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University of South Bohemia in České Budějovice

In České Budějovice, May 19, 2019

Opponent's review of diploma thesis **"Development and testing of computer models of phospholipid membranes"** by **Ricky Nencini.**

The thesis presents an extensive study of performance of existing + 2 newly developed force fields for ions interacting with lipid membranes. **The amount of work and scientific quality is exceptionally high** for a diploma thesis. Numerous properties are studied, their results presented in many graphs and nicely discussed. The motivation for the whole work and individual chapters, as well as summary of the results, are well written. The whole thesis is written in a logical structure and easy to read with a pleasure and understanding. I like the introduction which succeeded to introduce the key points of the experimental and simulation techniques in a reasonable number of pages. Considering the key discussion of the new force fields vs. the CHARMM36 with and without NBFIX correction, I'd appreciate explicit description of this correction in the thesis, not just a reference. The results are nicely written, starting from basic properties of a membrane not interacting with ions, to complex analyses of interactions of ions with membranes. I also appreciate the final discussion and conclusions, fairly admitting know limitations of the developed force-fields and outlook for future improvements and/or generalization of the approach to other systems. I'm glad that the author on page 101 brings into readers' attention the possible effect of charge modifications on dihedral angles (i.e. non-excluded bonded terms) and resulting structure.

The main idea behind this thesis, replacement of the poorly justified (in terms of molecular interactions) NBFIX corrections to CHARMM36 force field by a physically sound ECC scaling of charges is definitely **a big methodological step forward**, particularly for its more general application to other ions and species. Together with other works devoted to the ECC method**, this thesis has a potential to contribute significantly to the development of a new generation of force fields surpassing the current ones**.

The thesis is written in good English with only rare typos. The quality of graphs and tables is high, the discussions logical and capturing key features. Overall, the thesis is written in a way to attract the reader, guide him/her through the topic and the story of the research it covers. **Definitely it represents one of the best diploma theses I reviewed and without any reservations I evaluate it as excellent.**

Questions to the author:

1) Counter-intuitively from the geometric point of view, reduction of Lennard-Jones σ_{ij} parameters to 89% leads to increase of area per lipid. What is your explanation of this behavior?
2) The equilibration protocol on page 43 mentions: "The phosphate atoms are fixed in the x direction with a gradually decreasing force constant …" Why x (and not xy or z)?
3) Semi-isotropic barostat and boundary conditions deserve a more detailed description for the systems with a membrane in contact with aqueous solution. Was barostating in both xy and z applied, but using different compressibilities? How did you ensure that both the lateral and perpendicular components of the pressure tensor equilibrated at 1 bar?
4) The criterion for a "bound ion", i.e. a distance less than 0.325 nm, is constant regardless of the nature (size) of ion. Can you comment this choice vs. often used position of a first minimum in ion-O radial distribution function? I assume that particularly for the residence times such a general value might not be the best choice.

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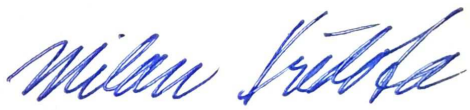
I also mention few deficiencies, which in most cases result from typesetting errors or omission during final proof-reading.

1) Symbol "X" in Table 1.1 is not explained. Symbol "q" in Eq. (2.2) is not explained. Symbol "N_α" in Eq. (3.30) is not explained.
2) Mismatch of "q" vs. "q_z" in Eq. (2.5). Missing "-" in the last leg of Eq. (3.1). Superscripted second exponential in Eq. (3.23). Absent subscript in "q_i" in Eq. (3.24). Inconsistent notation "A_b" vs "A_{xy}" in Eq. (5.5) and text.
3) Notation of vectors is inconsistent, cf. Eqs. (3.1), (3.14), (3.19), and particularly eqs. (3.25)-(3.28) which suffer from not identifying positions, dipoles, electric field and polarization as vectors.
4) In 3.2.2. "the cut-off cannot be smaller than half of the shortest box vector" should be "larger".
5) In the last line of page 31, I'd prefer "The *electronic* properties ..."
6) Legend of Fig. 3.1 does not describe the horizontal axes (concentration). Incorrect label of horizontal axis in Fig. 5.5. Incomplete caption to Fig. 6.22.
7) Footnotes 2 and 3 in Table 5.3 merged with spring constants are awkward (look like powers).
8) I disagree with the statement on page 80 (also caption of Fig. 6.18): "Both ECC-CHARMM36 and PN-model reproduces the X-ray scattering form-factor with a comparable accuracy to original CHARMM36, see Figure 6.18.". The performance of PN-model is clearly better, while that of ECC-CHARMM36 is worse.



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