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René Costard

Ultrafast Dynamics of Phospholipid- Water Interfaces

Studied by Nonlinear
Time-Resolved Vibrational
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Ultrafast Dynamics of Phospholipid-Water Interfaces

Studied by Nonlinear Time-Resolved
Vibrational Spectroscopy

Doctoral Thesis accepted by
Max-Born-Institut, Berlin, Germany

 Springer

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Supervisor's Foreword

Phospholipid molecules, the building blocks of cell membranes, consist of a hydrophilic headgroup and hydrophobic fatty acid chains of hydrocarbons. The ionic headgroup contains a phosphate unit and interacts strongly with water molecules, in particular through phosphate–water hydrogen bonds. The ionic groups generate strong electric fields in the plane of the membrane, leading to a spatial orientation of water molecules. While the structure and basic physical properties of phospholipids in thermal equilibrium are well understood, their dynamics in the time domain of molecular motions and related structural fluctuations represent topics of current research. Key issues are the fluctuating structure of the interfacial water layer, the influence of fluctuating electric fields on molecular dynamics and hydrogen bonding, and processes of energy exchange and dissipation in the hydrated system. The relevant timescales are in the femto- to picosecond range and, thus, methods of ultrafast spectroscopy hold a strong potential for unraveling the basic processes.

In his thesis, Rene Costard studied structural and vibrational dynamics of phospholipids with some of the most advanced methods of femtosecond infrared spectroscopy. His work extended the spectral range of two-dimensional infrared spectroscopy towards fingerprint vibrations of low frequency. As a model system, he investigated reverse micelles of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) which were filled with a water nanopool of variable size and embedded in a nonpolar liquid. Interactions between phospholipids and water were mapped via the ultrafast dynamics of vibrational excitations of either the phosphate groups or water molecules. Vibrational dynamics of the phosphate groups which are addressed here for the first time, are particularly sensitive to interfacial dynamics and, thus, spatially selective. In contrast, water vibrations provide information that is spatially averaged over the water pool.

Rene's work has given detailed new insight at the molecular level. Two-dimensional spectra of phosphate vibrations give evidence of structural disorder of the phospholipid surface, manifested in a distribution of vibrational frequencies. This disorder remains essentially unchanged on a timescale of the order of 10 ps,

both at a very low water level and under conditions of full hydration with up to six water molecules interacting with the phosphate groups. The phosphate–water hydrogen bonds remain intact during this time interval. The hydrated phospholipid structure undergoes limited fluctuations on a 300 fs timescale which originate from statistical motions of both the phospholipid and the interfacial water molecules.

Phospholipid–water interactions play a central role for energy dissipation in the system. The water pool can accommodate large amounts of excess energy stemming from the decay of vibrational and/or electronic excitations of the phospholipid. Redistribution and delocalization of excess energy over water molecules occur on a characteristic timescale of a few picoseconds and establish a heated ground state of the liquid. Rene has shown that a small shell of only three water molecules around a phosphate group is sufficient for implementing this basic and highly efficient mechanism of energy management.

Other key results show that the lifetime of both OH stretching and bending vibrations of water molecules depend on the size of the water pool. The OH stretching vibrations decays via the anharmonically coupled OH bending mode, whereas the OH bending mode relaxes directly into librational water motions. The OH bending lifetime depends sensitively on the energy mismatch with librational overtones.

In conclusion, Rene's thesis combines novel experimental techniques with a sophisticated research strategy to generate new insight into the dynamic properties of phospholipids. The conclusions drawn from the experimental results are supported by detailed theoretical calculations and simulations. This work paves the way for future studies of more complex biomolecular systems in an aqueous environment, the medium of life.

Berlin
February 2015

Prof. Thomas Elsässer

Abstract

Charged phosphate groups are the major hydration sites of biomolecules such as phospholipids and DNA. Hydration shells play a key role in the formation and stabilization of cell membranes and the DNA double helix structure. Here, we introduce phospholipid reverse micelles with variable water content (between one and sixteen water molecules per phospholipid) as a model system to study elementary phosphate–water interactions. The fastest processes at phosphate–water interfaces, e.g. hydrogen-bond dynamics and vibrational energy transfer occur on a femto- to picosecond timescale. Since molecular vibrations are sensitive local probes of the structure and dynamics, the use of femtosecond vibrational spectroscopy, in particular two-dimensional infrared spectroscopy (2D IR) and pump-probe spectroscopy in a broad spectral range, allow for the observation of microscopic phosphate–water interactions in real time. We present the first two-dimensional infrared spectra of phosphate stretching vibrations that represent true interfacial probes independent of the hydration level. Such spectra reveal that the fastest structural fluctuations of phospholipid headgroups occur on a 300-fs timescale, whereas phosphate–water hydrogen bonds are preserved for >10 ps. Vibrational dynamics of intramolecular water vibrations, i.e., the OH stretching and bending modes show that small water pools around the phosphate groups form when three or more water molecules per phospholipid are present. Such water pools act as efficient heat sinks of excess energy deposited in intramolecular vibrations of water or the phosphate groups.

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Chapter 1

Introduction

Cells are the machines of life for all living organisms from the smallest bacteria to large animals or plants [1]. Semipermeable cell membranes separate the cytoplasm from the extracellular space. Their basic structure consists of a bilayer of lipids (cf. Fig. 1.1) complemented by membrane proteins that account for selective transport of ions and molecules in and out of the cell as well as for cell signaling.

