

Review of a Doctoral Thesis entitled
“Molecular modeling of lipid membranes with fluorescent probes”

submitted by a PhD candidate
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The thesis of the PhD candidate is focused on the molecular dynamics simulations of fluorescent probes in phospholipid membrane at atomistic level. The study was focused on two types of probes: diphenylhexatriene (DPH) and pyrene (PYR) modified lipids.

The first probe was immersed in the phospholipid membrane with 5 or 20 mol % concentration of cholesterol. The probe effect on membrane properties such as area per lipid, mass density profiles, and chain order parameters were calculated. It was found that DPH induces short-range ordering of lipids, but the effect is smaller in cholesterol rich membranes that are already ordered by cholesterol. Interestingly, it was found that the Brownian rotational diffusion of DPH cannot be fitted well with Legendre polynomials up to four order in cholesterol rich membrane. This means that analysis of fluorescence anisotropy will fail to quantitatively describe the orientation distribution of DPH, which is an important finding.

Four different pyrene based probes were studied in the second part. The PYR moiety was placed at 4th, 6th, 8th, and 10th carbons of the aliphatic tails of DOPC lipid resulting in the probes testing different depth of the membrane. Apart from the PYR effects on the membrane the candidate calculated the dimerization rate of pyrenes and its relation to lateral pressure. Importantly, the dimerization rate was found to be independent on lateral pressure. Therefore, these probes cannot be used to measure lateral pressure profile as proposed before. Unfortunately, this result is discussed without display of the data (pressure profiles and dimer formation rates) or reference to it in the attached papers.

There are three attached papers to the thesis and the candidate is the first author on all of them. In general, the thesis contains substantial amount of work, it well organized and written. There are few typos in the text and the used version of Gromacs program should be mentioned for reproducibility. Other missing information are error bars of the tail order parameters in Figure 26 that are discussed on page 45 and related origin of smaller number of the samples for PYR6 is not explained.

I have following questions for discussion:

- It is mentioned that simulations were carried out in NPT ensemble (page 26). Was the pressure applied isotropically or anisotropically? Was there any tension in the membrane plane in the simulated setup and how this tension compares to experiments?
- It is shown that higher concentration of cholesterol results the shift of DPH from the bilayer center. On page 34 it is mentioned that DPH probe got also closer to the water phase with increasing amount of cholesterol. This is not obvious as the membrane thickness also increases with increasing cholesterol, was this taken into account?
- The employed parametrization for pyrene moiety has zero partial charges (ref 49). Is this parametrization suitable for pyrene-pyrene interaction? How well is described the interaction between aromatic rings (pi-electrons) and could the approximation lead to incorrect dimerization rates?

The candidate has proven to be capable of conducting independent research using molecular dynamics simulations. Therefore, I recommend the submitted thesis for further procedures in the process of granting a PhD degree to the candidate.

RNDr. Robert Vácha, Ph.D.
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