10TH POLISH-KOREAN CONFERENCE ON PROTEIN FOLDING:

THEORETICAL AND EXPERIMENTAL APPROACHES

GDAŃSK, 8-10 SEP. 2025

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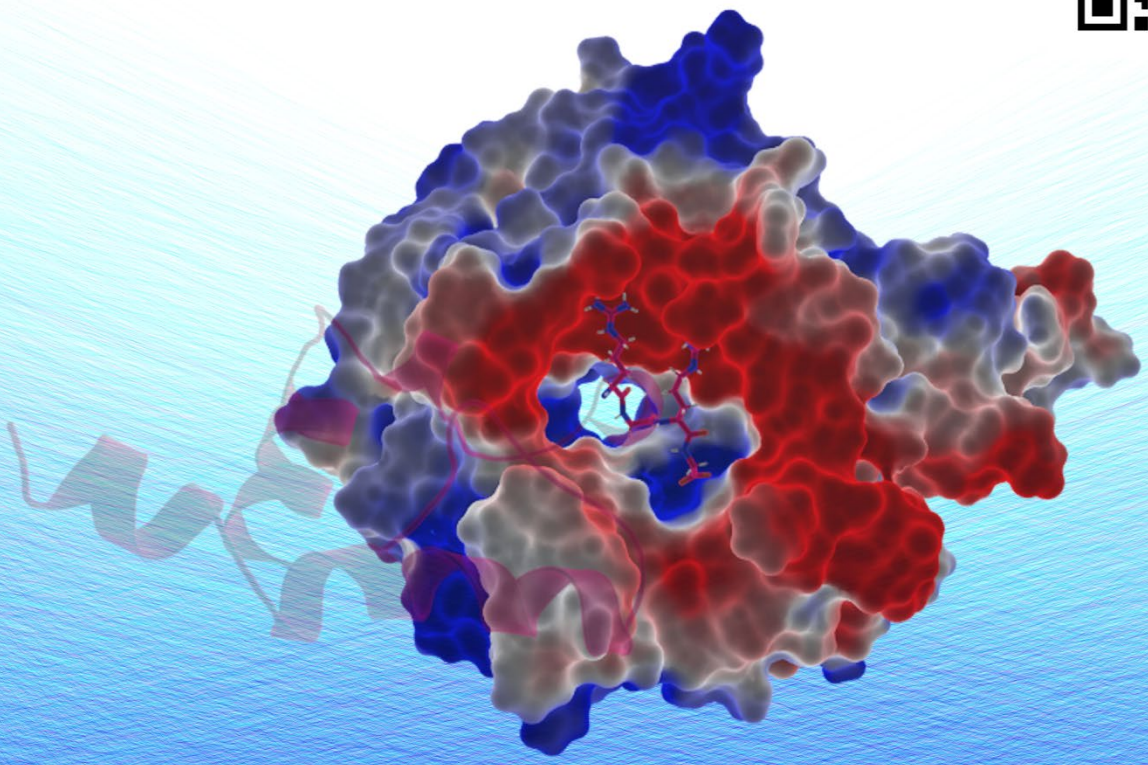

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Investigation of the folding and misfolding mechanism of proteins and other biological molecules is one of the most important task, to understand its role in the functioning of the living cells. Protein misfolding can lead to cancer and hereditary diseases; designing effective therapies and drugs against such diseases is one of the long-term goals of the research on protein folding. Due to the complexity of this problem, this research requires extensive collaboration between biochemists, biophysicists, chemical and theoretical physicists, theoretical chemists, and bioniformaticians. The researchers from the University of Gdańsk, Poland, and Korea Institute for Advanced Study (KIAS), Republic of Korea are very active in protein-folding research and the two institutions have a long-time history of collaboration in the field. This collaboration prompted the idea of organizing a series of conferences held in Seoul and in Gdańsk, on an alternating basis, to promote scientific collaboration and exchange between the scientists of the Republic of Korea and Poland, as well as other countries in Asia and Europe, on protein folding and related fields. This conference is the tenth from the series; see previous conferences' sites.

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P – Poster

L – Lecture

Conference program

Tenth Polish-Korean Conference on "Protein Folding:

Theoretical and Experimental Approaches"

08.09 – MONDAY

8:00 - 8:55 breakfast, conference registration
9:00 - 9:15 Conference welcome
Piotr Stepnowski, Beata Grobelna, Adam Liwo, Jooyoung Lee, Jejoong Yoo

Session I, chair: Adam Liwo

L1 (9:15 – 9:55) Jejoong Yoo (Korea I, South Korea**)**

L2 (9:55 – 10:35) Chang Yun Son (Seoul National U**)**

L3 (10:35 – 11:15) Gyu Rie Lee (KAIST, Korea)

11:15 - 11:55 coffee break

Session II, chair: GYU RIE LEE

L4 (12:00 – 12:40) Sebastian Kmiecik (Warsaw U, Poland)

L5 (12:40 – 13:10) Maciej Maciejczyk (Olsztyn U, Poland)

L6 (13:10 – 13:40) Agnieszka Lipska (Gdańsk U of Technology, Poland)

L7 (13:40 – 14:00) Soobon Ko (Chonnam Nat U Med, Korea)

14:00 - 14:55 lunch

Session III, chair: SEBASTIAN KMIECIK

L8 (15:00 – 15:40) Sergey Samsonov (Gdańsk U, Poland)

L9 (15:40 – 16:20) Krzysztof Bojarski (Gdańsk U of Technology, Poland)

L10 (16:20 – 16:40) Jacob A. Clark (Gdańsk U, Poland)

L11 (16:40 – 17:00) Pamela Smardz (PAS, Poland)

17:00 - 18:00 coffee & cookie

09.09 – TUESDAY

8:00 - 8:55 breakfast

Session IV, chair: STEVEN GROSS

L12 (9:00 – 9:40) Bernard Brooks (Nat I of Health, Bethesda, Maryland, USA)

L13 (9:40 – 10:20) Yoonji Lee (Chung-Ang U, South Korea)

L14 (10:20 – 11:00) Mai Suan Li (Warsaw I, Poland)

11:00 - 11:35 coffee break

Session V, chair: BERNARD BROOKS

L15 (11:40 – 12:15) Jyoung Lee (Seoul National U, Korea)

Enhanced drug candidate discovery using artificial intelligence and physical energy calculations....23

L16 (12:15 – 12:45) Woong-Hee Shin (Korea U C of Med, Korea)

Utilizing AlphaFold2 Multi-State Modeling for Improving Structure-Based Drug Discovery....24

L17 (12:45 – 13:25) Jongmin Park (Kangwon Nat U, Korea)

Discovery of E3 Ligase Ligands for Muscle Specific ProteinDegradation.....25

L18 (13:25 – 14:00) Patryk Wesołowski (Cambrigde U, U.K.)

Multilevel Frameworks for Computational Studies of BiomolecularSystems.....26

14:00 - 14:55 lunch

Session VI, chair: JONGMIN PARK

L19 (15:00 – 15:40) Steven Gross (California U, USA)

Anti-microbial peptides.....27

L20 (15:40 – 16:00) Elizaveta F. Petrusevich (Gdańsk U, Poland)

Effect of Multitorsional Potentials on Modeling the Structures of α-, β- and α+β-Proteins with the UNRES Coarse-Grained Force Field.....28

16:00 - 17:00 coffee & cookie

Session VII, chair: CEZARY CZAPLEWSKI

16:00 - 17:00 POSTER SESSION

17:00 - three options to choose from:

- stay at the faculty and work

- go to the shopping mall

- go to Gdańsk

10.09 –WEDNESDAY

8:00 - 8:55 breakfast

Session VIII, chair: MARIUSZ MAKOWSKI

L21 (9:00 – 9:40) Yuji Sugita (RIKEN, Japan)

L22 (9:40– 10:20) Yoonjoo-Choi (Chonnam Nat U Med, Korea)

L23 (10:20 – 11:00) Rafał Ślusarz (Gdańsk U, Poland)

11:00 - 11:35 coffee break

Session IX, chair: YOONJI LEE

L24 (11:40 – 12:20) Sang Hak Lee (Busan Nat U, Korea)

L25 (12:20 – 12:50) Mariusz Makowski (Gdańsk U, Poland)

L26 (12:50 – 13:15) Brice Korkmaz (CEPR, Tours, France)

L27 (13:15 – 13:30) Smita Pilla (Warsaw U, Poland)

L28 (13:30 – 13:45) Ayomide Fasemire (Warsaw U, Poland)

L29 (13:45 – 14:00) Nithin Chandran (Warsaw U, Poland)

14:00 - 14:55 lunch

Session X, MODERATOR: ARTUR GIEŁDOŃ

15:00 - 16:00 Protein folding, where do you go?
- Discussion panel
16:00 - 16:20 Closing Remarks
Adam Liwo, Jooyoung Lee, Jejoong Yoo

16:30 - 17:00 Faculty Tour

17:30 - 21:00 Conference Banquet
(at the Faculty of Chemistry)

Poster List

Abstracts

L01

Functional Roles of Intrinsically Disordered Regions in the Diffusion of Homeodomain and APE1 along DNA Revealed by Realistic All-Atom Simulations

Jejoong Yoo[1], Gyeongpil Jo[2]
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[2] Sungkyunkwan University, South Korea

Intrinsically disordered regions (IDRs) are prevalent in DNA-binding proteins and increasingly recognized as key regulators of target search and recognition dynamics. However, atomistic insights into their roles have been limited due to the inability of standard molecular dynamics (MD) force fields to reproduce experimentally consistent diffusion behavior. In particular, conventional force fields such as AMBER and CHARMM systematically overestimate protein-DNA electrostatic attraction, leading to artificially arrested diffusion.