The most abundant types of lipids in cell membranes are phospholipids, in particular phosphoglycerides that consist of a glycerol backbone linked to two fatty acids and a phosphate group by ester bonds (Fig. 1.2). The phosphate group is esterified with an alcohol that determines the nature of the head groups, the main types in mammalian membranes being phosphatidylcholines, phosphatidylethanolamines and phosphatidylserines (cf. Fig. 1.2c–e). All types of head groups are zwitterionic making them polar so that they can form hydrogen bonds with water [3, 4]. In contrast, the hydrocarbon chains of fatty acids are nonpolar. Thus, their composition defines the structure of a phospholipid's hydrophobic tails. Often, one of the chains is saturated and the other one has an unsaturated C-C bond causing a kink in that chain. One example is the mixed-chain phospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in Fig. 1.2c.

The amphiphilic character of phospholipids is of major importance for the spontaneous self-assembly into lipid bilayers in aqueous solutions. Hydrophobic effects cause the lipids to align in a way that the hydrocarbon chains form the interior of the bilayer that exposes the hydrophilic head groups to the aqueous environment. Water in the vicinity of such interfaces experiences an environment significantly different from the tetrahedral configuration in the bulk phase [5, 6]. Water molecules are aligned in the electrostatic potential of the lipid head groups and form hydrogen bonds with the phosphate and carbonyl groups. Due to this confinement, the motional freedom of water at lipid interfaces is reduced (the hydration structure of phospholipids is briefly reviewed in Sect. 1.1).

Water is not only essential for the formation of cell membranes but also determines the structure and function of other biomolecules such as proteins and DNA and is therefore a crucial component in cell biology [7, 8]. For example, it has been suggested that the interaction of water and proteins plays an important role in

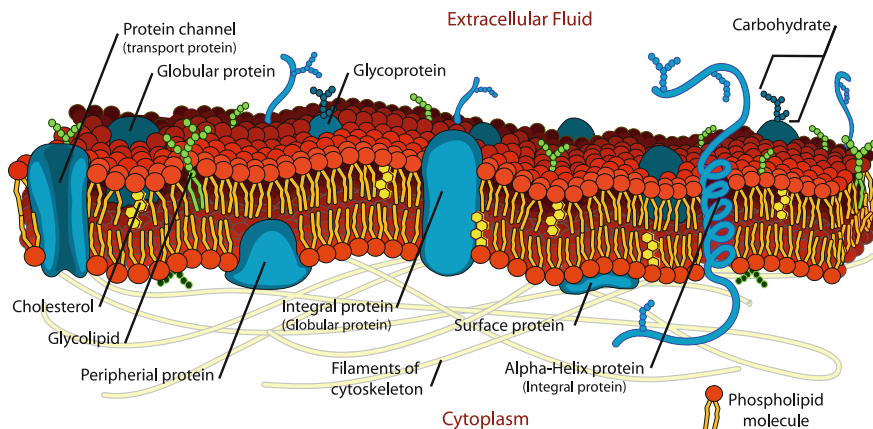


Fig. 1.1 Schematic of a lipid bilayer cell membrane. Modified from [2]

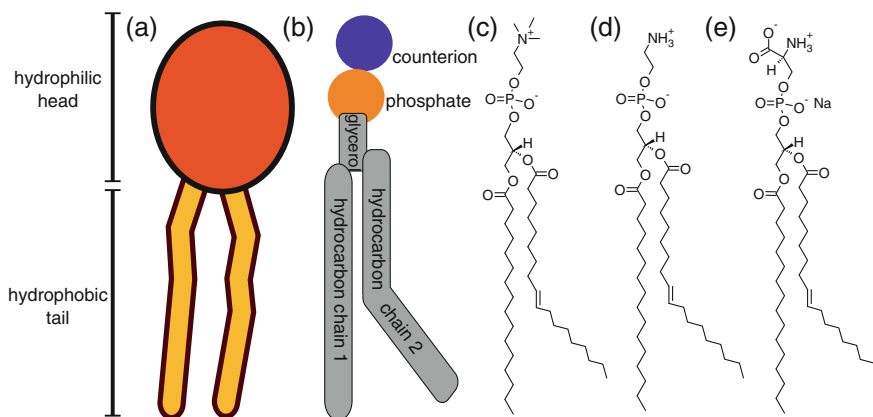


Fig. 1.2 Structure of a phosphoglyceride molecule. **a** Cartoon representing the hydrophilic head and hydrophobic tails as in Fig. 1.1. **b** Schematic of phosphoglyceride indicating its constituents. **c–e** Molecular structure of three phospholipids with different head groups as representatives for phosphatidylcholines, phosphatidylethanolamines and phosphatidylserines

protein folding and binding [9, 10]. X-ray diffraction studies have shown that the DNA double-helix conformation changes from the biologically important B-form to the A-form upon dehydration [11]. As for phospholipids, phosphate groups are major hydration sites of DNA [12, 13]. The characteristics of phosphate-water interactions therefore determine the properties of the hydration shells of a diverse class of biomolecules.

In this context it is of great relevance to obtain information about the distance from the interface up to which water properties differ from the bulk phase, i.e., to define a thickness of the hydration shell. In an extreme case, water is confined in small spherical phospholipid self-assemblies called vesicles. Organized molecular