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dr hab. **Maciej Maciejczyk**, prof. UWM
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Olsztyn, 26.05.2023

The review of the doctoral dissertation of MSc Małgorzaty Kogut-Günthel „Computational Approaches to Characterise Biologically Active Systems Containing Proteins, Carbohydrates, and Ions" prepared in Laboratory of Molecular Modelling, Department of Theoretical Chemistry, Faculty of Chemistry, University of Gdańsk under the supervision of dr hab. Sergey A. Samsonov, prof UG and dr Martyna Maszota-Zieleniak

The research problem and the scope of the thesis

The research topic covers several aspects of very important biochemical/biophysical problem of interactions of small and medium-sized molecules with proteins in aqueous environment. In particular special attention is paid to the role of ions in the investigated systems. The molecular systems investigated by PhD candidate are challenging and biologically important targets like: glycosaminoglycans (GAG) – long, charged oligosaccharides involved in many biological processes, occurring in the extracellular matrix, β-cyclodextrins – small cyclic oligosaccharides used in the pharmaceutical industry for drug delivery of water insoluble substances, bovine and human serum albumin (BSA and HSA) – dominant protein present in blood, performing many functions including drug transport and annexin – widely distributed and ubiquitous proteins involved in many physiological processes. Description of complex interactions involving the above-mentioned biologically-important molecules was the scope of the thesis submitted for the review.

Formal evaluation

The dissertation presented for review is written in English. I consider the layout of the doctoral dissertation appropriate and logic. It consists of 6 thematically coherent scientific

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research papers published in years 2021 and 2022, one mini-review paper and 115-pages study containing:

- List of abbreviations
- Abstract (English and Polish)
- Introduction
- Methods
- Goals of the research
- Summary of publications included in the PhD Thesis
- Conclusions and outlook
- Literature
- List of publications included in the PhD Thesis
- Appendix

The publications which constitute PhD Thesis were published in prestigious international JCR-indexed journals with impressive total impact factor 38.492 (2021) and total number of points of Polish Ministry of Education and Science equals to 790.

The PhD dissertation consists of the following publications:

- Kogut, Małgorzata M., Martyna Maszota-Zieleniak, Mateusz Marcisz, and Sergey A. Samsonov. 2021. "Computational Insights into the Role of Calcium Ions in Protein–glycosaminoglycan Systems." *Physical Chemistry Chemical Physics: PCCP* 23 (5): 3519–30.
- Kogut, Małgorzata M., Annemarie Danielsson, Sylvie Ricard-Blum, and Sergey A. Samsonov. 2022. "Impact of Calcium Ions on the Structural and Dynamic Properties of Heparin Oligosaccharides by Computational Analysis." Computational Biology and Chemistry 99 (August): 107727.
- Kogut, Małgorzata M., Ola Grabowska, Dariusz Wyrzykowski, and Sergey A. Samsonov. 2022. "Affinity and Putative Entrance Mechanisms of Alkyl Sulfates into the β-CD Cavity." *Journal of Molecular Liquids* 364 (October): 119978.
- Tesmar, Aleksandra, Małgorzata M. Kogut, Krzysztof Żamojć, Ola Grabowska, Katarzyna Chmur, Sergey A. Samsonov, Joanna Makowska, Dariusz Wyrzykowski, and Lech Chmurzyński. 2021. "Physicochemical Nature of Sodium Dodecyl Sulfate

Interactions with Bovine Serum Albumin Revealed by Interdisciplinary Approaches." *Journal of Molecular Liquids* 340 (October): 117185.

- Grabowska, Ola, Małgorzata M. Kogut, Krzysztof Żamojć, Sergey A. Samsonov, Joanna Makowska, Aleksandra Tesmar, Katarzyna Chmur, Dariusz Wyrzykowski, and Lech Chmurzyński. 2021. "Effect of Tetraphenylborate on Physicochemical Properties of Bovine Serum Albumin." *Molecules* 26 (21): 6565.
- Bertozo, Luiza de Carvalho, Małgorzata Kogut, Martyna Maszota-Zieleniak, Sergey A. Samsonov, and Valdecir F. Ximenes. 2022. "Induced Circular Dichroism as a Tool to Monitor the Displacement of Ligands between Albumins." Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy 278 (October): 121374.
- Kogut, Małgorzata M., Mateusz Marcisz, and Sergey A. Samsonov. 2022. "Modeling Glycosaminoglycan–protein Complexes." *Current Opinion in Structural Biology* 73 (April): 102332.

Six of these publications are original research papers and one is a mini-review. The PhD candidate is the first author of four publications (including mini-review). She contributed to six research papers by conducing the (computational) research, analyzing the data, writing and revising the manuscript and designed the research presented in one publication. In the mini-review she researched the literature, wrote and revised the manuscript. The PhD candidate had dominant contribution (80%) in preparation of two publications, but her input into the remaining five publications is also very significant – between 20 and 50%.

