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Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	RNDr. Mgr. Jozef Hritz, PhD.
Habilitation thesis	Dynamical Features of Biomolecular Complexes
Reviewer	doc. Ing. Vojtěch Spiwok, Ph.D.
Reviewer's home unit, institution	Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague

The habilitation thesis Dynamical Features of Biomolecular Complexes submitted by Jozef Hritz is based on 15 out of 29 publications that the applicant had published at the time of thesis submission. The applicant was the first author of six and the corresponding author of five of the 15 selected publications. This clearly shows creativity and deep knowledge of the applicant as well as his ability to lead his scientific team.

After his Ph.D. studies he was a post-doctoral fellow of Prof. Chris Oostenbrink at Vrije Universiteit Amsterdam and of Prof. Angela Gronenborn at University of Pittsburgh. The first stay focused his attention to development of advanced molecular modeling methods, namely advance docking algorithms, replica exchange molecular dynamics (REMD) methods (in particular Hamiltonian REMD, H-REMD) and “alchemical” methods. The second stay focused his expertise to the field of biophysics, in particular NMR research on flexibility of biomolecules. As the results of his stays he can carry out independent research in method development, he can apply his and other methods in collaboration with experimentalists and he can definitely initiate experimental studies by his own findings. The scope of methods he used indicates that he choose (and learns how to use) a method to be best for the given task rather than choosing the task to be best for a method he knows. He reached international recognition in the field as the result of both stays.

The applicant made an important contribution to the field of protein flexibility and other target heterogenities (e.g. water locations) in protein-ligand docking (works P1 to P4). He used molecular dynamics simulation in a combination with machine learning (already when machine learning was not so cool as it is now) to make it possible to dock ligands into different states of the target. This was done in a computationally efficient way. The methodology developed by the applicant is often used in academia as well as in industry and these articles are heavily cited. My question related to this field is rather general. Explicit evaluation of receptor flexibility and other target heterogenities (e.g. location of water molecules), on one hand, makes it possible to screen systems with important role of target flexibility and get hits that would be otherwise lost in rigid docking schemes. On the other hand, it often increases the complexity of the problem and adds too many degrees of freedom. What are the trends in the field of protein-ligand docking and virtual screening in modeling of target flexibility? Conformational changes in the target as a result of ligand binding may happen via induced fit or via conformational selection. Some researchers strictly

differentiate this, some see an overlap, some do not care. What is the opinion of the applicant on induced fit vs. conformational selection?

The applicant made an important contribution to the development of REMD algorithm (work P5, P6 and P7), in particular H-REMD development which combines REMD with “alchemical” methods (P5) or with distance field (P6, P7). My question related to H-REMD is related to availability of this method. I am a fan of metadynamics method. I feel that metadynamics is more often applied by researchers than H-REMD due to the fact that it can be easily ported to a number of simulation packages (Gromacs, AMBER, NAMD, various QM/MM packages) using Plumed and because there is a lot of analysis tools, training materials etc. From the physical point of view there no reason for any method to be better, maybe H-REMD may be more flexible thanks to its “alchemical” features, friendly paralelization etc. Is the applicant aware of any effort to make the H-REMD family of methods more available to researchers (including the “alchemical” features)?

Distance field is a great idea to avoid the ligand from “getting lost” at the complicated surface of the protein. As a concurrent method used in connection with metadynamics is restraining the ligand in a funnel-shaped area around the binding site (i.e. “funnel metadynamics”). What are in applicant’s opinion advantages and disadvantages of both approaches? In the distance field article I did not find explicit presentation of the method used to calculate distances field distances. I believe it was done by some sort of Dijkstra’s algorithm used prior the simulation. How computationally expensive is this part of the study? As far as I understand, the distance field definition does not change during the simulation, but conformational changes of the protein may cause that the true distance field changes. How big is this problem? Both, distance field and funnel metadynamics helps ligand not to “get lost” on the surface of the protein. However, “getting lost” is the driving force behind entropy. Is it possible that such factor is underestimated in both methods?

The applicant is also active in applications of “alchemical” methods (works P8 to P10), both, in their applications (P10) as well as in their development (P8, P9). Combined enhanced sampling – one-step perturbation method (ES-OS) is a clever approach developed with a significant contribution of the applicant. In principle, the ES-OS approach can be used not only to a pair of different molecule, but also on a molecule modeled at two levels of the theory (QM and MM) or using an accurate and inaccurate force field. Is it possible and if yes, is this approach applied?

The final series of works is related to combined molecular modeling and NMR studies on intrinsically disordered proteins (IDPs, works P11 to P15). I would like to ask the applicant, who has quite unique combination of experience in enhanced sampling techniques and NMR, what is his opinion on NMR-data-driven enhanced sampling simulations.

Habilitation of a candidate is an acknowledgment of his/her teaching abilities. I cannot evaluate teaching abilities of the applicant from a personal experience but from the Introduction of the thesis it is clear that he an experienced trainer who carefully thinks about ways how to deliver the knowledge and skills to the trainees.

Conclusion

The habilitation thesis entitled “Dynamical Features of Biomolecular Complexes” by Jozef Hritz **fulfils** requirements expected of a habilitation thesis in the field of Physical Chemistry.

In Úholičky, 19 September 2021

Vojtěch Spiwok