Interactions of Bispyridinium compounds with the DOPC Membrane as revealed by Constraint Dynamics Simulations and Rapid Cyclic Voltammetry

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Introduction

Bispyridinium compounds (BPCs) have important applications as potential pharmaceuticals and antidotes for nerve agent poisoning. These compounds consist of two pyridinium groups linked via the nitrogen atom by an alkyl chain of varying length, while t-butyl groups can substitute the hydrogen atoms of the pyridinium groups. The length of the alkyl chain and the presence or absence of t-butyl groups influence the interactions of BPCs with membranes. These selective interactions of the BPCs with membranes allow us to use the membranes as sensors to trace specific BPCs in blood, water, or other solutions. To confirm the selectivity of membranes to BPCs, we studied the interaction of six BPCs (i.e., having alkyl chain lengths of 1, 5, and 10 with or without t-butyl groups) with the 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) bilayer membrane when both are in water. We applied Rapid Cyclic Voltammetry (RCV) [1] to create rapid cyclic voltammograms and Constraint Dynamics Simulations to find the free energy profiles. Since BPCs have a positive charge of +2e, two negative anions (in our study, iodine anions I-) coexist in the solution. Different BPCs lead to differences in the RCV voltammograms (e.g., depression of the capacitance peaks) which would be used for their detection.

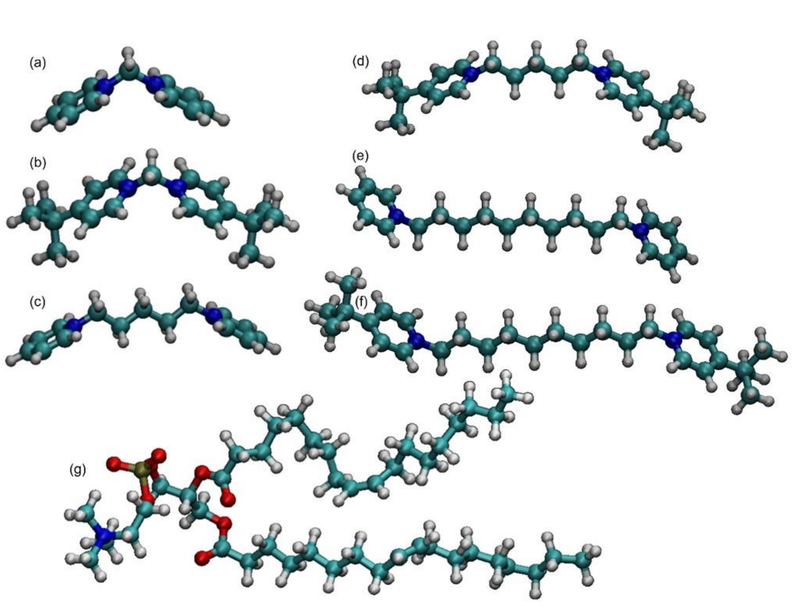

Fig.1: *Bispyridinium compounds (BPC) with (a) one carbon atom in the central carbon chain without t-Butyl groups at the ends (BPC-1-BTL-N), (b) one carbon atom in the central carbon chain with two t-Butyl groups at the ends (BPC-1-BTL-Y), (c) five carbon atom in the central carbon chain without t-Butyl groups at the ends (BPC-5-BTL-N), (d) five carbon atom in the central carbon chain with t-Butyl groups at the ends (BPC-5-BTL-Y), (e) ten carbon atom in the central carbon chain without t-Butyl groups at the ends (BPC-10-BTL-N), (f) ten carbon atom in the central carbon chain with t-Butyl groups at the ends (BPC-10-BTL-Y) and (g) 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). Nitrogen, Carbon, Oxygen, Phosphorus, Hydrogen atoms are illustrated with blue, cyan, red, brown and grey colors respectively.*

Implementation of Constraint Dynamics Simulations

In order to perform the molecular dynamics simulations of the previous systems, we first need to find their initial configuration (positions in space) and to decide which Force-Field (FF) we will apply in order to simulate their interactions. As Jämbeck and Lyubartsev [2, 3] have already invented a FF which describes well the properties of the DOPC membrane inside water, their FF has been chosen to be used in our simulations. In addition to FF parameters, Jämbeck and Lyubartsev [2, 3] provide the DOPC-water configurations, which were also selected to be our initial configuration for our simulations. This means that we used a simulation box consisted of 128 lipids, 40 H_2O/lipid (not illustrated in Figure 2 for clarity of the rest), two Iodine anions and one bispyridinium double-cation. We can see the properties of this simulation box in Fig. 2.

