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Oponentský posudek doktorské disertační práce

Mgr. Kamil Maláč: Počítačové modelování biomolekul – potenciálních chemoterapeutik

In his PhD thesis Kamil Maláč tries to understand and explain intermolecular interactions of chemically modified nucleic acids with RNA binding enzymes as are RNA- dependent RNA-polymerase, Ribonuclease H, Argonaute and Ribonuclease L using classical molecular dynamics simulations. In all three cases the underlying theme is the interpretation of available experimental data and a deeper understanding of the binding mode of these chemically modified nucleic acids that have been reported to be potential chemotherapeutic agents.

The thesis contains a huge amount of computational work, force field parametrization, extensive simulations and detailed characterization of the resulting complexes, and is backed by 2 papers already published in impacted journals and one manuscript submitted. In all 3 papers Kamil Maláč is the first author and the only co-author is his supervisor. As both published publications where published this year, there are no recorded citations in Web of Science yet. I am a bit puzzled by the fact that it took the candidate nine years of studies to publish his first paper. All three papers a purely computational and there are no co-authors/experimentalists that could be responsible for a delay in analysis and publication. This might indicate severe problems in the initial phase of the project, maybe wrong force field parameters, instability in the system, bad initial data, however, none of these is discussed in the thesis, therefore I would ask the candidate to explain the genesis of the thesis in more detail at the defense.

With this I would come to my main criticism, which is the thesis itself, not only that I cannot find nothing about the above mentioned, the thesis does not fulfill the criteria for a PhD thesis in several aspects. The candidate decided to write the thesis in a classical way plus attached publications. Such a thesis should tell a compact story, typically, one would expect to find in this 120 page thesis a comprehensive intro into the problematics, methodology and the observed systems, finishing with aims of the thesis. The following methods part should give all essential methodological details. Finally results should be presented and discussed and the story should culminate with conclusion. All these parts should follow the general story line and make it a true story, easy and understandable to read. However, this is not true for the thesis handed in by Kamil Maláč.

Instead of telling a story, the introduction is made from unconnected pieces of textbook knowledge, and to a large degree reads indeed like a textbook and not like a thesis. For example on six pages the authors describes second order methods for integrating differential equations, discusses differences, performances, modifications. After reading that I expected that the author later would describe how he modified, adapted, implemented or compared the different methods in his simulations and that a significant part of the work is obviously focused on numerical integration, however, numerical integration is not discussed in the results at all, and in his work Kamil Malác uses the standard methods implemented in the software packages used. It therefore seems doubtable why the reader should read this part of the introduction and get this information which is completely unrelated to the understanding of the concrete scientific work.

Another example is the discussion of the hydrogen atom and the Hartree equation, which is never used in the thesis. The protein systems used are presented as in an encyclopedia for structural biology. However, we are talking here about enzymes catalyzing reactions and about inhibitors preventing these reactions. I would have expected that the reactions would be shown in detail in a few schemes, to get a better understanding, but there is not a single reaction scheme in the thesis.
I could continue and go through the thesis step by step, but I rather prefer to sum up the general impression that the thesis in large parts reads like a compilate of various textbooks, missing a story line a giving non-relevant information, while it is very hard to distill the aims and motivation from what is written.
Additionally to the above said I have a couple of remarks that might serve for discussion:

1. As far as I am informed the first version of ACEMD was published in 2009. As ACEMD was used in the thesis for the microsecond simulations on GPUs, there might have been problems with the timescales of the expected conformational changes in the described systems (Simulations too short) before that date. Is this true? What are expected conformational changes in the described systems and on which timescales do they occur? Please comment and discuss.

2. In the norovirus polymerase simulations with ACEMD an untypical time step of 3fs is used. As I understood from the methods part, ACEMD uses so-called dummy hydrogens or virtual site hydrogens similar to Gromacs, however this should allow time steps of 4-5fs. Why 3fs if at least 4fs would be possible?

3. RNAse L has an ankyrin repeat domain containing nine repeats which is involved in enzyme activation, binding to 2'-5' oligoadenylate between the second and fourth repeat. Ankyrin repeats have long been implicated in protein-protein interactions, elasticity, and forming molecular springs, and the remarkable elastic properties of ankyrin repeats have been studied by steered molecular dynamics simulations [Sotomayor M, Corey DP, Schulten K (2005) In search of the hair-cell gating spring elastic properties of ankyrin and cadherin repeats. Structure 13:669–682; Serquera D, Lee W, Settanni G, Marszalek PE, Paci E, Itzhaki LS (2010) Mechanical unfolding of an ankyrin repeat protein. Biophys J 98:1294–1301], showing that the protein can extend over a range of 5- 10 nm while keeping structural integrity. Substrate or ion binding can alter the mechanical properties of this soft spring [Zayats et al, JMM 2012] How do the elastical properties of the ankyrin domain change upon 2'-5' oligoadenylate binding in your simulations?

4. The pdb structure used for the noroviros polymerase (pdb code 3bso) lacks residues 467-471. What happened to this gap in the simulations? There is nothing mentioned in the thesis about loop modeling or capping prior to the simulations.

5. The last paper was submitted to JMM recently. Is there already any feedback from the reviewers?

Despite having the above mentioned doubts and the strong criticism of the thesis itself, the scientific contribution of the candidate is evident and significant in the publications and certainly demonstrates the ability of Kamil Maláč to conduct research independently, the amount of work corresponds to a three years PhD study, research quality and publications fulfill the demanded criteria for a PhD at the Faculty of Physics and Mathematics, and thus I incline to recommend the thesis for further proceeding and to give the candidate a chance to defend his thesis in front of the commission.

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