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Review of doctoral thesis

Title: Deciphering the Molecular Basis of Specificity in Protein-Glycosaminoglycan Interactions: from All-Atom to Coarse-Grained Models
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Promotor: prof. dr. Sergey A. Samsonov

The formal page of the dissertation:

The thesis is presented as a collection of five published and thematically related scientific articles. The short introduction in the studied field and summary of the doctoral research precedes the attached publications. The introduction is well written and helps reader to understand the motivation of the presented research. Summary of the doctoral research contains the most important findings of the research done during the PhD study. The brief summary is written clearly, and reader can observe clear image of the accomplished work. Text and graphics of the submitted dissertation are prepared at the required level. The scope of the work is adequate to current standards in the field. The individual parts of the written work are clearly and logically structured. I have a few comments on the formal page of dissertation thesis presented in the Comments section.

The content page of the dissertation:

Presented dissertation thesis is focused on the study of the glycosaminoglycans (GAGs), their dynamics, interaction with different proteins and developing of the coarse-grained model suitable for the heparin polysaccharide. Within the first presented article the PhD candidate studied the changes in the electrostatic potential environment of the Interleukin-8 (IL-8) and SH2 domain of the Grb2 upon substrate binding. In this study, candidate used molecular dynamics (MD) simulations and APBS software to analyze the homodimeric NMR structure of IL-8 and its electrostatic potential (ϕENS). The IL-8's interaction with two GAGs, heparin (HP) and hyaluronan (HA), were modeled and examined how GAG binding affects ϕENS. The SH2 domain of Grb2 which recognizes phosphotyrosine peptides containing a pYXN motif (where X can be any residue; V, K or E in this study) served as an additional test system. MD results were compared with paramagnetic relaxation enhancement (PRE) NMR experiments, showing that changes in the electrostatic potential upon ligand binding aligned with predictions from Poisson-Boltzmann calculations.

In the second article the study of the interactions of the heparin with the IL-8 and compare its interactions with four designed highly charged decapeptides. This study was focused to selectivity of IL-8 to heparin compared to other possible charged substrates and influence of the highly charged substrate to the electrostatic potential of the IL-8 upon substrate binding. Within the study the candidate successfully employs several computational methods as molecular docking, molecular dynamics simulations or free energy calculations. All four peptide ligands interact with the identical epitope of IL-8, although their binding affinities are considerably lower, as demonstrated by 1H–15N HSQC NMR titration experiments. Complementary molecular docking

and molecular dynamics simulations provided additional atomistic insights into the interaction mechanisms of GAG and peptide ligands.

The third presented article focuses on extension of the UNIfied COarsegRaiNed (UNICORN) model of biological macromolecules and its SUGRES-1P force filed to heparin molecule. The NMR structure of HP obtained from the PDB database served as the template for constructing HP fragments utilized in Coarse-Grained (CG) simulations. HP molecules ranging from 6 to 68 residues in length were generated. Experimental data, including end-to-end distance (EED) and radius of gyration (*R*g), measured via analytical centrifugation and synchrotron X-ray scattering, were used as benchmarks to compare with the results from CG molecular dynamics simulations. This study also involved parameterizing the Energy-Term Weights and the Debye–Hückel Screening Factor to ensure the highest possible accuracy in MD simulations. Based on the observed outcomes and these parametrizations, the candidate successfully extended the UNICORN model to the heparin molecule.

The new coarse-grained modeling framework tailored to simulate protein–heparin interactions UNRES/SUGRES-1P is presented in a fourth article. This model integrates the UNRES force field for proteins with SUGRES-1P for polysaccharides, incorporating parameters for heparin's sulfation and electrostatics. Long-range electrostatic effects are modeled using optimized Debye–Hückel screening, enabling realistic treatment of heparin's charge. Validation against experimental data confirms the model's ability to replicate structural properties and binding modes in protein–heparin complexes. The framework developed by the candidate allows efficient, scalable simulations of these complexes at timescales inaccessible to all-atom approaches, supporting broad applications in studies of cell signaling, coagulation, and viral entry. The UNRES/SUGRES-1P developed by the candidate offers a precise and effective tool for analyzing the dynamics of proteins and heparin in complex biological systems.