Here, we show that CUFIX corrections to the AMBER force field—featuring fine-tuned nonbonded parameters for hydrogen bonds and electrostatics—enable realistic simulations of facilitated diffusion. Using CUFIX-AMBER, we investigated the one-dimensional diffusion of two representative IDR-containing proteins: the engrailed homeodomain and apurinic/apyrimidinic endonuclease 1 (APE1). Millisecond-scale MD simulations of the homeodomain revealed dynamic alternation between sliding and hopping, where both the N-terminal IDR and recognition helix cooperatively guided site recognition. For APE1, microsecond-scale simulations demonstrated that its disordered N-terminal tail actively modulates DNA contacts, facilitating initial binding and sustained sliding. Direct comparison with single-molecule diffusion measurements corroborates our findings and highlights the essential role of IDRs in modulating protein mobility. These results underscore the functional importance of disordered regions in DNA target search mechanisms and establish CUFIX-AMBER as a necessary framework for accurate, biologically relevant simulations of protein-DNA interactions.

L02

Controlling Biological Membranes for Materials Applications.

Son Chang Yun[1]

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Biological systems, including biomacromolecules and living cells, utilize interfacial interactions with external environments in many parts of their biological functions. Biological interfaces are characterized by amphiphilic boundary layer decorated with various functional moieties, which provide selective permeation or binding of other ingredients in a highly controlled fashion. Biological activity and function of biomaterials are often dictated by processes occurring at these biological charged interfaces. Designing drugs and their delivery systems requires control over the transfer of pharmaceutical compounds across the barriers of biological membranes and to activate their therapeutic function in selective fashion. Other examples are biosensing and bioimaging agents, which are injected into biological systems to detect target domains or species, or occurrence of certain biological processes in vivo. Non-destructive intrusion and selective/sensitive operations are desirable for either cases, requiring a sophisticated engineering strategy based on a systematic understanding of interactions at various biological interfaces.

In this talk, I'll present our ongoing efforts to control biological membranes for materials applications. Two major advances will be highlighted. First, I'll discuss about translocation of functional organic molecules through the lipid monolayer of lipid droplets, which can be utilized as cellular production and storage material in biorefinery process. Second example will discuss about design of selective ion channel across lipid bilayers using chemically modified G-quadruplex DNA sequence. These examples represent the complex interfacial systems abundant in biology. I'll cover both the development of new simulation methods and the applications of the methods toward biomolecular systems, illustrating the versatile utility of understanding the underlying physics for fundamental questions.

Reference list

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5. Jo, H.; Ju, S.; Kim, M.; Beon, J.; Jang, S.-Y.; Pack, S.P.; Son, C.Y.; Kim, J.-S.; Oh, S.S.* Aptamer-Guided, Hydrolysis-Resistant Deoxyoxanosine Enables Epitope- and Moiety-Selective Conjugation to Nonengineered Proteins Even in Complex Environments. J. Am. Chem. Soc. 147, 9328 (2025)

L03

De novo design of protein and chemical interactions using deep learning

Lee Gyu Rie[1]
[1] Korea Advanced Institute of Science and Technology (KAIST), Republic of Korea

The breakthrough in protein structural biology using Artificial Intelligence (AI) transformed protein structure prediction and protein design. However, problems that require atomic-level accuracy and high resolution modeling such as small-molecule binding protein design and enzyme design still remain challenging.

We tackled this problem by developing a new protein design computational method based on the state-of-the-art AI models. RFdiffusion All-Atom is a generative AI model trained using RoseTTAFold All-Atom. RoseTTAFold all-atom can predict complex structures of all types of life's molecules including proteins, chemical modifications, nucleic acids, metal ions, and small-molecules. The generative AI model RFdiffusion All-Atom was used to generate customized backbone structures for the target small-molecules. LigandMPNN enables generating sequences with given protein backbone and bound small-molecule atomic coordinates. By combining these methods with the Rosetta biomolecular software, we developed a next generation protein design platform that considers protein side chains or small-moleculesin all-atom level. Using the new method we designed binders for eight different small-molecule targets including metabolites and small-molecule drugs. The binders were readily expressed in E. coli, with binding affinities ranging from nanomolar to low micromolar, straight out from the computer. The design structure accuracy was confirmed with protein and ligand co-crystal structures of the cortisol binder and the apixaban binder being in very close agreement with the design models. The success in designing interactions between proteins and chemical groups now allows us to pursue developing de novo protein-based tools that can be used to study and modulate biological systems in very high resolution and accuracy. We are actively extending our methods for de novo enzyme design, sensor design, synthetic biology, and the study of protein and nucleic acid modifications in epigenetics.

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L04

Playing with Structural Flexibility: Revisiting the CABS-flex Web Server - What's New in Version 3.0

Sebastian Kmiecik[1]

[1] Biological and Chemical Research Centre, Faculty of Chemistry, University of Warsaw

Abstract:
CABS-flex has become a widely used tool for efficient simulations of protein structural flexibility. In its latest release, version 3.0, the server introduces several key improvements: new flexibility modes with direct integration of AlphaFold pLDDT restraints (Rigid, Flexible, Rigid-pLDDT, Unleashed), enhanced deep-learning-based all-atom reconstruction, extended options for modeling linear and cyclic peptides, and improved analysis and visualization tools. The server remains freely available and requires no registration. Overall, CABS-flex 3.0 provides a versatile resource that can support not only structural modeling but also functional studies of proteins. As a highlight, I will also briefly present our most recent studies on protein–peptide interaction prediction.

References:

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2. Integration of AlphaFold pLDDT scores into CABS-flex for enhanced protein flexibility simulations, *Comput Struct Biotechnol J*, 23: 4350–4356, 2024.

3. Structure prediction of linear and cyclic peptides using CABS-flex, *Brief Bioinform*, 25(2): bbae003, 2024.

L05

SimDNA: A Coarse-Grained Method for DNA Folding Simulations and 3D-Structure Prediction.

Maciejczyk Maciej[1,2], Moafinejad S. Naeim[2], Boniecki Michał J.[2], Bujnicki Janusz M.[2]
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[2] Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland

DNA, the blueprint of life, primarily forms a double helix but can also create structures like junctions, triplexes, and quadruplexes. These structures are essential for cellular functions, including gene expression regulation, replication, and the maintenance of genome stability.

Exploring DNA structure through methods like X-ray crystallography, NMR, and Cryo-EM spectroscopy is crucial but accompanied by challenges. These methods are often costly and time-intensive. X-ray crystallography captures static snapshots of DNA conformations, lacking dynamic insights. Moreover, NMR is restricted in its ability to analyze smaller DNA molecules, while achieving high-resolution Cryo-EM density maps are typically achieved only for larger biomolecules, such as those with 150 kDa.

SimDNA is a new computational tool based on SimRNA [1], that addresses these challenges. It predicts DNA 3D structures using a coarse-grained representation and the Metropolis Monte Carlo sampling technique - a statistical mechanics method that efficiently explores conformational spaces of the molecule by sampling from Boltzmann distribution.

This approach allows SimDNA to accurately fold DNA duplexes and shows promising results for junctions, and non-canonical structures like triplexes and G-quadruplexes, even without external restraints. Moreover, SimDNA enables guided simulations using data from experiments or other computational methods, providing a versatile tool for researchers. This flexibility allows user-defined restraints to focus simulations on specific interactions or structural configurations, facilitating the study of transitions between different DNA structures. Overall, SimDNA holds great promise for advancing our understanding of DNA behavior, offering insights into fundamental biological processes, and aiding in biomedical research and therapeutic development.

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Acknowledgments

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L06

Long-timescale simulations of biologically relevant systems using a coarse-grained approach.

Lipska A. G.[1], Narayanareddy B. R. J.[2], Sieradzan A. K.[3], Czaplewski C.[3], Lipińska A. D.[4], Ocetkiewicz K. M.[1], Proficz J.[1], Czarnul P.[5], Krawczyk H.[1, 5], Gross S.[2], Liwo A.[3],
[1] Centre of Informatics Tri-city Academic Supercomputer and Network (CI TASK), Gdańsk University of Technology, Poland
[2] Developmental and Cell Biology, University of California, USA
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[5] Faculty of Electronics, Telecommunications and Informatics, Gdańsk University of Technology, Poland

Molecular dynamics (MD) simulations have become an indispensable tool in structural biology, enabling the study of complex biomolecular systems. While conventional all-atom simulations are limited in time and system size, advances in coarse-grained models and high-performance computing now allow us to explore biologically relevant systems at unprecedented scales. In this work, we employ the UNitedRESidue (UNRES) physics-based coarse-grained model, which represents each amino acid with only two interaction sites. Owing to recent algorithmic optimizations, parallelization, and GPU/multi-GPU implementation, UNRES achieves simulation rates equivalent to ~1 ms/day for systems exceeding 200,000 residues. Because the model averages out fast internal degrees of freedom, this simulation rate corresponds to roughly 1 μs of all-atom or laboratory time. The reliability of the UNRES timescale has been previously validated by folding studies of small proteins, demonstrating consistency with both all-atom simulations and experimental data.

We illustrated UNRES applicability in two biologically relevant contexts: (i) the dynamic structure of human noroviruses, where strain-specific differences can be linked to biological activity, and (ii) kinesin–microtubule interactions, specifically the role of tubulin C-terminal tails in modulating motor binding.

Our results highlight the ability of UNRES to capture long-timescale dynamics of very large protein systems with high computational efficiency, opening the way to studies of complex biological processes.

Acknowledgments
This work was supported by the National Science Centre (Poland) grant Sonata UMO-2023/51/D/ST4/02288 andpartially supported by the infrastructure provided by European Funds—Smart Growth in a grant: EuroHPC PL—National Supercomputing Infrastructure for EuroHPC, POIR.04.0200-00-D014/20-00.

L07

Structure-based protein design for Simultaneous optimisation of Enzymatic activity and Immunogenicity

Soobon Ko[1], Sunghwan You[2], Yeojin Moon[2], Honglan Li[3], Junsu Ko[3], Woong-hee Shin[4*], Yoonjoo Choi[1*]

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Bacterial cancer therapy is one of the oldest, yet still promising, approaches for treating tumors[1]. However, using bacteria as living therapeutics presents major challenges, particularly their strong immunogenicity[2,3]. Reducing immune recognition is essential, but unlike conventional strategies where epitopes may be removed from any surface region, in this context the bacterial surface must largely remain intact to preserve essential interactions within the organism. This creates the need for a more comprehensive strategy: (i) redesigning internal regions rather than surface areas, (ii) reducing immunogenicity in ways that avoid disrupting external interactions, and (iii) maintaining overall protein stability and catalytic function.

