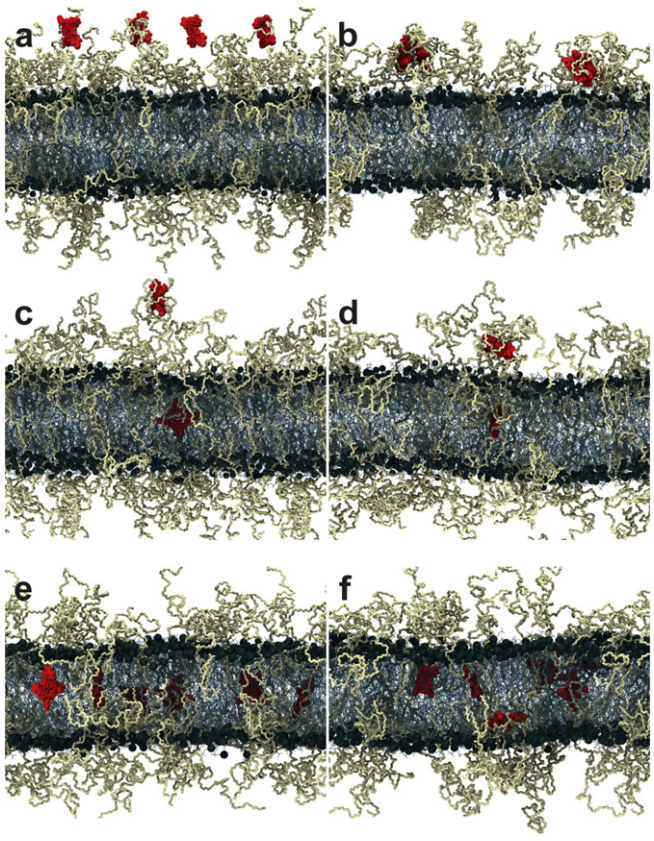

Fig. 1. Images of the systems consisting of p-THPP molecules in DLPC/DLPE-PEG bilayers. (a) Four p-THPP molecules (system M3) at $t = 0$ and (b) after 100 ns of simulation; (c) two p-THPP molecules (system M4) at $t = 0$ and (d) after 350 ns; and (e) six p-THPP molecules (system M5) at $t = 0$ and (f) after 350 ns. The porphyrin molecules are shown in red as a licorice representation. DLPC molecules are shown as blue sticks, with black spheres for phosphate groups. DLPE-PEG lipids are shown as beige sticks. For clarity, water and ions are not shown (reprinted with permission from Ref. [31]).