4-th Polish - Korean Conference on Protein Folding

Theoretical and Experimental Approaches

http://pkc2018.ug.edu.pl

9-13.09.2018

IŁAWA - POLAND

PERSPEKTYWA
FUNDACJA INICJATYW SPOŁECZNYCH I ZAWODOWYCH

SPONSORS & PARTNERS

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 fourth from the series:

The first conference (May 24 to May 28, 2015 in Seoul)

The second conference (May 28 – June 1 , 2016 in Gdańsk)

The third conference (5 – 9 February, 2017 in High-1 Resort, Korea)

Organizing committee

- Cezary Czaplewski (U of Gdansk, Poland)
- Jooyoung Lee (KIAS)
- Adam Liwo (U of Gdansk, Poland)
- Adam Sieradzan (U of Gdansk, Poland)
- Mariusz Makowski (U of Gdansk, Poland)
- Rafał Ślusarz (U of Gdansk, Poland)
- Bogdan Mierzejewski (PERSPEKTYWA)
- Agnieszka Gan-Skrzydlewska (PERSPEKTYWA)
- Artur Giełdoń (U of Gdansk, Poland) – conference secretary

IL – Invited Lecture

P – Poster

Conference program

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

09.09 – SUNDAY

18:00 – 21:00 – Registration, Conference Welcome

10.09 – MONDAY

Session I, chair: Marek Cieplak

IL1 (9:00 – 9:35) - Changbong Hyeon (KIAS, Seoul, Korea)

Energetic costs, precision, and transport efficiency of molecular motors ….……..8

IL2 (9:35 – 10:10) Marek Cieplak (PAN, Warsaw, Poland)

Empirical elements in coarse-grained models of proteins:

protein films at fluid-fluid interfaces & dual binding in cellulosomes…….……9

IL3 (10:10 – 10:45) Andrzej Koliński (Warsaw U, Poland)

Multiscale modeling of proteins and protein complexes……..…….…….....10

Session II, chair: Changbong Hyeon

IL4 (11:15 – 11:50) Juyong Lee (Kangwon U, Korea)

Finding multiple reaction pathways via global optimization of action……..……..…11

IL5 (11:50 – 12:25) Mai Suan Li (PAN, Warsaw, Poland)

Pulling for understanding biomolecular processes …….……...…….....……...12

IL6 (12:25 – 13:00) Hirokazu Yagi (Nagoya U, Japan)

Assembly and disassembly mechanisms of proteasome revealed by multilateral biophysical approaches……...…13

Session III, chair: Cezary Czaplewski

IL7 (20:00 – 20:35) Chaok Seok (Seoul U, Korea)

Prediction of protein and protein complex structure by GALAXY…….……14

IL8 (20:35 – 21:10) Andrzej Kloczkowski (Ohio U, USA)

Modeling Structure, Stability and Dynamics of Proteins and Protein Aggregates…….……15

11.09 – TUESDAY

Session IV, chair: Weontae Lee

Session V, chair: Joanna Sułkowska

20:00 – 21:00 – POSTER SESION

12.09 – WEDNESDAY

Session VI, chair: Jooyoung Lee

Session VII, chair: Adam Liwo

Poster List

Abstracts

IL01

Energetic costs, precision, and transport efficiency of molecular motors.

Hyeon Changbong[1], Hwang Wonseok[1]

[1] Korea Institute for Advanced Study, 02455 Seoul, Korea

An efficient molecular motor would deliver cargo to the target site at a high speed and in a punctual manner while consuming a minimal amount of energy. According to a recently formulated thermodynamic principle, referred to as the thermodynamic uncertainty relation, the travel distance of a motor and its variance are, however, constrained by the free energy being consumed. Here we use the principle underlying the uncertainty relation to quantify the transport efficiency of molecular motors for varying ATP concentration ([ATP]) and applied load (f). Our analyses of experimental data find that transport efficiencies of the motors studied here are semi-optimized under the cellular condition. The efficiency is significantly deteriorated for a kinesin-1 mutant that has a longer neck-linker, which underscores the importance of molecular structure. It is remarkable to recognize that, among many possible directions for optimization, biological motors have evolved to optimize the transport efficiency in particular.

References.

[1] Hyeon C, Hwang W (2017) Physical insight into the thermodynamic uncertainty relation using Brownian motion in tilted periodic potential. Phys. Rev. E. 96:012156

[2] Hwang W, Hyeon C (2017) Quantifying the Heat Dissipation from a Molecular Motor's Transport Properties in Nonequilibrium Steady States. J. Phys. Chem. Lett. 8:250-256

[3] Hwang W, Hyeon C (2018) Energetic Costs, Precision, and Transport Efficiency of Molecular Motors. J. Phys. Chem. Lett. 9:513-520

IL02

Empirical elements in coarse-grained models of proteins: protein films at fluid-fluid interfaces & dual binding in cellulosomes

Cieplak Marek[1]

[1] Institute of Physics, Polish Academy of Sciences, 02-668 Warsaw, Poland

We study the behavior of several proteins at the air–water and oil–water interfaces by all-atom molecular dynamics. The proteins are found to change orientation and get distorted when pinned to the interface. This behavior is consistent with the empirical way of introducing the interfaces in a coarse-grained model through a hydropathy-related force. Proteins couple to the oil–water interface stronger than to the air–water one. They diffuse slower at the oil–water interface but do not depin from it, whereas depinning events are observed at the other interface. The reduction of the disulfide bonds slows the diffusion down. We use the model to study interfacial protein layers and demonstrate existence of glassy effects as evidenced by slowing down of diffusion with increasing concentration of proteins. We also show that layers of two barley proteins, LTP1 and its ligand adduct LTP1b, flatten out at the interface and can make a continuous and dense film that should be responsible for formation and stability of foam in beer. The degree of the flattening depends on the protein - the layers of LTP1b should be denser than those of LTP1 – as well as on the presence of glycation and on the number of disulfide bonds.

The assembly of the polysaccharide degradating cellulosome machinery is mediated by tight binding between cohesin and dockerin domains. We have used an empirical model known as FoldX as well as molecular mechanics methods to determine the free energy of binding between a cohesin and a dockerin from *Clostridium thermocellum* in two possible modes that differ by an approximately 180^{o} rotation. Our studies suggest that the full-length wild-type complex exhibits dual binding at room temperature, i.e., the two modes of binding have comparable probabilities at equilibrium. However, single-point mutations or truncations of terminal segments in the dockerin result in shifting the equilibrium towards one of the binding modes. Each mode of binding leads to two kinds of stretching pathways, which may be mistakenly taken as evidence of dual binding.

References

[1] Cieplak M, Allen DB, Leheny RL, D. H. Reich DH (2014) Proteins at air-water interfaces: a coarse-grained approach. Langmuir 30: 12888-96

[2] Zhao Y, Chwastyk M, Cieplak M ~(2017) Topological transformations in proteins: effects of heating and proximity of an interface. Sci. Rep. 7: 39851

[3] Zhao Y, Cieplak M (2017) Structural changes in barley protein LTP1 isoforms at air-water interfaces. Langmuir 33: 4769-4780

[4] Zhao Y, Cieplak M (2017) Proteins at air-water and oil-water interfaces in an all-atom model. Phys. Chem. Chem. Phys. 19: 25197-25206

[5] Wojciechowski M, Różycki B, Huy Pham DQ, Li MS, Bayer EA, Cieplak M (2018) Dual binding in cohesin-dockerin complexes: the energy landscape and the role of short, terminal segments of the dockerin module. Sci. Rep. 8: 5051

IL03

Multiscale modeling of proteins and protein complexes

Andrzej Kolinski, Sebastian Kmiecik, Dominik Gront, Mateusz Kurcinski, Maciej Blaszczyk

University of Warsaw, Poland

The traditional, based on atomistic Molecular Dynamics, computational modeling of protein structure and dynamics remains difficult for many protein systems. It is mostly due to the size of protein conformational spaces and required simulation timescales. Shrinking the protein representation from all-atom to coarse-grained [1] extends the range of tractable systems. Two levels of coarse-graining and their applicability in the multiscale modeling strategies are discussed. Moderate resolution CABS (C-Alpha, Beta and Side-chain) enables quite efficient modeling of protein structure [2], dynamics [3-4] and protein-peptide molecular docking [5]. The model uses up to four united atoms for representations of main chain units and side chains of amino acid residues. The model of interactions is based on knowledge based statistical potentials and the sampling schemes employ Monte Carlo dynamics. CABS approach significantly speeds-up the modeling process and generates structures of sufficient resolution for realistic all-atom reconstruction. Unfortunately, CABS and related middle-resolution models are still computationally too expensive for the simulations of large (size and time) systems. For this reason we developed a lower resolution (SURPASS-Single United Residue per Pre-Averaged Secondary Structure fragment) model [6], that still maintains several protein-like structural features thanks to knowledge-based sequence specific multibody interaction. SURPASS enables very fast, although of low resolution, simulations of large proteins and protein systems [6-7]. Multiscale modeling strategies based on combination of these coarse-grained models are briefly discussed.

References

[1] S. Kmiecik, D. Gront, M. Kolinski, L. Wieteska, A. Dawid & A. Kolinski, "Coarse-grained protein models and their applications", *Chemical Reviews* **116(14)** 7898–7936 (2016)

[2] M. Blaszczyk, M. Jamroz, S. Kmiecik & A. Kolinski, "CABS-fold: server for de novo and consensus-based prediction of protein structure" *Nucleic Acids Research* **41(W1)**:W406-W411 (2013)

[3] Aleksander Kuriata, Aleksandra Gierut, Tymoteusz Oleniecki, Maciej Ciemny, Andrzej Kolinski, Mateusz Kurcinski, Sebastian Kmiecik, "CABS-flex 2.0: a web server for fast simulations of flexibility of protein structures", *Nucleic Acids Research*, **46(W1):**W338-W343 (2018)

[4] M. Kurcinski, T. Oleniecki, M. P. Ciemny, A. Kuriata, A. Kolinski & S. Kmiecik, "CABS-flex standalone: a simulations environment for fast modeling of protein flexibility" *Bioinformatics*, in press

[5] M. Kurcinski, M. Jamroz, M. Blaszczyk, A. Kolinski & S. Kmiecik, "CABS-dock: web server for flexible docking of peptides to proteins without prior knowledge of the binding site", *Nucleic Acids Research* 43**(W1)**: W419-W424 (2015)

[6] A. E. Dawid, D. Gront & A. Kolinski, "SURPASS low–resolution coarse–grained protein modeling", *J. Chem. Theory Comp.* 13**(11):**5766–5779 (2017)

[7] A. E. Dawid, D. Gront & A. Kolinski, "Coarse-grained modeling of the interplay between secondary structure propensities and protein fold assembly", *J. Chem. Theory Comp.* **14(4)**:2277-2278 (2018)

IL04

Finding multiple reaction pathways via global optimization of action

Lee, Juyong[1], Lee, In-Ho[2,3], Joung, InSuk[3,4], Lee, Jooyoung[3,4], Brooks, Bernard R.[5]

[1] Department of Chemistry, Kangwon National University, Republic of Korea

[2] Center for Materials Genome, Korea Research Institute of Standards and Science, Daejeon 34113, Republic of Korea.

