UNIWERSYTET WARSZAWSKI

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Prof. dr Sebastian Kmiecik
Biological and Chemical Research Centre,
Faculty of Chemistry,
University of Warsaw
email: sekmi@chem.uw.edu.pl

Review of the PhD Thesis

This review concerns the PhD thesis of **M. Sc. Annemarie Danielsson**, entitled: *"Deciphering the Molecular Basis of Specificity in Protein-Glycosaminoglycan Interactions: from All-Atom to Coarse-Grained Models"*, prepared under the supervision of prof. dr Sergey Samsonov and co-supervision of dr Martyna Maszota-Zieleniak at the Faculty of Chemistry, University of Gdańsk.

1. Character and Subject of the Thesis

The doctoral dissertation of M. Sc. Annemarie Danielsson constitutes a comprehensive study focused on the molecular mechanisms governing protein-glycosaminoglycan (GAG) interactions. This is a field of high biological relevance, given the critical role of GAGs in cell signaling, development, and pathology, yet it remains computationally challenging due to the flexibility and polyelectrolyte nature of these polysaccharides.

The thesis is constructed as a cohesive monograph based on a series of **five peer-reviewed publications** in recognized international journals (*Journal of Chemical Theory and Computation*, *Chemistry – A European Journal*, *Physical Chemistry Chemical Physics*, *Computational Biology and Chemistry*). In three of these articles, the Candidate is the first author, and in two, she shares the first authorship.

The scientific scope of the thesis follows a clear and logical progression from the microscopic to the mesoscopic scale. It starts with all-atom Molecular Dynamics (MD) simulations combined with NMR validation to characterize specific binding events. It then moves toward the development of coarse-grained (CG) models (SUGRES-1P and its integration with UNRES) that make it possible to simulate large protein–GAG assemblies. This transition from using existing methods to creating new computational tools highlights the strong methodological focus of the thesis.

2. Structure, Language, and Clarity

The thesis is written in English and follows the cumulative format. It encompasses an Abstract, Introduction, Aims, a summary of the included publications (Chapters 3.1–3.5), and a Bibliography. The Introduction provides a solid theoretical background on GAGs, experimental techniques, and computational methods, setting a clear context for the research.

The narrative structure is well-conceived. Rather than presenting disjointed papers, the Candidate presents them as a single, coherent line of research: from understanding the physics of binding (Papers 1 & 2) to building tools that capture this physics efficiently (Papers 3 & 4) and analyzing specificity descriptors (Paper 5). The language is precise, mature, and meets the highest standards of scientific communication.

It is also worth noting that the thesis has been prepared with exceptional diligence. The figures are aesthetically refined and informative, although including more of them could have further supported the presentation. Even the physical presentation of the document is of high quality and aligns with the overall professionalism of the thesis.

3. Scientific Evaluation

The research presented is of outstanding quality, characterized by methodological rigor and innovation. I have divided my evaluation into three key thematic blocks:

3.1. Synergy of Experiment and Simulation (Chapters 3.1 & 3.2)

The initial chapters demonstrate the Candidate's ability to integrate computation with experiment. **Chapter 3.1** (based on the publication in ***Chemistry – A European Journal***, 2024) introduces a novel approach using nitroxide-based paramagnetic cosolutes to detect protein binding interfaces. The Candidate's MD simulations were crucial for validating the experimental NMR data, showing that solvent paramagnetic effects can map binding hotspots even when classical Chemical Shift Perturbation (CSP) fails. Similarly, **Chapter 3.2** (based on the publication in ***Physical Chemistry Chemical Physics***, 2023) offers a comparative study of IL-8 binding to GAGs versus acidic peptides. Here, the Candidate used MD to decipher the electrostatic drivers of recognition, revealing that while mechanisms are similar, distinct binding geometries dictate specificities. These sections prove the Candidate's competence in applying MD as a tool for interpreting complex experimental data.

3.2. Methodological Innovation: Coarse-Grained Models (Chapters 3.3 & 3.4)

This section represents the core original contribution of the thesis. In **Chapter 3.3** (based on the publication in ***Journal of Chemical Theory and Computation***, 2023), the Candidate extended the

SUGRES-1P model to heparin. This involved a non-trivial parameterization of ellipsoidal beads and off-center charge sites to capture the kinks and persistence length of heparin, validated against experimental radii of gyration. Crucially, **Chapter 3.4** (based on the publication in ***Journal of Chemical Theory and Computation***, 2024) describes the integration of this heparin model with the UNRES protein force field. The Candidate successfully implemented the compatibility between protein and carbohydrate coarse-grained interactions, validating the model against atomistic MD and structural data. This provides the community with a computationally tractable framework for simulating large-scale protein-GAG complexes, addressing a significant gap in the field.