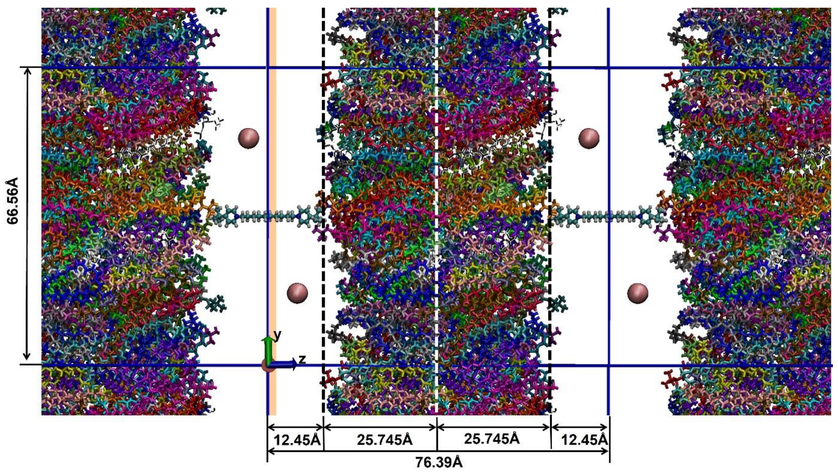

Fig. 2: *Simulation box (central box) at zy plane and its replications in space in order to understand the application of periodic boundary conditions on it. The blue lines, the black dashed lines and the white line illustrate simulation box edges, the border and the center of the DOPC membrane respectively. Iodine anions are illustrated with pink spheres, while each DOPC molecule of the membrane is illustrated with a different color. Water molecules have been omitted for clarity while the (f) BPC of Figure 1 is illustrated with the colors used in Figure 1. The length of the simulation box in x direction is 62.10Å. The light yellow lane has been used to illustrate that during constraint-dynamics the center of mass of the BPC is allowed to travel inside this area.*

Concerning the BPCs compound, an FF must be selected to describe their intramolecular interactions and their interactions with the DOPC membrane and the water molecules of the box. As we decided to use the CHARMM based S-lipids FF of Jämbeck and Lyubartsev [2, 3], we also selected a CHARMM FF [4] for the BPCs in order to be compatible with the S-lipids FF. As we are highly interest to understand the mechanism of the adsorption of the BPC on the DOPC membrane, the Local Free Energy profile of the BPCs along a reaction coordinate inside the simulation box of Fig. 5 must be found. To find this Local Free Energy profile, we decided to apply the strategy proposed by Carter et al [5] by using the formulation of Ciccotti and Ferrario [6]. According to Ciccotti and Ferrario [6], the Local Free Energy *A* can be calculated using the Eq. (1) after the integration of the potential of mean force derived from Eq. (2). Force, Potential energy, Reaction coordinate, Jacobian determinant are mentioned as *F, V, ξ, J* respectively. We can see the Local fee energy profiles in Fig. 3.

$$A(\xi) = A(\xi_0) + \int_{\xi_0}^{\xi} \frac{dA(\xi)}{d\xi} d\xi \qquad \text{Eq. (1)}$$

$$\left(\frac{dA(\xi)}{d\xi}\right)_{\xi=\xi_0} = \frac{\left\langle \left[\frac{\partial V}{\partial \xi} - k_B T \frac{\partial \ln|J|}{\partial \xi}\right] \delta(\xi - \xi_0) \right\rangle}{\langle \delta(\xi - \xi_0) \rangle}$$

$$\xrightarrow[\frac{\partial \ln|J|}{\partial R_{cm,z}}=0]{\xi = R_{cm,z}} \left(\frac{dA(R_{cm,z})}{dR_{cm,z}}\right)_{R_{cm,z}=R_{cm,z0}} = \frac{\left\langle \frac{\partial V}{\partial R_{cm,z}} \delta(R_{cm,z} - R_{cm,z0}) \right\rangle}{\langle \delta(R_{cm,z} - R_{cm,z0}) \rangle} \qquad \text{Eq. (2)}$$

$$\rightarrow \left(\frac{dA(R_{cm,z})}{dR_{cm,z}}\right)_{R_{cm,z}=R_{cm,z0}} = \sum_i -F_{i,z} = -F_{tot,BPC,z}$$

