Thomas Stockner, PhD
Institute of Pharmacology
Medical University of Vienna
Waehringerstrasse 13A
Austria

Vienna, 9th March 2013

Prof. RNDr. Thomáš Polívka, PhD.
Head of the Committee for PhD studies
Faculty of Science
University of South Bohemia
Branišovská 31, 370 05
České Budějovice
Česká Republika

Review of the PhD thesis

Molecular dynamics as a tool to study biological systems

submitted by

Žofie Sovová

Žofie Sovová used computational methods as investigative tool to study membranes and protein function. The PhD work is divided into three parts: a) development of coarse grained force field parameters for the main lipids of thylakoid membranes, b) simulation of the PsbI protein, a component of the photosystem II, and c) modelling of the NKR protein NRK-P1.
Here work resulted in contributions to six publications (four of them published): one, with Žofie Sovová as first author, has been published by J. Mol. Model., while two more, where she will be first author, are in preparation phase.

Žofie Sovová focused on the behavior of the PsbI protein, which is a small protein with a single transmembrane helix. The protein was intended to be studied in the natural environment of the thylakoid membrane. No parameters for these lipids are available. She therefore teamed up with S.J. Marrink, a well known expert in the field of parameter development for lipid simulations. A coarse grained description of the thylakoid lipids MGDG, DGDG, SQDG, and PG were developed and tested. These new parameters were used to create the quaternary mixture of the most common thylakoid membrane lipids. The behavior of these membranes could then be studied, a work carried out the first time *in silico*. Membrane properties were meticulously investigated and described.
In the next step, the PsbI protein was inserted into the thylakoid membrane and its behavior analyzed. The protein was stable, showed similar behavior in coarse grained and all atom representation, but some significant differences were observed. The coarse grained simulations were used to study long term behavior and aggregation properties, as well as membrane lipid interactions.

Modeling of NKR protein P1 was carried out in close collaboration with experimentalists. Homology models were thereby constructed and missing loops created. The models were scrutinized for correctness not only

by using standard scoring methods for homology model evaluation (which do have limited predictive power), but these models were much more thoroughly tested using MD simulations, exploring available phase space. Several mutations were investigated to predict dimerization behavior.

During the course of her PhD, Žofie Sovová gained a profound knowledge of computational techniques and used them to develop force field parameters, investigate membrane properties and study the behavior of proteins. Linking macroscopic experimental observations with microscopic details at the atomic level is difficult. The PhD candidate Žofie Sovová demonstrated in her PhD thesis to be able to successfully carry out these tasks. Further discussion of the methodological aspects relevant for the project as well as the pitfalls and their solutions would be welcome.

- Development of parameters for simulations is a very difficult and time consuming task. The correct choice of parameters to compare to is essential for success. A discussion of the rules applied to define the CG representation is encouraged. Which experimental properties are most suitable for comparison when developing parameters for membrane lipids? Which criteria were applied for their selection in this study?

- Molecular dynamics simulations is a powerful method that can be used to explore the energy hyper-surface of proteins, complexes and to measure the energetics involved. A requirement for analysis is ergodic sampling. Mobility was observed, but convergence was not always achieved. How was this property analyzed? What could be done to improve on this situation.

- PsbI association was observed, but as described, it was more indicative for a non-specific aggregation than for specific interactions. Further discussion of this observation would in encouraged.

Žofie Sovová has published four paper and is in the process of finishing two more as first author. This underlines here outstanding achievement. I can therefore assess the PhD thesis submitted by Žofie Sovová as very good and recommend Žofie Sovová for the admission to the PhD defense.

Best regards

Dr. Thomas Stockner

University of South Bohemia in České Budějovice
Faculty of Science
prof. RNDr. František Vácha, Ph.D., Dean

26 March, 2013

Reviewer's Report on Ph.D. Thesis entitled "Molecular Dynamics as a Tool to Study Biological Systems" by Žofie Sovová

The Ph.D. work contains introduction to molecular dynamics simulations and homology modeling. After the introduction, the core part of the work is divided into three chapters entitled: biological membranes, oxygenic photosynthesis and natural killer cells. The work is also accompanied by four papers published mostly in high-ranked journals.

The author analyzed several biologically important systems, i.e. thylakoid membranes, PsbI, PsbM and NKR-P1 proteins mostly by atomic and coarse-grained molecular dynamics simulations. She developed new parameters of glycolipids for coarse-grained simulations and co-authored a paper with one of the leading scientists in the field of coarse-grained simulations. In her studies focused on proteins, she constructed and ligated three protein systems using homology modeling and relaxed their structures by molecular dynamics simulations. The Ph.D. candidate co-authored four papers mostly published in high-ranked journals and her contribution was clear and significant. I am certain that the Ph.D. candidate displayed her ability to execute a research, which is considered as the main goal of Ph.D. study.

However, after this positive evaluation let me turn to some constructive criticism, which, I hope, would be useful for future scientific growth of the Ph.D. candidate.