PhD candidate is also co-author of four publications not included in the dissertation published in JCR journals with total IF=15.658 (340 points of Polish Ministry of Education and Science), nine conference posters and one oral presentation.

The thesis is prepared in good editing standard. The language is comprehensive and coherent, while errors and inaccuracies are relatively rare.

Evaluation of the content of the thesis

The first part of the study after the list of abbreviations and abstract is 21-pages long Introduction. The PhD candidate structured this section in a very logic way as it contains:

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- The biological relevance of macromolecules including description of the mechanisms of protein ligand binding processes and its basic thermodynamics.
- The systematic characterization of glycosaminoglycans (GAG's), role of various ions in GAG-containing systems
- characterization of cyclodextrins and the role of surfactants in cyclodextrin-containing systems
- The role of ions in albumin-containing systems

I found this section very valuable, because it allows the reader to get acquainted with all biological objects, which have been the subject of the research.

The next section (Methods – 38 pages) describes experimental and computational methods currently used for characterization of protein-ligand interactions. The following experimental methods are described: X-ray crystallography, nuclear magnetic resonance, mass spectrometry, circular dichroism, fluorescence spectroscopy, surface plasmon resonance, enzyme-linked immunosorbent assay and isothermal titration calorimetry. Three of the above mentioned techniques (circular dichroism, fluorescence spectroscopy and isothermal titration calorimetry) were used in four publication, which constitute the dissertation.

Theoretical and computational approaches used to characterize protein-ligand interactions are described in the next section. The following methods are described: electrostatic potential calculation with Poisson-Boltzmann equation, molecular docking, all-atom molecular dynamics, coarse-grained models and binding free energy calculations. All of these techniques except coarse-grained models were used by PhD candidate in her research. Special attention is paid to the description of challenges of the application of computational methods to the molecular systems investigated by PhD candidate.

There are some minor issues in Methods section. First – there should be either parenthesis or sign change in the argument of exponential function in equation 9 on page 60. Second – the equation 13 on page 62 is not Poisson-Boltzmann equation, but rather electrostatic contribution to the free energy of solvation. This equation utilize information about electrostatic potential determined with Poisson-Boltzmann equation, but it is not Poisson-Boltzmann equation itself.

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Next goals of the research are presented followed by summary of publications included in the PhD thesis. Next part of the review is the presentation of the content of the publications followed by my comments and questions.

In the first publication (D1) the influence of calcium ions on complexation of glycosaminoglycan with two important proteins namely annexin II and annexin V was investigated, by the application of various computational methods. Firstly, the important influence of the presence of calcium ions on the electrostatic potential generated around molecules was confirmed by the application of Poisson-Boltzmann equation in the presence and the absence of Ca^{2+} ions. Then heparin molecules were docked to proteins using classical docking tool and novel, recently developed, RS-REMD technique in the presence and absence of calcium ions. Although both methods were able to approximately localize crystal-structure binding site, short MD simulation performed for docked structures with and without ions proved their important influence on the stability of the HP-annexin system. The importance of ions was also partially confirmed by MM-GBSA and LIE end-point free energy calculations. The importance of particular calcium ions present in the binding site was investigated by more precise free energy perturbations method. The dissociation of GAG's from protein binding sites was proved to be more difficult in the presence of calcium ions as shown by the application of umbrella sampling method.

In the second publication (D2) with dominating input of PhD candidate the authors investigate the influence of calcium ions on conformation of two heparins built of 10 and 18 monomeric units. MD simulations performed with presence and absence of Ca^{2+} ions showed that calcium influence the compactness of the structure and frequency of formation of hydrogen bonds of both molecules, but the ring puckering, conformation of glycosidic linkage and flexibility of monosaccharides are relatively independent on the presence of Ca^{2+} ions. The investigation performed with two different sets of force-field parameters for calcium ions led to significantly different results and therefore another important conclusions arising from this work is the necessity of improvements in molecular mechanics parameterization of calcium ions. These results are very important, because it gives insight into the process which is very difficult to observe experimentally.

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In the third publication (D3) the entrance mechanism and thermodynamics of complexation of alkyl sulfates with β-cyclodextrins was investigated by means of experimental techniques (conductometry and isothermal titration calorimetry) supported by *in silico* analysis. The free energies of binding obtained with applied experimental techniques provided the same results within the experimental error and they are in qualitative agreement with the results of LIE and MM-GBSA end-point free energy methods. The authors show that binding affinity increases with increasing length of the chain, in agreement with the experimental data. Moreover authors propose the mechanisms of entrance of different ligands into β-cyclodextrin cavity. It was also shown that two possible configurations of ligands in the cavity are possible. This publication is a perfect example of very fruitful combination of experimental and computational methods, where the latter provided some details about investigated process, which are inaccessible to experimental techniques.