As a proof of concept, we selected Salmonella enterica PhoN, a class A acid phosphatase that is strongly immunogenic in infection yet enzymatically tractable[3]. We applied a structure-guided approach [4,5,6] to design variants computationally and screened them simultaneously for stability, immunogenicity, and enzymatic activity. Several redesigned variants showed reduced immune recognition while retaining or even enhancing catalytic performance. Computational analysis indicated that modest increases in flexibility could promote substrate access, with the best variant showing more than a two-fold improvement. These results demonstrate that careful targeting of non-surface regions can simultaneously mitigate immunogenicity and preserve activity in bacterial enzymes. The integrated framework of prediction, modeling, and experimentation provides a path toward safer and more effective bacterial cancer therapies.

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Acknowledgments

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L08

Breaking Glycosaminoglycan "Sulfation Code" with Molecular Simulations

Samsonov Sergey A.[1],Pągielska Marta[1], Clark Jacob[1]
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Glycosaminoglycans (GAGs), a class of linear anionic periodic polysaccharides, play crucial roles in various biologically relevant processes within the extracellular matrix. These processes involve cell signaling, development and proliferation, extracellular matrix assembly, coagulation and angiogenesis. GAGs mediate their functions through their interactions with specific proteins, which makes them attractive targets for regenerative medicine and drug design. At the same time, the molecular mechanisms underlying protein-GAG interactions remain poorly understood. Classical structure determination techniques face significant limitations when dealing with protein-GAG complexes [1]. This is due to GAGs' unique properties, including their extensive length, flexibility, periodicity, symmetry, multipose binding and the high heterogeneity of their sulfation patterns constituting the "sulfation code." As a result, only a limited number of experimental protein-GAG structures have been elucidated, potentially restricting the successful implementation of machine learning approaches to these systems. Nevertheless, theoretical approaches, complemented by the experimental data, hold particular promise for deciphering the code for deciphering this code and elucidating the structure-function relationships of these complex molecules. In our research, we focus on the particularities, challenges and advances of computational methods such as molecular docking, molecular dynamics and free-energy calculations when applied to GAG-containing systems [2]. These computational approaches offer novel valuable insights into the enigmatic world of protein-GAG interactions, paving the way for their enhanced understanding and potential therapeutic applications [3].

Reference list

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Acknowledgments

The National Science Centre of Poland, grant number UMO-2023/49/B/ST4/00041.

L09

Machine Learning Models for Prediction of (Pro)cathepsin-Glycosaminoglycan Binding Free Energies Based on Molecular Structure

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Machine learning (ML) offers a powerful, low-cost alternative to classical computational methods, capable of uncovering complex relationships between structural descriptors and biomolecular properties. In structural biology, ML enables rapid prediction of structural, energetic, and functional parameters from simulation- or experiment-derived features, making it increasingly attractive for systems that are otherwise difficult to characterize.

Here we focus on (pro)cathepsins, lysosomal proteases of the papain family, whose dysregulated activity contributes to cancer, arthritis, cardiovascular and bone disorders, neurodegenerative diseases, and more. These enzymes are synthesized as inactive precursors (procathepsins) and are tightly regulated by glycosaminoglycans (GAGs), highly sulfated, negatively charged polysaccharides that interact predominantly via electrostatics. Despite their biological relevance, the complexity of protein–GAG interactions poses significant challenges for both experimental and theoretical studies.

To address this, we trained eight ML models to predict MM-GBSA binding free energies from structural descriptors derived from MD simulations (ff14SB/GLYCAM06j) of six (pro)cathepsins in complex with six GAGs, covering four periodic states and six binding poses. Tested algorithms included ElasticNet, Linear Regression, LinearSVR (with and without RBFSampler), LightGBM, HistGradientBoost, Random Forest, and a Fully Connected Neural Network (FCNN). The best performance was achieved by a minimal FCNN architecture (no hidden layers, dropout 0.01, ReLU activation), outperforming more complex models.

This work provides the first proof-of-concept that ML can reliably capture binding energetics in protein–GAG systems. By bridging molecular dynamics and predictive modeling, it lays the foundation for the development of generalizable, structure-based ML predictors applicable to a broad range of biomolecular complexes.

Acknowledgments
This work was supported by the National Science Centre of Poland (Narodowe Centrum Nauki, grant number UMO-2023/48/C/ST4/00163). The molecular dynamics simulations were performed on the "Helios" cluster provided by the Polish Grid Infrastructure (PL-GRID, grant plgprotgag, PLG/2025/018351).

L10

Solvent Models and Charge Scaling: Benchmarking for Molecular Dynamics Simulations of Glycosaminoglycans

Jacob A. Clark[1], Sergey A. Samsonov[1],
[1] University of Gdansk, Poland

Glycosaminoglycans (GAGs) are highly negatively charged polysaccharides found extensively on the surface of cells and in the extracellular matrix. They possess a vast range of biological functions involved in cell signaling, cardiovascular health, tissue regeneration, inflammation and neurodegeneration [1]. The intrinsic properties of GAGs makes them difficult to study effectively, recent efforts to quantify the efficacy of various forcefields and water models within GAG simulations has highlighted promising routes to more accurate modelling of GAGs. Regarding forcefields, a novel approach of implicitly polarisable forcefields for GAGs has been demonstrated to overcome the limits of nonpolarisable forcefields and the computational demand of explicitly polarisable forcefields [2]. For the choice of water models, recent work has demonstrated the limits of the conventional TIP3P water model used and highlighted more appropriate alternatives [3]. By combining and comparing these approaches we have implemented the "charge-scaled" method for various implicit and explicit water models to identify the best compromise in terms of both computational efficiency and efficacy in simulating GAGs. In this work, both unbound heparin and heparin bound with basic fibroblast growth factor (FGF) were simulated with five implicit and six explicit water models to mirror previous work whilst also implementing charge scaling developed for the GLYCAM-ECC75 forcefield. From these simulations, we aim to identify the best approach for simulating GAGs within the context of both solvent model and forcefield choice.

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L11

Disulfide Bonds and Folding Pathways in Plant Non-Specific Lipid Transfer Proteins

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Disulfide bonds are critical post-translational modifications that influence protein folding, stability, and function. Our previous studies established their roles in Ribonuclease A mechanical stability [1], HVEM-derived peptide inhibitor binding affinity [2], and how introducing disulfide bonds into the amyloid beta peptide can explain observed experimental aggregation differences [3]. Plant non-specific lipid transfer proteins (nsLTPs) provide an important system to examine disulfide bond function, containing an evolutionarily conserved 8-cysteine motif that forms a disulfide network that stabilises the four-helix bundle. However, the precise contribution of disulfide bonds to folding pathways, conformational dynamics, and membrane interactions in nsLTPs remains incompletely understood.

We employed coarse-grained molecular dynamics simulations with the UNRES force field to examine how disulfide bonds influence nsLTP folding and membrane behaviour. Using soybean protein (pdb code 1HYP) as a model system, we found that nsLTPs can adopt native-like conformations without disulfide bonds, but these conformations are unstable and long-term structural integrity requires the complete disulfide network. Folding shows strong temperature dependence, with native-like structures forming more frequently at elevated temperatures where increased kinetic energy disrupts off-pathway non-native contacts. The disulfide network provides additional stabilisation that prevents protein unfolding under these conditions. We observed that folding involves dynamic formation and breaking of both native and non-native disulfide bonds, indicating that disulfides actively shape the folding energy landscape. Our simulations show that 1HYP can adopt both native nsLTP2-type and twin nsLTP1-type disulfide bond patterns while maintaining native-like folds.

These results demonstrate that disulfide bonds in nsLTPs function as dynamic regulators of protein folding rather than static structural constraints. The ability to fold through multiple disulfide patterns suggests evolutionary flexibility in folding mechanisms. Combined with our previous findings across different protein systems, this work establishes general principles governing disulfide-mediated protein folding and provides a foundation for understanding how these modifications contribute to protein evolution and stability in diverse biological contexts.

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L12

An overview of recent applications of machine learning and AI tools for problems in biophysics at NIH

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This presentation consists of a survey of our recent efforts to use machine learning and AI tools for applications in biophysics [1][2][3][4][5][6][7].

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L13

Exploring TM4SF5-mediated Protein Interactions: Insights from Structure Prediction and Molecular Simulations

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Small four-transmembrane (4TM) proteins, including tetraspanins and CD20-like proteins, are central to membrane organization and protein–protein interactions, yet the evolutionary and mechanistic relationships among their subfamilies remain unclear. To explore these differences, we carried out comparative structure modeling and molecular simulations of representative members from the genuine tetraspanin, CD20-like, and L6 membrane protein families. Sequence alignments and contact map analyses indicated that L6 proteins are evolutionarily closer to CD20-like proteins, while structural models revealed distinctive signatures in the large extracellular loop (LEL) of TM4SF5. Molecular simulations further identified specific interface residues that support the stability of TM4SF5 dimers, and targeted mutagenesis provided experimental validation of these predictions. Together, these results not only clarify how TM4SF5 achieves its atypical assembly behavior but also provide a mechanistic framework for understanding how its unique structural determinants contribute to membrane-associated functions.

Acknowledgments
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L14

Role of Translation Speed and Non-covalent Lasso Entanglements in Protein Misfolding and Dimerization

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According to the traditional view, the structure and functions of proteins are determined by their sequence. However, a new view, called the kinetic principle, has recently emerged. According to this view, the structure and function of proteins may depend on the rate of translation determined by synonymous mutations. For example, Zhou et al. showed that the stability of a protein dimer in solution depends on translation speed [1]. Using coarse-grained simulations of protein synthesis we were able to confirm their experimental result. Combining modellingandlimited proteolysis, we found that the structural and kinetic origin of this effect is associated with misfolded states containing non-covalent lasso entanglements, many of which structurally perturb the dimer interface, whose probability of occurrence depends on translation speed [2].