[3] Center for In Silico Protein Science, School of Computational Science, Korea Institute for Advanced Study, Seoul 02455, Republic of Korea.

[4] School of Computational Sciences, Korea Institute for Advanced Study, Seoul 02455, Republic of Korea.

[5] Laboratory of Computational Biology, National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health (NIH), Bethesda, Maryland 20892, USA.

In this talk, we will present the advances in computational methods to bridge the gap between the structures and dynamics of biomolecules. We will present a new computational approach, Action-CSA, to find multiple reaction pathways with fixed initial and final states through global optimization of the Onsager-Machlup action using the conformational space annealing method [1]. This approach successfully finds all possible pathways of small systems without initial guesses on pathways. Pathway space is efficiently searched by crossover and mutation operations of a set of pathways and preserving the diversity of the set. The search efficiency of the approach is assessed by finding pathways for the conformational changes of alanine dipeptide and hexane. The benchmarks demonstrate that the rank order and the transition time distribution of multiple pathways identified by the new approach are in good agreement with those of long Langevin dynamics simulations. We also show that the lowest action folding pathways of the mini-protein FSD-1 identified by the new approach is consistent with previous molecular dynamics simulations and experiments. In addition, we will discuss the future application of Action-CSA to finding binding reaction pathways of protein-ligand complexes based on the global organization of protein binding sites [2].

References

[1] Lee, J., Lee, I.-H., Joung, I., Lee, J. & Brooks, B. R. (2017) Finding multiple reaction pathways via global optimization of action. *Nat. Commun.* **8,** 15443

[2] Lee, J., Konc, J., Janežič, D. & Brooks, B. R. (2017) Global organization of a binding site network gives insight into evolution and structure-function relationships of proteins. Sci. Rep. **7,** 11652

Acknowledgments. We acknowledge helpful discussions with Attila Szabo and Richard Pastor. The authors wish to acknowledge Steven Gross for his critical reading of the manuscript. Juyong Lee and B.R.B. were supported by the Intramural Research Program of the NIH, NHLBI. I.-H.L., I.J. and Jooyoung Lee were supported by the National Research Foundation of Korea (NRF). I.-H.L. was also supported by Samsung Science and Technology Foundation.

IL05

Pulling for understanding biomolecular processes

Mai Suan Li

Institute of Physics, Polish Academy of Sciences, Al. Lonikow 32/46, 02-668 Warsaw, Poland

Binding affinity of a small ligand to a receptor is the important quantity in drug design and it might be characterized by different quantities. The most popular one is the binding free energy which can be estimated by several methods in conventional molecular dynamics simulation. So far in steered molecular dynamics (SMD) one can use either the rupture force or non-equilibrium pulling work as a measure for binding affinity. We have shown that the non-equilibrium binding free energy $\Delta G_{\mathrm{neq}}^{\mathrm{Jar}}$, obtained by Jarzynski's equality at a finite pulling speed, has good correlation with experimental data on inhibition constants implying that this quantity can be used as a good scoring function for binding affinity. In addition the rupture time as well as binding and unbinding free energy barriers are also good descriptors for binding affinity. Our observation is useful for fast screening of potential leads as the SMD simulation is not time consuming.

The fibril formation resulting from protein misfolding and aggregation is a hallmark of several neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Despite much progress in understanding the protein aggregation process, the factors governing fibril formation rates and fibril stability have not been fully understood. We showed that the kinetic stability of the fibril state can be accessed via mechanical stability in such a way that the higher mechanical stability or kinetic stability the faster fibril formation. This result opens up a new way for predicting fibril formation rates based on the mechanical stability which may be easily estimated by the SMD method.

We have also demonstrated that SMD is useful for studying ribosome stalling by antibiotics and for assessing the blood-brain barrier.

IL06

Assembly and disassembly mechanisms of proteasome revealed by multilateral biophysical approaches

Yagi Hirokazu[1], Yagi-Utsumi Maho[1], Satoh Tadashi[1], and Kato Koichi [1, 2]

[1] Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan

[2] Exploratory Research Center on Life and Living System and Institute for Molecular Science, National Institutes of Natural Sciences, 5-1 Higashiyama Myodaijicho, Okazaki 444-8787, Japan

Molecular organization in biological systems comprises elaborately programmed processes involving metastable complex formation of biomolecules. This is exemplified by the formation of the proteasome, which is one of the largest and most complicated biological supramolecular complexes. This biomolecular machinery comprises approximately 70 subunits including structurally homologous, but functionally distinct, ones, thereby exerting versatile proteolytic functions. In eukaryotes, proteasome formation is non-autonomous and is assisted by assembly chaperones, which transiently associate with assembly intermediates, operating as molecular matchmakers and checkpoints for the correct assembly of proteasome subunits. Accumulated data also suggest that eukaryotic proteasome formation involves scrap-and-build mechanisms. However, unlike the eukaryotic proteasome subunits, the archaeal subunits show little structural divergence and spontaneously assemble into functional machinery. Nevertheless, the archaeal genomes encode homologs of eukaryotic proteasome assembly chaperones.

Here we introduce the assembly and disassembly mechanisms of eukaryotic proteasome by using multilateral biophysical approaches such as mass spectrometry, X-ray crystallography and atomic force microscopy. In addition, we also introduce the recent structural and functional studies of archaeal homologs of eukaryotic proteasome assembly chaperones for understanding the evolution of molecular mechanisms involved in proteasome biogenesis. This knowledge in-turn provides a guiding principle in designing molecular machineries using protein-engineering approaches and de novo synthesis of artificial molecular systems.

References

[1] Yagi-Utsumi M, Sikdar A, Kozai T, Inoue R, Sugiyama M, Uchihashi T, Yagi H, Satoh T, Kato K (2018) Conversion of functionally undefined homopentameric protein PbaA into a proteasome activator by mutational modification of its C-terminal segment conformation. Protein Eng Des Sel.31:29-36. doi: 10.1093/protein/gzx066.Kozai T, Sekiguchi T, Satoh T,

[2] Yagi H, Kato K, Uchihashi T (2017) Two-step process for disassembly mechanism of proteasome α7 homo-tetradecamer by α6 revealed by high-speed atomic force microscopy. Sci Rep. 13:15373. doi: 10.1038/s41598-017-15708-8.

[3] Ishii K, Noda M, Yagi H, Thammaporn R, Seetaha S, Satoh T, Kato K, Uchiyama S. (2015) Disassembly of the self-assembled, double-ring structure of proteasome α7 homo-tetradecamer by α6. Sci Rep. 14:18167. doi: 10.1038/srep18167.

IL07

Prediction of protein and protein complex structure by GALAXY

GALAXY team and Chaok Seok*

Department of Chemistry, Seoul National University, Seoul 08826, Korea

*E-mail: chaok@snu.ac.kr

Association of proteins is abundant in nature and is intimately related with the physiological functions of proteins, such as in metabolism, signal transduction, or immunity. Information on the protein complex structure is therefore important to obtain a molecular-level understanding of protein functions and their regulation. In this presentation, I will introduce the softwares and web servers (available in http://galaxy.seoklab.org) for protein structure and protein complex structure prediction developed in our group such as GalaxyTongDock and GalaxyPPDock for ab initio docking, GalaxyPepDock for protein-peptide docking, and GalaxyHomomer for protein homo-oligomer structure prediction. We have observed that applicability and performance of the programs are improved further by combining the docking methods with template-based modelling (GalaxyTBM), loop modeling (GalaxyLoop), and refinement (GalaxyRefine) methods developed in the group. These methods have proven to be successful in blind prediction experiments such as CASP (Critical Assessment of techniques for protein Structure Prediction) and CAPRI (Critical Assessment of PRedictions of Interactions).

References

[1] M. Baek, T. Park, L. Heo, C. Park, and C. Seok, GalaxyHomomer: A web server for protein homo-oligomer structure prediction from a monomer sequence or structure, ***Nucleic Acids Res.*** 45 (W1), W320-W324 (2017).

[2] G. R. Lee and C. Seok, Galaxy7TM: Flexible GPCR-ligand docking by structure refinement, ***Nucleic Acids Res***. 44 (W1), W502-506 (2016).

[2] H. Lee, L. Heo, M. S. Lee, and C. Seok, GalaxyPepDock: a protein-peptide docking tool based on interaction similarity and energy optimization, ***Nucleic Acids Res***. 43 (W1), W431-W435 (2015).

IL08

Modeling Structure, Stability and Dynamics of Proteins and Protein Aggregates

Andrzej Kloczkowski

Nationwide Children's Hospital and The Ohio State University, Columbus OH, USA

Recent progress in modeling structure and dynamics of proteins and protein aggregates will be reviewed. Recent advancements in structure prediction such as development of better potentials and force-fields (including multibody potentials), and improved modeling of free energies will be presented. We significantly improved protein structure evaluations by considering the effects of amino acid variants on protein stability, and we have shown that the outliers in stability are typically aberrant proteins. Recently, we have made significant progress in understanding protein stability and dynamics by computing protein free energies extracted from structures to account for the high packing densities in proteins, including important novel evaluations of protein entropies. Preliminary results show large improvements over previous potentials for assessing protein stabilities. Our results demonstrate that there are substantial gains in specificity from combining the sequence with structural and protein dynamic data. These developments may significantly impact the advancement of precision/personalized medicine.

IL09

Intrinsic disorder in the extracellular matrix: does binding promote folding?