The overall text is written in a very compact form with a low level of structuring, which makes it very difficult to orient in the text. Numerous grammatical errors together with terse (definitely not self-explanatory) table and figure captions also do not help the reader to follow the text smoothly. I think that the introductory part might contain more illustrative figures and schemes. A crisp concluding paragraph is also missing.

In the Ph.D. thesis focused on molecular dynamics simulations, I would expect a part dealing with current limitations of classical force fields and some discussion on the performance of used force fields (namely those used for protein simulations, as performance of coarse-grained simulation of membranes is subject of chapter 2). Repeatedly, it was shown in literature that the results of molecular dynamics simulation depend on the force field and also on other parameters, e.g. choice of explicit solvent model, ionic parameters etc. The author states that: "The main aim of force field parameterization is to obtain the potential energy function, that is in agreement with the reality". Do we have any straightforward tool how to relate the empirical potential with a real potential of

a biomacromolecule in water? I suggest the author is more careful in such statements.

I have several questions concerning the PsbI protein study; Are the conclusions on agglomeration of PsbI biologically relevant? What is the expected concentration of PsbI on the membrane? Could the author display the orientation of PsbI on membrane (structures of the mentioned bundles would be also useful)? Is the radius of gyration a good structural parameter for description of a cylindrical molecule?

Despite all the above-mentioned comments I fully recommend Mgr. Žofie Sovová to be awarded the Ph.D. degree.

Doporučuji uvedenou práci k obhajobě jako podklad pro udělení titulu Ph.D.

prof. RNDr. Michal Otyepka, Ph.D.

Review of a Doctoral Thesis entitled
"*Molecular dynamics as a tool to study biological systems*"
submitted by a PhD candidate *Mgr. Zofie Sovová*

The thesis of the PhD candidate contains a wide range of *in silico* methods applied to proteins and membranes. In the first part candidate describes successful development of a new parametrization of glycolipids, which was consequently used to construct thylakoid membranes with a mixture of four different lipids. The compositions were chosen to correspond to two experimentally determined ratios from the membrane of cyanobacterium *Synechocystis PCC6803*. Membranes were simulated at two different levels of description (atomistic and coarse grained) and the obtained results are in good agreement.

In the second part candidate describes sequence analysis, homology modeling and molecular dynamics simulations of transmembrane protein PsbI. PsbI behavior in thylakoid membrane was analyzed and formation of dimers and larger oligomers described. In the last section candidate studied natural killer cell proteins. These results are described only briefly as they were already published in per-review journals.

There are in total four attached papers to the thesis and the candidate is the first author on one of them. I will focus on the first two parts, where the main results are still unpublished.

In general, the thesis contains substantial amount of work and it reads well even though there are small mistakes, e.g. wrong schematic structure of SQDG molecule (e.g. in Figure 2.3). I have two comments to analysis of membrane simulations. The first one is that the deuterium order parameter for hydrocarbon tails of lipids is typically defined or reported with absolute value as it cannot be negative. (It is proportional to quadrupole splitting which is zero or positive.) The second comment is that the effect of undulations on density profiles of large systems is well known and could be removed by analysis of intrinsic surface.

I have following questions for discussion:

- It is common to test new components of MARTINI force filed with respect to the partitioning between water and oil. Were sugars parametrized in the thesis tested as well? Were any other tests performed, e.g. accuracy of sugar-sugar interactions which can be crucial for domain formation or rotation states around glycosidic bond in DGDG?

- Was there a water freezing observed in MD simulations using MARTINI force field, especially when membrane phase transitions was studied at low temperatures (a common problem of MARTINI force field)?

- Previous all atom studies with simple phospholipids (e.g. DPPC) reported adsorption of sodium ions at phosphate and/or carbonyl region. [Bockmann, et al. Biophys. J. 2003, 85, 1647–1655.; Pandit, S. A. et al. Biophys. J. 2003, 84, 3743–3750; Gurtovenko, A. A. et al. J. Phys. Chem. B 2008, 112, 1953–1962.] What is the candidate explanation for the different location of the ion density peak (above the membrane) in simulations with atomistic model (page 34)?

- The results of oligomerization of PbsI protein is very interesting. Is it possible to determine what is the most stable size of oligomers in simulation with 16 proteins? A top snapshot of the most stable conformations of dimers and larger oligomers would be helpful and could clarify the crucial contacts between the peptides.

- Simulations of protein in SPC water with 5 fs time step are reported in unpublished results. Large times scales as well as short thermostat coupling can lead to temperature drift or violation of equipartition theorem (different amounts of energy in translational and rotational degrees of freedom). Was any of those observed?

All the described topics are biologically relevant and candidate's results bring new biophysical insight. The candidate has proven to be capable of conducting independent research with impressive range of mastered methods including molecular dynamics simulations, force field development, homology modeling, and bioinformatics analysis. 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.
20th March 2013, Brno