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Review of Doctoral Thesis

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Modeling of biomembranes

In this thesis, Mgr. Martin Šrejber tackles two important aspects related with pharmacology in lipid bilayer membranes: drug permeation through the lipid bilayer, and structural aspects of enzymes (two cytochromes and cytochrome P450 reductase).

The thesis is well structured and written in good English, on 79 pages plus three publications in the appendices. Mgr. Martin Šrejber is the coauthor of 7 publications in journals with international readership, 3 of which are directly related to this thesis, 1 in first author, and 3 in second author, reflecting an important scientific production.

After a short introduction, sections 2 to 4 briefly introduce basic concepts about membranes and membranes proteins, necessary for the understanding of the research work presented. Section 5 summarizes the main concepts behind molecular mechanics and molecular dynamics, details the enhanced sampling techniques used in this work, and introduces equations to tackle drug permeabilities. Overall, these introduction sections are well presented, and clearly explain the essential concepts, without delving in small details. This highlights the pedagogical skills of Martin Šrejber.

Section 6 – the heart of the thesis – presents 5 scientific *in silico* studies, 3 of which are part of the publications in the appendices. The topics are presented in a gradual increase of the complexity of the methods used, facilitating the reader's understanding. The studies are related to: (i) the MolMeDB database which compiles data from the literature on molecule-membrane interactions, with case studies showcasing the database usefulness; (ii) the effect of lipid bilayer composition of the permeation of cyclosporin A; (iii) a method to identify compounds suitable for heat-mediated liposome release ; (iv) the interaction of the mitochondrial cytochrome CYP11A1 with lipid bilayer membranes ; (v) MD simulations of conformational sampling of the cytochrome P450 reductase and its interaction with CYP3A4. Some of my questions and comments about these studies were answered in the publications in the appendices. The remainder are listed in the following page. The short conclusion to the thesis summarizes the findings of each study. The future perspectives for each field could be emphasized.

The wide diversity of the topics (drug-membrane, protein-membrane, and protein-protein interactions) and methods in these studies (from database interface building to enhanced sampling MD simulations of a membrane protein, via quantum calculations) is striking, and denotes the great scientific versatility of Martin Šrejber. Each the studies presented expands the knowledge in their respective fields by answering important and currently relevant questions. Overall, the quality of this thesis meets the international standards for obtaining a PhD. I therefore recommend the thesis to be presented for the disputation and award of a doctoral degree.

Limoges, July 28th, 2023

Dr. Gabin Fabre

Questions to the author:

On section 6.2 (Cyclosporin A permeation study):

- You observed key conformational changes of CsA between water and hexane. Was the same behavior also measured in umbrella sampling simulations in and out of the membrane?
- In figure 19, no error estimation seam to appear, nor did several replicas seem to have been performed. Could you comment on the statistical significance of the differences observed?
- In umbrella sampling calculations, did you observe significant changes in orientation of CsA, either during each window, or between neighboring windows?
- The title of section 6.2 mentions permeation, and in page 43, you mention permeability calculations. However, I could not find the results of permeation coefficients in the text. A discussion about these data would be valuable, and would help strengthen the conclusions of the study, currently only based on an appreciation of free energy barriers. If these coefficients were obtained, were the D(z) values computed with equation (29)?

On section 6.3 (temperature-responsive liposome formulation):

- In this study, you calibrated the release of the drug at 60-65°C. How can such temperatures be reached in the body of patients without damaging tissues?
- Page 46, you write that the limits "for permeatbility coefficients were recalculated into apparent permeabilities". However, even in the publication, it is unclear how, since no value for P_{aq} is provided. I also have trouble making the link between the criteria "$\log(f_{HA}) + \log P_{erm}^{298K} < -9$" and the results of calculations and experiments. Could you please elaborate on that?

On section 6.4 (CYP11A1):

The difference between DOPC and mitochondrial membranes seems to be well rationalized, this study raises some methodological questions.

- The crystal structure used for homology modelling was in complex with cholesterol. Cholesterol is a substrate of CYP11A1. Even though the fraction of cholesterol is low in mitochondrial membranes, why did your model not include it?
- In the absence of a membrane anchor, what factors drove the choice of the initial placement of the protein in/near the membranes? Was any equilibration/thermalization simulations done prior to production? Could the behavior observed in figure 23 be the result of the system equilibrating?
- g_membed was used to assist the initial insertion of the protein in the membrane. Did the procedure result in an imbalance of the number of lipid molecules from one leaflet to another?
- The radial distribution function in figure 26 could have been normalized by the amount of lipids in the upper leaflet to better assess the local enrichment in some lipids.
- Section 6.4.4 about the interaction with adrenodoxin would benefit from a figure, that would help the reader visualize the interaction and provide some support to the hypothesis.

On section 6.5 (CPR):

This is a very interesting study, which tackles the very difficult problem of modeling interaction between two large membrane proteins. The text and figures in the publication really help understanding the text of the dissertation.

- The methodology for this section does not appear in the main text, and should be included.
- Was the 20 ns MTD simulation long enough to obtain a converged free energy profile? What was the free energy estimation between the two states?
- The two redox partners still end up far from each other. Could the residues identified between the $FMNH_2$ and heme form an electron transfer pathway?