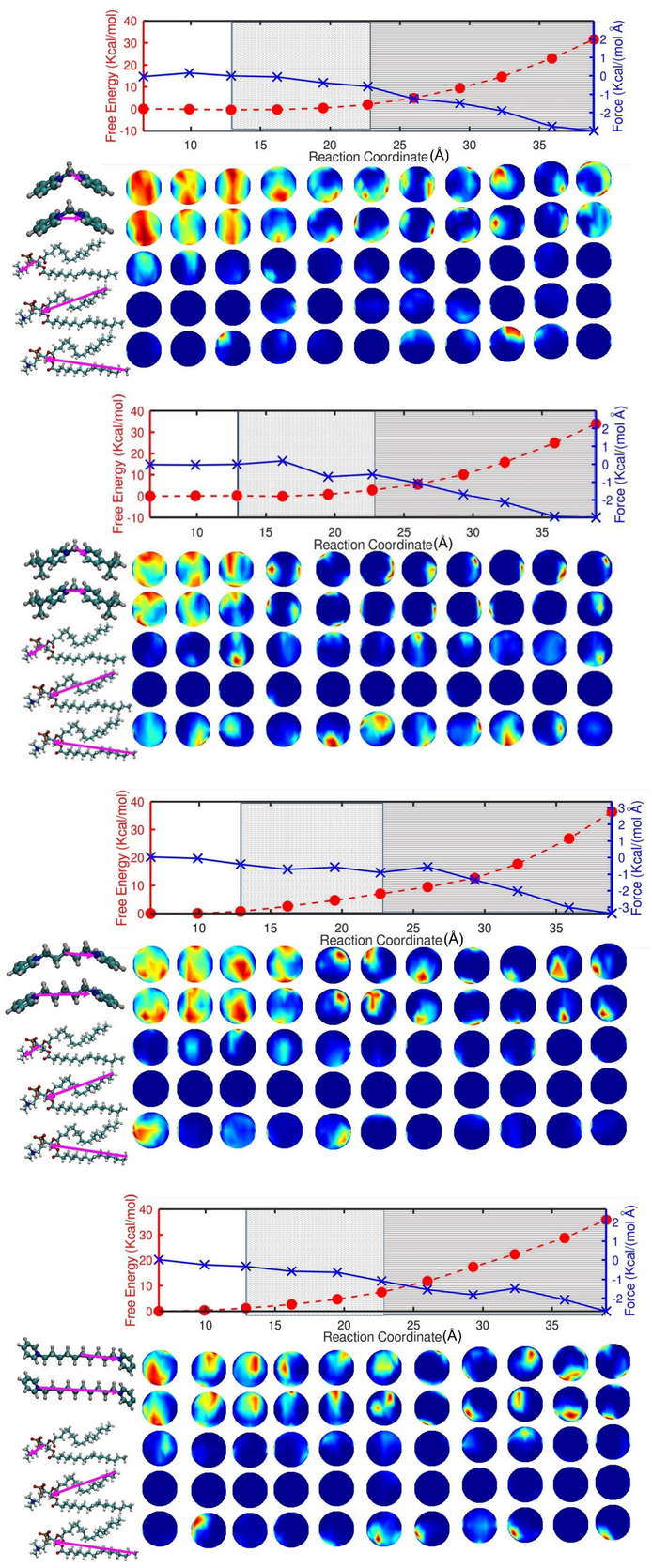

Fig. 3: *Free Energy (red color) and Force compound (blue color) along the reaction coordinate. The curved lines outline illustrates the reaction coordinate values where the hydrophilic part of the DOPC molecule is expected to be according to its structure. Similarly, the straight lines outline illustrates the reaction coordinate values where the hydrophobic part of the DOPC molecule is expected to be according to its structure. Probability density spheres of the arrowhead of the pink arrows defined at the bottom left. Red and blue color show high and low probability respectively. Each sphere corresponds to a specific z position (=reaction coordinate direction) of the graph at the top and the view of the sphere is on its projection at zy plane of Figure 2.*

