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## Review report on the Ph.D. thesis submitted to Uniwersytet Gdanski

Author: MChem Malgorzata Marzena Kogut-Günthel

Title: Computational approaches to characterise biologically active systems containing proteins, carbohydrates, and ions

Promotor: Prof. Dr. hab. Sergey A. Samsonov

The submitted thesis covers the topic which has high relevance as well as vast importance in biomolecular recognition. In order to get deeper mechanistic insights into the topic "biomolecular recognition", the author used computational tools - mainly classical molecular dynamics simulations - but she also closely worked together with experimentalists that enabled her to have a good balance of theory and experiments as well as their correspondences in her thesis. Neither of these methods alone can derive sound conclusion on these sophisticated scientific topics. The topics of the thesis are timely (also somewhat associated to the COVID-19 pandemic) and her way of approach has its own uniqueness. Also, the methodologies applied in this thesis are overall state-of-the-art. In some instances, different methodologies or parameter sets have been tested.

The thesis includes 7 scientific papers published in peer-reviewed journals that constitutes vast majority of the scientific body of the thesis (chapter 7). Additionally, introduction (chapter 1), methodology (chapter 2), scientific goal (chapter 3), summary of each publication (chapter 4), and conclusion/outlook (chapter 5) are also included. Each section is written in a concise manner that makes it easy to read and to digest.

In Chapter 1, first, biological relevance of the studied topics are explained, followed by brief explanations on individual systems. In Chapter 2, all methodologies are explained including experimental methods involved. The majority of this chapter is devoted to the description on molecular docking and molecular dynamics simulations, that are the major focus of the thesis. In Chapter 3, the goals of the research in a broader sense and also for individual systems are described. In Chapter 4,

summary of each publication is presented individually as a digest. Full texts of the publications are provided as Chapter 7. In Chapter 5, conclusions and outlook are written. Besides the conclusions for individual systems, general conclusion on the robustness and the limitation of computational tools are mentioned here.

Subtopics were all well-selected, for each of which computational chemistry can greatly contribute to improve our understanding of the systems. She has demonstrated the relevance of her results for these areas.

I have 4 specific questions and I wish the answers to be presented in the occasion of the Ph.D. defense.

1. In subsection 4.2 (paper **D2**), the impact of applying different $Ca^{2+}$ parameters have been tested. Although a clear difference between the two parameter sets are demonstrated, it appears not straightforward to digest these results and to conclude “which one is better”. Did the author also consider applying 12-6-4 Lennard Jones potentials (the method is briefly mentioned in subsection 2.2.5)? Since the system is not extremely large, it could eventually be calculated using quantum chemical method (such as density functional theory) to obtain an *in silico* “real” structure. In essence, the impact of using different parameters have indeed been tested but what would be a conclusion out of it?

2. In all systems except for the ones with only carbohydrates, interacting energies are discussed in terms of electrostatic terms and van der Waals interactions. In these systems, aromatic hydrocarbons are heavily involved and pi-interactions (pi-pi and ion-pi) become important. In cyclodextrin systems (subsection 4.3, paper **D3**) pi-pi interactions are important (as written in subsection 1.2.3), and for BSA stabilization through tetraphenylborate (subsection 4.5, paper **D5**) phenyl group is playing key role to stabilize BSA through BSA-$[B(Ph)4]^-$ interaction. These interactions are not explicitly discussed in the thesis. They are generally weak interactions but at least appears to be playing key role in the BSA system. What is the author’s conclusion on pi-interactions in the studied systems and how good is MD simulations to reproduce these interactions?

3. In subsection 4.4 (paper **D4**) the effect of pH on the binding affinity of BSA is discussed. However, the difference is not traced down to amino acid level. It therefore remains unclear which residues are committed to this phenomenon. Is it due to the deprotonation of particular residues?

4. In subsection 4.6 (paper **D6**), BSA and HSA are discussed in a comparative manner and their difference is highlighted. On the other hand, BSA is widely used as a model substance for HSA (subsection 1.3.2). Is the author implying that BSA is eventually not a good model for HSA?

Overall all the materials are presented clearly and logically, and the thesis is in high standard also in terms of language and technical presentation. The author successfully published 7 papers during the last 2 years (despite of the difficult time in the pandemic), out of which 4 papers as the first author and in prestigious journals. This thesis is great achievements of works and is a proof that the author has capability to carry out scientific works independently and I acknowledge and recommend that the author be given an opportunity to defend her Ph.D thesis orally.

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