Ricard-Blum Sylvie[1]

[1] Institute of Molecular and Supramolecular Chemistry and Biochemistry, UMR 5246, CNRS - University Lyon 1, Villeurbanne cedex, France

The extracellular matrix (ECM) determines the shape and organization of tissues, provides them with mechanical properties, and regulates numerous cellular processes. ECM assembly and functions are mediated by protein-protein and protein-glycosaminoglycan (GAG) interactions. The aims of this work were *i*) to build interaction networks of individual proteins and GAGs, and of the human ECM, *ii*) to determine how these networks are rewired in physiological and pathological contexts with a focus on the role played by intrinsic disorder, and *iii*) to build a comprehensive 3D network of the ECM.

Proteoforms, proteolysis, growth factors, mutations, and conformational changes contribute to the rewiring of ECM networks *in vivo*. Indeed, the ECM proteome contains numerous intrinsically disordered regions, which can adopt a broad range of conformations and are prone to interactions [1]. We focused on the structure-interaction-function relationship of one intrinsically disordered bioactive ECM fragment, the propeptide of lysyl oxidase, and of the ectodomains of syndecans 1-4, which contain a large amount of disorder. We have shown by several biophysical techniques (circular dichroism, small angle X-ray scattering, dynamic light scattering and multi-angle light scattering) that the propeptide of lysyl oxidase is monomeric, flexible and may adopt six major conformations. We have identified 17 new binding partners of the propeptide by label-free binding assays, including heparin [2] and 17 new partners. We have built its interaction network, which comprises ECM proteins and GAGs (43%), matrisome-associated (21%), and membrane or intracellular (36%) proteins, using data stored in MatrixDB database [3], and other databases of the International Molecular Exchange consortium. Using the above approaches and ESI-mass spectrometry, we have identified two major populations of the intrinsically disordered ectodomain of syndecans, which may have different roles, mediated by different partners, in ECM-cell interplay and cell signaling. Given that the propeptide of lysyl oxidase and syndecans are able to fold and form α-helices in certain conditions, the next step is to integrate the intrinsically disordered regions, predicted by IUPred, in the human ECM interactome (~ 40 000 experimental protein-protein and 990 protein-GAG interactions) to identify the most structurally flexible and dynamic zones of the network able to induce local and/or global rewiring of the interactions.

References

[1] Peysselon F, Xue B, Uversky VN, Ricard-Blum S (2011) Intrinsic disorder of the extracellular matrix Mol Biosyst 7:3353-3365.

[2] Vallet SD, Deddens L, Vonarburg A, *et al.* (2017) Strategies for building protein-glycosaminoglycan interaction networks combining SPRi, SPR, and BLI. In: Schasfoort RBM (ed) Handbook of Surface Plasmon Resonance, 2nd edn. Royal Society of Chemistry, pp 398-414

[3] Launay G, Salza R, Multedo D, Thierry-Mieg N, Ricard-Blum S (2015) MatrixDB, the extracellular matrix interaction database: updated content, a new navigator and expanded functionalities. Nucleic Acids Res 43(Database issue):D321-327.

IL10

Computational Insights into the Glycosaminoglycan-mediated Molecular Mechanisms Underlying Cell Signaling

Samsonov Sergey[1], Bojarski Krzysztof Kamil[1], Uciechowska-Kaczmarzyk Urszula[1]

[1] Faculty of Chemistry, University of Gdańsk, Poland

Glycosaminoglycans (GAGs) is a class of long unbranched negatively charged polysaccharides formed by a repetitive disaccharide unit made up of an aminosugar and an uronic acid [1]. They bind their protein targets in the extracellular matrix (ECM) and at the cell surface such as chemokines and growth factors and so actively participate in cell signaling processes affecting cellular adhesion, proliferation and communication [2]. At the same time, molecular mechanisms underlying GAGs-mediated processes in ECM are not fully understood, and application of experimental techniques is not sufficient alone for gaining insights into the interactions occurring at the molecular level. In these lines, theoretical approaches not only complement but also beneficially contribute to the elucidation of the role of GAGs by bringing fundamentally crucial though experimentally inaccessible details [3].

We established and tested a set of computational approaches, which we successfully applied for characterization of protein-GAG interactions in particular systems to complement, support and rationalize available experimental data. We observe the dependence of protein-GAG interactions on GAG net sulfation, type and length. GAGs can be involved in the interactions of their protein targets with their receptors by different mechanisms: directly blocking receptor binding sites; shifting probability distributions in conformational ensemble of the protein influencing, in turn, the probability of binding the receptor; repulsing receptors via long-ranged electrostatic interactions; supporting interactions via formation of sandwich structures; predetermining the sequence of binding events in protein tertiary complexes.

The data obtained in our studies widen the fundamental knowledge of protein-GAG molecular recognition, which adds to the theoretical rationale in the further development of advanced and efficient approaches for tissue regeneration.

References

[1] Esko JD, Kimata K, Lindahl U (2009) Essentials of Glycobiology, 3rd edn; Cold Spring Harbor Laboratory Press: Cold Spring Harbor (NY)

[2] Perrimon N, Bernfield M (2000) Specificities of heparan sulphate proteoglycans in developmental processes. Nature 404: 725-728

[3] Almond A (2018) Multiscale Modeling of Glycosaminoglycan Structure and Dynamics: Current Methods and Challenges. Curr Opin Struct Biol 50: 58-64

Acknowledgments

This work was supported by the National Science Center of Poland (Narodowe Centrum Nauki, grant UMO-2016/21/P/ST4/03995; This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 665778).

IL11

Sweet spots on cysteine cathepsin S:

how glycosaminoglycans and derivatives modulate its proteolytic activity

Lecaille Fabien[1], Sage Juliette[1,2], Renault Justine[1], Samsonov Sergey[3], Leblanc Emmanuel[2], Schnebert Sylvianne[2], Nizard Carine[2], Kurfurst Robin[2], Lalmanach Gilles[1]

[1] INSERM, UMR 1100, Centre d'Etude des Pathologies Respiratoires, Université de Tours, Tours, France

[2] LVMH-Recherche, Saint Jean de Braye, France

The epidermal basement membrane (BM) is a key player in adhesion between epidermis and dermis, and in controlling epidermal differentiation. It is commonly known that major constituents in the BM architecture (type IV collagen, laminins, nidogen-1) are damaged in sun-exposed and aged skin. Although matrix metalloproteinases (MMPs) and serine proteases (kallikreins) are believed to be involved in the extracellular matrix (ECM) BM breakdown, there are converging evidences that cysteine cathepsin S (CatS), which is secreted by keratinocytes, participates in distinct physiological and pathophysiological cellular processes, by degrading several major constituents of the ECM and BM, including collagen IV and nidogen-1, a key protein in BM assembly. Thus, CatS is a protease of therapeutical interest. Developing drugs for CatS has proved challenging in recent years. This in part could be due to issues such as the difficulty of achieving selectivity when targeting its active site. Here, we evaluated the capacity of natural products (ie. glycosaminoglycans and flavonoids) to inhibit CatS, through kinetics, chromatography, biophysic, docking and molecular dynamic techniques. Overall, the mechanism of action established for these natural products provides a new outlook in the search for drugs against CatS.

IL12

Bacterial DNA replication initiation proteins – structure, stability and replication control

Katarzyna Bury[1], Rafael Giraldo[2], Marta Gross1, Anna Karlowicz[1], Maria Moreno[2], Marzena Nowacka[3], Marcin Nowotny[3], Asmar Nayis[4], Małgorzata Ropelewska[1], Urszula Uciechowska[1], Katarzyna Wegrzyn[1], Elżbieta Zabrocka[1], Martin Zacharias[4], and Igor Konieczny[1]

[1] Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland,

[2] Centro de Investigaciones Biológicas (CIB), Madrid, Spain,

[3] International Institute of Molecular and Cell Biology (IIMCB), Warsaw, Poland,

[4] Technische Universität München, Germany

Plasmid and chromosomal replication initiators are key elements for DNA stably maintenance in bacterial cells. Although, chromosomally encoded DnaA proteins and plasmid encoded replication initiators (Reps) are structurally different and are responsible for replication initiation of different replicons, the previously reported research and our recent analyses indicate that both class of proteins have similar features and function in a very similar way. Both DnaA and plasmid replication initiators cause local destabilization of the DNA unwinding element region (DUE). We demonstrated that Reps specifically interact ssDNA of DUE as it was also shown for DnaA. The interaction networks of both replication initiators contribute helicase loading and replisome assemble. We have recently obtained new structural data on Rep complex with ssDNA, and, data on Rep complex accommodating both ssDNA and dsDNA. Also, our recent investigations on the stability of DnaA and Rep proteins indicate the new role of the specific proteases in DNA replication control in bacteria.

IL13

Eukaryotic transcription factors can track and control their target genes using DNA antennas

Victor Muñoz

Centro Nacional de Biotecnología (CNB-CSIC) Darwin 3, Madrid, Spain

Eukaryotic transcription factors (TF) function by binding to short, 6-10 bp DNA recognition sites located near their target genes, which are scattered through vast genomes. Such process surmounts enormous specificity, efficiency and celerity challenges using a molecular mechanism that remains poorly understood. Combining biophysical experiments, theory and bioinformatics, we dissected the interplay between the DNA-binding domain of Engrailed, a *Drosophila* TF, and the regulatory regions of its target genes. Remarkably, Engrailed binding affinity is enormously amplified by the DNA regions flanking the recognition site, which contain long tracts of degenerate recognition-site repeats. Such DNA organization operates as an antenna that attracts TF molecules in a promiscuous exchange between myriads of comparatively weaker binding sites. The antenna ensures a local TF supply, enables gene tracking and fine control of basal site occupancy. This mechanism illuminates puzzling gene expression data, and suggests novel engineering strategies to control gene expression.

Toward Understanding Architectures and Molecular Sociology of Life

SONG Ji-Joon[1]

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

Proteins are molecular machines in cells to maintain life. These molecular machines are often composed of many different proteins. However, little is known about the complexity and diversity of protein complexes and supramolecular structures in-cell and their functions.

My research focuses on three areas 1) to understand molecular mechanism of epigenetic gene regulation focusing on nucleosome assembly, modification and recognition, 2) to understand the mechanism by which neurodegenerative disease proteins cause the diseases, and lastly 3) to understand structural and molecular sociology of protein complexes in diverse organisms and cells via a comparative and comprehensive analysis.

