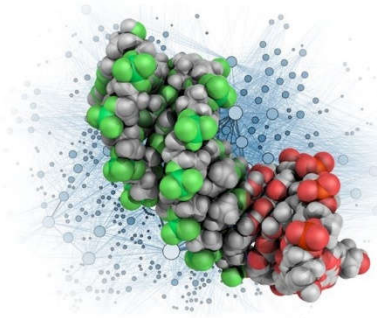

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Effect of protonation state on the interaction of Hoechst 33342 with lipid membranes – An experimental and computational study

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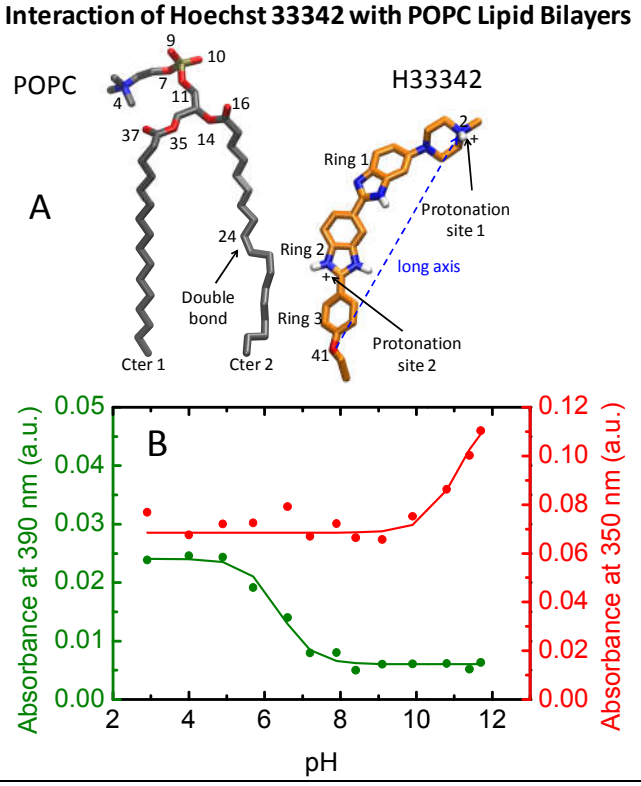
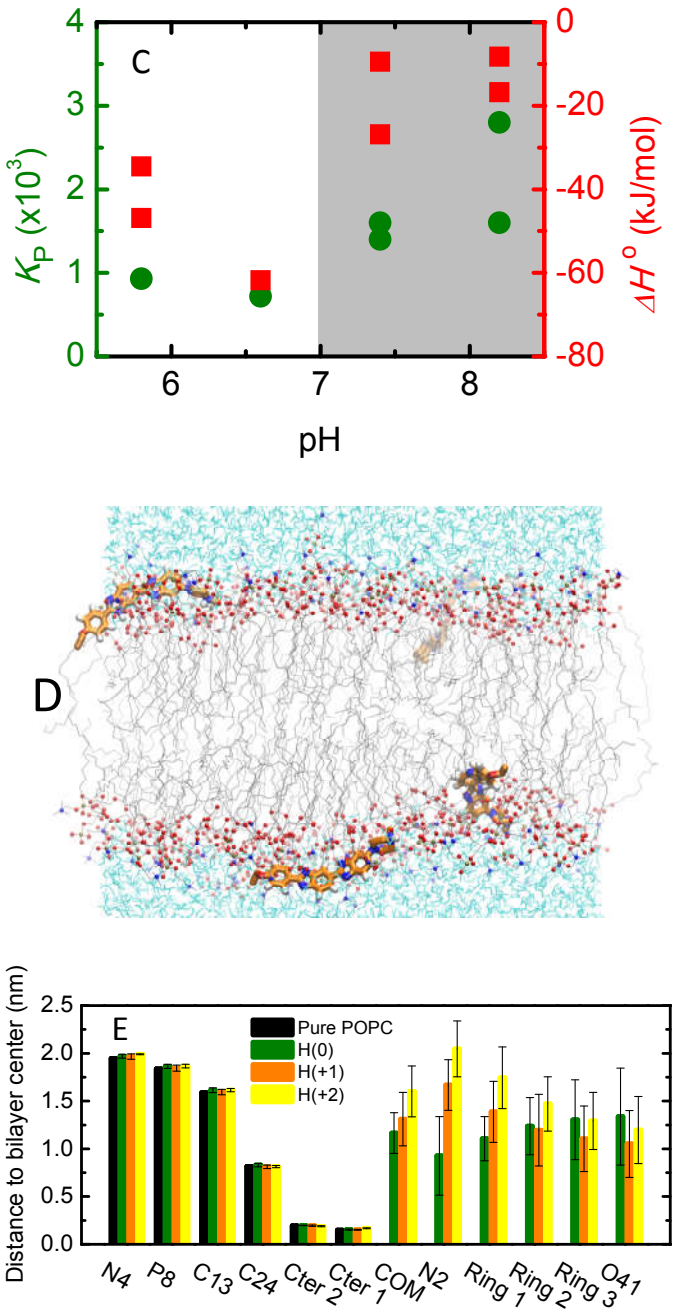
Graphical Abstract



Abstract.

Hoechst 33342 (H33342), panel A, is a fluorescent probe that stains the DNA of living cells, permeating through cell membranes.[1] However, the influence of the probe ionization state [2] in this process is poorly characterized. The knowledge of H33342 ionization state in lipid bilayers will help to predict and interpret its passive permeation through cell membranes. In this work we characterized the acid/base properties of the interaction of H33342 with POPC bilayers using an experimental and computational combined approach.

H33342 pK_a values in aqueous solution of 6.4 and 11.1 were measured by its UV/Visible spectra at different pH, panel B. H33342 partition coefficient (K_p) to POPC bilayers at different pH was measured by isothermal



titration calorimetry (ITC). An increase of K_p for higher pH values was obtained, indicating stronger interaction with membranes for the less charged or neutral forms of the probe, panel C. The enthalpy variation (ΔH^o) for the partition to the bilayer was negative at all pH values, with higher absolute values at low pH. This may indicate that when H33342 is more protonated, it adopts a more external position in the bilayer, being able to make favorable interactions in this membrane region. Detailed characterization of H33342-membrane interactions was also obtained through Molecular Dynamics (MD) simulations, panel D. This allowed to support experimental results by the calculation of membrane transverse location, panel E, and preferential orientation of the H33342 in different protonation states, as well as possibility of hydrogen bonding between the probe and the membrane.

We conclude that at physiological pH H33342 presents a high fraction of the neutral form while associated with POPC bilayers, justifying the fast permeation observed through cell membranes.

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