3.3. Data-Driven Analysis of Specificity (Chapter 3.5)

In **Chapter 3.5** (based on the publication in ***Computational Biology and Chemistry***, 2022), the Candidate applied a data-centric approach to understand the specificity of 3-O-sulfated heparan sulfate. By extracting physicochemical descriptors (H-bonds, RMSF, entropy) from MD trajectories of 27 hexasaccharides and linking them to binding affinities via multivariate analysis, the study supports the concept of conformational selection. This demonstrates the Candidate's versatility in using statistical and machine learning tools alongside physics-based simulations.

3.4. Assessment of the Candidate's Contribution

Across these thematic blocks, the Candidate's contribution to the multi-author works is clearly defined and substantial. According to the contribution statements, in the three first-author publications (**Chapters 3.3–3.5**), Ms. Danielsson was responsible not only for the core methodological developments and execution of simulations but also for data analysis, interpretation of results, and drafting the manuscripts. In the remaining co-authored studies (**Chapters 3.1 and 3.2**), she performed the molecular dynamics simulations that provided the essential atomistic context for the experiments, and participated in the analysis of these results and preparation of the relevant manuscript sections. This confirms her leading role in the computational aspects of the presented research.

4. Remarks and Questions for Defense

The Candidate's publication record is very strong. Serving as the first author on three publications—including two in the *Journal of Chemical Theory and Computation*, a leading venue in the field—demonstrates a very high level of technical and scientific proficiency. Additionally, holding joint first authorship on two other papers highlights her crucial role in collaborative projects, particularly those involving direct interaction with experimental groups. Furthermore, the thesis highlights successful international collaborations, notably with the Huster and Künze laboratories, as well as productive

cooperation with the groups of Professors Liwo and Sieradzan. This reflects a high level of versatility, spanning from the interpretation of experimental data, through classical all-atom MD, to advanced coarse-grained modeling. I am impressed by the substantial workload required to master and integrate such a diverse array of techniques. The portfolio confirms that M. Sc. Annemarie Danielsson has acquired the skills to conduct independent research at an internationally competitive level.

While the scientific substance is excellent, the "Outlook" or concluding section regarding the broader biological implications could be more expansive. The thesis excels at the "how" (methods) and "what" (molecular mechanism), but is somewhat brief on the "so what?" – i.e., the direct translational potential. To stimulate discussion during the defense, I propose the following questions:

1. **Therapeutic Applications**: Your work provides deep mechanistic insights into protein-GAG specificity. From a translational perspective, how do you envision applying the computational framework and knowledge you have gathered in a practical drug discovery pipeline? Specifically, do you see your tools being scalable for screening libraries of GAG mimetics, or are they best suited for refining the mechanisms of already identified lead compounds?
2. **Model Transferability**: In developing the SUGRES-1P model, you focused heavily on Heparin. To what extent are the parameters derived in your work transferable to other classes of Glycosaminoglycans? Would their modeling require a complete re-parameterization, or is the current framework sufficiently robust to handle these distinct GAG types?
3. **AI vs. Physics-Based Models:** In the era of AlphaFold 3 and rapidly advancing AI models that are beginning to tackle protein-ligand complexes, how do you view the future role of physics-based Coarse-Grained methods? Is there still a distinct niche where CG remains indispensable, or will AI eventually replace these simulations? Furthermore, how do you foresee AI being integrated specifically into the de novo design of GAG-based therapeutics?
4. **Vision:** Your thesis represents a massive workload, impressively bridging experimental NMR data with multiscale modeling. Looking back, which specific element of this integrative approach did you find the most challenging - both technically and conceptually - and why? With the knowledge you have now, are there specific choices you would have approached differently?

5. Final Evaluation

The doctoral dissertation of M. Sc. Annemarie Danielsson is a mature, scientifically rigorous, and methodologically innovative body of work. It ranks among the best dissertations I have encountered. The integration of diverse computational scales with advanced experimental validation represents a breadth

and depth of research that is rarely encountered at this stage of a scientific career. This is confirmed by five publications in recognized journals, in which the Candidate made a significant contribution. The thesis successfully bridges the gap between experimental data and theoretical modeling, contributing novel and practical computational tools to the community. It meets the highest standards expected of a PhD dissertation.

In light of the above, I state that the submitted PhD thesis fulfills all requirements specified in Art. 187 of the Act of 20 July 2018 – Law on Higher Education and Science. **I recommend its acceptance and move to admit M. Sc. Annemarie Danielsson to the public defense**. Furthermore, given the high quality of the research and the considerable scope of work undertaken by the Candidate, **I recommend that the thesis be awarded a distinction**.

Electronically signed by

Sebastian Kmiecik; Uniwersytet Warszawski

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