Among these research programs, here, I will present our on-going integrative structural studies on a neurodegenerative disease protein-Huntington's disease protein. Huntington's disease (HD) is caused by mutation causing polyglutamine (PolyQ) tract expansion in huntingtin protein. We analyzed the structural and functional changes caused by PolyQ expansion in protein via an integrative structural approach including cryo-electron microscopy, mass spectrometry, small angle x-ray scattering and molecular dynamics modeling as well as biochemical approaches. Our data shows that the polyQ expansion at the N-terminus of the protein affects global structural and functional changes in the protein, which is involved in the HD pathology. Furthermore, we are seeking to develop means to regulate the structure and function of huntingtin protein, which might lead to develop therapeutics for the devastating HD.

References

[1] Jang et al., unpublished

[2] Jeong et al., unplublisehd

Acknowledgments

This work is partially supported by Korea National Research Foundation.

IL15

Structure and Dynamics of Membrane Proteins by NMR, X-RAY Crystallography and Femtosecons Laser

Ji-Hye Yun, Jae-Hyun Park and Weontae Lee*

Department of Biochemistry and 2Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, Korea
Email: wlee@spin.yonsei.ac.kr

Membrane proteins have been considered as most interesting and challenging targets for biologists because of their importance of biological functions as well as drug development. The crystallization and NMR technologies for membrane proteins have become more advanced, resulting in the accumulation of membrane protein structure information. Recently, with the development of LCP injectors, membrane protein structure determination has become possible from microcrystals at room temperature using serial femtosecond crystallography (SFX) with X-ray free-electron lasers (XFELs). Structural determination of membrane proteins using XFELs showed that XFEL-derived structures are comparable to synchrotron-derived structures. However, understanding of the structural and dynamics differences between structures determined using SFX at XFEL facilities at room temperature and those determined using the conventional macromolecular crystallography method at synchrotrons in a cryogenic state remains limited, along with the implications for molecular functions. We determined the non-cryogenic structure of chloride-pumping rhodopsin at a resolution of 1.65 Å using serial femtosecond X-ray crystallography (SFX). Strikingly, the XFEL-derived structure revealed that the all-trans retinal (ATR) region and positions of two coordinated chloride ions have a distinct location to those of the synchrotron-derived structure. This demonstration of the non-cryogenic structure of membrane proteins and NMR approach for dynamics could serve as a starting point for gaining a better understanding of the functional modulation at room temperature.

Proteins' knotty problems

Joanna I. Sułkowska

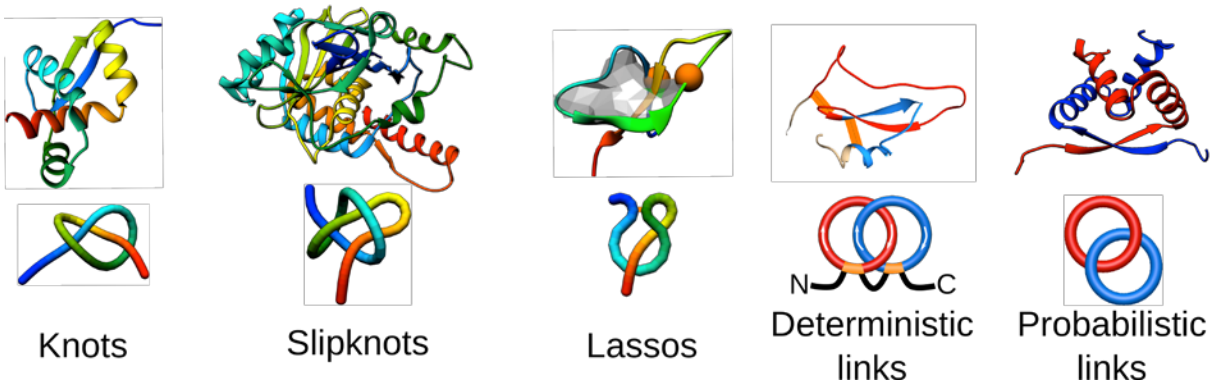
Centre of New Technologies and Faculty of Chemistry, University of Warsaw

Knotted proteins are believed to be functionally advantageous and to provide extra stability to protein chains [1]. Twenty years of their investigation suggests that they fold via a slipknot conformation, across the native twisted loop. During the talk I will show that diversity of identified folds of knotted proteins and their locations in cells is still growing and surprising us.

Moreover recently we went one step further and found that proteins can be even more entangled than knots – they also form lassos and links, which consist of several components. Based on the search through the entire Protein Data Bank we identified several sequentially nonhomologous chains that form a Hopf link, a Solomon link [2], and various types of lassos. I will show that topological properties of these proteins are related to their function and stability. Finally I will explain how the presence of links affects folding pathways of proteins and present new reaction coordinate to study entangled proteins.

During my talk I will also present knotted TrmD which is the leading antimicrobial drug target owing to its essentiality for bacterial growth, its broad conservation across bacterial species, and its substantial differences from the human and archaeal counterpart Trm5. I will also present our achievements in designing selective inhibitor for TrmD, based on combining theoretical and experimental methods [3].

All entangled proteins – 7% of proteins deposited in the PDB – are collected in databases: KnotProt, LassoProt and LinkProt.



[1] JI Sulkowska, E Rawdon, KC Millett, J Onuchic, A Stasiak, PNAS (2012) 109: E1715-23

[2] P Dabrowski-Tumanski, JI Sulkowska, PNAS (2017) 114, 3415–3420

[3] T Christian, et al., Nature S&MB (2016) 23, 941-948

IL17

Efficient refinement of predicted protein-protein and peptide-protein complexes

Martin Zacharias

Physics Department, Technical University of Munich

James Franck Str. 1, D-85747 Garching, Germany

Protein and peptide structure can change conformation upon association. Such changes can range from local adjustments of side chain conformation and global domain movements to refolding of entire peptide segments. In the ***ATTRACT*** protein-protein docking approach [1] one can account for conformational changes during complex formation using a multi-copy representation of side chains, energy minimization in collective normal modes or by including a conformational ensemble representation of partner molecules. However, many predicted complex geometries require further refinement including realistic representation of conformational flexibility and of the surrounding solvent. Preferably, refinement steps should be accurate but also rapid enough for applications that involve hundreds or thousands of complexes. In the ***iATTRACT*** method [2] the interface region of a docked complex is described in atomic detail including full flexibility of side chains and backbone. The local motion is efficiently coupled to global translational and rotational movements and possible displacements in soft normal mode directions. Recently, we developed a Hamiltonian replica exchange based method that employs a biasing potential to penalize the smallest contact distance between two partner molecules by means of an ambiguity restraint [3]. By modifying this ambiguity restraint along the replicas, a gradually increase in the average distance between the ligand and the receptor is achieved. While moving in close proximity to the protein surface in the reference replica, ligands can rotate and translate freely at slightly larger distances around the receptor surface in the higher replicas. This avoids wasting time in trapped transiently stable states. The application of the methodology to test systems of different complexity will be presented.

References

1. Zacharias M (2003) Protein-protein docking with a reduced protein model accounting for side-chain flexibility Protein Science. 12, 1271.

2. Schindler, C, de Vries S, Zacharias M (2015) iATTRACT: Simultaneous global and local interface optimization for protein-protein docking refinement. Proteins 83, 248-258.

3. Ostermeir, K.; Zacharias, M. (2017) Accelerated flexible protein-ligand docking using Hamiltonian replica exchange with a repulsive biasing potential. PloS one 12 (2), e0172072.

IL18

Recent Advances in Protonation State Modeling and Constant pH simulations

Bernard R. Brooks

Laboratory of Computational Biology, National Heart, Lung, and Blood Institute,
National Institutes of Health, USA

This presentation focuses on our recent efforts to develop improved methods for modeling systems at constant pH by including multiple explicit protonation states. By extending simulation methods to constant pH simulations using explicit waters and discrete charges, we significantly also improve our abilities to accurately calculate free energies. Two main methods will be presented, one is based on Enveloping Distribution Sampling (EDS) coupled with Replica-Exchange Methods (REM), and the other is based on a Virtual Mixture of Multiple States (VMMS). Applications to several protein system will be presented. These approaches can be combined with both enhanced sampling techniques that preserve the canonical ensemble and applied to problems in structural biology and biophysics and drug discovery efforts. Results and perspective from our participation in the recent SAMPL6 pKa and binding free energy prediction challenge will be presented.

IL19

Deltabodies - Application of *E. coli* inclusion body expression and refolded dimeric Fv based antibodies.

Xiao-Dong Su
Biodynamic Optical Imaging Center (BIOPIC)
School of Life Sciences, Peking University

A new formula of monoclonal Antibody production using E. coli inclusion body expression, and followed by in vitro refolding into homo-dimeric tags plus Fv hertero-dimeric proteins, the so-called deltabodies will be described, and their advantages and drawbacks will be presented and compared with other forms of native- or re-folded forms of antibodies. Our short term aim is using these deltabodies for modern optical imaging and diagnostic applications, the long term goal can be to apply these form of easy and low-costs protein production for therapeutic purposes.

IL20

From disordered chain to fibrils – SAXS and spectroscopic studies of protein fibrillation process

Maciej Kozak[1], Maciej Gielnik[1], Michał Taube[1], Zuzanna Pietralik[1], Aneta Szymańska[2], Igor Zhukov[3], Michał Nowakowski[4], Wojciech Kwiatek[4]

[1] Department of Macromolecular Physics, Adam Mickiewicz University, Poznań, Poland

[2] Faculty of Chemistry, Gdańsk University, Gdańsk, Poland

[3] Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warszawa, Poland

[4] Institute of Nuclear Physics Polish Academy of Sciences, Kraków, Poland

Many proteins that are involved the development of neurodegenerative diseases (Alzheimer, Creutzfeldt-Jakob diseases or Hereditary cystatin C amyloid angiopathy), undergo conformational changes, form oligomers or amyloid deposits, because of their flexible structure [1-4]. The flexible fragments of their polypeptide chains allow rapid rearrangement of protein structure, oligomerization and the formation of fibrillar structures.

