

BISPYRIDINIUM COMPOUND SCREENING USING PHOSPHOLIPID TARGET SENSOR ELEMENTS

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BACKGROUND, MOTIVATION AND OBJECTIVE

Pyridinium is the cationic conjugate acid of pyridine. It is synthesised from the electrophilic attack on the pyridine nitrogen atom [1]. The lone electron pair on the nitrogen atom is not delocalised and hence pyridine can be easily protonated to form the pyridinium ion. Compounds with pyridinium inclusion are known to hydrogen bond to the phosphate moiety of lipid layers such as dimyristoyl phosphatidylcholine (DMPC) bilayers [2]. Bispyridinium (BP) compounds have important applications as potential pharmaceuticals and antidotes to nerve agent poisoning [3-5]. As a result, in order to understand the molecular initiating events in the biological activity of BP compounds, it is essential as a first step to elucidate their interactions with biological membrane models. Fig. 1(i) summarises the structures of the compounds investigated.

STATEMENT OF CONTRIBUTION/METHODS

1. Compounds at 20 $\mu\text{mole dm}^{-3}$ in PBS at pH 7.4 were screened using the HISENTS dioleoyl phosphatidylcholine (DOPC) membrane sensor element on microfabricated Pt/Hg electrodes in an online high throughput flow system [6,7]. This technology screens compounds for their ability to bind to, modify and damage a fluid DOPC structure as interference in two electrically induced lipid phase transitions shown as depressions in two voltammetric peaks.
2. Molecular dynamics (MD) simulations of BP compounds interacting with a DOPC membrane in water were carried out with the aid of Enalos Demokritos and Asclepios KNIME nodes. The simulation informed on the energetics of the compound in vertical orientation as it approaches the DOPC membrane.

RESULTS AND DISCUSSION

Fig. 1(i) shows that BP compounds with intermediate length carbon (C) chains exhibited insignificant interactions with DOPC layers, whereas those with only one and two C linker(s) interacted to the greatest extent. 4-t-Bu groups on the pyridinium ring enhanced the interaction with the DOPC layers. Voltammograms (Fig 1(ii)) at high sensitivity showed that a translocation of the longer C chain-linked BP compounds within the DOPC layer was identified as a cathodic and anodic displacement current peak. MD simulations indicated that the BP compounds with shorter C link were repelled at closer distance to the membrane whereas those with longer C link are attracted to the membrane layer (Fig. 1(iii)).

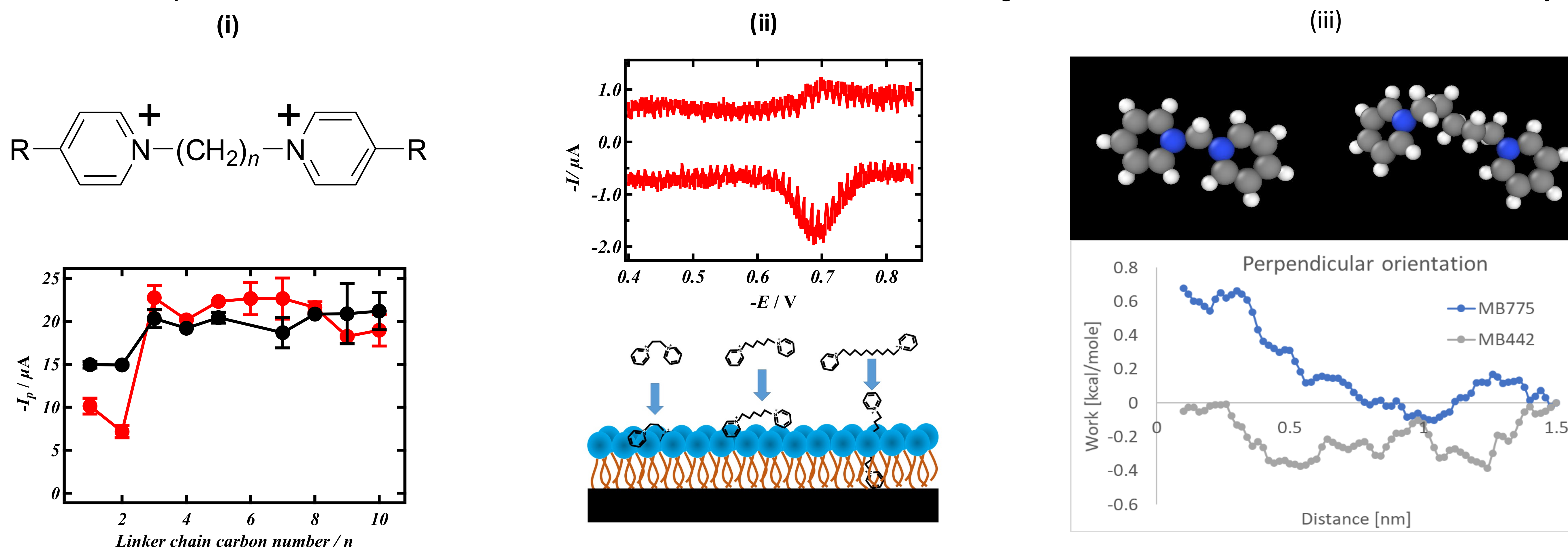


Figure 1: 1(i) Top, BP structures, R=t-but or H and n=1 to 10, bottom, output of sensor where red is t-but- and black is H-derivative and current depression indicates interaction, 1(ii) top, voltammogram of 9-t-but-BP on DOPC showing displacement current peaks, bottom, model of BP/DOPC interaction from combined sensor and MD evidence 1(iii) top, MB 775 one C and MB 442 five C links respectively, and, bottom, energetics of their respective approach to the membrane.

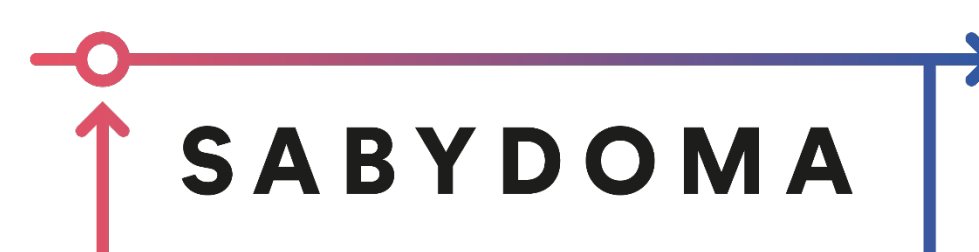
CONCLUSION

BP groups connected by short C chain initiate interaction with the polar groups of the DOPC causing the molecule to adsorb on the surface whereas the BP molecules with longer C linker chain have greater overall affinity for the membrane and penetrate the membrane vertically instead of adsorbing on the surface (Fig. 1(ii)).

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