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Molecular modeling of lipid probes and their influence on the membrane☆

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ABSTRACT

In this review a number of Molecular Dynamics simulation studies are discussed which focus on the understanding of the behavior of lipid probes in biomembranes. Experiments often use specialized probe moieties or molecules to report on the behavior of a membrane and try to gain information on the membrane as a whole from the probe lipids as these probes are the only things an experiment sees. Probes can be used to make NMR, EPR and fluorescence accessible to the membrane and use fluorescent or spin–active moieties for this purpose. Clearly membranes with and without probes are not identical which makes it worthwhile to elucidate the differences between them with detailed atomistic simulations. In almost all cases these differences are confined to the local neighborhood of the probe molecules which are sparsely used and generally present as single molecules. In general, the behavior of the bulk membrane lipids can be qualitatively understood from the probes but in most cases their properties cannot be directly quantitatively deduced from the probe behavior. This article is part of a Special Issue entitled: Biosimulations edited by Ilpo Vattulainen and Tomasz Róg.

1. Introduction

A wide variety of experiments in biomembranes needs specially designed lipids with probes chemically attached to perform the experiment and only the behavior of the probe molecules is seen in the experiments [1–13]. Regularly it is assumed that the probe molecules behave essentially the same way as the lipids and membranes they are probing. But it is easy to see that this cannot exactly be the case. Assume for example a diffusion experiment where the probe molecule (often a fluorescent probe) has a much larger molecular weight than the surrounding lipids as the lipid has to carry the fluorescent marker with it. Phase information or information on the type of diffusion can normally be reliably obtained from such an experiment. However, the actual quantitative diffusion coefficient of the probe molecule will be different from the surrounding lipids even in the case of ideal mixing. The phase of the lipids can often be deduced as at the phase transition the diffusion drops by several orders of magnitude such that small quantitative differences are not crucial. But for a correct calibration of the diffusion dynamics or other properties we need to understand how probes are behaving differently from lipids.

Molecular modeling can help bridge the gap between the experimental data gained on the probe and obtaining information on the bulk lipids if experiments and simulations are performed in tandem. Molecular Dynamics (MD) simulations are regularly used to capture the behavior of biological macromolecules in full atomic detail, but their computational demands, combined with the challenge of appropriately modeling the relevant physics, sometimes restrict their reliability and accuracy. Dramatic recent improvements in speed and the development of better models have enabled atomic level simulations on timescales up to milliseconds that capture key biochemical and biophysical processes. MD can serve as a computational microscope, revealing biomolecular mechanisms at spatial and temporal scales that are difficult to observe experimentally [14] such that it is an ideal counterpart to real microscopes. MD is well–established for biomolecular studies. It has been used widely to study the behavior of lipids and their interactions [15–22]. Most simulations on biomembranes and lipid assemblies are performed in atomistic detail where every (at least non-hydrogen) atom is represented, this is the level of detail needed for studying probe lipids as we largely expect local deviations from bulk behavior.

This review aims to summarize simulation efforts on lipid probes over the last decade in order to inform the reader on the current state of the art. Another review of a part of this field has been published a few years ago [23] which, however, focused on fluorescent probes exclusively. Here we focus particularly on the effects of the probes on the other lipids and differences in behavior between probe lipids and bulk lipids and we discuss also EPR active probe molecules. This review is focused on classical molecular dynamics. There are many other techniques including electron structure calculations or Monte Carlo which are not discussed here.

There are a few experimental studies which investigated the influence of lipid probes on the system. In a study of fluorescence quenching lipid–probe interactions between the non-fluorescent substrate and the lipid, which affect the observed rate of change of fluorescence after addition of lipids to DHR (dihydrorhodamine 123) and

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DCFH (dihydrodichlorofluorescein) (for definitions see Fig. 1) was found [24]. These interactions depend on a large variety of parameters including sample collection and storage, types and concentrations of lipid and fluorescent probe, as well as pH. One assay yielded reproducible measurements despite fluorescence quenching, while the other had rather large experimental variability. Furthermore, the lipid–probe interactions varied according to the introduced level of inflammation. In another study using deuterium nuclear magnetic resonance spectroscopy (^{2}H NMR), it was found that trace amounts of the carbocyanine probe DiIC$_{12}$ which is used as NMR marker were enough to alter the phase coexistence of a 30:30:35 DPPC:DOPC:cholesterol membrane, while other probes like Laurdan, Naphthopyrene, and another carbocyanine probe DiOC$_{18}$, did not affect the membrane appreciably [25]. These experimental results make it clear that there is no generic behavior of probe lipids in a membrane and detailed MD studies can be very useful to interpret experiments.

Fluorescent or EPR-spinactive molecules or moieties are very common tools to study the behavior of lipids in membranes. They can be attached to lipids or proteins or added to the membrane as a separate component and can then be monitored with a variety of fluorescence microscopy and optical spectroscopy techniques [26–29], with EPR [30–36], and NMR techniques [37,38]. Fig. 1 shows examples of such probe moieties which are abundant in experiments and some of which have been studied computationally. Typically, the focus of a fluorescence experiment is not the fluorophore itself, but the other molecules that make up the lipid membrane. The probes can be thought of as an impurity, a "necessary evil" that allows the measurement of static and dynamic membrane properties of interest. This of course only makes sense if we can assume that the probe does not alter the behavior of the membrane molecules in a dramatic way.