In the fourth and fifth publication (D4 and D5) interactions of sodium dodecyl sulfate (SDS) and tetraphenylborate with bovine serum albumin were investigated. Several experimental techniques were employed complemented with docking and molecular dynamics simulation of the binding process. As a result two binding sites for sodium dodecyl sulphate and tetraphenylborate were located and it was found that one binding site is common for both ligands. Also binding affinities of tetraphenylborate ions and SDS molecules to two protein binding sites was estimated with end-point free energy LIE method and MM-GBSA. The other important conclusion pertains to the second binding site of both molecules. Not only it is different for two ligands, but also physico-chemical nature of the binding process is different – entropy driven for tetraphenylborate and enthalpy driven for SDS. Again, in both publications application of computational techniques significantly widened the picture of the binding process.

The previous to last publication in the PhD dissertation (D6) is devoted the problem of displacement of two ligands – divanillate and divanilline – between human and bovine serum albumins. The induced circular dichroism (ICD) experiments showed that both compounds have higher affinity to HSA than to BSA. The change of the shape of ICD spectra observed when investigated compound were released from BSA and bound to HSA were partially explained by combination of molecular dynamics, umbrella sampling and end-point free energy

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estimation techniques. The change of CD spectra calculated with quantum chemistry methods of investigated molecules bound to HSA and BSA was in qualitative agreement with those measured experimentally.

The last publication is the mini-review devoted to the important and difficult problem of modeling the interactions between proteins and glycosaminoglycans.

The next part of the dissertation "Summary and outlook" includes brief description of the most important outcome of the work divided into three thematic sections,

Finally the Literature section contains impressive number of 563 references. The selection of references confirms good theoretical preparation and orientation of PhD candidate in the subject of the doctoral dissertation.

It should be stressed that in all publications the molecular modeling methods applied by PhD candidate provide very important insight into the molecular association mechanisms at the atomistic level, which is inaccessible (or hardly accessible) to the experimental methods.

I have the following comments and questions:

- In four publications (D1, D4, D5, D6) the interactions of small molecules with proteins were investigated. What was the assumed protonation state of all titratable residues present in proteins of the investigated systems? I assume that the most probable protonation (model) states for the solvent-exposed model residues at neutral pH have been assumed. In many protein-ligand systems, the protonation state of the titratable groups can have a significant effect on the arrangement of the ligand in the binding pocket and, of course, on the association constant (*Proteins* (2011) **79**: 304–14). Has the PhD candidate considered the possibility of using numerical titration methods to prepare proteins for MD simulations? In the opinion of the PhD candidate, can the introduction of numerical titration significantly change obtained results or the effect should be rather small? In the publication D4 MD simulations were performed at pH 5 and 7. How modeling of different pH conditions was incorporated in the molecular docking and MD simulation procedures? Was the constant-pH explicit-solvent MD methodology applied? I couldn't find this information in the Methods section of the publication D4.

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- In the majority of publications (all but D5) of the dissertation the medium-precision end-point MM-GBSA method was applied. I assume that AMBER's default 1A-MM-GBSA methodology (as defined in *Exp. Opin. Drug. Disc. (2015)* 10: 449–61) was used in all cases. This approach is very practical, because it causes cancellation of some energy terms, decreasing the statistical error of calculated free energies, but it ignores conformational reorganization free energy of the ligand. This term can be significant for highly elastic molecules (like GAGs) which rather exist in a dynamic conformational equilibrium in solution. It was shown that inclusion of ligand reorganization energy can significantly improve accuracy of MM-GBSA method (J. Am. Chem. Soc. (2009) 131:13709-13721). Does PhD candidate think that inclusion of the free energy of ligand reorganization could improve obtained results?
- In the Table 3 of publication D3 Gibbs free energies of binding obtained from numerical pulling experiments are reported. These values seems to depend not only on the initial and final state of the system (Complex 1 and Complex 2), but also on the direction of pulling (C-pull path and S-pull path). Theoretically, as the free energy in an equilibrium quantity, it should only depend on initial and final configuration of the system. Can PhD candidate comment on this result?

Minor issues

- Page 8 line 11 – "są" should be removed
- Page 23 Table 2 – unit of solubility is missing
- Page 30 line 5 – Mm should be replaced by mM
- Page 30 line 10 – I think that "pairs" should be removed

Final statement

In my opinion the dissertation submitted for review was carefully planned, performed and written. I rate its scientific value very high. It is original elaboration of scientific problem, which provides atomistic-level insight into biologically important processes and enriches the broad field of biomolecular simulations. It contains elements of scientific novelty. The candidate demonstrated the ability to conduct in-depth studies, planning, performing and

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analyzing *in silico* experiments and critically assessing facts and formulating conclusions. In my opinion MSc Małgorzata Kogut-Günthel is a scientist fully prepared to conduct independent research.

To sum up, I declare that the PhD thesis submitted for review meets the requirements for doctoral dissertations according to the point 187 of the act of Law on Higher Education and Science (July 20, 2018, uniform text. Journal of Laws of 2022, item 574, as amended). Therefore, I apply to Scientific Council of the discipline Chemical Sciences of University of Gdańsk for admission of MSc Małgorzata Kogut-Günthel to the next stages of the PhD procedure.

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