In the lecture, special attention will be paid to combined use of the methods of small angle X-ray scattering (SAXS), X-ray absorption spectroscopy (XAS) techniques and synchrotron radiation, in the study of the protein fibrillation process in the presence of divalent metal cations (e.g. Zn, Cd). These studies are focused on characteristics of the fibril formed by the unstructured fragment of the N-terminal domain of the human prion protein (huPrP). The SAXS technique alone was also used for analysis of structure and overall conformation of selected variants of huPrP and human cystatin C (HCC) in solution. Therefore in the second part of the lecture, the project aimed at establishment of the experimental beamline for Polish synchrotron (NCPS Solaris) will be presented. This beamline, dedicated to BioSAXS and protein crystallography, has a real chance to be constructed in the next few years [5].

References

[1] P. Brundin, R. Melki, R. Kopito (2010) Prion-like transmission of protein aggregates in neurodegenerative diseases. Nature Rev. Mol. Cell Biol. 11:301–307.

[2] D. Eisenberg, M. Jucker (2012) The Amyloid State of Proteins in Human Diseases. Cell 148:1188-1203.

[3] K.J. Wolfe, D.M. Cyr (2011) Amyloid in neurodegenerative diseases: Friend or foe? Seminars in Cell & Developmental Biology 22:476-481.

[4] P.P. Liberski (2014) Prion, prionoids and infectious amyloid. Parkinsonism & Related Disorders 20, S1: S80-S84.

[5] M. Kozak, W. Rypniewski, M. Jaskolski (2012) Koncepcja budowy linii pomiarowej MX/SAXS/XRD w NCPS SOLARIS. Synchr. Radiation Nat. Sci. 11:5-9.

Acknowledgments

The studies were supported by research grant UMO-2017/27/B/ST4/00485 from National Science Centre and international grant POLTUR2/3/2017 from National Center of Research and Development.

IL21

Champaign-Urbana nbFIX (CUFIX): improved AMBER and CHARMM parameters for accurate calculations of charge–charge and hydrophobic interactions

Yoo Jejoong[1], Aksimentiev Aleksei[2],

[1] Institute for Basic Science, South Korea

[2] University of Illinois at Urbana-Champaign, USA

Most biomolecules such as proteins and nucleic acids obtain their defined functions by folding into highly ordered three-dimensional structures. The key to the folding process is a delicate balance among several competing molecular interactions such as hydrogen bonds, charge–charge interactions, and hydrophobic collapses. Purely relying on the physics-based atomistic model, the force fields, the molecular dynamics (MD) simulation method proved its power through the successful folding simulations of monomeric proteins. However, as the field is moving toward new challenges, e.g., intrinsically disordered peptides and multimeric complexes, the force fields are revealing previously unknown problems. Importantly, it turned out that the force fields overestimate attractive charge-charge interactions and hydrophobic collapses, promoting artificial aggregation of all biomolecules including proteins and nucleic acids. In this talk, I will discuss a route towards improving the force fields, the NBFIX approach. In this approach, the intermolecular forces are calibrated against thermodynamic quantities such as osmotic pressure by making atom pair-specific adjustments to the non-bonded interactions. Test MD simulations of various systems including folded and unfolded proteins, electrolyte solutions, dense DNA condensates, Holliday junctions, and lipid bilayer membranes—demonstrate that the NBFIX approach is promising.

IL22

Global Optimization by Conformational Space Annealing and its Applications to Protein Structure Prediction/Determination and Machine Learning

Jooyoung Lee

School of Computational Sciences, Korea Institute for Advanced Study 85 Hoegiro, Dongdaemun-gu,

Seoul 130-722, Korea

E-mail: jlee@kias.re.kr

Web-page: http://lee.kias.re.kr

First, I will discuss our recent progresses on the protein structure prediction using the global optimization method of Conformational Space Annealing (CSA) as illustrated in the CASP11/12 competitions held in 2014/2016. We will demonstrate that this method can be applied to difficult MR (molecular replacement) targets to determine X-ray crystallography structures of proteins and protein complexes, which could not be solved using conventional MR methods. We will also discuss the possible application of our method to the high throughput NMR structure determination of large proteins (over 20 kDa) and membrane proteins.

If time is allowed, I will also discuss the optimization issue in the study of machine learning (ML). A preliminary study indicates that proper application of CSA to ML can provide a solution to the overtraining problem in ML. I will share the progress of our attempt to build our own AlphaGo in this respect.

P01

Covalent knots in proteins

Pawel Dabrowski-Tumanski[1,2], Pawel Rubach[3], Joanna Sułkowska[1,2]

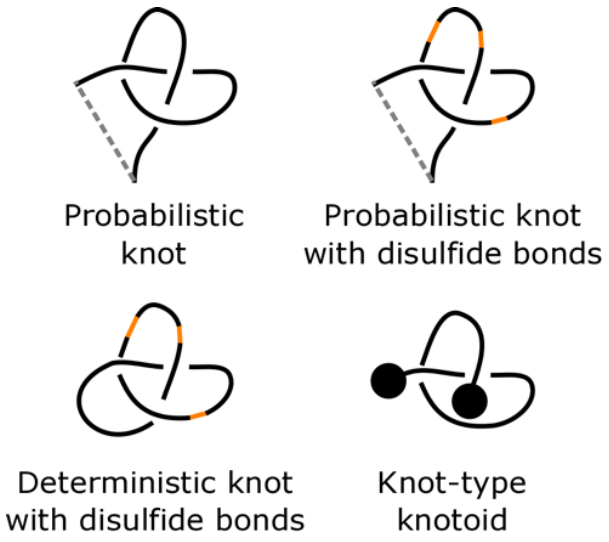
[1] Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093, Warsaw, Poland

[2] Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097, Warsaw, Poland

[3] Warsaw School of Economics, Al. Niepodległości 162, 02-554 Warsaw, Poland

When connecting the chain termini, it turns out that backbones of some proteins are knotted. In fact, almost 2% of all protein structures possess the knotted main chain. These knots are believed to be functionally, as well as structurally beneficial for the protein [1]. However, the proteins' topology may be much more complicated, if non-backbone connections of the residues are also taken into account. In particular, the covalent loops closed by disulfide bridges may be as well knotted.

In this work, we show the results of an extensive search for knotted covalent and ion-based loops in proteins (see Fig.). We identify various kinds of knots and study their folding pathways. Moreover, to describe the topology of the main chain better, we describe it using the mathematical concept of knotoids - planar, "open-chain knots" (see Fig.) [2]. This in total allows us to reach the full mathematical classification of proteins, taking into account the non-backbone interactions.



Moreover, discussed will be the implementation of the methods created during the project in the newest version of the KnotProt database [3], collecting information about knots in proteins. This gives the users the ability to determine the entanglement type of own proteins, taking into account also the interactions via ions and the disulfide bridges.

References

[1] Christian T, Sakaguchi R, Perlinska AP, Lahoud G, Ito T, Taylor EA, Yokoyama S, Sulkowska JI, Hou YM (2016). Methyl transfer by substrate signaling from a knotted protein fold. Nature structural & molecular biology, 23:10, 941.

[2] Dorier J, Goundaroulis D, Benedetti F, Stasiak A (2018). Knoto-ID: a tool to study the entanglement of open protein chains using the concept of knotoids. Bioinformatics, 1, 3.

[3] Jamroz M, Niemyska W, Rawdon EJ, Stasiak A, Millett KC, Sułkowski P, Sulkowska JI (2014). KnotProt: a database of proteins with knots and slipknots. Nucleic acids research, 43(D1), D306-D314.

P02

Is the ribosome crucial for protein's knotting?

Piejko Maciej[1,2], Dabrowski-Tumanski Pawel[1,2], Niewieczerzal Szymon[2], Stasiak Andrzej[3,4], Sułkowska Joanna I. [1,2]

[1] Faculty of Chemistry, University of Warsaw, Poland

[2] Centre of New Technologies, University of Warsaw, Poland

[3] Centre for Integrative Genomics, University of Lausanne, Switzerland

[4] Swiss Institute of Bioinformatics, Switzerland

Great efforts have been put in the search for a proper folding mechanism of knotted proteins throughout the years. On one hand, a self-tying event was never observed directly in the experiment. On the other hand, even theoretical studies fail to solve this puzzle [1]. There are two major concepts in the literature: knotting occurs either before or after the chain collapse [2]. In order to prove which mechanism describes knotting we chose the most deeply knotted protein recently discovered and deposited in the KnotProt database. Its 3-domain structure resembles some already thoroughly studied fused proteins [2]. It is thus a perfect object for knotting mechanism investigation. Nevertheless, after having studied both mentioned mechanisms we claim that both fail to deliver an ultimate characterization of knotting. Combining all data known about knotted proteins we have created a simple model of the ribosome exit tunnel to study a new type of mechanism in which the ribosome plays a crucial role. In this work we present the results of a holistic study of this protein's folding features and an evaluative look at all three mechanisms. The mechanism involving the ribosome opens new possibilities for describing the knotting of proteins.

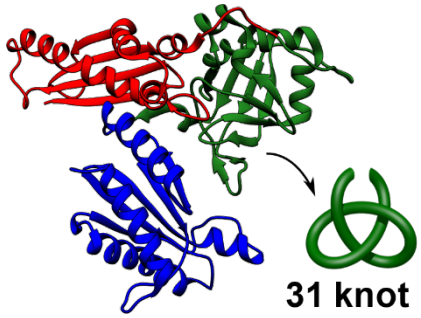


Fig. 1 The structure of the most deeply knotted protein. The middle domain (green) forms the knotted core.

References

[1] Sulkowska JI, Rawdon EJ, Millett KC, Onuchic JN and Stasiak A (2012) Conservation of complex knotting and slipknotting patterns in proteins, Proc. Natl. Acad. Sci. U.S.A. 109, E1715–E1723

[2] Lim, N. C., & Jackson, S. E. (2015). Mechanistic insights into the folding of knotted proteins in vitro and in vivo. Journal of molecular biology, 427(2), 248-258

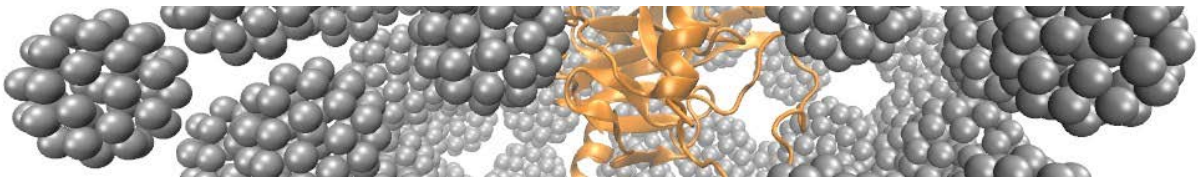
This work was supported by National Science Centre #2012/07/E/NZ1/01900 (to JIS) and #2016/21/N/NZ1/02848 (to PDT), and EMBO Installation Grant #2057 (to JIS).

