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Characterization and validation of a membrane mimetic with magnetic orienting capabilities and natural phospholipids content

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The cellular membrane in a highly complex structure with variable and inhomogeneous composition which makes the direct study of the sole lipid bilayer a very difficult task, this introduces the necessity of membrane mimetics, that is, lipid bilayer structures that behave as close as possible to the cellular membrane. In this article we present a membrane mimetic with a high phospholipid content, the main component in natural lipid membranes, and the capability of orienting itself when exposed to an external magnetic field, this enables it for studies of its dynamics and mobility employing ^{2}H-NMR. We validate its permeability properties as membrane mimetic by reproducing the membrane-permeating ability of Benzocaine, and the inability of Levodopa to do so. We also present a molecular dynamics simulation model of the membrane mimetic, calibrated to reproduce experimental ^{2}H-NMR results.

1 Introduction

The cell is a highly complex unit present in all living organisms: it constitutes the building block of life. Essentially, consists in a closed domain containing smaller organelles in a highly complex and crowded aqueous solution, all enclosed by a bilayer made of mainly pospholipids and containing fatty acids, sugars, cholesterol and proteins, among others. This bilayer is called cell membrane or cytoplasmic membrane. Membranes itself are very complex molecular organizations with variable and inhomogeneous composition, and its atomic level understanding is a very difficult task. For this reason, the employment of membrane mimetics and models has become common practice.[1]

Membrane proteins play a significant role in human pathologies[2,3]. About 30% of human genes code for membrane proteins[4] and they are targeted by more than 50% of drugs[5,6]. Therefore, most drugs have to cross membrane interfaces to reach their active site, and consequently, the activity of these drugs depends, among other factors, on their ability to perform this task. This is particularly true for local anesthetics (LA). For more than hundred years it has been observed that the effectiveness of many LA correlates positively with lipophilicity[7–10], showing the importance of becoming incorporated into the bilayer. It is widely accepted that inhibition of voltage gated Na^+ channels is directly involved in the mechanism of LA[11,12] and three possible pictures have been proposed: (a) LA directly binds the pore of the channel blocking the transit of ions, (b) LA reaches the active site by one of the lateral cavities filled with hydrophobic membrane components, and (c) the presence of LA near the interface modifies the structure and dynamics of the bilayer itself, perturbing the conformational dynamics and functioning of the channel[13–21]. Despite which mechanisms are actually taking place, crossing membrane interfaces to become incorporated into the hydrophobic domain appears to be a crucial step for most drugs.

Benzocaine is a well known LA for topical use. It has been widely employed anesthetizing the oropharynx for trans-esophageal echocardiography, bonchoscopy, esophagogastroduodenoscopy, in cold sores, mouth ulcers, toothache, sore gums and denture ache among others[22]. A ignificant number of cases of Benzocaine induced cyanosis (methemoglobinemia) have been reported along the years[23], however it still remains in use.

Benzocaine has been subject of a significant number of studies, including free energy transfer from water to the interior of different membrane mimetics[24–27], estimations about location and orientation in different bilayers and monolayers[28–31], interactions with a variety of solvents[32–36] and encapsulation in different structures for controlled delivery purposes[37–41] among others. All the evidence confirms that Benzocaine is able to cross the interface of membrane mimetics to become incorporated into the hydrophobic bilayer to finally be located at the inner interface.

Contrary to most LA, Levodopa (or L-DOPA), the precursor of the neurotransmitter dopamine, commonly used in treatment of Parkinson's disease, is able to cross membrane interfaces only via active processes[42]. Theres is evidence that neurotransmitters

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