Several mechanisms are known to cause monomeric protein misfolding. Coarse-grained simulations have predicted an additional mechanism exists involving off-pathway, non-covalent lasso entanglements, which are long-lived kinetic traps and structurally resemble the native state. We further show these misfolded states are consistent with the structural changes inferred from limited proteolysis and crosslinking mass spectrometry experiments. Our results indicate that misfolded states composed of non-native entanglements can persist for long timescales in both all-atom simulations and experiments [3]. If time permits, the role of entanglement in the mechanism by which soluble misfolded proteins bypass chaperones at the molecular level will be discussed [4].

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L15

Enhanced drug candidate discovery using artificial intelligence and physical energy calculations

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The integration of artificial intelligence (AI) and molecular dynamics (MD) simulations is rapidly transforming the drug discovery process. Recent advancements demonstrate that state-of-the-art computational techniques are accelerating the discovery of drug candidates across various modalities, including small molecules, peptides, and antibodies.

This presentation will explore recent progress in AI models applied to drug screening and candidate generation, with several case studies illustrating their practical applications. First, I will present our findings on the discovery of novel inhibitor candidates for autotaxin using AK-Score, an AI-based scoring function designed to predict protein-ligand interactions. Next, I will discuss the identification of new E3 ligase ligands for targeted protein degrader, PROTAC discovery, leveraging a combination of molecular docking, molecular dynamics simulations, and free energy calculations. Finally, our work on designing novel nanobody sequences using a protein generative model combined with empirical binding free energy calculations will be presented and discuss the status of protein generative models in drug discovery. In all three cases, the success rate for identifying novel candidates was significantly higher compared to traditional high-throughput screening approaches, underscoring the practical advantages of AI-driven strategies in modern drug discovery.

L16

Utilizing AlphaFold2 Multi-State Modeling for Improving Structure-Based Drug Discovery

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As the name suggests, structure-based virtual screening is contingent on the receptor structure employed. The receptor conformation is generally treated as rigid, despite the high flexibility of proteins. The resolution of this issue is achieved through the implementation of an ensemble screening method, which employs a multitude of structures obtained from the Protein Data Bank (PDB) and/or molecular dynamics simulations. The structures for the ensemble screening should be as diverse as possible to cover the conformational space.

Recent advancements in structure prediction methods, such as AlphaFold, were anticipated to address this challenge by providing more precise models. However, it has been observed that the predicted structures exhibit a bias toward the conformations that are present in the PDB. Recently, a methodology referred to as multi-state modeling was presented, which furnishes a template of a specific conformational state to AlphaFold2 to facilitate the attainment of a desired structure. In this talk, the results of the multi-state modeling will be presented and discussed in the context of structure-based drug discovery for kinases, a prominent target family characterized by its diverse structural complexity.

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L17

Discovery of E3 Ligase Ligands for Muscle Specific Protein Degradation

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Current PROTAC development relies on two E3 ligases, cereblon (CRBN) and von Hippel-Lindau (VHL), for targeted protein degradation (TPD). However, this reliance presents several challenges. First, the expression levels of CRBN and VHL vary depending on tissue types, making it difficult for current PROTACs to effectively degrade the desired target proteins in all cases. Additionally, unintended protein degradation in non-target tissues by PROTACs may lead to side effects. Another recently reported issue is the development of drug resistance during disease treatment with PROTACs. To overcome this drug resistance, the discovery of various E3 ligase ligands is necessary. Beyond the known CRBN and VHL ligands, more than 600 E3 ligases exist in cells, with expression patterns that vary depending on human cells, tissues, and diseases. Therefore, identifying E3 ligase ligands specific to certain tissues or pathological cells can facilitate the development of tissue-, cell-, and disease-specific targeted protein degradation (TPD). The discovery of such novel E3 ligase ligands will serve as an important foundation for the development of next-generation therapeutics for intractable diseases using PROTAC technology.

In this study, we successfully identified a muscle-specific E3 ligase ligand, c-EN10, through virtual screening and developed a muscle-specific degrader based on it. The c-EN10-based PROTAC demonstrated excellent muscle-specific protein degradation efficacy at nanomolar concentrations. Muscle-specific protein degraders are expected to be effectively utilized in the treatment of muscle-specific diseases, such as sarcomas.

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L18

Multilevel Frameworks for Computational Studies of Biomolecular Systems

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Proteins, which constitute over 50% of dry cell mass, are essential to nearly all biochemical processes, with their functionality depending on correct folding into native structures. Accurately simulating their structural dynamics over biologically relevant timescales remains beyond the reach of quantum mechanical methods. To address this challenge, mechanical force fields and coarse-grained models have been developed to reduce the number of interaction sites. While advances like AlphaFold have improved native structure prediction, understanding thermodynamic stability and folding pathways remains a major challenge.

This work introduces multilevel computational frameworks that integrate diverse theoretical approaches to analyse protein energy landscapes. A key development is the lwONIOM library, a freely accessible, multilevel, multicentre tool enabling accurate and efficient analysis of complex proteins over extended timescales. Applied to the bovine pancreatic trypsin inhibitor (BPTI), lwONIOM achieves a balance between computational cost and precision, providing deep insights into structural stability and dynamic behaviour [1].

In parallel, the UNitedRESidue (UNRES) coarse-grained potential was integrated into the Cambridge energy landscape software, enabling efficient modelling of large biomolecular structures. This method preserves accuracy while substantially reducing computational cost, yielding results consistent with experiment and all-atom models [2]. UNRES includes a dynamic disulphide bond potential, allowing bond formation and breakage to be explored within a single landscape, and has recently been extended to model explicit lipids within the UNICORN framework.

Additionally, we applied machine learning potential, coarse-grained energy landscape searches, and structural analysis to study amyloid monomers associated with Alzheimer's disease [3]. These multilevel frameworks thus provide robust tools for the study of complex biomolecular systems, with potential applications in drug discovery and health-related research.

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L19

Anti-microbial peptides

Steven Gross[1]
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Anti-microbial peptides help protect the body from bacterial invasion, and frequently work in conjunction with histones to kill bacteria.

I will discuss this synergistic function and highlight how specific adaptations allow both better efficacy and decreased self-harm.

L20

Effect of Multitorsional Potentials on Modeling the Structures of α-, β- and α+β-Proteins with the UNRES Coarse-Grained Force Field

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Despite the recent strong progress of knowledge-based methods in the protein folding prediction [1], physics-based approaches are still valuable for their insights on physical nature of intra- and intermolecular protein interactions. Simulations should account for numerous factors, such as hydrogen-bonding, local interactions, hydrophobic interactions, steric repulsion, to name a few. Due to high computational costs, physics-based all-atom models are limited to simulations of small proteins, while coarse-grained models, which combine atomic groups into sites, are enabling simulations of big systems. Recently, new cooperative terms corresponding to the coupling between local conformational states of consecutive residues were proposed by our research group based on the scale-consistent theory of coarse graining [2].

In the current study we implemented the new multitorsional terms recapturing helical [3] and extended turn-bordered patterns to UNRES coarse-grained force field. The new potentials were parametrized using maximum likelihood approach based on the training set of 1,092,517 helical segments and 190,577 extended turn-bordered segments of protein structures of the Protein Data Bank. The new approach was assessed by *ab initio* multiplexed replica-exchange molecular dynamics simulations of the 28 α-, 24 β-, and 24 α+β-proteins with size from 20 to 126 residues and with different topologies. Simulations were performed without the secondary structures constrains, the ``state-of-the-art'' UNRES simulations were used as reference. Introduction of the new terms resulted in a remarkable improvement of the first (top-probability) models for 15 studied proteins, (ΔGDT_TS>5), observed improvement is the result of enhancing of the α-helices and β-sheets and the fragments following their ends, thus enabling correct folding. The improvement is observed for the whole distribution of GDT_TS for the modeled structures. The most significant improvement (by more than 30 GDT_TS units) was obtained for the 1K40 protein, which has never been predicted correctly by the previous versions of UNRES.

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L21

The Distortion-Push Mechanism for the γ subunit rotation in F_1-ATPase

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F_1-ATPase is a cytoplasmic part of the F_oF_1 ATP synthase and produces ATP in the cell. It consists of the stator $\alpha_3\beta_3$ subunits, and the rotator γ subunit can work as a rotatory molecular motor without F_o and other subunits in F_1. The relationship between the γ-subunit rotation and conformational changes of the stator $\alpha_3\beta_3$ is essential to understanding the chemo-mechanical coupling in F_1-ATPase. Though F_1-ATPase has long been studied structurally and functionally, recent structures determined with cryo-electron microscopy (cryo-EM) provided new insight into the γ-subunit rotation mechanisms.

We utilized these recent cryo-EM structures and conducted extensive molecular dynamics (MD) simulations with enhanced sampling and free-energy analysis. Our simulations include target MD simulations, the mean-force string method simulations, and the umbrella sampling. The last two simulations involved 64 copies of the original system, each comprising more than 500,000 atoms, and were conducted using the GENESIS software on the supercomputer Fugaku. The results from our simulations and analysis of the 80° substep rotations of the γ-subunit are summarized in the distortion and push model, which indicates that the distortion of the stator α3β3 subunits occurs during the early process. Then, the β-subunit interacts directly with the γ-subunit to complete the substep rotation. This result can resolve a long-term debate on the rotation mechanisms in F_1-ATPase, such as the steric hindrance vs the electrostatic interaction, and explain the existing experimental data.

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L22

Structure-based protein design for simutaneousoptimisation of enzymatic activity and immunogenicity

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Neoantigens are ideal targets for cancer immunotherapy because they are expressed de novo in tumor tissue, but not in healthy tissue, and are therefore recognized as foreign by the immune system [1]. Advances in next-generation sequencing and bioinformatics have enabled the rapid identification and prediction of tumor-specific neoantigens; however, only a small fraction of predicted neoantigens are immunogenic. To improve the accuracy of immunogenic neoantigen predictions, we developed in silico neoantigen prediction workflows incorporating the physical binding of pMHC [2], paired with T cell receptors (TCRs) from putative tumor-reactive $CD8^+$ tumor-infiltrating lymphocytes (TILs) through deep learning [3]. We validated these workflows both in vitro and in vivo in patients with hepatocellular carcinoma (HCC) and in a B16F10 mouse melanoma model. The predictive accuracy of the pipeline was confirmed in a validation cohort of 8 patients with HCC. Of 118 predicted neoantigen candidates, 48 peptides were selected. In vitro validation revealed that 13 of these 48 peptides were immunogenic. In vivo assessment of the antitumor efficacy of the candidate neoepitopes in the mouse model showed that vaccination with the predicted neoepitopes induced neoantigen-specific T cell responses and promoted the trafficking of neoantigen-specific $CD8^+$ T cell clones into tumor tissue, leading to tumor suppression. This study demonstrates that integrating tumor-reactive TIL TCR-pMHC ternary complex data improves the prediction of immunogenic neoantigens.

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L23

Conformational freedom and binding affinities of selected glycopeptide antibiotics

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In this study four glycopeptide antibiotics (dalbavancin, teicoplanin, telavancin and oritavancin) are compared to vancomycin – a glycopeptide antibiotic used in the treatment of serious and multidrug resistant infections caused by Gram-positive bacteria. Vancomycin is composed of a disaccharide (D-glucose and vancosamine) and a heptapeptide core. Dalbavancin, telavancin and oritavancin are semisynthetic derivatives of vancomycin, teicoplanin is a naturally occurring antibiotic with a structure similar to that of vancomycin.

These five antibiotics bind to D-alanyl-D-alanine stem terminus on the bacterial cell wall peptidoglycan precursor [1]. This binding inhibits cross-linking between stem peptides which prevents bacterial cell wall synthesis.

Unrestricted molecular dynamics (MD) run in explicit water in AMBER [2] on complexes of selected antibiotics and their homologs (five homologs of dalbavancin and teicoplanin) with natural bacterial peptidoglycan representative (Ala-D-iGlu-Lys-D-Ala-D-Ala pentapeptide) made it possible to identify conformational differences and preferred conformations in each of the antibiotics.

MD trajectories and resultant structures were analyzed in detail and advantages and disadvantages of conformational restrictions resulting from structural modifications were discussed.

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L24

Chemical Framework for Understanding Protein Condensation in Neurodegenerative Diseases

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The study of non-bonding interactions has transcended the exclusive domain of physical chemists employing spectroscopy and computer simulations. With the advent of molecular biology, non-bonding interactions have emerged as pivotal factors in comprehending the structures and functionalities of biomolecules, including DNA and proteins. Among these non-bonding interactions, ionic interactions stand out as the most robust forces mediating interactions between anionic and cationic molecules.

When scrutinizing the intracellular milieu, non-bonding interactions, particularly those of the ionic nature, wield significant influence over protein-protein and DNA-protein interactions. Consequently, we hypothesized that protein aggregation or phase separation, known contributors to neurodegenerative diseases such as Alzheimer's, Parkinson's, and Lou Gehrig's diseases, may also be governed by these ionic interactions. Given the highly charged nature of disease-related proteins, a substantial charge disparity exists, making self-aggregation in the absence of cofactors a formidable challenge. Our research has yielded a compelling insight: small (negatively or positively) charged biomolecules play a pivotal role in facilitating the formation of protein condensates through ionic interactions within cellular environments.

L25

Metal–Ligand Coordination Compounds in the Fight Against Therapeutic Resistance

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The growing threat of antimicrobial resistance (AMR) and the increasing incidence of hard-to-treat cancers, especially those resistant to existing therapies or diagnosed at advanced stages, are among the most pressing problems facing medicine today. Traditional small-molecule drugs often fall short, and the limited progress in developing entirely new classes of antibiotics further complicates the situation.

As part of our recent work, we have been investigating the biological activity of coordination compounds formed by *d*-block metal ions such as Rh(III), Ru(III), Os(III), Ir(III), and Cd(II) with selected bioactive ligands. Recently, we observed that one of the coordination compounds, based on Rh(III) and bipyridyl sulfonamide, demonstrated encouraging selectivity, as it was cytotoxic to breast cancer cells (SKBr3) but had little effect on healthy epithelial cells (HB2). Mechanistic studies suggested it affected mitochondrial function and interfered with Rac1/VASP signaling pathways linked to cancer cell survival and migration.

We have also turned our attention to a cadmium(II) coordination compound with carboxyimidazole-derived ligand, which appears to have selective biological effects. Early findings suggest its antifungal activity, as well as distinct interactions with microbial cells that distinguish it from traditional antimicrobial agents.

Altogether, these studies reinforce the idea that coordination compounds of transition metal ions, especially when paired with thoughtfully designed ligands, can offer new therapeutic strategies for overcoming resistance in both cancer and infectious diseases.

L26

Experimental and theoretical approaches to investigate the inhibition of neutrophil serine proteases by pharmacological targeting of cathepsin S

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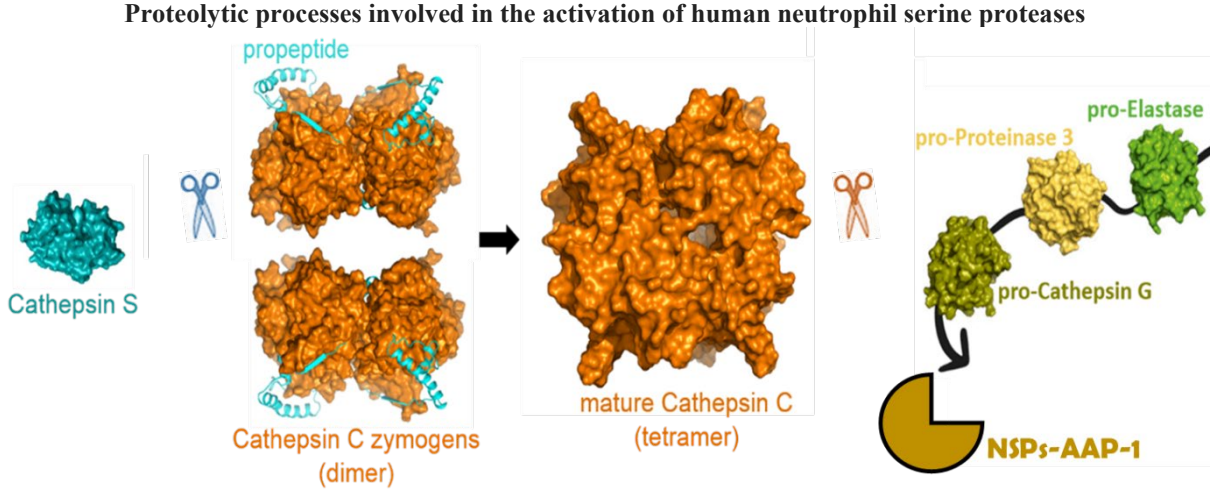
Neutrophil serine proteases (NSPs, elastase, proteinase 3 and cathepsin G) are synthesized as inactive zymogens (proNSPs) containing an N-terminal dipropeptide, which maintains the zymogen in its inactive conformation and prevents premature activation. Activation of proNSPs occurs through the removal of the N-terminal dipropeptide by a cysteine protease called cathepsin C (CatC) (responsible for ~90% of proNSP activation) and NSPs-AAP-1 (responsible for ~10% of proNSP activation). An uncontrolled activity of NSPs contributes to neutrophil-mediated inflammatory diseases. Therefore, CatC represents a promising pharmacological target in NSP-mediated diseases. CatC is first synthetized as an inactive zymogen containing an intramolecular chain propeptide, the dimeric form of which is processed into the mature tetrameric form by proteolytic cleavages.

We showed that cathepsin S (CatS), a potent elastinolytic enzyme that has been identified as a therapeutic target in chronic inflammation-associated tissue injury, is the physiologic converting protease of human proCatC in neutrophilic precursor cells. We further established a proof of concept for the indirect inhibition of NSPs by pharmacological targeting of CatS during human neutrophil differentiation using a nitrile inhibitor, **IcatS**$_{\#54}$ (Domain et al., 2024, *BiochemPharmacol*). Preventing ofproNSP activation by a CatS inhibitor, alone or in combination with a CatC/NSPs-AAP-1 inhibitor, therefore represents a promising therapeutic approach to efficiently control the extend of tissue damage in neutrophil-mediated chronic inflammatory and auto-immune disorders. Molecular modeling analysis of **IcatS**$_{\#54}$ analogs, with different pharmacomodulations at the P3 position for exposition in the bone marrow will be presented.

Proteolytic processes involved in the activation of human neutrophil serine proteases



L27

Practical insights from CASP15: Guidelines for RNA structure refinement using molecular dynamics

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The application of molecular dynamics (MD) simulations for enhancing computationalRNA structure predictions has gained attention, though optimal implementation strategiesremain poorly defined. We conducted a comprehensive evaluation using 61 diverse RNAmodels from the CASP15 assessment [1], employing Amber simulations with the ff99bsc0χOL3RNA forcefield [2].

Across 61 models representing diverse targets, we find that short simulations (10–50ns) can provide modest improvements for high-quality starting models, particularly bystabilizing stacking and non-canonical base pairs. Conversely, poorly predicted structuresdemonstrated limited improvement and frequently degraded during simulation. Extendedsimulations beyond 50 ns consistently led to structural drift and decreased accuracy.

Based on these findings, we provide practical guidelines for selecting suitable inputmodels, defining optimal simulation lengths, and diagnosing early whether refinement is viable.MD functions most effectively as a refinement technique for near-native RNA structures ratherthan a general correction mechanism. This work establishes practical frameworks to guide theRNA modeling community in effective MD implementation. Overall, MD works best for fine-tuning reliable RNA models and for quickly testing their stability, not as a universal correctivemethod.

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L28

Testing CABS-flex 3.0 for Protein–Protein Contact Dynamics: The Case of the IL-1 Receptor–Antagonist Complex

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CABS-flex 3.0 is the latest release of our multiscale simulation framework for exploring protein structural flexibility, featuring enhanced control of flexibility restraints, improved all-atom reconstruction, new peptide modeling options, and extended analysis tools [1]. Among its important new features is the ability to conveniently analyze the flexibility of protein–protein interfaces and their dynamic contact networks.

We benchmarked this functionality against extensive (1-μs) all-atom molecular dynamics (MD) simulations of the interleukin-1 receptor (IL-1R1) and its antagonist IL-1Ra, a key regulator of inflammation [2]. While crystallographic studies showed that IL-1Ra does not engage all receptor regions [3], MD revealed that it reshapes the receptor's energy landscape: the unbound receptor samples mainly one conformation, whereas the antagonist stabilizes an alternative inactive state through a two-state equilibrium. This supports a conformational-selection mechanism, with IL-1Ra locking IL-1R1 into a signaling-inactive form without undergoing significant structural changes itself.

Importantly, CABS-flex 3.0 reproduced residue-level flexibility patterns and interfacial contact networks observed in MD, confirming its utility for capturing receptor–antagonist dynamics at a fraction of the computational cost. Beyond this case study, the approach is now being systematically tested on a broader set of protein–protein complexes to further assess its applicability.

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L29

Advancing RNA structure prediction: Lessons from benchmarks and community challenges

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Accurate RNA structure prediction is increasingly recognized as essential for understanding biomolecular function and guiding therapeutic design. Here, we report systematic evaluations of state-of-the-art RNA 3D prediction methods, informed by benchmarking studies and participation in international community challenges, including RNA
Puzzles, CASP15, and CASP16.

Benchmarking standalone tools revealed clear differences between machine-learning and physics-based approaches: ML-based methods excel in predicting overall RNA folds, whereas physics-based approaches, particularly when supported by experimental restraints, better capture local interactions and fine structural detail. Notably for drug-design applications, ligand-binding site geometry often remains accurate even when the global model is of moderate quality [1].

Results from recent community challenges highlight substantial progress in RNA structure prediction. In CASP16, our LCBio group achieved a first-place ranking in multimeric RNA prediction, with models explicitly commended for their accurate RNA–RNA interface modeling [2]. Together with systematic method evaluation, these achievements offer practical guidance for selecting computational strategies tailored to specific research goals, while also outlining the current frontiers and remaining challenges in RNA structure prediction.

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P01

Impact of glycosaminoglycans binding on proteins fluctuations in ff14sb/GLYCAM06j and CHARMM36m force fields

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Glycosaminoglycans (GAGs) are negatively charged, linear polysaccharides present in the extracellular matrix and lysosomes, where they play key roles in protein function through electrostatic interactions[1]. Due to their structural and electrostatic complexity, modeling GAG-containing systems with *in silico*approaches remains challenging. This study evaluates the predictive performance of two force fields: ff14SB/GLYCAM06j[2] and CHARMM36m[3] by examining three experimentally validated protein-GAG complexes: FGF-2 HP dp6 (PDB ID: 1BFC), FGF-1 HP dp6 (PDB ID: 2AXM) and Cathepsin K-C4-S dp6 (PDB ID: 4N8W).The protein-GAG interaction trajectories obtained using AMBER and CHARMM force fields were analyzed with a focus on conformational dynamics, particularly root-mean-square fluctuations (RMSF)[4] in both bound and unbound protein states. RMSF was calculated across all simulation frames and atomic positions, and the results were compared to experimental B-factors. Simulations were based on protein-GAG complex and their unbound counterparts: FGF-2(PDB ID: 1BFG), FGF-1(PDB ID: 2K43) and Cathepsin K(PDB ID: 5TUN). Findings indicate that AMBER more accurately captures overall flexibility changes upon GAG binding. However, both force fields tend to overestimate loop flexibility, which affects RMSF accuracy. Because visual assessment of fluctuation patterns can be misleading, statistical comparison with experimental data was performed. Correlation analyses suggest that while AMBER better reflects general structural flexibility, CHARMM offers slightly more precise predictions of GAG-induced changes.

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P02

Exploring the Binding Affinity of Alkyl Gallates to Human Albumin

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Human serum albumin (HSA) is an abundant protein in the blood plasma, which principal role is transportation of a wide variety of compounds, such as fatty acids, phenolic compounds and drugs. There are nine known fatty acids binding sites in albumin [1]. Previously fluorescence experiments for alkyl gallates, that are gallic acid derivatives, showed significant increase in emission upon complexation with HSA. In this study, we investigated the interaction of these gallates and natural fatty acids, with HSA, using *in silico* approaches. The molecular dynamics simulations showed that these alkyl gallates interact with the known fatty acid bind sites of HSA, in particular FA1, FA5 and FA6.

The affinity of interactions were quantified by the free binding energy calculations performed with MMGBSA [2] and Linear Interaction Energy [3] methods. The gallates with longer chain (octyl (G08) and dodecyl gallate (G12)) showed strongest interactions. Concerning the van der Waals energy contribution of G08 and G12 they are comparable to natural fatty acids, which may be related to the more abundant hydrophobic interactions with the protein, which is consistent with the long alkyl chains of gallates. To analyze the energetic barrier required to displace the ligands, we used umbrella sampling and potential of mean force [4], and the results obtained showed that the energy required is approximately 3 to 4 kcal/mol, which indicates stable interaction with the protein, particularly with FA5.

With these results, we have gained important insights for the fatty acid binding sites of albumin, and how gallates are capable to interact with these sites with high affinity.

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P03

EXPLORING PROTEIN INTERACTIONS IN THE P53 PATHWAY: POTENTIAL BIOMARKERS AND THERAPEUTIC TARGETS IN CANCER

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The tumor suppressor p53 is a central regulator of cellular stress responses and is frequently inactivated in human cancers. This study investigates the intricate network of p53 protein-protein interactions (PPIs), with a focus on key partners involved in DNA repair, apoptosis, and cell cycle regulation. A primary objective is to identify alternative regulatory proteins to MDM2 that exhibit structural or functional similarities but may circumvent the resistance and toxicity issues often associated with MDM2-targeted therapies [1].A comprehensive bioinformatics workflow was implemented, incorporating molecular docking, molecular dynamics simulations, and mutational analysis to assess the stability and modulation of p53 through its interactors. Negative regulators such as TOPBP1, TOPORS and BCL2 were found to attenuate p53 function, whereas activators like ATF3, ATM and CHEK1 contributed to its stabilization and activation. The effects of cancer-associated mutations and environmental conditions on these PPIs were also evaluated to understand context-specific behavior [2].Further, docking analyses using experimentally validated ligands/drugs revealed critical binding residues and highlighted the pharmacological role of several p53-interacting proteins using Autodock4 [3] . These findings were complemented with mutational hotspot data and structural refinement, offering mechanistic insights into protein-ligand interaction dynamics and domain-specific p53 regulation. This integration of structural and computational analysis uncovers novel regulatory mechanisms within the p53 interactome. It can open promising therapeutic avenues for reactivating p53 function in tumors, particularly those with intact but functionally suppressed p53 pathways.

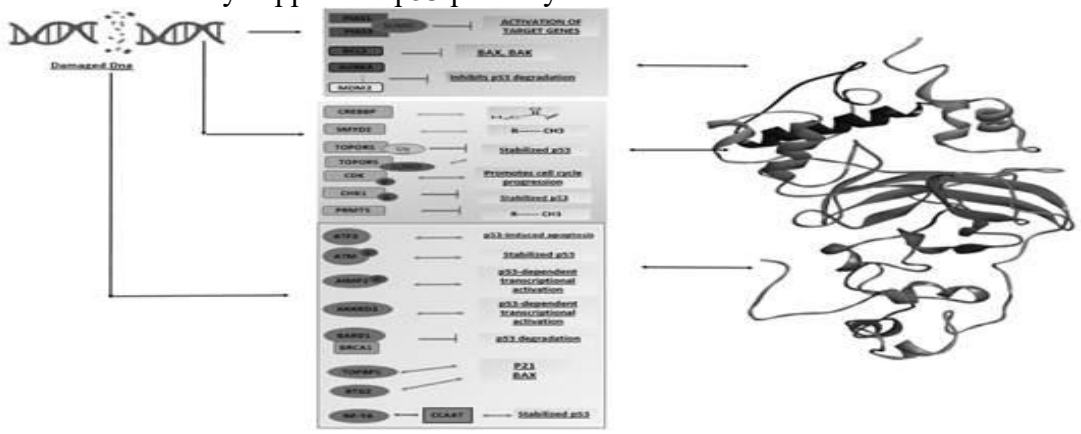

Figure 1: The figure shows interactions of different proteins to p53 and its functionality and mechanism in context to p53

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P04

Molecular bushings: Predicting the structure of different LP-rings

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Flagellar motors are pieces of molecular machinery build by a number of different proteins. Their structure is quite similar to regular electric motors [1]. The stator converts the potential energy of a proton or ionic gradient into kinetic energy – the rotation of the rotor [2]. Also, similarly, to minimize the "friction" between the rotor and the biological membrane, bushings are put in place. The bushings consist of two rings: the L-ring and the P-ring, interacting with one another [3]. The different flagellar motors have different sizes and structures of those rings.

In my work I am using a computational approach to construct all-atom models of the LP-rings of several bacterial species. I am using existing cryo-electron microscopy maps, known protein assemblies to fit protein models/structures to them. The process consists of protein-protein docking and protein-protein cyclic docking assays. With the obtained models I'm able to predict the conformations of those molecular bushings of different symmetries. With the information from the CRYO-EM map, I'm able to assess the probability of each symmetry.

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P05

Molecular mechanism of recognition of the herpesviral protein UL49.5 by Kelch domain-containing protein 3 - molecular dynamics study

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UL49.5 is a transmembrane protein from the bovine herpes virus 1 (BoHV-1) conserved throughout the Herpesviridae family. UL49.5 binds to the transporter associated with antigen presentation (TAP) and blocks transport of the viral peptides from the cytosol into the cytoplasmic reticulum. In consequence, the viral antigenic peptides are not loaded onto major histocompatibility complex class I (MHC I) and not presented to the cytotoxic CD8+ T-cells. Moreover, UL49.5 also triggers TAP degradation via recruiting an E3 ubiquitin ligase CRL2 to TAP. The first, crucial step of this process is recognition of the UL49.5 by Kelch domain-containing protein 3 (KLHDC3) being a substrate receptor of CRL2. It has been determined that the mutations within the C-terminal ^{93}RGRG^{96} sequence of UL49.5 affect binding of UL49.5 to KLHDC3. The wild type UL49.5 and its R95K mutant (RGKG) both bind KLHDC3, while R93K (KGRG) and G96D (RGRD) mutants do not.

The molecular docking and subsequent molecular dynamics (MD) simulations of KLHDC3 in complexes with four UL49.5 C-terminal decapeptides (one native protein and three mutants) revealed the activity is closely correlated with the conformation which they adopt in KLHDC3 binding cleft. Moreover, to analyze the interaction between the wild type UL49.5 and KLHDC3 in detail, long MD of the UL49.5-KLHDC3 complexes embedded into the fully hydrated all-atom lipid membrane model has been performed. The network of polar interactions has been determined to be responsible for the C-degron recognition and binding in KLHDC3. Among these residues, the most important has been declared the "RSR triad".

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The computations were carried out using the computers of Centre of Informatics Tricity Academic Supercomputer & Network.

P06

In silico structure prediction and binding mechanism assay of lysin-motif-containing protein of *Medicago truncata*, critical for arbuscular mycorrhiza

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As advances in the understanding of the genes responsible for various interactions between organisms progress, it is important that analogous advances in the understanding of the mechanisms of those interactions follow. Among these interactions, mycorrhiza is worthy of note as the interaction which nutritionally underpins a significant majority of ecosystems based on the productivity of land plants. In this process, plants exchange nutritious products of photosynthesis in return for mineral salts and other soil-assimilated substances from fungi.

Among the types of mycorrhiza observed in contemporary plants, arbuscular mycorrhiza is present in the vast majority of species. In it, fungal cells intrude into plant root cells, creating arbuscules – branching structures which maximise the area of contact between the hyphae and roots. In order to establish such a connection, biochemical communication must take place first.

At least in some cases, this communication is dependent on proteins containing lysin motifs (LysM). They have been known to be secreted by fungi, but it has been established recently that arbuscular mycorrhiza is not formed or formed less reliably in the absence of LysM proteins secreted by the plant [1]. As such, LysM proteins are revealed as another key component in arbuscular mycorrhiza formation.

Although the genetic sequences of the proteins in question are typically well-known, their structure and binding mechanism are less so. Therefore, in this work, the sequence of a LysM protein from *Medicago truncatula* was used as the basis for the construction of a potential structure by use of *in silico* methods. Afterwards, a molecular dynamics simulation was conducted using the predicted structure, examining the interaction between the protein and a chitin-derived molecule to observe the formation of the expected protein-chitin complex and determine the mechanism of molecular recognition.

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P07

How glycosaminoglycans mediate procathepsin K maturation: new insights from computational studies

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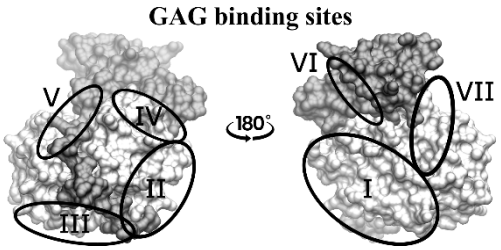
Procathepsin K is the precursor of cathepsin K, a key proteolytic enzyme involved in bone resorption and extracellular matrix remodeling. The allosteric regulation of cathepsin activity by glycosaminoglycans (GAGs) has recently gained scientific interest, yet its exact mechanisms remain unclear. Overexpression or overactivity of cathepsin K is linked to serious health conditions, including osteoporosis, pycnodysostosis, and various tumors, primarily due to its role in bone degradation. The maturation of inactive cathepsin forms is crucial for their activation at the right time and place. GAG binding can induce conformational changes that influence propeptide flexibility, either accelerating maturation or stabilizing structures where the proregion blocks the active site. However, little is known about the allosteric regulation of procathepsin K by GAGs.



The aim of this study was to identify the most promising glycosaminoglycan candidates from an initial set with varying chain lengths, charges, sulfation patterns, and disaccharide units, as well as their corresponding binding sites, that form the most stable complexes with procathepsin K. The set of glycosaminoglycan binding poses obtained from docking was narrowed down to a few of the most promising ones with potential significance for the allosteric regulation of procathepsin K activity. By performing PCA analysis of 1 ms molecular dynamics simulations, we wanted to check whether the best candidates from first stage 100 ns MD simulations,are able to induce conformational change of the proregion of procathepsin K upon binding. To quantify the effect of GAG binding on the conformational transition of procathepsin K leading to exposure of the active site, we performed Umbrella Sampling simulations combined with Potential of Mean Force (PMF) calculations with the reaction coordinate defined as the distance between Cα atoms of selected cathepsin and propeptide residues. Additionally, 100 ns MD simulations were conducted on complexes of procathepsin K with an accessible active site, generated using the UNRES coarse-grained model.

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P08

Computational design of inhibitors of the HVEM/LIGHT complex based on LIGHT protein-binding fragments

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HVEM (Herpesvirus Entry Mediator) and TNFSF14/LIGHT (Tumor Necrosis Factor Superfamily member 14) are immune checkpoint proteins that play an important role in regulating immune responses. Their interaction triggers T cell activation, proliferation, differentiation, and cytokine secretion. Sustained expression of LIGHT and persistent T cell stimulation may contribute to chronic inflammation And thus to the development of autoimmune diseases. In this work, we aimed to interfere with HVEM/LIGHT complex formation using peptide-based inhibitors.

Our approach relied on the design of peptides modelled from binding structural motifs within LIGHT protein, with the goal of selectively binding to HVEM and preventing complex formation. Compounds were based on the crystal structure of the HVEM/LIGHT complex (PDB ID: 4RSU). For the designed compounds, we carried out molecular dynamics (MD) simulations 200 ns long and, for selected systems, extended simulations to 1000 ns. Binding affinities were evaluated using MM-GBSA (Molecular Mechanics Generalized Born Surface Area) analysis, and per-residue and pairwise energy decomposition was performed to determine the individual energy contributions of amino acids. Thermodynamic properties, such as entropy contributions to complex stability, were also calculated. In addition, Steered Molecular Dynamics (SMD) simulations were applied to selected peptide–protein systems to probe binding strength, dissociation pathways, and intermolecular contacts.

This computational strategy led to the identification of peptide candidates with strong binding potential towards HVEM, providing a promising basis for the development of innovative immunomodulatory therapeutics.

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P09

Development of Improper Torsional Potentials for Coarse-Grained Simulations of Amino-acid Racemization

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Improper torsional potentials can describe the racemisation of amino acid residues at the course-grained level [1]. The potential of mean force (PMF) for a serine residue from its ketone form to its enol form as an intermediate state to the racemisation of the serine residue have been determined and the respective terms in the UNRES coarse-grained force fields parametrisation [2]. To achieve this objective, we conducted quantum mechanics (QM) calculations at the PM7 level to investigate the enolisation and to determine the PMF associated with the keto-enol conversion of serine.

We started from the reaction path of enolisation determined in our earlier study [1]. We defined the coarse-grained coordinates as the Cα...Cα...Cα virtual-bond angle θ, and the angles α and β of the spherical coordinate system that define the location of the side-chain center with respect to the Cα...Cα...Cα frame. We sampled the configurational space around each point of the reaction path, obtaining a total of 256254 points. The Boltzmann sums at T=298 K were calculated in the three-dimensional bins in the angles and the PMF was obtained by Boltzmann inversion.

The obtained PMF surface was used to parameterise the analytical expressions for the improper torsional potentials of the UNRES coarse-grained model of proteins. Including the potentials in UNRES will enable us to study peptide-residue racemisation, which is important in, e.g, the aging of human crystallins.

The optained improper- torsional potentials were implemented in UNRES.

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P10

De novo design of peptide binders with generative deep learning and molecular dynamics simulations

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Designing peptides with high affinity for a given region on a protein surface is a significant challenge. The goal of this project is to develop a versatile method for rapidly generating peptide sequences with affinity for a manually defined region on a protein surface. In this work I present a method for generating peptide sequences based on transformer – a deep learning architecture originally designed for natural language translation – and a method for filtering the generated sequences based on their properties, largely based on molecular docking by coarse-grained replica-exchange molecular dynamics simulations using UNRES-Dock [1][2]. To demonstrate the method's capabilities, I designed five peptide sequences with potential affinity for the Programmed cell death protein 1 (PD-1), an important molecular target in several anticancer therapies.

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P11

Smoothing MD: Energy Landscapes without Geometry Optimisation

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We introduce an open-source software that converts molecular dynamics (MD) trajectories into disconnectivity graphs [1][2], offering a compact and interpretable summary of explored energy landscapes. The method applies Savitzky–Golay smoothing [3] to per-frame thermodynamic traces (potential energy in NVE/NVT or enthalpy in NPT), identifies local extrema as proxy minima and transition states, and generates graph-ready outputs on the fly, without geometry optimisation or saddle searches. It is ensemble-agnostic and works for both all-atom and coarse-grained simulations. On benchmark systems spanning chemical space, our approach processes 10^4–10^5 frames in seconds on a standard laptop, reproducing the branching topology of conventional quench-and-search workflows from the Cambridge energy-landscape framework [4][5] while achieving orders-of-magnitude speedups. The resulting graphs capture thermally broadened basins and pathways sampled under the chosen simulation protocol, providing a readily interpretable view of conformational hierarchies and kinetic connectivity. By detecting extrema from the trajectory,wevisualise the organisationof the explored landscape only.

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P12

Benchmarking UNRES Simulations of Intrinsically Disordered Proteins by Tuning the WSCP Parameter

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Intrinsically disordered proteins (IDPs) play vital roles in regulation and disease, but their high structural flexibility makes them challenging to characterize. In this study, we benchmarked the UNRES coarse-grained model for nine IDPs (Hst5, ACTR, Sic1, A1LCD, CoINT, K19, Nup153, p53, and α-synuclein). Simulations were performed at 300 K using two UNRES force fields, OPT-WTFSA-2 and NEWCT-9P, each with three trajectories per system and different random seeds.

To refine agreement with experimental data, we systematically varied the WSCP parameter from 1.3 to 2.5 for NEWCT-9P force field and from 1.3 to 2.0 for OPT-WTFSA-2 force field. For each trajectory, the radius of gyration (Rg) was computed and averaged, and model accuracy was quantified by the mean absolute error (MAE) relative to reference experimental values.

Our results show that the default WSCP values systematically overestimated Rg, but tuning WSCP significantly improved accuracy. Optimal WSCP values were force field– and protein–dependent, with the lowest MAEs observed around WSCP 1.7 for NEWCT-9P force field and 1.6 for OPT-WTFSA-2 force field. This demonstrates that parameter tuning can enhance UNRES performance for IDPs and provides a pathway to more reliable coarse-grained modeling of flexible proteins.

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P13

Efficient Superposition of Like-Molecule Assemblies without Exhaustive Permutation Search

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Measures of the similarity of the structures of a given molecule or a molecular assembly are necessary to dissect the ensembles obtained in molecular simulations or generated by bioinformatics approaches (e.g., MassiveFold[1]) into families of similar structures and to compare the representative structures with their reference (usually experimental) counterparts. These measures are widely implemented in the Community-Wide Experiments on the Critical Assessment of Techniques for Protein Structure Prediction (CASP)[2].However, superposing such assemblies is computationally challenging, as it typically requires evaluating all N! permutations of molecule-to-molecule assignments to determine the minimum root mean square deviation (RMSD). This becomes rapidly intractable even for moderately sized systems. In our work[3], two novel algorithms were developed that address this challenge without resorting to exhaustive permutation. The first, Like-Molecule Assembly Distance Alignment (LMADA), performs an initial mapping based on grid search in quaternion space, followed by rigid body alignment using Singular Value Decomposition. The second, Like-Molecule Assembly Gaussian Distance Alignment (LMAGDA), minimizes a Gaussian-weighted distance-based objective function using the LMADA orientation as a starting point. Both methods scale quadratically with the number of molecules ($O(N^2)$) and achieve RMSDs comparable to the best permutation-based alignments. LMADA is effective at minimizing global RMSD, while LMAGDA better preserves local geometric features of the assemblies, making it particularly useful for identifying conserved structural motifs in biomolecular condensates or fibrillar aggregates. These tools open the door to high-throughput structural comparison of simulated and experimental assemblies, and may facilitate the classification of aggregation patterns in phase-separating peptides or amyloid-forming systems. Their implementation supports ongoing efforts in rational peptide design and the interpretation of biomolecular condensation.

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P14

Combination of OGF Activity and Topoisomerase II Inhibitors in Novel Peptide Conjugates with Anticancer Potential

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers, characterized by high mortality and limited therapeutic options. Difficulties in its treatment arise mainly from late diagnosis, drug resistance, and the toxicity of commonly used chemotherapeutics. One promising approach may involve the use of the opioid growth factor (OGF), an endogenous pentapeptide which, together with the OGFr receptor, regulates the cell cycle by inhibiting cancer cell proliferation [1], [2], [3]. The aim of the ongoing project is to develop new therapeutic strategies based on OGF analogs with enhanced stability, as well as conjugates that combine two anticancer mechanisms: inhibition of proliferation through the OGF–OGFr axis and inhibition of topoisomerase II—an enzyme crucial for maintaining genome integrity, whose excessive activity promotes DNA fragmentation and chromosomal instability. Stability tests in human plasma demonstrated an extended half-life of selected analogs. Preliminary enzymatic and cellular assays indicate their potential efficacy. Computational modeling in an aqueous environment enabled the determination of the spatial arrangement of the obtained constructs and the prediction of their interactions with the OGFr receptor.

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P15

Structure-Guided Disruption of MAD2 Binding in Cytomegaliavirus IE2 via MD and MM-GBSA Analyses

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Cytomegalovirus is one of the most commonly found viruses in the human population. Around 70% to 100% of the population is reported to be infected with HCMV/HHV-5 [1]. Upon infection, it becomes latent in the human body most of the time. While for most people the infection is asymptomatic, it is also suspected to play a role in the growth and spread of cancer cells in the human body, possibly also playing a part in crossing the blood-brain barrier [2]. Intermediate early protein (IE1) is suspected to play a key role in the HCMV life cycle [3]. It participates in transactivating viral early genes and activating its own promoter. Its expression is believed to be essential for lytic infection and reactivation from the latency state. However, the binding partner of IE1 responsible for binding the centromere remains unknown.

The aim of our work is to explore the suspected interaction between the aforementioned protein and human MAD2 protein, a component of the spindle-assembly checkpoint that prevents the onset of anaphase until chromosomes align on the metaphase plate.

First, we wanted to establish if the described interaction takes place, using full-atom molecular dynamics, employing HADDOCK for docking and then simulating the evolution of the system using the AMBER force field. MM-GBSA analysis of the acquired simulation allowed us to establish the strength of the interaction and determine which amino acids participated. Once the interaction had been confirmed to exist in the simulation, based on MM-GBSA per-residue decomposition we developed IE1 mutants possessing lower affinity towards the MAD2 protein. Our work, therefore, may lead to a better understanding of the cytomegalovirus and help to develop a vaccine for human cytomegalovirus.

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P16

Over 5000 Simulations Later: Finding Allostery in the Database of Cathepsin-GAG Interactions

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Cathepsins are a family of protease enzymes essential for protein degradation in all living organisms. Their enzymatic activity is known to be regulated by glycosaminoglycans (GAGs) – linear periodic anionic polysaccharides essential for numerous biological processes [1]. Experimental and computational studies indicate that GAGs can critically influence the structure, activity and stability of cathepsins through direct interactions, promotion of oligomerization and allosteric modulation [2]. Allosteric regulation in proteases remains particularly poorly understood mechanism affecting catalytic activity and specificity. Here, we present a database of *in silico* cathepsin–GAG interactions, comprising 11 cathepsins and 6 classes of GAGs, resulting in over 300 unique complexes and more than 5000 conventional and replica exchange all-atom as well as coarse-grained molecular dynamics (MD) simulations. This dataset enables in-depth analysis of protein–ligand interaction patterns and conformational landscapes. While the MD simulations are short for fully converged Markov State Models (MSMs), the established computational pipeline allowed to extract a range of structural descriptors with most informative identified through MSM-based feature analysis. These descriptors offer a rich feature space for machine learning approaches aimed at classifying and predicting allosteric effects. The database thus serves both as a source for cathepsin-related hypothesis generation and as a benchmark dataset for other computational methods development.

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P17

β-cyclodextrin saves lysozyme from the clutches of 1-alkylsulfonates

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Reversible protein folding induced by molecular crowding can be controlled by introducing ligands that compete with the protein for small molecules [1,2]. One example is lysozyme, which unfolds in the presence of ligands that contain hydrophobic groups in their structure, such as 1-alkylsulfonates (KXS). Series of experimental methods, namely circular dichroism spectroscopy, isothermal titration calorimetry, conductometric titration and differential scanning calorimetry, supported by *in silico* analysis, have been applied to characterise the process of reversibility changes of lysozyme structure in the presence of 1-alkylsulfonates with different hydrophobic chain lengths and *β*-cyclodextrin (*β*-CD) used as a strong competitive KXS-binding ligand. The observed structural changes can be reversed by introducing *β*-CD into the system, which, due to its higher affinity for KXS in comparison to lysozyme, effectively binds small ligands and so allows the protein to refold. It can be speculated that *β*-CD may also be considered as a potential protective factor against the unfolding processes of proteins exposed to the damaging effects of hydrophobic group-containing small molecules that bind *β*-CD [3, 4].

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P18

Analysis of interactions between cytosol-penetrating antibody and cell membranes through MD simulation

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Antibodies are essential for targeting extracellular and membrane-bound antigens, but they have the limitation of being unable to penetrate the cell membrane to access intracellular targets. This limitation is particularly important in diseases where the target molecule is located within the cytosol or organelles, such as cancer or viral infections. This study introduces a new antibody derived from human IgG designed to improve its interaction with and ability to cross cell membranes [1]. The antibody, called in2CT1.1, is modified with arginine (Arg), tryptophan (Trp), and glutamic acid (Glu) to improve its ability to cross cell membranes at acidic pH levels. The corresponding in2CT1.1w/o E lacks the Glu modification; at pH 7.4, in2CT1.1 is unable to cross the cell membrane, but at pH 5.5, due to protonation of the Glu patch (E patch), it crosses the cell membrane, which is expected to facilitate endosomal escape and improve therapeutic efficacy. We evaluated the ability of the CH3 domain of the wild-type (WT), in2CT1.1, and in2CT1.1w/o E antibodies to interact with the POPC 80%, POPS 20% membrane at pH 7.4 and pH 5.5 through molecular dynamics (MD) simulations. The results showed that the protonated E patch of in2CT1.1 significantly enhanced membrane interaction, contributing to membrane insertion. This study provides valuable insights into how antibodies engineered through MD simulations can interact with and penetrate cell membranes under neutral and acidic conditions. Understanding these interactions is essential for designing next-generation antibody therapies that target proteins in the cytosol.

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University of Gdańsk

The University of Gdańsk is a dynamic and modern academic institution that successfully integrates a rich academic tradition with forward-looking development. It offers comprehensive education across nearly all academic disciplines, with a strong emphasis on fields that are in high demand on the labor market. Students benefit from state-of-the-art facilities located on one of the largest university campuses in the Pomeranian region, spanning the cities of Gdańsk, Gdynia, and Sopot. The university was officially established on March 20, 1970, through the merger of the Higher School of Economics in Sopot and the Higher School of Education in Gdańsk. Later, it also incorporated the Higher Teacher Training College. The origins of the Higher School of Economics can be traced back to the Maritime School of Commerce in Sopot, founded in 1945, which issued its first diplomas as early as 1947, continuing the education of third-year students. March 20, 2025, marked the 55th anniversary of the University of Gdańsk. Today, it is the largest institution of higher education in northern Poland and a key contributor to the advancement of modern polish science, education, and society. The university consists of eleven faculties and serves nearly 22,000 students, doctoral candidates, and postgraduate participants. Its academic staff includes more than 1,800 scholars and educators. Several programs—such as biology, biotechnology, chemistry, oceanography, quantum physics, pedagogy, psychology, law, and economics—are nationally recognized for their excellence.