P03

Molecular dynamics simulations of enzymes under crowding - closer to reality and faster?

Natalia Ostrowska[1,2], Michael Feig[3], Joanna Trylska[1]

[1] Centre of New Technologies, University of Warsaw, Poland
[2] College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, Poland
[3] Department of Biochemistry and Molecular Biology, Michigan State University, USA



MD simulations of biomolecules are conducted in water with ions in physiological concentrations, whereas the cell in which the enzymes function is filled with other macromolecules like metabolites, proteins, nucleic acids and lipids. This crowd occupies up to 30% of cytoplasmic volume[1]. Simulations of crowding are known to consume large amounts of computational power[2], but representing the crowd by coarse-grained structures can actually
decrease the simulation time, by decreasing the number of atoms in the system. Our research aims at determining how crowding affects the dynamics of viral proteases. Specifically, we investigate the dynamics of one of the hepatitis C virus (HCV) proteases, named NS3/4a, essential for viral replication. The protease, water and ions are represented at atomistic details. To speed-up the simulations of crowding we designed a crowder as a spherical structure composed of pseudo-atoms: 42 carbon sized pseudo-atoms distributed on a sphere with an additional atom in the centre, ensuring the crowder's stability. Our model was inspired by the laboratory experiments involving crowding, in which the crowd is often mimicked by adding polymers like polyethylene glycol (PEG). Their only aim is to exert an exclusion volume effect that is thought to cause the effects of crowding. To enable the simulation of this model of the crowder in NAMD, bonds between crowder's atoms were replaced with nonbonded potential. Interactions with explicit water molecules were modified to eliminate strong hydrophobicity of uncharged atoms. We show what it takes to run a simulation of a coarse-grained model in NAMD and why this model of crowder has been found to be optimal for this.

References

[1] Minton AP (2001) The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. J. Biol. Chem. 276:10577–80
[2] Yu I, Mori T, Ando T, Harada R, Jung J, Sugita Y, Feig M (2016) Biomolecular interactions modulate macromolecular structure and dynamics in atomistic model of a bacterial cytoplasm. eLife 5:e19274

Acknowledgments

NO and JT acknowledge the National Science Centre, Poland (OPUS, DEC-2016/23/B/NZ1/03198)

P04

Computational analysis of interactions of endostatin with glycosaminoglycans

Urszula Uciechowska-Kaczmarzyk[1], Sylvain D. Vallet[2], Agnieszka Karczyńska[1], Sylvie Ricard-Blum[2], Sergey A. Samsonov[1]

[1]Laboratory of Molecular Modeling, Department of Theoretical Chemistry, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk, Poland.

[2] Univ Lyon, University Claude Bernard Lyon 1, CNRS, INSA Lyon, CPE, Institute of Molecular and Supramolecular Chemistry and Biochemistry, UMR 5246, F-69622 Villeurbanne cedex, France.

Glycosaminoglycans (GAGs) play a key role in a variety of biological processes in the extracellular matrix (ECM) via interactions with their protein targets. Due to their high flexibility and electrostatics-driven nature of their interactions, GAG-containing systems are very challenging for *in silico* approaches. In this study, we characterized the interactions of endostatin, a naturally occuring proteolytic C-terminal fragment of collagen XVIII known to be an anti-angiogenic and GAGs of different type, length and sulfation pattern. We applied molecular docking and molecular dynamic simulations to obtain the stable conformations of protein-GAG complexes. MM-GBSA approach was used to calculate the binding free energies and to characterize the amino acid residues of endostatin which have the most favourable contribution to GAG binding. We also investigated the influence of endostatin-Zn^{2+} binding on a conformational change in endostatin-heparin (HE) complexes using computational approaches. We detected particular Asp residues in endostatin that unfavourably contributed to the binding to all analyzed HE and heparan sulfate (HS) ligands. Several mutations on Asp residues were analyzed using *in silico*. We are expressing recombinant simple, double and triple endostatin mutants to perform binding assays, calculate the affinity of the mutants for HE and HS and validate the *in silico* findings.

P05

Computational study on phospholyrated glycosaminoglycans

Bojarski Krzysztof Kamil, Samsonov Sergey A.

Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, Gdańsk 80-308, Poland

Glycosaminoglycans (GAGs) are a group of long unbranched negatively charged polysaccharides consisting of repeatable disaccharide units made up of an aminosugar and an uronic acid. GAGs are located in the extracellular matrix and are involved in many cruacial cell signaling-related processes via interactions with various protein targets. Since GAGs are negatively charged, their interactions are electrostatic-driven. Sulphate group is a core element of GAGs when taking into account its impact on the overall charge of monosaccharide units and the involvement in the intermolecular interactions. This functional group can be artificially substituted with a phospate group which can have two different protonation states (Figure 1). Such change results in an increase of the overall negative charge of a GAG which, in turn, can lead to the increase of biologically relevant complex stability formed by protein with such a phospholyrated glycosaminoglycan (pGAG) in comparison to the complex with a natural GAG.

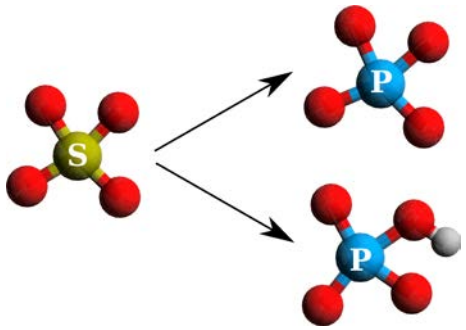


Figure 1. In the pGAGs, a sulfate group is substituted into a phosphate group with two possible protonation states.

Up to date little is known about pGAGs from the experimental point of view. However, these molecules are promising for the studies aimed to understand and to control tissue regeneration: phosphates are weak eletrolytes in comparison to sulfates and, therefore, are potentially more efficient in organization of complex H-bonding networks; phosphate groups have different charge characteristics in comparison to sulfates, and this could be used in a better controlled regulation of specificity of their interactions with proteins; phosphorylated GAGs can not be substrates for classical GAGs-specific glycosidases, which make their potential applicability substantially different and attractive for biological systems; ^{31}P is a more conventional nucleus for NMR approaches than ^{33}S, which makes analysis of phosphorylated GAGs binding more straightforward in comparison to sulfated ones. All this makes pGAGs an attractive molecular target.

In our study, we parametrized and characterized pGAGs both unbound and in complex with their respective receptors (FGFs, cathepsin K) applying computational methods such as molecular docking, molecular dynamics, free energy and conformational analysis. **The obtained data are important for getting fundamental insights into the understanding molecular basis of GAGs phosphorylation in terms of their conformational space and complexes with proteins.**

Acknowledgement. This work was supported by the National Science Center of Poland (Narodowe Centrum Nauki, grant UMO-2016/21/P/ST4/03995; This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 665778).

P06

Probing the binding selected metal ions to the antimicrobial peptide LL-37 using ITC measurements and MD simulations

Wyrzykowski Dariusz, Barwicka Izabela, Makowska Joanna

Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, 80-308 Gdańsk, Poland

The focus of this research is on LL-37, the only cathelicidin-derived antimicrobial peptide found in humans [1]. LL-37 is a 37-residue, amphipathic, helical peptide found throughout the body and has been shown to exhibit a broad spectrum of antimicrobial activity. In the presented results, isothermal titration calorimetry (ITC) [2] supported by MD simulation were used to study the interactions between copper(II), zinc(II) and nickel(II) ion and the LL-37. The stoichiometry, conditional stability constants and thermodynamic parameters for the pertinent complexation reactions were determined. It was found that LL-37 has coordination properties only for Cu(II) and Zn(II) ions.

References

[1] Ulrich H.N. Dürr, U.S.Sudheendra, Ayyalusamy Ramamoorthy (2006) LL-37, the only human member of the cathelicidin family of antimicrobial peptides Biochimica et Biophysica Acta (BBA) - Biomembranes 1758: 1408-1425

[2] Ernesto Freire, Obdulio L. Mayorga, and Martin Straume (1990) Isothermal titration calorimetry Anal. Chem. 62:950A-959A

Acknowledgments

The studies were financially supported by the grant DS530-8238-D743-18

P07

Simulations of calcium-ion binding by an amyloid forming peptide

Lipska Agnieszka G.[1], Platts Jamie[2], Sieradzan Adam K.[1],

[1] Faculty of Chemistry, University of Gdańsk, Poland

[2] School od Chemistry, Cardiff University, United Kingdom

Amyloids formation lead to many diseases, such as Alzheimer and Parkinson diseases, type II diabetes, and a number of systemic amyloidosis [1]. It is hypothesized that calcium cations play important promoting role in formation of deposits [2].

In this study we performed multiplexed replica exchange molecular dynamics (MREMD) simulations of Ab1-40 with the use of UNRES force field [3]. Calcium-amino-acid potentials [4] were introduced into unresf90 software [5] and calculations with and without cations were performed. To maintain proper cation concentration (2mM) periodic boundary conditions [6] were used. The box sixes were 200 Å, 300 Å, 400 Å and 500 Å for monomer, dimer, trimer and tetramer, respectively. The simulations were performed in 250-480K. After simulation weighted histogram analysis and clustering was performed. Additionally, radial distribution function was calculated between Ca2+ and amino acid side-chains and peptide groups. Our results suggest that cations significantly promote formation of associated forms. This confirm role of calcium in deposits creation.

[1] Chiti F, Dobson CM (2017) Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. Ann Rev Biochem 86:27-68

[2] Ahmad A, Staratton CM, Scemama J-L, Muzaffar M (2016) Effect of Ca^{2+} on Aß40 fibrillation is characteristically different. Int J Biol Macromol, 89:297-304

[3] Liwo A, Baranowski M, Czaplewski C et al (2014) A unified coarse-grained model of biological macromolecules based on mean-field multipole-multipole interactions. J Mol Mod 20:2306

[4] Khalili M, Sunders JA, Liwo A, Ołdziej S, Scheraga HA (2004) A united residue force-field for calcium–protein interactions. Protein Sci, 13:2725-2735

[5] Lubecka EA, Liwo A (2016) New UNRES force field package with Fortan 90. TASK Quaterly, 20:399-408

[6] Sieradzan AK (2015) Introduction of periodic boundary conditions into UNRES force field. J Comput Chem, 36:940-946

Acknowledgments . This work supported by Preludium 2016/21/N/ST4/03154 (to AGL) form the National Science Centre (Poland).