The lastly presented fifth article concentrates on identifying a series of molecular dynamics-based descriptors for unbound Heparan Sulfate hexasaccharides, which serve to characterize glycosaminoglycans (GAGs) and elucidate their binding affinities to various protein receptors. This study considered a previously synthesized and analyzed library of 27 heparan sulfate (HS) hexasaccharides. Reference binding affinity data were collected between these 27 HS hexasaccharides and nine functionally diverse proteins that recognize 3-O-sulfation to different extents. Molecular dynamics (MD) simulations of the unbound HS hexasaccharides were performed using the AMBER16 program suite. For each unbound HS, 35 physico-chemical properties ("descriptors") were extracted from the MD trajectories. These descriptor values underwent various analyses, including Pearson correlation coefficients, machine learning approaches, linear regression, and statistical and cluster analysis. Using experimental GAG-protein binding affinity data, the candidate was able to further elucidate the interaction characteristics and contribute to the development of predictive models for GAG-protein binding specificity.

The PhD candidate published altogether five publications in highly impacted journals as *Journal of Chemical Theory and Computation*, *Chemistry—A European Journal* or *Computational Biology and Chemistry*. All publications are a part of the presented dissertation thesis. I really appreciate the amount of the computational experiments done and the amount of the data which had to be analysed. All used computational methodologies are high quality and PhD candidate was able to develop very efficient computational procedures eligible for the glycosaminoglycans and their interactions with proteins simulations.

Comments:

1. Figure 1-1 includes several mistakes, namely, the glycosidic linkage presented for the Hyaluronic acid is GlcA-β1,4GlcNAc, the correct linkage is GlcA-β1,3GlcNAc. Similarly, for the Heparan sulphate and Heparin the β glycosidic linkage is presented instead of α in case of GlcNAc. Also, presentation of the IdoA in the $^{1}C_{4}$ ring conformation would be more appropriate instead of the $^{4}C_{1}$ ring conformation. Moreover, in the presented oligomeric representation of the structures should be linking oxygen presented only on one side.
2. In Table 1-1 the chemical representation of the Keratan sulfate is presented as GlcNAc- β1,4Gal- β1,4 instead of GlcNAc- β1,3Gal- β1,4. In case of Heparan sulfate where GlcA might be substituted by IdoA would be more appropriate include both chemical representations while IdoA is in α configuration, however the GlcA is present in β configuration in the structure.
3. The paragraph on page 25 addressing the differences between ab initio and DFT quantum mechanical approaches contains some inaccuracies. For instance, the wave function should be described as a linear combination of single-electron wave functions rather than as a single many-electron wave function. Additionally, the self-consistent field procedure is applied in both methods, not exclusively in DFT.
4. Table 1-2 on page 47 is cut on one side.
5. In the description of Figure 3-1 is incorrectly described the position of the presented plots, top instead of left and bottom instead of right.
6. In the chapters 3.1 – 3.5 some references in the text to the appropriate figures are missing.

Questions:

1. In the Publication 1 is mentioned that the system was equilibrated for the 100ns. I would like to ask whether it is so, because it is quite unusually long equilibration before the production MD run. And if so, what was the reason for such a long equilibration?
2. The topic in the Publication 1 and 2 is very similar, however you used different force fields sets, ff14SB onlySC in Publication 1 and ff14SB in Publication 2. I would like to know your opinion whether and if so, how could influence the used force field the results when you would like to compare them between the Publications.
3. In the Conclusions of the Publication 4 you mentioned you plan to develop back mapping software for the GAGs Coarse-Grained model. I would like to ask, what is the current status of the back mapping software.

Overall assessment:

In the final evaluation of the submitted dissertation, I appreciate the huge amount of implemented computer experiments that the PhD candidate conducted. The obtained results are an indisputable benefit not only in the specific area of glycosaminoglycans simulations but also have a methodological significance for the application of sophisticated theoretical methods, which is also indicated by the number of works published by the PhD candidate.

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In conclusion, I conclude that the set goals of the dissertation were fully met, and the PhD candidate demonstrated the ability to do independent scientific work. Due to the significant contribution to the field of *in silico* modelling of GAGs and their interactions with proteins I recommend the presented dissertation thesis of M. Sc. Annemarie Danielsson to the award of a distinction.

In summary, I conclude that the doctoral dissertation submitted by M. Sc. Annemarie Danielsson, entitled "Deciphering the Molecular Basis of Specificity in Protein-Glycosaminoglycan Interactions: from All-Atom to Coarse-Grained Models", meets the requirements set forth in the Act – Law on Higher Education and Science (Article 187, paragraphs 1-3) of 2018, as amended. Therefore, I submit to the Council of the Discipline of Chemical Sciences of the University od Gdansk a recommendation to allow M. Sc. Annemarie Danielsson to proceed to the subsequent stags of the procedure for the awarding the academic degree of Doctor.

In Bratislava, 23. 01. 2026 Mgr. Stanislav Kozmon, Ph.D.