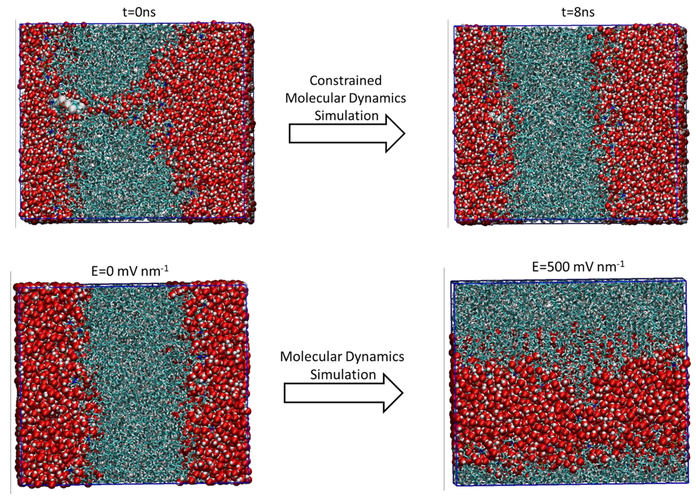

Fig. 4: *a) Initial configuration (left) after a continuous 0.2Å displacement of the BPC center of mass from right to left. For every displacement we applied 40ps constraint dynamics simulation. Final Configuration (right) after 8ns of constrained dynamics simulation having started form the initial configuration. b) Effect of DOPC membrane orientation because of electric field of 500mV/nm. Nitrogen, Carbon, Oxygen, Phosphorus, Hydrogen atoms are illustrated with blue, cyan, red, brown and grey colors respectively.*

Implementation of Rapid Cyclic Voltametry experiments

Examples of the effects of the interactions of the bispyridinium compounds with the DOPC monolayer on the RCV plots are shown in Fig. 5 where capacitance current peaks 1 and 2 are labelled in Figure 5(a). Significantly, interaction of the DOPC layer with compounds with a single ethylene linker suppress the capacitance current peaks. In addition the tert-butyl (t-Bu) substituents on the C-4 atom of the pyridinium ring effect a stronger suppression of the current peaks. Bispyridinium compounds with a 5-carbon linker chain show an insignificant effect on the RCV plot following interaction with the DOPC layer. Interestingly the t-Bu substituted bispyridinium compound with a 10-carbon linker effects a response in the capacitance current peak profile following interaction with DOPC similar to that of aromatic hydrocarbon interaction. Also interesting is the "hump" in the low capacitance minimum which is more obvious on the return anodic scan. It is noted that the capacitance current "humps" are observed on both the cathodic and anodic scans and are seen for scans obtained from interactions of bispyridinium compounds, C8 to C10 and t-bu C6 to C10 with the lipid sensor layer. This "hump" arises from a displacement current of the movement of the bispyridinium compound in a fluctuating electric field which can only take place if the compound is embedded within the apolar region of the lipid monolayer.

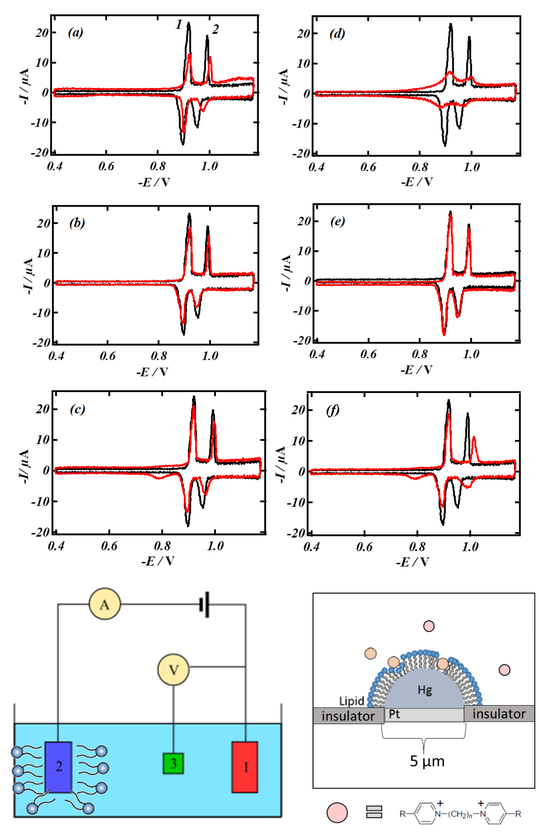

Fig. 5: *RCVs recorded at 40 Vs^{-1} of a DOPC coated Pt/Hg electrode in PBS at pH 7.4 (black line) in the presence of 20 mol dm^{-3} bispyridinium compounds (red line): (a) C2, (b) C5, (c) C10, (d) t-Bu C2, (e) t-Bu C5, (f) t-Bu C10.*

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