Molecular dynamics simulation is perfectly suited to address the questions associated if probe molecules correctly represent the "average" behavior of a lipid membrane or if the probe alters its environment or even changes the phase behavior of the lipids around it. Molecular dynamics can provide atomistic detail over length scales of individual to hundreds or thousands of lipids and time scales from picoseconds to hundreds of nanoseconds. We expect that the largest influence of the probe molecules is in the local neighborhood of a given lipid, thus we need full spatial resolution which leads to atomistic simulations. Knowing how a fluorescent molecule interacts with the molecules of actual interest – the lipids around it – is particularly critical in single molecule studies [39] as one tries to deduce typical behavior from the observation of one individual molecule which is even of another chemical species. In single molecule tracking experiments [6,40] a single fluorescent molecule is imaged in time sequence to determine its rotational and translational diffusion behavior. The advantage of such single molecule studies over ensemble techniques is their ability to reveal the statistical distribution of behavior that is averaged over in ensemble measurements [41]. But to interpret such experiments reliably it is important to know that subtle features in the data are caused by the membrane characteristics and not by the fluorophore itself.

Fig. 2 shows a visualization of a typical simulation system where one probe lipid is embedded in a bilayer of "regular" lipids. The thick black lines represent one Texas Red marked DHPE lipid. Texas Red is a very abundantly used fluorophore. The purple balls mark the phosphate groups of the non-fluorescent lipids to identify the interface between the aqueous region and the hydrophobic region. It is clear that the chemistry of the fluorophore which often bases on fused aromatic rings is different from any other chemistry in the system. It is known that aromatics prefer the interfaces between water and oil (alkanes) [43]. So we would expect as is seen here that the aromatic dye segments locate at the interface and may disrupt the lipid packing in their vicinity.

2. Fluorescent probes

Fluorescent probes which enable fluorescent microscopy techniques are arguably the most abundant type of probe lipids used experimentally so we will first discuss this molecular class. Fluorescent probes can, e.g., be used to study the phase behavior of lipid mixtures because many probe molecules partition preferentially to one of the lipid phases which allows visualization of the phase separation as now the phase with the higher abundance of fluorophores is lighter and the other phase is darker [3,8,9,29]. However, it has experimentally been reported that attachment of a fluorescent label to higher order lipids and eliminate a molecule's ability to partition with other higher order lipids [44] and therefore it might be that one gets un-intuitive phase identification. In order to avoid this, simulations of the probes with different neighborhoods are needed to determine which lipids (or mixtures) are preferred by a given probe.

Many fluorescent molecules, particularly the ones which are used in single molecule studies, are of the xanthene family of dyes [45]. These are polycyclic aromatics and can be attached chemically to a lipid

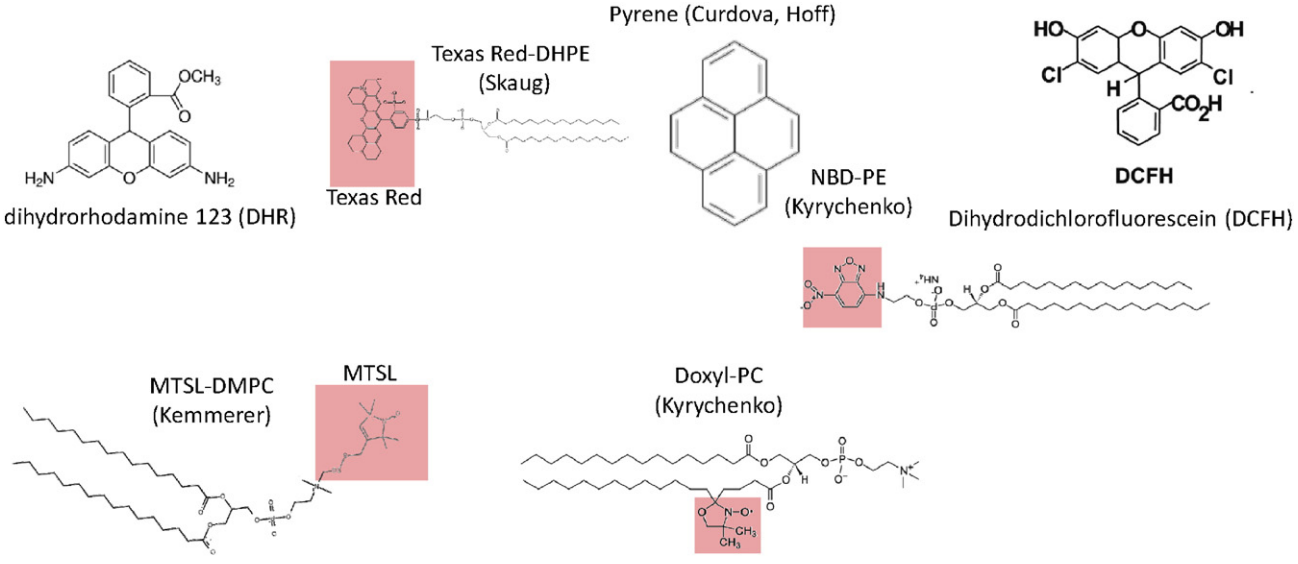

Fig. 1. Some of the molecules and moieties used as probes in membranes and discussed in this review. The fluorescent or EPR active moieties connected to other molecules are marked with a pink background. Top row: fluorescent probes including Dihydrorhodamine 123, Texas Red DHPE, NBD-PE, Pyrene and Dihydrodichlorofluorescein, bottom row: EPR spin probes. Pyrene has also been studied in NMR experiments. The names in parentheses are the researchers who studied the corresponding systems computationally.