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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	Martin Zacharias, PhD
Reviewer's home unit, institution	Technical University of Munich, Physics Department

Understanding the driving forces that lead to structure formation and association of biomolecules is of fundamental importance for all areas biophysics and biophysical chemistry. The experimental characterisation of proteins and nucleic structures gives typically only a static picture of distinct conformational states of biomolecules supplemented by a thermodynamic analysis of equilibrium properties such as stability of a folded protein or binding equilibrium constants of a biomolecular complexes. Computer simulation methods, especially Molecular Dynamics (MD) simulations, allow one to study structure formation and binding at atomic detail and at very high time resolution. In addition, molecular simulations provide the basis for a statistical mechanical description of biomolecular systems and provide access to thermodynamic quantities for changes in the studied systems calculated directly as ensemble averages.

In his habilitation thesis Dr. Jozef Hritz put together the most important scientific contributions that he worked on during the time period of 2007-2020. It includes an impressive number of 15 published manuscripts in the field of biomolecular simulations covering a broad range of simulation methodologies and applications.

In a first chapter he introduces into the basic molecular simulation methodology and how one can extract thermodynamic quantities like free energy changes. Also, the principles of the replica-exchange (REMD) method as an example of an advanced sampling approach are explained.

The prediction of how biomolecules associate and how small drug-like compounds bind to cavities on the surface of protein molecules is of major importance in the area of drug design. The standard scheme for predicting compound (ligand) binding to proteins is the use of docking methods. In these approaches one searches the surface of proteins for putative binding sites for potential ligand molecules. During these computational searches the protein is usually kept rigid and also often for the ligand only limited flexibility is allowed. In addition, simple scoring functions based on surface complementarity are used neglecting the solvent environment. These approximations allow for fast exploration but are of limited reliability. In chapter 3 of the habilitation thesis Dr. Hritz presents new approaches to include receptor flexibility during docking based on representing a protein structure as an ensemble of conformations during the docking process. This smart approach is applied for the prediction of substrate binding to Cytochrome P450 and other systems demonstrating improved performance

compared to docking to single structures. In addition, the role of water molecules for the substrate binding is also studied. Dr. Hritz provides here very interesting new approaches to the community to overcome the two most critical deficiencies of docking methods, namely, the realistic inclusion of flexibility and solvent effects at modest additional computational costs.

The focus of the next chapter is on the development of new advanced sampling methods mostly based on the Hamiltonian Replica Exchange Method (H-REMD). A major general problem of MD simulations is the limited maximum simulation time that can be achieved with current computer systems. The statistically relevant states of a biomolecule are often separated by large energy barriers that are not crossed in standard continuous MD simulations on accessible time scales. Even with an extension of the maximum simulation time one can waste a large amount of computer time in sampling only a limited range of conformations. In this regard advanced sampling methods aim at accelerating the sampling but still representing an appropriate thermodynamic ensemble in order to minimize the necessary computational effort. Dr. Hritz has made important contributions for the development of new smart H-REMD approaches. This includes the idea of soft-core scaling of interactions along the exchanging replicas or the use of distance-field restraints. These very powerful approaches were proven to be very useful to improve the sampling of relevant states for binding pathways and conformational transitions. Very interesting is also the design of a method to systematically optimize REMD simulations (fast mimicking) introduced by Dr. Hritz. The chapter also includes a very careful and comprehensive description of the simulation of the ligand binding pathways of the 14-3-3ζ protein.

Alchemical relative free energy calculations are of major practical importance for example in the area of drug design. Such free energy simulations are complex calculations that are highly demanding and nevertheless suffer often from insufficient convergence during available computer times. In alchemical calculations typically a chemical group is transformed in several steps into another group and the associated free energy change is extracted from the simulation. Jozef Hritz achieved remarkable progress in such free energy calculations by designing an approach that basically requires only one step for the transformation. This can dramatically decrease the necessary computational demand. In addition, he designed an approach to better sample multiple conformational states during alchemical transformations.

The last chapter of the habilitation thesis covers the study of intrinsically disordered proteins by combining experimental NMR data with simulations. The focus is on the most recent research projects of Dr. Hritz. A large percentage of proteins in humans and other organisms do not form a single stably folded structure but form an ensemble of mostly disordered conformations. Understanding this ensemble and investigating its properties is of major importance. Unfortunately, experimental methods give only limited information on the molecular details of disordered proteins. On the other hand, most current molecular mechanics force fields are well tested for the application on folded proteins and often show limited performance for describing the structures of unfolded or disordered protein conformations. In a series of papers Dr. Hritz has investigated the dynamical and interaction properties of intrinsically disordered proteins. He found that not only the force field but especially the water model used during the simulations has a critical influence on the outcome and performance of the simulation studies. His studies give very useful recommendations for force field and

water models that one can use to study disordered proteins and also to investigate the effect of chemical modifications such as phosphorylation.

In summary, Dr. Hritz has made important and outstanding contributions to the field of biomolecular simulations ranging from improvements of biomolecular docking, advanced sampling H-REMD methods to new free energy simulation methods and systematic studies of intrinsically disordered proteins. His habilitation thesis is an impressive collection of very fine molecular simulation studies.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

1. Recently, machine learning and artificial intelligence methods have also entered the field of quantum chemistry as well as simulations. I am wondering how Dr. Hritz assesses the value and opportunities of these techniques for force field developments and simulation methods in the future?
2. Dr. Hritz studied also disordered proteins. These are quite common in many organisms. Why are there so many disordered proteins in biological systems. One could argue that well-structured proteins are useful for a specific function. What could be the advantage of "using" disordered proteins instead?
3. Free energy simulations had so far only modest impact on drug development, why? What are the bottlenecks?

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.

16.9.2021

Date: