8th Polish-Korean Confrence on Protein Folding:

Theoretical and Experimental Approaches

Jastrzębia Góra, 24-28 September 2023

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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 eighth from the series.

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IL – Invited Lecture

P – Poster

SL - Short Lecture

SPL – Sponsor Lecture

Conference program

Eighth Polish-Korean Conference on "Protein Folding: Theoretical and Experimental Approaches"

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IL21 (11:35 – 12:10) David Wales (Cambridge U, UK)

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Abstracts

IL01

Structure and function of G protein coupled receptors/Gproteins/agonist complex by Cryo-EM

Ji-Hye Yun[1] , Chankyo Kim[1] , Ji-Joon Song[2] and Weontae Lee[1]*

[1] Structural Biochemistry and Molecular Biophysics Laboratory, Department of Biochemistry, Yonsei University, Seoul, Korea;

[2] Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea

G protein-coupled receptors (GPCRs) are the largest and most diverse family of membrane proteins in eukaryotes, which regulate diverse biological and physiological processes through orthosteric and allosteric ligand interaction. Somatostatin is a peptide hormone that regulates endocrine systems by binding to G-protein-coupled somatostatin receptors. Somatostatin receptors (SSTRs) are involved in variety of human diseases, such as highly implicated in hormone disorders, cancers and neurological diseases. Here, we report the high resolution cryo-EM structures of full-length human SSTRs bound to the agonist somatostatin (SST-14) in complex with inhibitory G proteins. Our structural and mutagenesis analyses reveal that seven transmembrane helices form a deep pocket for ligand binding and that SSTR recognizes the highly conserved Trp-Lys motif of agonist at the bottom of the pocket. Furthermore, our structural analysis of other SSTR isoforms provide a structural basis for the mechanism by which SSTR family proteins specifically interact with their cognate ligands. This work uncovers the molecular mechanism of somatostatin receptor family and plays a crucial role to develop therapeutics targeting somatostatin receptors

IL02

The DMSO-quenched H/D-Exchange 2D NMR Spectroscopy and Its Applications in Protein Science

Kunihiro Kuwajima[1]

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Hydrogen/deuterium (H/D) exchange combined with two-dimensional (2D) NMR spectroscopy has been widely used for studying the structure, stability, and dynamics of proteins. When we apply the H/D-exchange method to investigate non-native states of proteins such as equilibrium and kinetic folding intermediates, H/D-exchange quenching techniques are indispensable, because the exchange reaction is usually too fast to follow by 2D NMR. In this lecture, I will describe the dimethylsulfoxide (DMSO)-quenched H/D-exchange method and its applications in protein science [1, 2]. In this method, the H/D-exchange buffer is replaced by an aprotic DMSO solution, which quenches the exchange reaction. We have improved the DMSO-quenched method by the use of spin desalting columns, which are used for medium exchange from the exchange buffer to the DMSO solution [1, 2]. This improvement has allowed us to monitor the H/D-exchange of proteins at a high concentration of salts or denaturants. I describe methodological details of the improved DMSO-quenched method, and present two case studies: (i) studies on the H/D-exchange behavior of *Escherichia coli* co-chaperonin GroES [3]; and (ii) studies on the H/D-exchange behavior of unfolded proteins in 6 M guanidinium chloride [4, 5]. In both of these, the spin desalting column was effectively used.

Reference

[1] Chandak MS, Nakamura T, Takenaka T, Chaudhuri TK, Yagi-Utsumi, M, Chen J, Kato K, Kuwajima K (2013) The use of spin desalting columns in DMSO-quenched H/D-exchange NMR experiments. Protein Sci 22:486-491

[2] Kuwajima K, Yagi-Utsumi M, Yanaka S, Kato K (2022) DMSO-quenched H/D-exchange 2D NMR spectroscopy and its applications in protein science. Molecules 27:3748 (17 pages)

[3] Chandak MS, Nakamura T, Makabe K, Takenaka, T, Mukaiyama A, Chaudhuri TK, Kato K, Kuwajima K (2013)The H/D-exchange kinetics of the *Escherichia coli*co-chaperonin GroES studied by 2D NMR and DMSO-quenched exchange methods. J Mol Biol 425:2541-2560

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[5] Yanaka S, Yagi-Utsumi M, Kato K, Kuwajima K (2023) The B domain of protein A retains residual structures in 6 M guanidinium chloride as revealed by hydrogen/deuterium-exchange NMR spectroscopy. Protein Sci 32:e4569 (15 pages)

IL03

Accurate prediction of protein-ligand interactions by combining structure-based graph neural networks and physics-based functions

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Accurate prediction of protein-ligand interactions is crucial for understanding biological processes and advancing drug discovery. Although various structure-based deep learning approaches have been proposed for protein-ligand affinity prediction, their accuracy in ligand screening remains limited due to the restricted size of complex datasets. Here, we present a novel hybrid model that integrates graph neural networks with docked conformations and physics-based scoring functions to enhance prediction accuracy.Our model comprises three distinct graph neural networks for predicting protein-ligand interactions. The first network serves as a binary classifier, determining whether a protein-ligand pair is active without relying on docked conformations. The second and third networks are regression models that predict binding affinities and root-mean-square deviations (RMSDs) from the native conformation, respectively. The model also incorporates information from physics-based scoring functions. Our results demonstrate that combining graph neural network predictions with physics-based estimations is essential for improving prediction accuracy.Testing our model with the PDBBind dataset reveals that it surpasses other deep learning-based approaches in screening power, highlighting its potential for facilitating advancements in drug discovery and understanding complex biological processes.

IL04

On the systematic construction of motion trees for dynamics simplification of molecular systems

Sergei Grudinin[1]

[1] LJK CNRS Grenoble, France

Identification of rigid parts in dynamic systems has multiple biological applications ranging from protein domain identification [1] to automatic coarse-graining of molecular dynamics simulations [2, 3]. There has been extensive research in this area. Current solutions include examination of connectivity, cliques calculation [4], or pebble game [5] from the graph theory, analysis of the covariance patterns in elastic network models [6], or more recent machine-learning (ML) based approaches [7]. Yet, we propose a novel solution based on rigid-body dynamics. Our method deterministically constructs a motion tree in O(N log N) time with respect to the number of particles in the system by progressively rigidifying the system and simplifying its dynamics. The tree construction is guided by the consecutive identification of system parts with the most similar dynamics measured by the kinetic energy before and after the dynamics simplification step.

Our approach has multiple advantages over previous research. It is extremely fast and deterministic. It is physics-based and can be used as a part of ML pipelines. The method is grounded on the provided information about velocities or fluctuations of the particles in the system. Thus, it can be readily applied to simplify essential dynamics computed from normal mode or principal components analysis methods. The method can also be used to identify non-contiguous protein domains from a single structure or a conformational ensemble.

Reference

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[7] J. Wells, A. Hawkins-Hooker, N. Bordin, C. Orengo, and B. Paige, “Chainsaw: protein domain segmentation with fully convolutional neural networks,” bioRxiv, pp. 2023–07, 2023.

IL05

Modeling structure and dynamics of linear and cyclic peptides

Sebastian Kmiecik[1]

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

In our laboratory, we have developed, or tested, a number of protocols for modeling protein-peptide interactions, including linear and cyclic peptides. I will present the modeling results with the use of coarse-grained modelling, in various configurations, and also some tests of the state-of-the-art deep learning models. Furthermore, I will discuss results of modeling protein flexibility and structure-based prediction of protein aggregation properties. These results have been recently utilized in the aggregation predictions on the proteomes scale.

IL06

Analytical Approaches for Deriving Friction Coefficients for Selected α-Helical Peptides Based Entirely on Molecular Dynamics Simulations

Robert Szoszkiewicz[1] , Aleksandra Wosztyl[1] , Krzysztof Kuczera[2] ,

[1] University of Warsaw, Poland.

[2] University of Kansas, Lawrence, KS, USA.

In this talk, I will show our recently published study to derive analytically from molecular dynamics (MD) simulations the friction coefficients related to conformational transitions within several model peptides with α-helical structures [1]. We studied a series of alanine peptides with various length from ALA5 to ALA21 as well as their two derivatives, the (AAQAA)3 peptide and a 13-residue KR1 peptide that is a derivative of the (AAQAA)2 peptide with the formula GN(AAQAA)2G. We used two kinds of approaches to derive their friction coefficients. In the local approach, friction associated with fluctuations of single hydrogen bonds was tackled. In the second approach, friction coefficients associated with folding transitions within the studied peptides were obtained. In both cases, the respective friction coefficients differentiated very well the subtle structural changes between studied peptides and compared favorably to experimentally available data.

Reference

[1] Wosztyl A, Kuczera K, Szoszkiewicz R (2022) Analytical Approaches for Deriving Friction Coefficients for Selected α-helical Peptides and Based Entirely on Molecular Dynamics Simulations. Journal Physical Chemistry B, 126: 8901-8912 DOI: 10.1021/acs.jpcb.2c03076 Open access.

Acknowledgments

The work was supported by the National Science Center, Poland, grant no. 2018/30/M/ST4/00005. Molecular dynamics simulations were carried out on the computer cluster at the Biological and Chemical Research Centre (CNBCh) at the University of Warsaw (UW). Trajectory analyses were performed on computer workstations supported by the General Research Fund at the University of Kansas as well as within the CNBCh at UW.

IL07

Biophysical characterization of lysyl oxidase, a moonlighting protein

Sylvie Ricard-Blum[1]

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Lysyl oxidase (LOX) is a copper-dependent amine oxidase. This extracellular matrix (ECM) enzyme contributes to ECM stiffness by catalyzing the first step of the covalent cross-linking of the ECM proteins collagens and elastin [1]. However, LOX has also been detected in the nucleus of several cell types, interacts with intracellular and extracellular proteins, andacts on non-ECM substrates[2].LOX is secreted as a proenzyme, and the proteolytic cleavage of the propeptide, which is intrinsically disordered [3], releases the 32-kDa disulfide-bonded catalytic domain, which contains a lysyltyrosylquinone cofactor. We have built a 3D model of the catalytic domain of LOX [4], and our goal is to experimentally determine its 3D structure in order to determine how LOX accommodates its various substrates, and to decipher the mechanisms underlying its non-catalytic functions.

We have designed three constructs of the full-length and truncated catalytic domain of LOX, expressed them in E. coli, and set up a refolding protocol. LOX ismostly comprised of -strands, random coil, and turnsas shown by circular dichroism, and contains several molecular species including large aggregates partly dissociated in 6 M urea. The three LOX forms retain most of their secondary structure in 6 M urea. They form dimers to hexamers depending on the forms and experimental conditions as shown by dynamic light scattering and mass photometry. Small particles of uniform shape (~ 9 nm diameter), consistent with oligomer formation,have beenvisualized by negative staining electron microscopy for the truncated LOX form lacking seven amino acid residues predicted to promote aggregation. Preliminary experiments in presence of thioflavin T suggest that LOX might form amyloid-like fibrilsin vitro. Further experiments usingnegative stain electron microscopy and small angle X-ray scattering are needed to determine the oligomerization state and shape of LOX.

Reference

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Acknowledgments

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IL08

Tautomeric equilibrium of selected purine analogs and their binding to Purine Nucleoside Phosphorylase

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[1] Department of Physics and Biophysics, University of Warmia and Mazury in Olsztyn, Poland

8-azaGuanine (8AG) and 8-azaisoGuanine (8AIG) are Guanine derivatives in which carbon at position 8 is replaced with nitrogen. This modification significantly increases the intrinsic fluorescence properties of these molecules, which can be utilized for monitoring their interactions with biological polymers like proteins or nucleic acids. In particular, these molecules are substrates for ribosylation processes catalyzed by Purine Nucleoside Phosphorylase protein (PNP). To better understand protein-ligand interactions, it is important to understand the ground-state tautomeric equilibrium as well as the possible excited-state proton transfer processes that can take place in these compounds.

The ground-state tautomeric equilibrium of 8AIG was revealed in our previous publications [1,2]. It was shown that, besides the dominant tautomer protonated at positions 3 and 8, some minor tautomers might be present in the water solution. Moreover, methylation of 8AIG at position 9 surprisingly significantly increases the population of enol forms of the molecule. For the 8AG molecule, tautomeric equilibrium seems much simpler, as tautomers protonated at positions 1 and 9 dominate both in water and in the gas phase [3]. Methylation of the molecule at position 9 does not lead to any important shifts in tautomeric equilibrium, with the dominant form protonated at position 1. The results obtained from population analysis are also supported by vertical absorption energies, which are in very good agreement with the experimental data.

The binding of selected purine analogs to PNP was investigated with molecular dynamics-based techniques and compared to available experimental data [4].

Reference

[1] Pyrka M, Maciejczyk M (2015) Theoretical study of tautomeric equilibria of 2,6-diamino-8-azapurine and 8-aza-iso-guanine. Chem. Phys. Lett. 627:30-35.

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IL09

Understanding the role of protonation states for ion channel selectivity via ion modulated changes of selectivity filter pKa values

Bernard R. Brooks[1], Ada Chen[1], Ana Damjanovic[1]
[1] Laboratory of Computational Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, USA

In bacterial voltage-gated sodium channels, the passage of ions through the pore is controlled by a selectivity filter (SF) composed of four glutamate residues. The mechanism of selectivity has been the subject of intense research, with suggested mechanisms based on steric effects, and ion-triggered conformational change. Here, we propose an alternative mechanism based on ion-triggered shifts in pKa values of SF glutamates. We study the NavMs channel for which the open channel structure is available. Our free-energy calculations based on molecular dynamics simulations suggest that pKa values of the four glutamates are higher in solution of K+ ions than in solution of Na+ ions. Higher pKa in the presence of K+ stems primarily from the higher population of dunked conformations of the protonated Glu sidechain, which exhibit a higher pKa shift. Since pKa values are close to the physiological pH, this results in predominant population of the fully deprotonated state of glutamates in Na+ solution, while protonated states are predominantly populated in K+ solution. Through molecular dynamics simulations we calculate that the deprotonated state is the most conductive, the singly protonated state is less conductive, and the doubly protonated state has significantly reduced conductance. Thus, we propose that a significant component of selectivity is achieved through ion-triggered shifts in the protonation state, which favors more conductive states for Na+ ions and less conductive states for K+ ions. This mechanism also suggests a strong pH dependence of selectivity, which has been experimentally observed in structurally similar NaChBac channels.

Reference

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SL1

Water Models for Molecular Dynamics of Protein - Glycosaminoglycan Complexes: A Benchmark Study

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Glycosaminoglycans (GAGs) are anionic linear periodic polysaccharides with vast structural diversity facilitating various functions by thousands of interactions in the extracellular matrix, at the cell surface, and within the cells.[1] Such interactions occur mainly through electrostatic forces and are crucialfor the biological activity of these proteins. The influence of the solvent medium is vital in enabling these interactions and thus it is important to have the most appropriate solvent model for the computational studies involving such protein-GAG interactions. Although there are several explicit and implicit water models exist, TIP3P model remains as the most widely used one. Hence it is pivotal to analyse and evaluate different water models to find the most appropriate one for the protein-GAG interactions in terms it of its capability of yielding the agreement with the experimental data. Our previous study has shown a comparative analysis of several water models in the molecular dynamics of unbound GAGs.[2] In the present work, we focuse on comparison of interactions in three different sytems *viz.* basic fibrobalst growth factor complexed with heparin hexamer (pdb:1BFC), CD44 in a Type A complex with an hyaluronan 8-mer (pdb: 2JCQ), and cathepsin K - chondroitin sulfate complex (pdb: 4N8W) with the following water models: implicit IGB = 1, 2, 5, 7, and 8 and explicitTIP3P, TIP4P, TIP4PEw, TIP5P, SPCE, and OPC.

Reference

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Acknowledgments

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SL2

Membrane curvature sensing by model biomolecular condensates

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Biomolecular condensates (BCs) are fluid droplets that form in biological cells by liquid-liquid phase separation. Their major components are intrinsically disordered proteins. Vast attention has been given in recent years to BCs inside the cytosol and nucleus. BCs at the cell membrane have not been studied to the same extent so far. However, recent studies provide increasingly more examples of interfaces between BCs and membranes which function as platforms for diverse biomolecular processes. Galectin-3, for example, is known to mediate clathrin-independent endocytosis and has been recently shown to undergo liquid–liquid phase separation, but the function of BCs of galectin-3 in endocytic pit formation is unknown. Here, we use dissipative particle dynamics simulations to study a generic coarse-grained model for BCs interacting with lipid membranes. In analogy to galectin-3, we consider polymers comprising two segments – one of them mediates multivalent attractive interactions between the polymers, and the other one has affinity for association with specific lipid head groups. When these polymers are brought into contact with a multi-component membrane, they spontaneously assemble into droplets and, simultaneously, induce lateral separation of lipids within the membrane. Interestingly, we find that if the membrane is bent, the polymer droplets localize at membrane regions curved inward. Although the polymers have no particular shape or intrinsic curvature, they appear to sense membrane curvature when clustered at the membrane. Our results indicate toward a generic mechanism of membrane curvature sensing by BCs involved in such processes as endocytosis.

Reference

[1] Midhun Mohan Anila, Rikhia Ghosh, Bartosz Różycki (2023). Membrane curvature sensing by model biomolecular condensates. Soft Matter 19(20): 3723-3732

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IL10

Tracking single-proteins in a living cellby photoconversion of cyanine dyes

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Cyanine5 (Cy5) is one of the most widely used far-red organic dyes in biophysical research. Due to its high quantum yield and the photophysical capability of reversible photoswitching, this dye has been extensively utilized in single-molecule and superresolution imaging. Here, I will present the mechanism of Cy5 photoconversion to to Cyanine3 (Cy3) upon photoexcitation during single-molecule measurements in vitro and superresolution localization imaging of living cells [1,2]. A combination of optical spectroscopy, high-resolution mass spectrometry, and 1H NMR spectroscopy unambiguously confirms the identity of the fluorescent photoproduct of Cy5 to be Cy3. The excision of a two methine unit from the polymethine chain, which results in the formation of blueshifted products, also occurs in other cyanine dyes, such as Alexa Fluor 647 (AF647) and Cyanine5.5, even with exposure to room light. The formation of a blueshifted congener dye can obscure the multicolor fluorescence imaging and lead to potential misinterpretation of the data. The otherwise undesirable photoconversion, however, provides a new photoactivation method for high density single-particle tracking in a living cell without using UV illumination and potential cell-toxic additives. Using this method, we observed the diffusion of EGFR at the single-protein level.

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IL11

Enhanced Conformational Sampling for Protein Dynamics and Function

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Molecular dynamics (MD) simulation is an essential tool to investigate protein conformational fluctuations and dynamics related to their molecular functions. To extend the limitations of available simulation time scales, we have developed various enhanced conformational sampling methods and applied them to atomistic MD simulations of protein dynamics and functions. Generalized Replica Exchange with Solute Tempering (gREST) [1], which was extended from REST2 [2-4], is very useful for simulating conformational dynamics of large multi-domain proteins. Recently, we applied it to RBD motions in spike protein on the surface of SARS-CoV-2 [5]. The two-dimensional replica-exchange method, gREST/REUS, applies to protein-ligand binding effectively, allowing many binding/unbinding processes [6]. Our recent simulations on SrcKinase-inhibitor binding/unbinding processes suggest the importance of conformational fluctuations in the kinase and inhibitors.

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IL12

Using high-throughput sequencing (HTS) to probe TF-dsDNA recognition --Anchoring elements revealed by KaScape

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HTS technology has changed many ways we study and understand biology and biomedical research in the recent 10 years, however, the majority of genomic sequencing-oriented studies have been focusing on in vivo genomic, transcriptomic, or epigenomic research, creating a huge amount of "big data" for later analyses, whereas, in this work, we would like to demonstrate that the HTS technology could be designed and applied for in vitro biochemical and biophysical experiments with defined thermodynamic equilibrium conditions. By taking advantage of the high- throughput, high-efficiency, and low-cost HTS, we can develop and optimize methods such as this KaScape for studying DNA-protein recognition and interaction in general.

In this talk I will first review the current methods and knowledge to study and understand transcription factors (TF) and double stranded DNA (dsDNA) recognition, then introduce a novel concept of anchoring elements (AE) as the main contributor to TF– dsDNA interaction. The AE concept is mainly revealed by a new method currently developed in our lab, i.e. KaScape: a high-throughput sequencing-based method for global characterization of protein-DNA binding affinity.

IL13

Dynamic Dance of Sugars and Proteins: Unveiling the Secrets of Protein-Carbohydrate Complex Structures with Molecular Dynamics

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Carbohydrates represent the most diverse among biologically active classes of molecules found in living organisms [1]. Despite their significance, the understanding of their functions is still relatively limited compared to proteins and nucleic acids. Both experimental and computational tools face significant challenges in dealing with the unique physico-chemical properties of carbohydrates. At the same time, elucidating the molecular mechanisms of many biochemical processes is impossible without considering this complex class of molecules. Carbohydrates play a crucial role in affecting the structure, dynamics, and functional activity of numerous proteins, either through non-covalent interactions or as constituents of proteoglycans. In this talk, I will demonstrate the various effects of carbohydrates on proteins and how they can be modeled *in silico*. I will present the computational study proposing the molecular mechanism underlying the mediation of cathepsin protease maturation by glycosaminoglycans, anionic linear periodic polysaccharide [2]. Then, I will discuss the role of Ca^{2+} ions in the active site of the carbohydrate-processing enzyme mannuronan C-5 epimerase AvAlgE6 [3]. Lastly, I will showcase the impact of glycan chains on the behavior of proteoglycan molecules syndecans. These examples highlight the critical importance of considering carbohydrate molecules in the analysis of biologically relevant multimolecular systems.

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IL14

Protein-protein interaction stabilization and inhibition studied by molecular simulations

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Protein-protein interactions (PPI) are essential for many biological processes including signal transduction, immune reactions and many diseases. Inhibition of protein-protein interactions by drug-like compounds is a common basis for therapeutic approaches. However, due to the large and flat interface of most protein-protein complexes, it is often difficult to find compounds that bind specifically to a partner and inhibit binding. However, complex formation often results in new pockets at the interface, and binding of complex stabilizing compounds to such cavities is an alternative but so far much less explored strategy. We have systematically analyzed known PPI stabilizers and associated protein complexes.For most cases a dual-binding mechanism, meaning a similar interaction strength of the stabilizer to each protein partner, was found as an important prerequisite for effective stabilization. Some stabilizers follow an allosteric mechanism by indirectly stabilizing the bound structure of one protein partner to increase the PPI. For a large set of known complexes we find interface cavities suitable for binding of drug-like compounds and describe a computational protocol to identify such compounds by drug-screening approaches. In addition, I will also present work on identifying PPI inhibitors based on computational selection of suitable cyclic peptides and applications to example cases.

IL15

Investigating protein-protein interaction dynamics

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One of the key outstanding problems in studying protein structure and function is to understand the dynamics of protein-protein interactions, both from an experimental and theoretical perspective. We have been studying kinesin, the microtubule motor, as a test case to attempt to both experimentally understand the kinetics of binding, and also model this interaction theoretically. I will present a brief overview of our experiments, and some of the progress we are making explaining these findings theoretically.

IL16

Effect of the environment on the structure and dynamics of proteins

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The intricate interplay between proteins and their surrounding environment shapes their structure and dynamics, playing a pivotal role in cellular processes. This presentation focuses on the influence of varying environments on protein behavior, drawing from a series of studies that employ extensive Molecular Dynamics (MD) simulations to investigate protein interactions in both aqueous and lipid environments. It was found that not only the sole presence of the lipid bilayer drastically impacts the protein structure, but also the lipid bilayer composition can have tremendous influence on the observed effects. These findings will be presented based on the recent articles about the oligomeric and fibrillar forms of the amyloid beta peptide in proximity of the lipid bilayer, herpesviral UL49.5 proteins embedded into both homogeneous and heterogeneous lipid bilayers, impact of serotonin molecules on lipid bilayers of various compositions and role of the redox environment on the disulfide-bond and structure stability of the ribonuclease A and non-specific lipid transfer proteins [1-6].

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SL3

Computational analysis of the interactions between glycosaminoglycans and small molecules

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Glycosaminoglycans (GAGs) are long anionic polysaccharides made up of repeating disaccharide units [1]. They play essential roles in cellular communication, adhesion, and proliferation within the extracellular matrix through interactions with protein targets [2,3]. Changes in protein-GAG interactions can lead to various pathologies, including cancer, Alzheimer's and Parkinson's diseases [4]. GAGs also enhance peptide integration into membranes and modify drug activity through their direct or indirect interations with small draggable molecules [5,6]. Despite their significant pharmacological potential, our understanding of the molecular mechanisms underlying their interactions with small molecules is in particular far from being complete. Experimental methods are not always able to provide sufficient information about the nature of interactions in GAG-small molecule systems, and the corresponding available structural data are limited. Consequently, computational chemistry approaches such as molecular docking, molecular dynamics, and free energy analysis have become necessary. By studying over 20 drug molecules (e.g., ellipticine, berenil, pentamidine, surfen, tacrine, thioflavin T), we gained valuable insights into the molecular aspects of GAG-small molecule interactions. Our findings were further supported by experimental data [7-11] and represent the initial steps for the understanding of molecular mechanisms underlying protein-GAG-small molecule multicomponent systems.

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SL4

Energy landscapes for proteins described by the UNRES coarse-grained potential

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The self-assembly of proteins is encoded in the underlying potential energy surface (PES), from which we can predict structure, dynamics, and thermodynamic properties. However, the corresponding analysis becomes increasingly challenging with larger protein sizes, due to the computational time required, which grows significantly with the number of atoms. Coarse-grained models offer an attractive approach to reduce the computational cost. In this Feature Article, we describe our implementation of the UNited RESidue (UNRES) [1,2,3,4] coarse-grained potential in the Cambridge energy landscapes software [5,6,7]. We have applied this framework to explore the energy landscapes of four proteins that exhibit native states involving different secondary structures. Here we have tested the ability of the UNRES potential to represent the global energy landscape of proteins containing up to 100 amino acid residues. The resulting potential energy landscapes exhibit good agreement with experiment, with low-lying minima close to the PDB geometries and to results obtained using the all-atom AMBER [8]force field. The new program interfaces will allow us to investigate larger biomolecules in future work, using the UNRES potential in combination with all the methodology available in the computational energy landscapes framework.

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IL17

Drect experimental observation of blue-light-induced conformational change and intermolecular interactions of Cryptochrome

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Cryptochromes (CRYs) are blue light receptors that mediate circadian rhythm and magnetic sensing in various organisms. A typical CRYconsists of a conserved photolyase homology region (PHR) domain and a varying carboxyl-terminal extension (CTE) across species. The structure of the flexible CTE and how CTE participates in CRY's signaling function remain mostly unknown. In this study, we uncovered the missing link between CTE conformational changes and downstream signaling functions. Specifically, we discovered that the opening of CTE exposes an active surface of *Cra*CRY (*C. reinhardtii* animal-like CRY), which interacts with Rhythm Of Chloroplast 15 (ROC15), a circadian-clock-related protein. Our finding was made possible by two technical advances. First, we directly observe, for the first time, the opening of CTE upon blue light excitation using single-molecule Förster resonance energy transfer technique. Second, we obtain the dark and lit structures of full-length CraCRY, including CTE, through computation and small-angle X-ray scattering (SAXS). Our results provide insights into how CRY performs diverse functions upon light activation.

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IL18

Condensation of Intrinsically Disordered Proteins and DNA Demonstrated by Molecular Dynamics Simulations

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During the last decade, condensation of biomolecules has emerged as an essential research topic in biophysics because it was demonstrated that various intrinsically disordered proteins (IDPs) and nucleic acids could form droplet-like condensates through liquid-liquid phase separation in living cells. However, the inner making of the condensates remains elusive because of the lack of experimental methods to track down dynamically moving biomolecules inside the condensates. Thus, computational methods such as molecular dynamics (MD) simulations can play a valuable role in characterizing the inner making of biological condensates. Here, I will present MD simulations of various biological condensates. First, I will demonstrate that the state-of-the-art MD simulations can quantitatively reproduce the polyamine-mediated condensation of DNA, which has been experimentally well-characterized. Then, I will apply the newly developed MD simulation technique to biologically relevant systems such as DNA-histone tail and tau-polyamine complexes. Our simulation results dramatically visualize how the amino acid sequence and chemical modifications control the condensation behaviors of those systems.

IL19

Protein structure at extreme conditions – how it can be observed

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The study of the structure at high pressures, ultra-low and very high temperatures and other extreme conditions is now the standard in the characterization of new materials and their applications.

A protein macromolecule is a sophisticated structure stabilized by a network of hydrogen bonds that ensure its proper folding so that a given molecule can perform its functions (enzymatic, receptor, transport, etc.). Sometimes these molecules have to function in conditions that we can consider extreme, at least from the point of view of the stability of biological structures.

Many enzymes used in the food processing industry are subjected to high pressure or elevated temperatures in order to obtain optimal production conditions from the point of view of given processes. Also proteins functioning in extremophilic organisms must maintain their functional structure at high temperatures, extreme pH conditions or high pressures.

Summing up, the behavior of the protein structure in extreme conditions, although not as extreme as in the case of material research, is extremely interesting. In order to understand these changes, it is necessary to use a set of methods ranging from spectroscopic techniques, through methods of scattering synchrotron radiation and neutrons to protein crystallography.

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IL20

Selected Experimental and Theoretical Aspects of Working with Sulfonamides and Their Complexes with Trivalent Metal Ions

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Antibiotic resistance is a global problem, and one promising solution to overcome this issue is using metallodrugs, agents containing metal ions and ligands. It seems that a good example of proper coordination is the combination of a trivalent metal ion of Ru, Rh, Os, and Ir with selected sulfonamide derivatives.These complexes may be able to interact with molecular drug targets. In the context of drug development, a molecular target is a specific biological molecule or process that a drug is designed to interact with in order to produce a desired therapeutic effect. Molecular targets can include proteins, enzymes, receptors, and other cellular components that are involved in disease pathways or physiological processes. The identification and validation of molecular targets is a key step in the drug discovery process.

In our project, we focus on the synthesis and studies of Ru, Rh, Ir, and Os -sulfonamide complexes, their bioinorganic profiles determination, biological properties, and activity characterization as well as the methods used to determine the biomolecules' interaction modes with them. During the lecture, approaches with specific solutions of selected problems using experimental and theoretical methods will be presented.

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IL21

Energy landscapes: new insights for biomolecules

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The potential energy landscape provides a conceptual and computational framework for investigating structure, dynamics and thermodynamics in atomic and molecular science. This talk will summarise some recent applications to biomolecules. First, mutational basin-hopping will be introduced as a biological realisation of generalized basin-hopping for structure prediction and design. The focus will then shift to multifunnel landscapes, which are contrasted to the single funnel landscapes expected for biomolecules that have evolved to perform a single function. The hypothesis that multifunnel landscapes are associated with intrinsically multifunctional systems has motivated new theory to analyse the corresponding emergent thermodynamic and kinetic signatures. Multiple heat capacity peaks are associated with broken ergodicity and thermodynamic transitions between competing morphologies, which can be assigned by a detailed analysis of the occupation probabilities as a function of temperature. Multifunnel landscapes also feature multiple relaxation time scales, which can be assigned if it is possible to calculate the first passage time distribution. For large kinetic transition networks this assignment first requires renormalisation to reduce the dimension of the state space. The mean first passage time, associated with an overall rate constant, is exactly preserved by the renormalisation. However, for multifunnel landscapes, the mean value hides the existence of alternative relaxation processes, which are properly resolved if the full first passage time distribution can be calculated. The renormalised landscape can also be directly translated into a free energy disconnectivity graph, and this construction has been used to understand the organisation of light harvesting in photosystem II.

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SL5

A coarse-grained approach to NMR-data-assisted modelingof protein structures

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The remarkable performance of DeepMind AlphaFold2 group in the CASP14 experiment has set a new standard for protein structure modeling[1].However, these approaches are of limited use in studying the conformations of flexible proteins and protein dynamics. Physics-based approaches that use force fields are quite successful in this area. To treat larger systems, coarse-grained models are particularly useful. Because of inaccuracies inherent in force field, including even sparse information from experiments in modeling is beneficial. In this talk, the extension of physics-based coarse-grained UNRES model of polypeptide chains [2] to data-assisted modeling with the use of information from nuclear magnetic resonance (NMR) experiments will be presented. The ESCASA algorithm for analytical estimation of proton positions from coarse-grained geometry has been developed for this purpose [3]. This algorithm allows to use NMR data directly with coarse-grained simulations. Moreover a penalty function with the shape of intersecting gorges was developed to treat ambiguous distance restraints, which automatically selects consistent restraints [4]. Our results have shown that the new implementation of UNRES can handle ambiguous and contradictory NMR restraints, and gives better results than the standard CYANA software [5] when only very poor quality NMR data are available.

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IL22

Design, synthesis and biological evaluation of peptide-based immune checkpoint inhibitors immune system in cancer therapy

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Due to the increasing incidence of cancer (19.3 million new cases diagnosed in 2022 new cases) there is a constant need to search for and develop new therapies anti-cancer therapies. Currently, high hopes in the fight against cancer are associated with immunotherapy. The idea of using the immune system to fight cancer cells cancer cells was pioneered by Rudolf Virchow as early as 150 years ago. In contrast, in 2018, Tasuku Honjo jointly with James P. Allison were awarded the Nobel Prize in Physiology or Medicine, for their discovery of an anti-cancer therapy based on negative inhibition of the of the immune system and, in particular, on blocking the formation of a complex between the proteins PD-1 and PD-L1, which are among the so-called immune system checkpoints. The PD-1 protein is a receptor (located on the surface of T lymphocytes) that forms a complex with its ligand, PD-L1 protein (found on the surface of antigen-presenting cells and tumor cells tumor cells). Formation of the PD-1/PD-L1 complex results in inhibition of T-lymphocyte proliferation and the production of cytokines, resulting in the immune system's failure to fight and eliminate cancer cells. It has been shown that blocking the formation of the PD-1/PD-L1 complex leads to the activation of the immune response. Many other checkpoints act in a similar manner including, for example, BTLA/HVEM. Numerous studies are currently underway to find inhibitors of the binding of the above-mentioned proteins. These include antibodies, compounds low molecular weight or peptides.

The presentation will discuss the results of work on peptide inhibitors of PD-1/PD-L1 and BTLA/HVEM immune checkpoints

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SPL

PeptAIm Bioinformatics Platform

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PeptAIm is a unique, artificial intelligence-powered web-based bioinformatics platform. It relies on an innovative and experimentally validated algorithm, supported by computer simulations using the coarse-grained UNICORN[1] force field, as well as advanced machine learning[2] (ML) and artificial intelligence[3] (AI) methods.PeptAIm enables the accurate identification of peptides that exhibit the highest affinity and spatial compatibility with the target protein. The selection of such peptides is essential for the development of molecular diagnostic tests that would allow for the selective detection of specific proteins. This software boasts high computational accuracy and significantly reduces the cost, time, and computational power required for such analyses. The PeptAIm platform is user-friendly, requiring you to specify the number of amino acid residues in the peptide and then upload your own protein structure or provide the protein ID from the Protein Data Bank[4] database. The result of the analysis is a concise and straightforward report tailored to the needs of individuals without specialized bioinformatics knowledge.The software has a wide range of applications, including the diagnostics of viral and bacterial pathogens, as well as the diagnosis of cancer, neurodegenerative, and autoimmune diseases.

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P01

An application of the coarse grain methods for the prediction of the flexibility X-RAY and NMR ensembles of the protein structure.

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Proteins to be functional need to have dynamical properties. It has been discovered that the flexible fragments of many proteins have a significant influence on their function [1].
In this work, we evaluated the accuracy of the flexibility prediction by four computational methods: UNRES (UNitedRESidue) [2], UNRES with secondary structure restraints based on DSSP (Define Secondary Structure of Proteins) [3], CABS-flex (C-Alpha, C-Beta, and Side chains flexibility) [4] and NOLB (NOn-Linear rigid Block NMA approach) [5]. Those methods allow us to obtain reasonable results, extremely fast.
We employed a set of 92 proteins, which had previously been utilized to assess the predictive capacity of the UNRES force field [6], which encompasses a diverse array of protein structures, derived from various experimental techniques.
We computed the average Pearson's or Spearman's correlation coefficients, and their dependence on the protein length, structure type, and other properties.
We found that CABS-Flex aligns well with NMR and X-ray ensemble fluctuations, while NOLB performance was worst among tested methods, however, in some cases, NOLB was revealed to be the best method.

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P02

Towards Understanding the Role of Disulfide Bonds in Non-Specific Lipid Transfer Proteins

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Disulfide bonds are essential post-translational modifications found in proteins [1], influencing protein structure, stability, and function. Disulfide bonds are covalent bonds between sulfur atoms located within inter- or intra-molecular cysteine residues, and their presence or absence is associated with redox intra- and extracellular environment. Our investigation focuses on elucidating their role in plant non-specific Lipid Transfer Proteins (nsLTPs), a crucial protein family found in terrestrial plants. NsLTPs have been reported to be crucial to membrane stabilization, cell wall organization, signal transduction, resistance to biotic and abiotic stresses, plant growth and development, as well as seed development and germination [2]. Despite their significance, the precise function of disulfide bonds in nsLTPs remains uncertain. This study employed all-atom and coarse-grained molecular dynamics (MD) simulations, utilizing AMBER ff19SB, CHARMM36m, SIRAH2.0, and UNRES force fields, to explore the impact of various disulfide bond combinations on nsLTP behaviour. These investigations encompassed a range of conditions, including changes in salinity, temperature, pH, pressure, membrane proximity and the presence or absence of a ligand.

Our research has shown that in the absence of disulfide bonds, the nsLTP maintains its overall shape, although a noticeable increase in flexibility is observed, especially at the C- and N-termini. Interestingly, the presence of a specific single disulfide bond can provide greater structural stabilization than certain combinations of two or even three disulfide bonds, suggesting potential variations in their roles within the protein. Two out of four disulfide bonds in nsLTP, which are located between the helices forming a cavity, can cause internal tension that lowers stability, the remaining two bonds restrict labile termini. Moreover, MD simulations conducted under challenging conditions, such as thermal stress, highlight a more pronounced influence of disulfide bonds on nsLTP stability, indicating a closer association with protein destabilization and enzymatic degradation during stress rather than the sole maintenance of stability within typical physiological contexts.

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P03

Solvent Model Benchmark for Molecular Dynamics of Glycosaminoglycans

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There has been very little discussion about the role of solvent models in the molecular dynamics simulations of glycosaminoglycans (GAGs), a group of anionic, periodic linear polysaccharides. In the computational studies of GAGs, TIP3P explicit water model is used almost exclusively while other numerous alternative implicit and explicit solvent models are omitted. This lack of the data regarding the use of water model types in the studies of GAGs is surprising considering the immense importance of solvent-mediated interaction for GAG dynamic and structural properties. Therefore, it would be of great interest for the GAG community to establish the solvent model that is suited the best in terms of the quality of theoretically accessible GAG description parameters and, at the same time, would be reasonably demanding in terms of computational resources required. In this study, heparin and hyaluronic acid oligomers were simulated using 11 different solvent models (6 explicit and 5 implicit). GAGs' molecular descriptors were analyzed to assess the effects of each of water model on GAG behaviour to find the most suitable one.

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P04

Cracking the Glycosaminoglycan "Sulfation Code" with Molecular Dynamics-based Approaches

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Glycosaminoglycans (GAGs) are linear anionic periodic polysaccharides containing disaccharide repetitive units. Each unit consists of glucuronic (GlcA) or iduronic (IdoA) acid and N-Acetylglycosamine (GlcNAc) or N-Acetylgalactososamine (GalNAc). GAGs are found in extracellular matrix where they play crucial role in biochemical processes such as angiogenesis, anticoagulation or cell adhesion [1]. Therefore they are important participants in carcinogenesis, Alzheimer's or Parkinson's diseases [2]. GAGs are challenging molecules both for experiments and computation due to their chemical heterogeneity, flexibility and periodicity. The amount and particular positions of the sulfation group constitute ***"sulfation code"***, which determines the conformational, dynamic, thermodynamic and recognition properties of GAGs, and, consequently, defines their biological activity mediated by their interactions with proteins and other biomolecules [3]. In this work, we are taking a step towards deciphering "sulfation code" for chondroitin sulfates-4,6 (CS4, CS6) and dermatan sulfate (DS). CS4 and CS6 vary by the position of sulfation in GalNAc residue. Difference between CS and DS is within uronic acid unit – GlcA is present in CS while IdoA in DS. Aiming to find dissimilarities regarding structural, conformational and dynamic properties, we applied MD-based analysis of these GAGs in dimeric, tetrameric and hexameric forms. As descriptors we used the following ones: RMSD, R_{gyr}, EED, molecular volume, glycosidic linkages, puckering conformational space, internal electrostatic tension, interactions with counterions and H-bonding propensities. Obtained data show that CS4, CS6 and DS are substantially different concerning above-mentioned properties [4]. Understanding the molecular basis of the GAG "sulfation code" and how are they distinct while binding to proteins may play significant role in developing GAG-based drugs and therapies.

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P05

Analysis of the interactions between lysozyme protein and selected compounds of natural origin

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Lysozyme is the most thermally stable at pH around 5.0. However, in an alkaline environment, it quickly loses its activity. Previous studies of various factors on the properties of lysozyme have shown that the reaction of the environment in which the enzyme is dissolved, temperature and its complexing abilities have a huge impact on its activity [1]. In this work, the interaction of lysozyme protein with compounds of natural origin, i.e., vitamin C, caffeine, epinephrine and trigonelline, was investigated using the techniques of isothermal calorimetric titration (ITC), fluorescence spectroscopy, differential scanning calorimetry (DSC) and circular dichroism (CD) as well as calculations using molecular docking simulationsby using EADock program [2]. The studies allowed to determine changes in the thermal stability of lysozyme at physiological pH under the influence of these substances and possible interactions that may result in the deactivation of the enzyme. It was observed that only in the case of vitamin C, some effects could be observed in the area responsible for the activity of the protein, i.e. in the vicinity of the Glu-35 glutamic acid residue. It should be noted that any modifications in this area affect the enzymatic activity of the lysozyme. Our study is in good agreement with published resultsofthe lysozyme structure [3].

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P06

Extension of the SUGRES Coarse-Grained Model of Polysaccharides to Heparin

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The glycosaminoglycan heparin (HP) is a challenging target in all-atom molecular dynamics (MD) simulations due to its size, flexibility and the predominantly electrostatic nature of its interaction with proteins. Therefore, application of coarse-grained approaches is potentially promising to model HP-containing molecular systems. We have extended the coarse-grained SUGRES-1P model of polysaccharides [1] to HP and modified the interaction energy function to account for a shift of the interaction centers and to enable a direct modification of the electrostatic energy term weight. The implemented parameters were previously obtained using all-atom MD simulations [1,2] with the GLYCAM06 force field [3]. With this modification, we were able to apply the SUGRES-1P force field in microsecond-long MD simulations of free HP oligosaccharides ranging from degree of polymerization 6 to 68. The modeled HP chains exhibited remarkable similarity to experimentally-determined HP molecules [4,5] in terms of their global structural characteristics. We conducted a comprehensive analysis of the constituent energy term weights and ion concentration, represented by the Debye-Hückel parameter κ. Our findings suggest that long HP chains are characterized by coiled conformations governed predominantly by electrostatic interactions established between the charged residues. The SUGRES-1P model, integrated into the coarse-grained UNICORN model, marks a significant milestone as it is the first time a "bottom-up" physics-based approach has been employed for coarse-grained modeling of HP chains while maintaining compatibility with other biomolecule classes within the UNICORN modeling package.

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P07

Design of compounds based on HVEM protein fragments to inhibit the formation of the HVEM/LIGHT complex

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LIGHT (TNFSF14) and HVEM (Herpesvirus Entry Mediator) proteins are responsible for regulating the immune response in the human body [1]. Overexpression of the LIGHT protein and its prolonged entanglement in a complex with the HVEM protein may be the cause of a number of autoimmune diseases, such as rheumatoid arthritis [2]. Preventing the formation of this complex, for example with peptides, is a way to cure these diseases. Therefore, our goal was to design as short as possible peptide with the highest affinity towards LIGHT protein, which would retain high selectivity.HVEM consists of three domains: CRD1 (Cysteine Rich Domain), which can interact with the BTLA protein and inhibit the body's immune activity, and CRD2 and CRD3, whichinteractwith the LIGHT protein, activating the response. For this reason, when designing the peptide sequence, we based on the native domains of the HVEM protein. Designed compounds differed in the number and location of disulfide bonds as well as the number of amino-acid residues taken from the HVEM molecule. Based on the prediction of the binding free energies by MM-GBSA method and structural analyses, we selected one of the peptides for further research, characterized by the good stability in solution and complex, as well as satisfactory affinity to LIGHT. In order to improve affinity to LIGHT, we designed a series of deleterious and substitutional mutants: amino-acid residues having a negative impact on the stability of the complex, based on their chemical properties and binding energy, were exchanged by different residues or removed completely. Two of the finally designed mutants are characterized by a significantly higher affinity for LIGHT and may be used as potential drugs candidates against immune diseases and in organ transplantations.

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P08

The interactions in ionic liquids dependent on their atomic point charges

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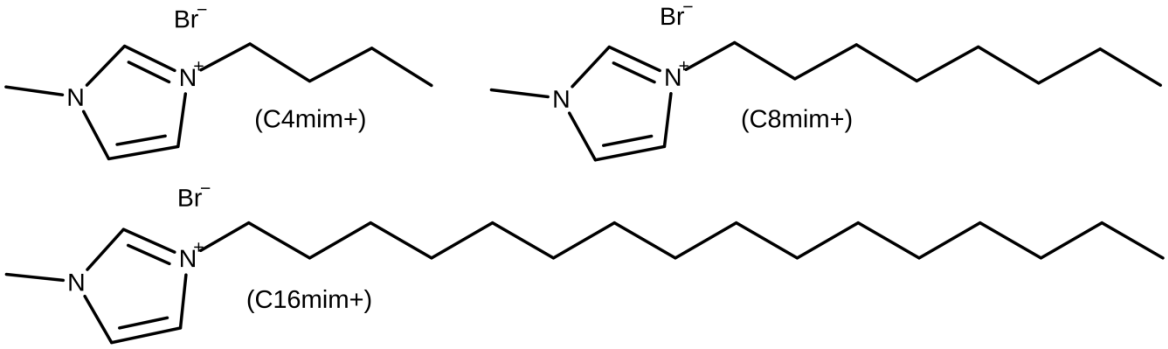
To clarify the role of the side chain of ionic liquids (ILs) on the formation of $Bi_4O_5Br_2$ and BiOBr crystallites in dependence on the solvent used, imidazolium cation with an alkyl chain containing from 4 to 16 carbon atoms (C4mim+, C8mim+, C16mim+) were built and compared. The molecular dynamics (MD) calculations were carried out in ethylene glycol (EG) and explicit water. The main goal of this investigation was to confirm that the imidazolium cation addition was modifying the ethylene bonds network, but during the construction of MD sets and discussion on proper point charges on imidazolium cation atoms the need for scaled (0.8 *e*) [1] or unscaled (1.0 *e*) [2] atomic point charges of imidazolium cations has been questioned. The number of hydrogen bonds formed during MD simulation and the interaction forces between individual system elements were determined.



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Acknowledgments

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

P09

Molecular dynamics study on the influence of C-terminal sugar substitution on dynamics and conformation of vancomycin derivatives

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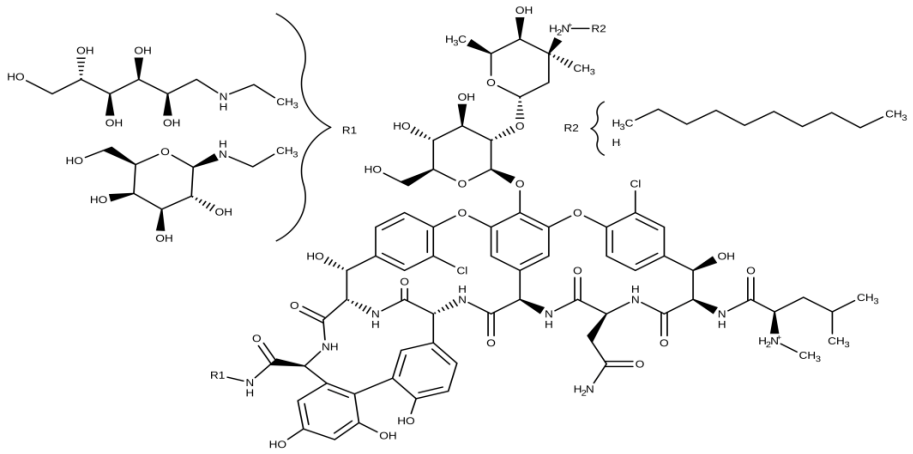
The effect of modifications of vancomycin (Van) on the interactions of its derivatives with the peptidoglycan (PG) fragment (Ace–Ala–D-iGlu–Lys–D-Ala–D-Ala) was studied. Complexes of a pentapeptide representing PG and unmodified Van or Van with selected substituents (chain (D-glucitol) or cyclic (ß-D-galactopyranose) sugar and/or decane) were constructed. Molecular dynamics (MD) and evaluation of hydrogen bond lengths determined to be critical for Van recognition and activity [1] in complexes with the physiological PG of the bacterial cell wall were performed.

Conformational analysis of complex components and control distances indicated energetically or conformationally favorable and unfavorable Van substitutions and the effect of the substitutions studied on Van–PG binding strength.



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Computations were carried out using the computers of Centre of Informatics Tricity AcademicSupercomputer & Network.

P10

Molecular modeling of the vasopressin V1b receptor and its interaction with a selective antagonist

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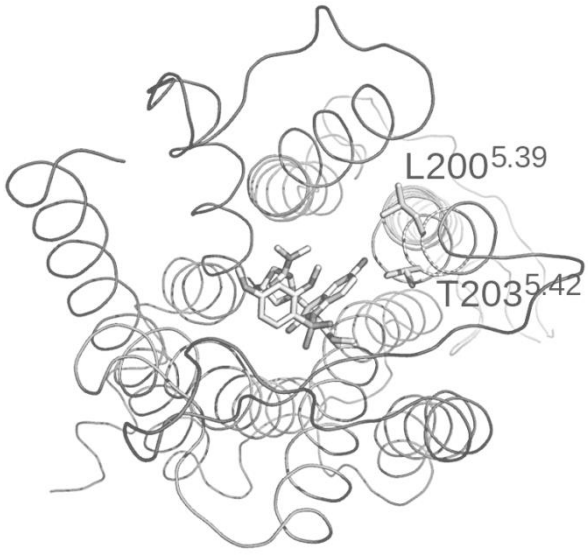
The human V1b receptor (V1bR) belongs to the G protein-coupled receptors. The activation of V1bR induces the secretion of adrenocorticotropin hormone (ACTH) from the anterior pituitary cells which, in turn, stimulates the production of cortisol via the adrenal cortex. The chronic dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis is correlated with several psychiatric disorders. Therefore, the inhibition of the V1b receptor and normalizing the HPA axis activity is a promising approach to the treatment of stress-related disorders such as anxiety and depression. Nelivaptan is a selective V1bR antagonist which may be used for treating depressive and anxiety disorders. It is also an excellent model molecule to study V1bR interaction with antagonists. In recent years the experimental structures of vasopressin V2 and oxytocin receptors were solved, providing excellent templates for homology modeling of V1bR. To study receptor-antagonist interactions, 1.5 μs all-atom molecular dynamics of a V1bR-nelivaptan complex in a fully hydrated lipid bilayer has been conducted. The receptor-ligand interaction has been analyzed in detail, revealing the the molecular mechanism of antagonist binding to V1bR, namely a probable contribution of $L200^{5.39}$ and $T203^{5.42}$ to binding selectivity.



Acknowledgements

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

P11

Structural characterization of the arginine-rich gemini lipopeptides containing p-xylene or biphenyl bridge

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Ultrashort cationic lipopeptides (USCLs) are potent antimicrobial and antifungal agents. Lipopeptide antibiotics, such as daptomycin and polymyxins are often used as drugs of last-resort in the treatment of systemic infections caused by susceptible strains of multidrug-resistant (MDR) organisms such as *Pseudomonas aeruginosa* or *Staphylococcus aureus.* The mechanism of lipopeptides' action is still not fully understood. In general, they interact with the bacterial or fungal cell membrane and disrupt the membrane integrity, initiating a series of events that eventually leads to the cell's death. Understanding this mechanism at the molecular level is crucial for designing novel lipopeptide agents with improved antimicrobial activity.
In this work,two arginine-rich gemini lipopeptides containing p-xylene or a biphenyl bridge have been studied. They are bothgemini surfactants consisting of two surfactant molecules bonded together by a spacer. To determine the antimicrobial activity of the investigated lipopeptides against*S. aureus*, *P. aeruginosa* and *C. albicans*, minimal inhibitory concentrations (MIC) testing was performed. To assess the toxic side effects of the compounds against human cells, standard hemolysis assays were performed. The lipopeptide structures was constructed *de novo* and energy minimized. The self-assembly tendency of the lipopeptides was tested using molecular dynamics in explicit water with trifluoroacetate anions as the counterions. All-atom molecular dynamics simulations were performed to investigate the mechanism of interaction of the lipopeptides with bacterial and fungal membranes.

Acknowledgements

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

P12

In silico prediction of the structure of the VAMP72a SNARE protein of Lotus japonicus, critical for forming mycorrhizal interactions

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Mycorrhiza is a critically important form of interaction between plants and fungi, which has yet to be fully understood. While itsidentification and quantification are for the most partattainable in routine study, the molecular mechanismsupon which it depends remain largely unknown. This research aims to further this field by predicting the structure of the LjVAMP72a protein, which, along with LjVAMP72b, was determined to be essential for the formation of mycorrhizal interactions of the model legume *Lotus japonicus* with fungi [1]. Its sequence was known [2], but its structure was not. Therefore,an *in-silico* simulation was conducted to determine the structure of LjVAMP72a. The obtained resultsmay be an important steppingstone into further insights into the mechanisms of its binding and interactions, especially as is compares to other members of the VAMP family and will prove highly useful when research pertaining to the fungus side of this interactionemerges and the time comes to conduct molecular dynamics between them.

Reference

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P13

Unveiling the Phase Separation Behavior of Ultrashort peptides using UNRES coarse-grain force field

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The significance of protein phase separation in physiological and pathological processes is becoming increasingly evident. The liquid-liquid phase separation (LLPS) of proteins plays a crucial role in the formation of membraneless compartments in cells[1], but aberrant LLPS and liquid-solid phase separation (LSPS) can lead to the formation of neurotoxic aggregates[2,3]. Understanding the sequence determinants and molecular mechanisms underlying phase separation is vital for designing therapeutic targets to address protein-aggregation disorders.

In this study, we investigated the phase separation of many peptides, with chain length from 2 to 7 residues, using the UNRES coarse-grainforce field. Each simulated system consisted of 320 peptide molecules. The size of the simulation box was adjusted so that in each simulation there was the same volume per amino acid residue. Through Multiplexed-Replica Exchange Molecular Dynamics(MREMD) simulations, we found that various peptides spontaneously phase separate into solid- or liquid-like condensates. Our predictions were verified through transmission electron microscopy(TEM). This study marks the successful prediction of the LLPS behavior of peptides, which has been challenging to investigate using existing theoretical and computational methods designed for larger proteins. Our findings shed light on the minimalistic building blocks and sequence determinants crucial for protein phase separation, offering new insights into the design of therapeutic strategies targeting protein-aggregation disorders.

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P14

Structural Characterization and Degradation of a Double Knotted Protein CnTrmD-Tm1570 from Calditerrivibrio nitroreducens

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Knots in proteins can vary in complexity, ranging from simple knots, such as trefoil knots, to more elaborate and intricate arrangements. The knotted protein formation can be attributed to a combination of factors, including the folding pathway, the sequence of amino acids, and the presence of certain structural motifs. The study of knotted proteins provides valuable insights into the folding process, stability, and function of these biomolecules. The main aim is to study most complex knotted structures in proteins, which we have discovered in the SpouT superfamily methylotransferaze (SAM). These structures involve the presence of two knots and they are found in pathogens and in organisms living in extreme conditions. The first crystal structure is composed by TrmD and Tm1570 and the double knotted protein derived from Calditerrivibrio nitroreducens (Cn). The CnTrmD-Tm1570 protein comprises two domains, both containing a single trefoil knot within their respective active sites. Notably, these domains exhibit independent functionality across different organisms. Our investigations reveal that CnTrmD-Tm1570 forms a homodimeric complex, with binding affinity for SAM and Mg2+ ions. Intriguingly, each protein backbone forms a granny knot, positioning this protein as one of the 296 possible doubly knotted proteins within the SPOUT family. Furthermore, our study delves into the degradation dynamics of this unique double knotted protein and its individual domains, CnTrmD and CnTm1570. Through experimental analysis, we demonstrate that the ClpXP degradation system effectively degrades the double knotted protein as well as its constituent domains. To gain insights into the underlying mechanisms governing degradation rates, we employ numerical simulations. Our findings indicate that the knot core location and the degree of local frustration play crucial roles in determining the degradation rate. In more detail, in this study we aim to determine how double knotting affects folding of proteins, biological activity, and proteasome degradation. Our analysis was based on computer simulations and supported by experiments.

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P15

Estimation of positions of protein side-chain hydrogens based on coarse-grained geometry using artificial intelligence methods

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Artificial intelligence methods have achieved a remarkable success in protein structure prediction, as shown by e.g. the DeepMind AlphaFold2 group in the 2020 CASP14 experiment[1]. However, these approaches are of limited use in studying the conformations of flexible proteins and protein dynamics, whereas physics-based approaches that use force fields are quite successful in this area. For example, coarse-grained UNRES force field was used with success to study protein folding, free-energy landscapes, and to solve a variety of biological problems [2]. Recently, a new implementation of UNRES to handle ambiguous and contradictory NMR restraints was created [3,4]. The use of NMR data with coarse-grained UNRES was made possible through a numerical method to estimate atom positions based on coarse-grained geometry [5]. Recently, we have presented that for the Hα and the Hβ protons long short-term memory recurrent neural networks give better results than analytical formulas (using the same data - Cα trace). In this study, we have used neural networks to predict positions of side-chain hydrogens. We have tested different approaches, from dense neural networks, through convolutional neural networks, recurrent neural networks to transformers. The results of these studies will be presented.

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P16

Heparin Interaction with Calcium Cations: Potential of Mean Force for 2-O-Sulfo-L-Iduronic Acid

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Heparin is a biologically important glycosaminoglycan (GAG) used pharmaceutically as an anticoagulant. Due tothe high negative charge density, electrostatic force plays a significant role in its interactions with proteins. These interactions are often solvent- or cation-mediated[1].

Due to their high charge, flexibility, and variable length, GAGs are difficult to study experimentally [2]. Simulations using traditional all-atom force fields are also limited to short GAG fragments as longer chains are computationally demanding. To overcome this challenge coarse-grained models have been developed, significantlyreducingcomputational costs.

As part of the UNICORN family of coarse-grained force fields,SUGRESisdedicated to saccharide simulationand has recently been extended to heparin subunits [3,4]. Here we present the early results of work aiming to introduce interactions between heparin monosaccharide units and calcium cations.Based on QM calculations of 2-O-sulfo-L-iduronic acid – one of the heparin components - we derived a potential of mean force which will serve as a basis for the corresponding energy term in the SUGRES force field.

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P17

UNRES-MARTINI

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In this work, we present a new force field that combines two well-established force fields: the UNRES force field for proteins [1,2] and the MARTINI force field for lipids [3,4]. We computed the potentials of mean force (PMF) for interaction between peptide groups and side chains in UNRES model representation and 18 types of MARTINI lipid centers. The PM7 quantum mechanical method was employed, along with the implicit solvent model SMD, which accurately captures solvation effects. After computation of the PMFs we fitted the analytical function derived in previous works [5] and implemented it into the UNICORN software (the UNRES offspring which incorporates all different types of molecules). The new code required memory improvements which were described for the UNRES force field in earlier work [6]. We applied the obtained software to simulate the mu-opioid receptor, a representative of G protein-coupled receptors (GPCRs), and found that C-terminal is very flexible and can even penetrate the membrane. Our simulations also demonstrated that the structure and dynamics of the transmembrane helices is well preserved. Additionally, we simulated the Gramicidin A peptide and its behavior in the membrane.

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P18

Kinesin-microtubule binding insight.

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Kinesin is a motor protein that uses energy derived from ATP hydrolysis to move organelles along microtubules (MT)and plays a role in tasks like cargo transport and mitosis[1]. The kinesin-1 subfamily mainly exists in the nerve axons and form a dimer structure *in vivo*[1,2]. The entire structure of kinesin-1 can be divided into three domains, i.e., the motor domain, the tail domain and the stalk domain. The motor domain (also called motor head), which contains the nucleotide-binding and microtubule-binding sites, is highly conserved among the kinesin family. The tail domain is used to bind with the "cargo". The motor domain and the tail domain are connected by a single long α-helix, which is called the stalk domain[1,2].

The contribution of the C-terminal tails of MT and different nucleotide-binding stateswere investigated in this work. The coarse-grained molecular dynamic simulations of kinesin motor and stalk domains interacting with the MT were performed with the use of multi-GPU version of UNRES software [3]. The 50 events for each of the nucleotide-binding state and MT type were obtained. Each simulation lasted for 25 ns (25 μs in laboratory time scale).

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P19

Application of heterobifunctional cross-links pseudopotentials in UNRES

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This work is the next step in the determination of cross-links pseudopotentials [1]. Five heterobifunctional cross-links have been examined. All particles are composed by succinimidyl 4,4′-azipentanoate (SDA) with Lysine on one side. On the other side of cross-links five different side chains are attached (aspartic acid, glutamic acid, serine, threonine, tyrosine). The pseudopotentials have been derived with help of all-atom molecular dynamics simulations in Amber ff14SB force field. Subsequently, the potentials have been applied for coarse-grained simulations in UNRES. The impact of cross-links presence in protein into its folding process have been examined for selected proteins. Some of the results have been compared to the experimental results. Moreover, the pseudopotentials have been replaced by potential well restraints. When compared cross-links results to no-restraints ones, it has been revealed that the eficiency is in favor of the former one, but not significantly. It has been also shown that the cross-links pseudopotentials efficiency is either close to the potential well or even less.

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P20

Protein-glycosaminoglycan interactions in different force fields

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Glycosaminoglycans (GAGs) constitute a class of long, linear, and negatively charged carbohydrates composed of recurring dimeric units. Each GAG, with the exception of keratan sulfate, consists of an amino sugar and a uronic acid [1]. GAGs typically consist of 5 to 30,000 disaccharide units, depending on the type of GAG. Each of these polysaccharides has a specific distribution of sulfate groups, known as a sulfate code. Currently, there are six main types of GAGs: chondroitin sulfate (CS), dermatan sulfate (DS), hyaluronic acid (HA), heparin (HP), heparan sulfate (HS), and keratan sulfate (KS). GAGs are present in the extracellular matrix and lysosomes, where they are involved in numerous biologically relevant processes, including angiogenesis, anticoagulation, signaling cascades, cell adhesion, cell proliferation, and many others [2]. GAGs participate in these biological processes primarily through electrostatic interactions with various protein targets, including growth factors and cathepsins. Despite playing a crucial role in proper physiology, these systems present substantial challenges in terms of detailed analysis, both computational and experimental. Therefore, novel methods, protocols, and approaches are being developed to address these challenges [3].

The computational analysis of these systems, allowing for the description of specific protein-GAG interactions, involves standard and specific approaches, including the calculation of electrostatic potential maps, molecular docking, molecular dynamics (MD), free energy calculations, and conformational analysis. One of the most critical steps, MD analysis, is typically performed using either the ff14SB/GLYCAM06j or CHARMM36m force fields. Here, protein-GAG interactions in these two fields are compared, considering various energetic and conformational properties such as binding free energy, ring pucker and glycosidic linkage analysis, contact maps, RMSD, and RMSF. Both force fields provided coherent results. Minor differences were observed for specific parameters such as glycosidic linkages, ring puckers, RMSD and RMSF.

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Jastrzębia Góra

The initial mentions of Jastrzębia Góra date back to the interwar period of the 20th century, a time when Poland had regained its independence, and the first dwelling was erected in this region. The name "Jastrzębia Góra" was bestowed upon it by Polish settlers who had arrived from outside the immediate vicinity. Prior to this, the territory that now constitutes Jastrzębia Góra was desolate and difficult to access, lacking any established roads, and it primarily served as pastures for the grazing of geese. This is why the Kashubian name for Jastrzębia Góra is "Pilëce," as "pila" in Kashubian refers to a small goose, and "Pilëce" signifies a place where geese graze.