

# On the investigation of the bilayer functionalities of 1,2-di-oleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) large unilamellar vesicles using cationic hemicyanines as optical probes: A wavelength-selective fluorescence approach

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## Abstract

The behavior of the cationic hemicyanines trans-4-[4-(dimethylamino)-styryl]-1-methylpyridinium iodide (HC) and 4,4-(dihexadecylamino)-styryl-*N*-methyl-pyridinium iodide (DIA) were studied in large unilamellar vesicles (LUV) of 1,2-di-oleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) using absorption, emission, depolarization and time resolved spectroscopies. Also, thorough spectroscopic studies were performed in homogeneous media to investigate the different interactions that the dyes can experience with its microenvironment. These results help us to comprehend the dye performance under different media and, consequently find interesting features of the DOPC membrane properties. The studies in homogeneous media analyzed by the Kamlet and Taft's solvatochromic comparison method demonstrate, for the first time, that the cationic hemicyanines undergo specific interactions with the medium through the solvents ability to donate an electron pair as measured by the  $\beta$  parameter. Thus, the absorption bands shifts bathochromically with  $\beta$  while, the emission band shifts hypsochromically. In addition, for the relaxed hemicyanines the 00 energy,  $\nu_{00}$ , is invariant with the solvent properties. The results in LUV of DOPC show that, DIA undergoes a strong association with the vesicle bilayer while HC partitions between the water and the bilayer pseudophases. To monitor directly the microenvironment and dynamics around HC and DIA inside the DOPC bilayer, we use the wavelength-selective fluorescence approach, which is based on the red edge effect in fluorescence spectroscopy, in addition with the  $\nu_{00}$  energy of the hemicyanines. The results show that the fluid state of the DOPC bilayer resembles the microenvironment of sodium bis (2-ethylhexyl) sulfosuccinate (AOT) reverse micelles at  $W = [\text{H}_2\text{O}]/[\text{AOT}]$  below 10 where there is no free water forming the water pool. Moreover, it is demonstrated for the first time, that the region of the bilayer close to the polar head of DOPC is a powerful electron donor environment.

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## 1. Introduction

Organized molecular assemblies, such as vesicles or liposomes, can be considered as large cooperative units with characteristics very different from the individual structural units, which constitute them [1]. Phospholipids form the fundamental

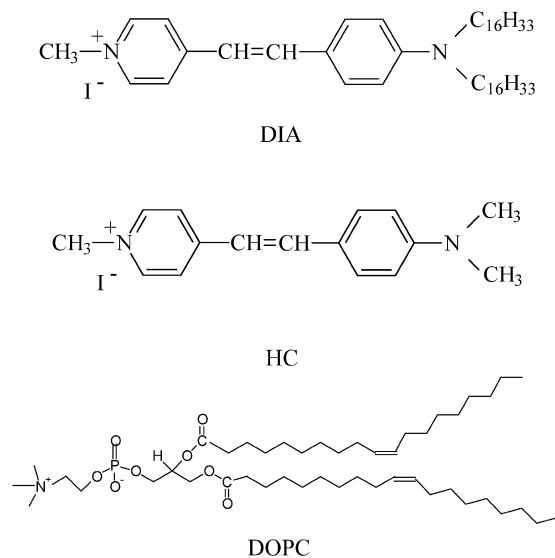
matrix of natural membranes and represent the environment in which many proteins and enzymes display their activity [2]. Results obtained from small unilamellar vesicles (SUV) are often controversially discussed as model systems for cells because of their small size and bilayer defects due to their high curvature. For this reason, it is common the use of large unilamellar vesicles (LUV) to get closer to cell-like structures [3–6].

On the other hand, interactions of small molecules with membranes are important issues in membrane biology. Understanding their role in modulating the structure and function of

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Scheme 1. Molecular structure of the molecular probes used: DIA, HC, and the phospholipid DOPC.

biological membranes, require the knowledge of the location of the molecules in the membranes and the degree of perturbation of membranes caused by these molecules [7–10]. Among small molecules, dyes based on the hemicyanine (aminostyryl pyridinium) chromophore were investigated in the past to establish the relation between molecular structure and their solvatochromism [11]. Today, they are studied not only for the effect of solvent polarity on their optical properties, but also because of possible applications in the field of nonlinear optics. Hemicyanine containing halogen, are dyes used in physiology for the research on transport phenomena through cell membranes. Depending on the electric potential inside and outside the cell, charge separation can occur in the hemicyanine dyes bonded into the cell membrane and, this charge movement results in a change of the optical properties [12–14]. Many aspects of this subject were investigated in model membrane using different spectroscopic techniques to characterize the membrane structure and dynamics. Among others, fluorescence spectroscopy has several advantages including high sensitivity, noninvasive nature, intrinsic time scale and excellent response to physical properties of the membrane [1,15,16].

In general, for a fluorophore in a bulk nonviscous solvent, the dipolar relaxation of the solvent molecules around the probe in the excited state is much faster than its fluorescence lifetime. Thus, the wavelength of maximum emission usually is independent of the excitation wavelength. However, it does show excitation wavelength dependence if the dipolar relaxation of the solvent molecules is slow in the excited state, such that the relaxation time is comparable to or longer than the probe's fluorescence lifetime. Such a shift in the maximum emission toward higher wavelengths, caused by a shift in the excitation wavelength toward the red edge of the absorption band, is known as the red edge excitation shift (REES) [17–20]. Wavelength-selective fluorescence comprises a set of approaches based on the red edge effect in fluorescence spectroscopy. This approach can be used to directly monitor the microenvironment and dynamics around a fluorophore in a motion restricted me-

dia such as organized media like reversed micelles or vesicles [1,21,22].

It is very important the choice of a suitable probe in order to infer properties of the membrane through the wavelength-selective fluorescence approach. Thus, we present first the solvatochromic study of the molecular probes used in this contribution to understand its behavior in relative simple environments. Then, we used these studies to investigate the probes behavior in the phospholipids bilayer.

The dyes shown in Scheme 1: 4,4'-(dihexadecylamino)styryl-*N*-methylpyridinium iodide (DIA) and trans-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (HC) have been studied in homogeneous media as well as incorporated in large unilamellar vesicles (LUV) of the phospholipid 1,2-di-oleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC, Scheme 1). The behaviors of these dyes were investigated using absorption and emission spectroscopy in addition to the steady-state and time-resolved fluorescence emission techniques. The results, compared with those obtained in homogeneous media, help us to comprehend the dye performance under different environments and consequently find interesting features of the DOPC membrane properties.

We have previously reported the behavior of the molecular probe PRODAN (6-propionyl-2-(dimethylaminonaphthalene) in LUV of DOPC. The results show that PRODAN forms aggregates in water but, inside the DOPC bilayer vesicles exists completely incorporated as monomer and sense two different microenvironments within the bilayer: a polar region in the interface near the water and a less polar and also less viscous environment, between the phospholipids tails. The absorption and emission bands of PRODAN were used to measure the value of the micropolarity of the different sites where the molecule exists [9]. The results in the present work introduce HC and DIA as very good probes to study the dynamics of confined environments through the wavelength-selective fluorescence approach. The results show that, DIA and HC's lifetimes values increase and its fluorescence anisotropy ( $r$ ) values considerable

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