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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	F. J. Luque, Full Professor in Physical Chemistry
Reviewer's home unit, institution	University of Barcelona

In his habilitation thesis Dr. J. Hritz discusses the description and analysis of the dynamical behaviour of biomolecular complexes. Understanding the role of dynamics in biomolecular systems is absolutely necessary for exploring the relationships between structure and function. Protein flexibility plays a fundamental role in a vast variety of biological processes, such as enzymatic catalysis, the diffusion of ligands to the active sites, the allosteric modulation of protein function by small molecules, the formation of macromolecular assemblies, and the folding of proteins. All these processes involve an enormous range of spatial, energetic and temporal scales. Overall, this adds complexity to the research efforts undertaken by means of experimental and computational techniques to provide a comprehensive analysis of the structure-dynamics-function relationships.

Molecular dynamics (MD) simulations, along with a range of complementary computational approaches, have become valuable tools for investigating the role of protein dynamics. These techniques can now be routinely applied in the investigation of a wide range of dynamic properties and processes, including structural biochemistry, biophysics, enzymology, molecular biology, pharmaceutical chemistry, and biotechnology. In conjunction with statistical mechanics, they provide a rigorous framework that relate the distributions and motions of atoms and molecules to macroscopic observables. Furthermore, accelerated MD methods extend the conformational sampling characteristics, enabling extended (effective) time scales to be accessed, and hence the study of rare dynamic events.

In this context, the habilitation thesis provides a brief but comprehensive analysis of advances in computational methods, particularly regarding Dr. Hritz's contributions, to gain deeper insight into specific molecular recognition processes. After a succinct **Introduction** to the relevant role of protein dynamics, Dr. Hritz discusses two relevant concepts in **Chapter 2**: the generation of an ensemble of structures representative of the system under specific simulation conditions, and the suitability of Replica Exchange Molecular Dynamics (REMD), either in the form of temperature REMD or Hamiltonian REMD, as an enhanced techniques to accelerate the sampling of molecular complexes.

In **Chapter 3** Dr. Hritz faces the specific problem of docking between biomolecules, specifically addressing two major challenges: the inclusion of structural changes induced upon binding of the interacting partners, and the inclusion of water molecules that bridge the interaction between the partners. The two questions still remain unsolved and, although several strategies have been proposed, finding a practical, efficient solution for the practical inclusion of conformational flexibility and water-mediated bridges in docking calculations remains elusive. The approach undertaken by Dr. Hritz combines ensemble ligand docking

and MD simulations in order to account for the plasticity and flexibility of the protein, exemplified with the pharmaceutically relevant case of cytochrome P450. Remarkably, the method was found to yield to a significant increase in the reliability of predicted binding poses.

Chapter 4 deals with methodological refinements implemented in Hamiltonian-REMD. Tho refinements are discussed. In the first case Dr. Hritz examines the modulation of the soft-core treatment of selected van der Waals and electrostatic interactions in order to enhance the conformational sampling of a selected region of the system. This permits to perturb only those parts of the Hamiltonian that contribute most to a local free energy barrier, while keeping an affordable computational cost. In the second case attention is paid to the usage of distance restraints applied to the ligand with respect to its binding site in Hamiltonian-REMD simulations. In essence, the method combines the enhanced sampling provided by Hamiltonian-REMD with distance restraints that guide the (un)binding of ligand by the shortest sterically possible pathway. This method is successfully used to follow the binding of phosphopeptides to 13-3-3 protein.

Chapter 5 is focused on a distinct subject related to the calculation of relative binding affinities between structurally related ligands, which is a typical scenario in the design of novel materials and in drug discovery. These calculations typically involve the progressive alchemical transformation between two ligands through a number of intermediate states. The main methodological contribution (enhanced sampling – one-step perturbation method; ES-OS) of Dr. Hritz consists of defining a suitable reference state in conjunction with a single MD simulation and the usage of two sets of soft-core interactions. This simplified approach sufficed to explore the conformational distributions of chemically similar compounds and to determine the relative binding affinity. The method has general applicability and can be utilized for exploring changes between related ligands and site-directed mutagenesis.

Finally, **Chapter 6** deals with the computational study of intrinsically disorded proteins (IDPs), which represents a relevant challenge for current biomolecular simulations. This obeys not only to the problem of insufficient sampling associated with the large conformational space available for IDPs, which can be at least in part alleviated resorting to experimental (NMR) data, but also to doubts about the reliability of current force fields to deal with both well folded proteins and IDPs. In turn, this raises questions about the best strategy to simulate proteins containing both globular parts as well as disordered regions, a question addressed by Dr. Hritz in his studies. In particular, the sensitivity of NMR data (chemical shifts, relaxation parameters) to the choice of force field parameters was used to calibrate the merits of different force field and water models. These studies can be valuable not only to provide guidelines in the refinement of biomolecular force fields, but also for their application to a diverse number of pathologies associated with misfolded proteins.

The habilitation thesis of Dr. Hritz encompasses a selection of 15 scientific papers collected after completion of the PhD thesis. Though this choice demonstrates the innovative ideas and sound theoretical background of the applicant, it is worth noting that the Dr. Hritz's scientific outcome is reflected in a larger number of papers (> 30), which can be grouped in the areas of Biochemistry and Molecular Biology, Chemistry, Biophysics and Computer Science. His track record reflects a regular production since 2001. Remarkably 8 papers have accumulated more than 50 citations, which is indicative of the relevance of his contributions. This is also supported by other indexes, such as an H-index of 17, an average number of 27 citations / paper, and a total number of citations close to 1000, excluding self-citations. He is first author in 8 publications, and corresponding author in 6 scientific publications. Taken together, these comments suffice to give strong support to the candidate, demonstrating both the achievement of scientific maturity and independence as a well-

established researcher and a consolidation of his research in the simulation of biomolecular systems.

Finally, besides the scientific evaluation, it is also worth stressing the current involvement of Dr. Hritz in teaching academic, particularly in the courses: Fundamentals of Biophysical Chemistry, Experimental Methods of Biophysical Chemistry I, Advanced Theoretical Methods of Biophysical Chemistry, Experimental methods of Biophysical Chemistry II, and Protein Expression and Purification courses. I am convinced that the sound research background achieved by Dr. Hritz will be an excellent basis to provide a balanced explanation of the main concepts of these disciplines, highlighting the relevance of dynamics in the function of proteins. This is well illustrated by the different 'pedagogical notes' found along the thesis, which show the ability of the applicant to communicate in a fresh, imaginative way the key concepts of these courses.

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

1. Deciding whether a water molecule is really implicated in assisting ligand binding is not easy, as this depends on the nature of both pocket and ligand. What are the best parameters that should be considered (i.e., density relative to bulk water, residence time, number of hydrogen bonds formed with protein,...) to account for the presence of bridging water molecules in docking calculations?
2. The optimal λ settings (i.e., number of replicas and value of λ) in Hamiltonian-REMD seem to be highly dependent on the chemical features of the simulated system. How robust is the optimized protocol proposed for fixing the best set of parameters in the soft-core treatment? Have the general applicability of this protocol been assessed in other systems?
3. Simulations of disordered proteins have highlighted the need to keep a balanced treatment of protein hydration. Should we attempt to develop a novel water model beyond the 'general-purpose' 4-point rigid water? What novel physical features should this model incorporate?

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.

Date: September 20, 2021 Signature: F. Javier Luque