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Review of PhD Thesis "Computational Approaches to Characterize Biologically Active Systems Containing Proteins, Carbohydrates, and Ions" submitted by Mrs. Małgorzata Kogut-Günthel

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Spectabilis,

Thank you very much for letting me review the PhD Thesis of Mrs. Małgorzata Kogut-Günthel.

Biological macromolecules such as proteins, nucleic acids, fats, and carbohydrates represent the building blocks of all biological matter. While the structures of the first three classes have been well characterized by experimental techniques such as crystallography and nuclear magnetic resonance spectroscopy, our structural knowledge of macromolecular carbohydrates is very limited. Experimental techniques fail because of the high mobility of polysaccharides and/or their highly repetitive nature. While polypeptides or nucleic acids can only form linear polymers, sugar moieties can engage many different glycosidic linkages giving rise to a much richer polymorphism that is very difficult to describe. Similarly, weakly binding ligands are often not providing very clear signatures in spectroscopic analysis and require other techniques for a comprehensive characterization.

An alternative to experimental methods are computer approaches, which have advanced significantly over the last decade. In addition to structure prediction and calculation, molecular dynamics simulations can easily cover 10s of microseconds of evolution of relatively large systems. Furthermore, molecular docking has become very reliable to study the interaction of molecular partners and to predict binding poses. The current thesis exemplifies opportunities and limitations of these approaches on rather complex and divers systems, involving (*i*) highly complex acidic glycosaminoglycans (GAGs) in interaction with metal ions (Ca^{2+}), (*ii*) interactions of small molecules with proteins (bovine and human serum albumin, BSA and HSA, respectively), and (*iii*) cyclodextrins (CD) with anionic surfactants of varying chain length (SXS). Mrs. Kogut-Günthel has built models for these systems on the computer and studied their properties and interactions under various biologically relevant conditions, such as pH, molecular modifications, and concentration.

In her thesis, Mrs. Kogut-Günthel first introduces the topic quite comprehensively with a specific focus on protein-ligand interaction considering all relevant literature and models. I must say, however, that I would have appreciated more pictures to illustrate the individual systems under study.

It follows a technical section that gives a very instructive overview of the methods employed in the field. I much appreciate the very honest discussion of the current challenges in the field at the end of this chapter which underlines the very high scientific standards of the author.

The results part of the thesis comprises 7 (!) papers in well recognized and all peer reviewed journals, the candidate is first author all of these publications (sometimes shared first authorship). I consider this an extremely good scientific output of a PhD thesis, also considering the Mrs. Kogut-Günthel is coauthor on 4 other scientific publications.

In the first study, the candidate investigates the interaction of GAGs with two proteins of the annexin family in the absence and presence of Ca^{2+} ions. Ca^{2+}-binding increases the amount of heparin bound by increasing the electrostatic surface potential towards more positive values. In the second study, the influence of Ca^{2+} ions on heparin oligosaccharides is investigated. In the presence of Ca^{2+}, the radius of gyration of the GAGs decreases, the hydrogen bond pattern changes, but other characteristics such as ring puckering, glycosidic linkage or monomer flexibility remain unchanged.

In the third study, a host/guest system is analyzed in close collaboration with experimentalists comprising CD and anionic SXS detergents with varying chain length (between 8 and 12 carbons). Remarkably, simulations allow determining a putative entrance mechanisms of the guest, an analysis of the energetics of binding, and the dissociation pathway.

Studies 4 and 5 look in detail into the interaction of serum albumins with SDS detergents. Here, simulations are performed to complement experimental experiments in an orthogonal fashion. Simulations and docking approaches reveal details of the interaction of SDS with the proteins and describe the energetics of binding also in competition with a small organic ion. Simulations also help to develop the tool induced circular dichroism to monitor the displacement of ligands as shown in the sixth study.

Finally, the seventh paper represents a comprehensive review on the computational approaches to model GAG-protein complexes published in the very distinguished journal Current Opinion in Structural Biology (IF 7,8).

I suggest the following questions to be discussed during the defense:

1. Very often, the term ‚hydrophobic interaction(s)' is used. The author should specify what is exactly meant by that. What is the difference between hydrophobic effect and hydrophobic interactions, what is the theoretical description of either of these phenomena?
2. The interactions of GAGs with proteins or membrane surfaces often uses the model of calcium bridging. Could such a mechanism be identified in the classical docking/MD approaches carried out for the annexin/calcium/GAG system? What kind of simulations could represent such a scenario?
3. Would it be possible to perform MD simulations of heparin also in an excess of Ca^{2+}? What would be expected under such conditions? Have you considered counter ion condensation of the heparin polyelectrolyte?
4. In the CD/SXS system, could one determine the decrease in free energy per methylene group and compare it with the 0.8 kcal/mol determined for partitioning into an organic solvent by Tanford?
5. I would like the author to specify the interaction of SDS with BSA: what is the contribution of electrostatics to binding? Is SDS-association mainly driven by the hydrophobic effect?
6. In paper D6 page 2, the authors state that the hydrophobic cavities of HSA "present some degree of specificity". It is generally agreed that both HAS and BSA very unspecifically bind many different targets and I would like the issue of specificity to be discussed in more detail.

Taken together, I must say that I am very impressed with the sheer amount of work that has been done during the conclusion of this thesis. Mrs. Kogut-Günthel has impressively demonstrated that she can solve open scientific questions with a broad arsenal of theoretical tools. Her research tackles a lot of current topics in biophysical research. She also seems very familiar with many experimental methods allowing her to build very strong and convincing cases as outputs of her scientific work. I conclude that she has a very detailed knowledge of her field, great knowledge on the application of computational methods and very elaborate communications skills as the thesis is written in high class English language. I was very pleased with the thesis and her level of understanding such that I fully concur with the award of the degree philosophical doctor The thesis submitted by Mrs. Kogut-Günthel fulfills all criteria required for the award of this academic title and I recommend the Faculty of Chemistry of the University of Gdańsk to accept it.

If there is the opportunity, a few minor errors should be corrected:

- Pg: 30: mM, not Mm.
- Pg: 34. X-ray beam is diffracted upon interaction with electrons, not atoms.
- Pg: 39: assay, not essay
- Pg: 50: monosaccharide blocks

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