P08

Theoretical model of DnaA protein dimer – implication on DnaA oligomerization in the presence of double-stranded DNA

Ołdziej Stanisław[1], Thiel Marcel[1], Zamłyńska Beata[1], Artur Giełdoń[2]

[1] Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdansk, Poland

[2] Faculty of Chemistry, University of Gdańsk, Poland

Replication of DNA is a key process in any living cell and it involves many enzymes and proteins. In bacteria the DnaA protein plays a crucial role in the initiation of DNA replication. Among many molecular features DnaA possesses the ability to form functional oligomers. It is a well-known experimental fact that DnaA forms one type of oligomers with the ability to bind single stranded DNA (ssDNA) [1]. On the other hand, DnaA forms oligomers of unknown structure when bound to double-stranded DNA (dsDNA), and it is possible that DnaA can oligomerize without DNA (single or double stranded) presence. Using molecular modeling tools (docking, structure prediction) and data coming from bioinformatic analysis (multiple sequence analysis) we proposed a three-dimensional structure of the DnaA dimer which could be a basic building block of the DnaA oligomers which are able to bind to dsDNA. Dimer stability as well as its internal dynamics were investigated by molecular dynamics simulations using all-atom (AMBER) and coarse-grain (UNRES) force-fields.

References

[1] Richardson T.T., Harran O., Murray H. (2016) The bacterial DnaA-trio replication origin specifies single-stranded DNA initiator binding. Nature 534:412-416

Acknowledgments

Computational resources used in this project were provided by the Informatics Center of the Metropolitan Academic Network (IC MAN-TASK) in Gdańsk.

P09

DesiRNA - RNA Secondary Structure-Based Sequence Design

Tomasz K. Wirecki[1], Grzegorz Łach[1,2], Gaja Klaudel[2], Przemysław Gierski[1], Janusz M. Bujnicki[1,3]

[1] Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, Warsaw, Poland

[2] Institute of Theoretical Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland

[3] Laboratory of Bioinformatics, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland

Ribonucleic acid (RNA) molecules are master regulators of cells. They are involved in a variety of molecular processes: they transmit genetic information, they sense and communicate responses to cellular signals, and even catalyze chemical reactions [1]. These functions of RNAs depend on its ability to assume one or more structures, which is encoded by the ribonucleotide sequence. One of the fundamental challenges of biology and chemistry is to design molecules that form desired structures and carry out desired functions. The computational design of RNA requires solving the so-called RNA inverse folding problem: given a target structure, identify one or more sequences that fold into that structure (and do not fold into any other structure). Designing RNA sequences with specific folding properties and with desired functions has already proven useful in a number of applications in the areas such as the development of probes and sensors, molecular medicine, and material science. Nonetheless, RNA design is very difficult, especially for molecules with complex structures.

Currently, existing methods for RNA design methods exhibit many severe limitations. Typically, they do not check for the potential of the RNA sequence to oligomerize, and few methods allow for designing RNA molecules comprising several different chains or ones that fold into several alternative structures.

In this work, we present a prototypical method – DesiRNA – program for designing RNA sequence based on the provided secondary structure and additional restraints [2]. DesiRNA is capable of designing not only single molecules (taking into account the potential of RNA to oligomerize) but also oligomers or chains that fold into several alternative structures.

References

[1] Atkins JF, Gesteland RF, Cech TR (2011) RNA Worlds: From Life's Origins to Diversity in Gene Regulation. Cold Spring Harbor Laboratory Press Cold Spring Harbor, New York.

[2] Klaudel G. (2016) Wykrywanie konfliktów w projektowaniu sekwencji RNA. M.Sc. Thesis, University of Warsaw, Warsaw.

Acknowledgments. This work has been supported by the European Research Council (ERC) StG grant RNA+P = 123D and National Science Centre (NCN) OPUS grant UMO-2017/25/N22/01294.

P10

Function approximation of DFIRE statistical potential using machine learning

Min, Jiyeon[1], Kim, Hyunjin[1], Joo, Keehyoung[2], and Lee, Jooyoung[1,2]

[1] School of Computational Sciences, Korea Institute for Advanced Study, Republic of Korea

[2] Center for Advanced Computation, Korea Institute for Advanced Study, Republic of Korea

Knowledge-based statistical potentials with diverse reference states have been developed and widely used for protein folding. The potential using distance-scaled, finite ideal-gas reference (DFIRE) state is most popular and its variants, having partly modified reference state, show high-performance. Nowadays, the more number of PDB files exists, the more information of proteins is available. Taking advantage of given numerous information, we apply machine learning algorithm to approximate the DFIRE statistical potential. In details, we extract features from protein 3D structures and DFIRE potential energy values. We compare the machine-based potential with the original one by checking the average z-score for decoy sets.

Reference

[1] Zhou H. and Zhou Y. (2002) Distance-scaled, finite ideal-gas reference state improves structure-derived potentials of mean force for structure selection and stability prediction. Protein Science 11:2714-2726

P11

Initial protocol for Protein-protein docking by MREMD simulations with the coarse-grained UNRES force field.

Pawel Krupa [1,2] , Agnieszka S. Karczynska [2], Adam Liwo [2] and Cezary Czeplewski [2]

[1] Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, PL-02-668 Warsaw, Poland.

[2] Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland.

Many proteins in living organisms create multimeric systems and also it is often crucial for them to be functional. It is very important to find their interaction sites and how their structure is changing during oligomerization. While some of the proteins are not subjected to significant conformational changes during this process, others may need to refold. To dock one protein to another, that information is crucial to chose a proper method of docking. For first mentioned instance the docking method is called rigid-body docking and the other, flexible docking. Rigid-body docking is mostly relying on searching the translational and rotational space of two proteins to find the position where the potential energy of the complex is the most favorable. The biggest role here is playing by the electrostatic and hydrophobic interactions. The flexible docking is much more complicated and can be divided on more subclasses. Main of them are when part of the structure of polypeptide chain is changing or when whole domain of protein is moving. In our lab, for this moment, we are developing a protocol for rigid-body docking. Because protein-protein complexes can be too large in case for computational and time capabilities of present supercomputers, we are utilizing a coarse-grained representation and therefore, some simplifications in calculations.

The physics-based UNRES model developed in our lab assumes only two interaction sites per amino-acid residue: united peptide group and united side chain. Our protocol is based on multiplexed replica exchange molecular dynamics (MREMD) with coarse-grained UNRES force field starting from multiple protein orientations also called as poses. Poses are generated depends on how much information we can find about the target complex in the databases. If we have enough information, we can generate several poses which suppose to be close to native one. If we don't have a relevant information, we are randomly generating 20 models with the highest diversity to improve the sampling.

The protocol was designed and tested recently for various protein-protein complexes with a chain length from 51 to 586 amino-acid residues (rigid-body docking; 20 complexes). The results show that the UNRES force field is able to predict the structures of protein complexes with good quality given sufficient sampling. The protocol was already utilized in CASP/CAPRI experiments by our group.

P12

Coarse-grained and all-atom studies of structure and dynamics of amyloid beta 42 (Aβ42) monomers and oligomers

Krupa Paweł[1], Huy Pham Dinh Quoc[1], Nguyen Hoang Linh[2], Li Mai Suan[1]

[1] Institute of Physics Polish Academy of Sciences, Al. Lotników 32/46, 02-668 Warsaw, Poland

[2] Institute for Computational Science and Technology, SBI Building, Quang Trung Software City, Tan Chanh Hiep Ward, District 12, Ho Chi Minh City, Vietnam

Amyloids Aβ40 and Aβ42 are likely connected to the Alzheimer disease (AD) and formation of the oligomeric and fibril structures, which is the core process in AD. The structures of amyloid fibrils are known, while the structures of monomeric Aβ and small oligomers, which are more toxic than fibril, are difficult to study due to their transient nature.[1] We used coarse-grained simulations in physics-based UNited RESidue (UNRES) force field to study the folding, dynamics and aggregation of amyloid oligomers and five popular all-atom force fields (three Amber variants and two CHARMM) to study monomeric Aβ42. Due to the simplified representation of the polypeptide chain and reduction of the interaction sites, UNRES force field allows to speed-up the calculations in comparison to all-atom force field of at least 3-4 orders of magnitude. It allowed us to extensively study the folding process, dynamics and aggregation of amyloid Aβ40 and Aβ42 monomers, and 2-7mers, with special emphasis on the Aβ42 tetramers, using Replica Exchange Molecular Dynamics (REMD) simulations. Analysis of the simulations shows that due to the disordered character of monomers and small Aβ oligomers obtained results are highly dependent on used sampling method and force field. Our studies demonstrate that newer versions of the all-atom force fields provide results, which are closer to the experimental observations (e.g. chemical shifts and secondary structure content) and that use of the coarse-grained force field for larger system can significantly speed-up the calculations without comprising quality. We also observed that with the oligomerization of Aβ increase of the β-content can be observed and that structures of the hexamers and heptamers cores start to resemble the mature fibril.

References

[1] Nasica-Labouze J, Nguyen PH, Sterpone F et al. (2015) Amyloid β Protein and Alzheimer's Disease: When Computer Simulations Complement Experimental Studies. Chem. Rev. 115 (9), 3518–3563.

Acknowledgments

This work was supported by Department of Science and Technology at Ho Chi Minh city, Vietnam, and the Polish NCN grant 2015/19/B/ST4/02721, Poland.

P13

Implementation of Nucleoside-Cation Interactions in UNRES 4

Golon Łukasz[1], Sieradzan Adam K.[1], Liwo Adam[1,2],

[1] Department of Chemistry, University of Gdańsk, Poland
[2] School of Computational Sciences, Korea Institute for Advanced Study, 85 Hoegiro, Dongdaemun-gu, Seoul 130-722, Republic of Korea

While DNA is most commonly found in the B form, its flexibility enables it to adopt many other conformations. The G-quadruplex is one of the most important secondary structures of DNA, being partially responsible for the mechanical durability of telomeres. The stability of G-quadruplexes is dependent on interaction with metal cations – lack of cations has been shown to destabilize the structure[1].

