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Review report on the habilitation thesis of RNDr. Michal H. Kolář, Ph.D., entitled "Atomistické počítačové simulace ribozomu"

The habilitation dissertation presented by RNDr. Michal H. Kolář, Ph.D., is focused on the work of the candidate on computational modeling of ribosomes. In particular, it covers atomistic molecular dynamics (MD) simulations of the ribosome and ribosome fragments performed using direct MD, free energy MD, and MD-based Markov State Models.
The dissertation presents the research completed, or ongoing, in the last five years. The thesis is based on two published original research papers, three review articles, and unpublished results, some of which are part of diploma theses done under the supervision of Dr. Kolář.

The importance of Dr. Kolář's research work stems from the fact that the ribosome, despite being one of the crucial components of life itself, is relatively unexplored at the molecular level. And without molecular-level insight, our understanding of natural protein synthesis, not to mention the possibilities of controlling it or tweaking, for instance, by newly designed drugs, is inadequate. Our limited knowledge of ribosome's molecular-level function and properties is caused by its relatively big size and the long timescale of the processes involved. Therefore, there is an urgent need for MD simulations of ribosomes at realistic length and time resolution. Dr. Kolář actively works on this timely topic using state-of-the-art computational approaches.

The thesis is written in Czech, which is relatively untypical for habilitation dissertations in natural and physical sciences. The author explains such a choice by his motivation to use the text as tutorial material for students, and I accept this argument. On the other hand, the usefulness is limited to Czech-speaking students.

The dissertation opens with a preface in which Dr. Kolář briefly explains a general philosophy of molecular simulations and his motivation for using this method. It is followed by a chapter introducing the ribosome, protein synthesis, and MD simulations for ribosome-related research. The next part of the text contains a chapter describing the methodology. It is an essential part of the dissertation, dedicated to a general description of the MD approach and several very practical issues. To mention some, it contains information about the building of the systems for simulations, various phases of the simulation process, and leading methods

for MD data analysis. This chapter will indeed be helpful for students willing to start with MD modeling of ribosomes or large biomolecular complexes.

The longest chapter is dedicated to describing the scientific results obtained by Dr. Kolář in the past several years. It contains relatively brief subchapters discussing published data (references 81 and 81). The results presented here are related to arrest caused by the regulatory peptide VemP in the ribosome tunnel (the work published in the Nucleic Acids Research) and to deformations of the tunnel caused by peptide deformylase which is an enzyme that binds to the ribosome surface (the work recently published in the Biophysical Journal). Two subchapters (3.2 and 3.4) contain not yet published material. Here, the author gave a more comprehensive description of the projects and the results. In my opinion, this material can be easily published in good peer-reviewed biological or biophysical journals. Some of the parts within the results chapter are based on three review papers published recently or accepted for publication (in Chemické Listy, Current Opinion in Structural Biology, and Annual Reviews in Biophysics). Apart from the already described results, on several occasions, the author also discusses future research plans and ideas. Overall, the results presented in the dissertation clearly prove that the research work of Dr. Kolář, the methodology he uses, and the problems he studies are at a very solid top scientific level.

The thesis is summarized in the very brief chapter in which the importance of MD simulations for studying ribosome-related phenomena is stressed. As a side note, I do not share mentioned therein author's worries about the future of science – I agree that the new generation of students and young scientists is sometimes doing things differently, but this is how new horizons can be opened up.

Overall, the habilitation thesis is well prepared and redacted. The equations, figures, and simulation snapshots are well presented and discussed. I found only a few typos and minor editing errors. The literature is well-chosen and adequately cited. However, it would be helpful to give the papers' titles in the References chapter.

The published and unpublished results selected as a basis of the habilitation thesis are a good representation of the main research topic of Dr. Kolář and his newly created scientific group at VŠCHT. This research direction is very timely, connecting the simulations with experimental findings and having great potential for explaining fundamental biological issues. Dr. Kolář is active in his research field, with very good publication and citation records despite his relatively early carrier stage. The review papers co-authored by Dr. Kolář prove that he is already being recognized in the field (I noted, however, that he is not the main author of the two most essential reviews). Dr. Kolář is also very dynamic in teaching and supervising, with a significant share of the presented results coming from the work completed with his students. The number of theses finalized or undergoing under his supervision (thirteen) is well above the average for his position.

I also want to stress the strong and positive role of Dr. Kolář in popularizing science, preparing novel educational materials, the organization of courses, and various science popularization events and initiatives. Nowadays, all these are important, although often underestimated, elements of scientists' role in modern society.

I suggest that the following issues can be discussed during the defense:

1. What is the role of kinetic and thermodynamic effects in the processes occurring in ribosomes? For instance, in chapter 3.1.1, slowing down the protein formation in the tunnel is mentioned – is it a kinetic effect? Can current atomistic MD simulations address these issues?
2. The simulated systems containing ribosomes are extensive, while the trajectories are relatively short, and hence replicas should be used. In the presented work, on several occasions, a re-generation of initial velocities was used to construct replicas. Was it enough to generate qualitatively different trajectories in the considered systems?

Summary:

In my opinion, the academic activity of RNDr. Michal Kolář, Ph.D., and the presented habilitation thesis fulfill the requirements of the habilitation procedure. Therefore, I support his candidacy for obtaining the docent appointment by the Scientific Council of the Faculty of Chemical Engineering of the University of Chemistry and Technology in Prague.

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