UNRES 4 is the latest version of the UNRES package capable of running coarse-grained simulations of both proteins (using the UNRES model) and nucleic acids (using the NARES-2P model[2]). NARES-2P was parameterized to reproduce the melting temperature of DNA oligomers based on sequence and can be used to predict the general fold of DNA and RNA molecules[3]. Using the NARES-2P model with steered molecular dynamics our group studied how the duplex form of telomeric repeat sequences responds to mechanical stress[4]. However, due to the lack of nucleoside-cation interaction potential, performing correct simulations of G-quadruplexes was impossible so far.

To extend the applicability of NARES-2P to ion-dependent systems we derived and implemented the required potentials. We calculated MP2/6-31G** potential energy maps for eight model complexes (each of the four DNA deoxynucleosides paired with Na^+ and K^+) and integrated them to obtain potentials of mean force. We used these potentials of mean force as target to fit the parameters of physics-based model potentials which we later implemented in the UNRES 4 package. We tested the ability of new potentials to maintain total energy of cation-DNA system in microcanonical simulations, and performed canonical MD to verify the stability of quadruplex structures.

References

[1] Bhattacharyya D, Arachchilage GM, Basu S (2016) Metal cations in G-quadruplex folding and stability. Front Chem 4:38
[2] He Y, Maciejczyk M, Ołdziej S, Scheraga HA, Liwo A (2013) Mean-field interactions between nucleic-acid-base dipoles can drive the formation of a double helix. Phys Rev Lett 110:098101
[3] Sieradzan AK, Golon Ł, Liwo A (2018) Prediction of DNA and RNA structure with the NARES-2P force field and conformational space annealing. Phys Chem Chem Phys 20:19656-19663
[4] Sieradzan AK, Krupa P, Wales DJ (2017) What makes telomeres unique? J Phys Chem B 121:2207-2219

Acknowledgments

This work was supported by University of Gdańsk Young Researcher Grant nr 538-8370-B740-17 and Polish National Science Center Grant nr UMO-2017/27/N/ST4/01907. Calculations were carried out at the Academic Computer Centre in Gdańsk (CI TASK) and in the Wrocław Center for Networking and Supercomputing (http://www.wcss.pl), grant no. 196.

P14

Implementation and tests of carbon nanoparticles in coarse-grained UNRES force field

Magdalena A. Mozolewska[1], Adam K. Sieradzan[2]

[1] Institute of Computer Science, Polish Academy of Sciences, Poland

[2] Faculty of Chemistry, University of Gdańsk, Poland

Nanoparticles are relatively new, but a rapidly increasing area of interest by scientists. Due to their unique properties, Carbon Nanotubes (CN) and fullerenes can be used in a variety of fields e.g. an industry, military or in a medicine, as carriers of small ligands into target cells e.g. in a cancer therapy, or CNs themselves can be used as ligands, interacting with receptors as their antagonists.[1] Very high computational cost of all-atom force fields limits severely a time-scale of the simulations and a size of the investigated systems. Therefore, in this work, we introduce the implementation of CNs and fullerenes into the physics-based, coarse-grained UNited RESidue (UNRES) force field,[2] which due to the reduction of the number of interaction sites, provides 3-4 order of magnitude speed-up comparing to the all-atom force fields.[3] We simplified representations of CNs and fullerenes to the immobile outer field with adjustable dimensions, which interacts with proteins through Kihara potential. Simulations of protein-nanoparticle systems were tested for the ability to keep constant energy and influence of nanoparticle on temperature stability of the thermostats. Then new potential was utilized to study interactions of various sizes of CNs and fullerenes with different proteins. Our results show that the extended UNRES force field can predict binding of these proteins to CNs correctly and observed influence of carbon nanoparticles on the protein-structure stability is in a good agreement with the experimental observations.[4,5]

References

[1] Ahmed L, Rasulev B, Kar S, Krupa P, Mozolewska MA, Leszczynski J (2017) Inhibitors or toxins? Large library target-specific screening of fullerene-based nanoparticles for drug design purpose, Nanoscale, 9:10263–10276.

[2] Liwo A et al. (2014) A unified coarse-grained model of biological macromolecules based on mean-field multipole-multipole interactions, J. Mol. Model., 20:2306-2014.

[3] Liwo A, Khalili M, Scheraga H (2005) Ab initio simulations of protein-folding pathways by molecular dynamics with the united-residue model of polypeptide chains, Proc. Natl. Acad. Sci. U. S. A., 102: 2362–2367.

[4] Prato M, Marchesan S (2015) Under the lens: carbon nanotube and protein interaction at the nanoscale, Chem. Commun., 51:4347–4359.

[5] Sieradzan AK, Mozolewska MA (2018) Extension of coarse-grained UNRES force field to treat carbon nanotubes, J. Mol. Model., 24:121.

Acknowledgments.

The work was supported by the grant UMO-2015/17/N/ST4/03935 from the National Science Center of Poland.

P15

Introduction of implicit solvent mimicking lipids into United Residue Force Field

Adam K. Sieradzan,[1], Karolina Zięba[1], Magdalena Ślusarz[1], Rafal Ślusarz[1],

Adam Liwo [1]

[1] Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland

UNRES (United RESidue) coarse-grained force field is a well-established tool for protein modeling (protein structure prediction, protein association and dynamics) [1]. However, the current UNRES version is limited only to water environment and simulations of membrane proteins are not possible while, lipids are, along with proteins, sugars and nucleic acids, one of most important cellular components. Lipids perform many important functions in the living cells such as, e.g., keeping the cell shape [2], maintaining the desired concentration of various components [3], separation of various cellular organellae [4], and protecting against pathogens[5].

In this presentation an extension of the UNRES force filed introducing a potentials mimicking lipid bilayer is implemented. New potentials as well as function strengthening electrostatic interaction were derived for the lipid environment. The new software was tested for total energy conservation and thermostat behavior. Additionally, to validate the new potential, 10 short helical low similarity membrane proteins [6] were simulated starting from extended structure. The UNRES force field was able to predict correctly the overall fold of these proteins. The UNRES force field with lipid membrane is a promising approach for further development toward modeling of large membrane protein systems.

References

[1] Czaplewski C, Karczyńska A, Sieradzan AK, Liwo A, UNRES server for physics-based coarse-grained simulations and prediction of protein structure, dynamics and thermodynamics, Nucl Acid Res, 46:W304-W309

[2] Svetina S, Zeks B, Membrane bending energy and shape determination of phospholipid vesicles and red blood cells, Eur Biophys J, 17:101-111

[3] Lundbek JA, Regulation of membrane protein function by lipid bilayer elasticity—a single molecule technology to measure the bilayer properties experienced by an embedded protein, J Phys Cond Mat, 18:S1305

[4] Benning C, Mechanisms of Lipid Transport Involved in Organelle Biogenesis in Plant Cells, Ann Rev Cell Dev Biol, 25:71-91

[5] Hamosh M, Protective function of proteins and lipids in human milk, Neonat, 74:163-176

[6] Pulawski W, Jamroz M, Kolinski M, Kolinski A, Kmiecik S, Coarse-Grained Simulations of Membrane Insertion and Folding of Small Helical Proteins Using the CABS Model, J Chem Inf Model, 56:2207-2215

Acknowledgments

This work was supported by National Science Center Poland UMO-2017/27/B/ST4/00926.

P16

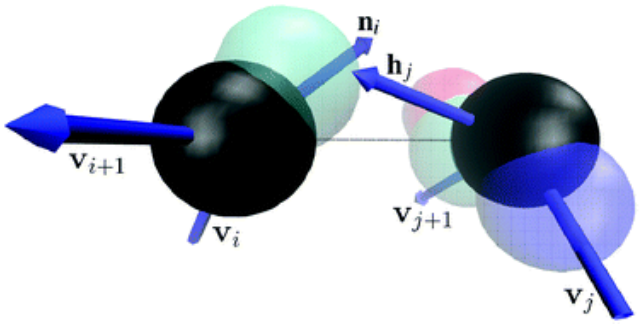
Disordered peptide chains in an α-C-based coarse-grained model

Mioduszewski Łukasz[1], Cieplak Marek[1]

[1] Institute of Physics, Polish Academy of Sciences, Poland

We construct a one-bead-per-residue coarse-grained molecular dynamics model to describe intrinsically disordered proteins at significantly longer timescales than in the all-atom models. In this model, inter-residue contacts form and disappear during the course of time evolution. The contacts may arise between the sidechains, the backbones or the sidechain and backbone of the interacting residues. The model takes into account the directionality of these interactions, despite being only α-C-based. Amino acid specificity and electrostatic effects are also included. The solvent is implicit.

The model yields results that are consistent with many all-atom and experimental data on intrinsically disordered proteins. We demonstrate that the geometrical properties of various homopeptides differ substantially in this model. In particular, the average radius of gyration scales with the sequence length in a residue-dependent manner.



References

[1] Mioduszewski Ł, Cieplak M (2018) Disordered peptide chains in an α-C-based coarse-grained model. Phys. Chem. Chem. Phys. 20:19057-19070

[2] Hung NB, Le D-M, Hoang TX (2017) Sequence dependent aggregation of peptides and fibril formation. J. Chem. Phys. 147:105102.

[3] Enciso M, Rey A (2010) A refined hydrogen bond potential for flexible protein models. J. Chem. Phys. 132:235102.

Acknowledgments

We appreciate fruitful discussions with B. Różycki as well as receiving help of G. Matyszczak and K. Wołek with the calculations. MC has received funding from the National Science Centre (NCN), Poland, under grant No. 2014/15/B/ST3/01905 and by the EU Joint Programme in Neurodegenerative Diseases project (JPND CD FP-688-059) through the NCN grant 2014/15/Z/NZ1/ 00037. He was also supported by the NCN grant no. 2016/21/B/ NZ1/00006. The computer resources were supported by the PL-GRID infrastructure and also financed by the European Regional Development Fund under the Operational Programme Innovative Economy NanoFun POIG.02.02.00-00-025/09.

P17

Replica Exchange Coarse-Grained Molecular Dynamics Simulations of Proteins with Distance Restraints

Lubecka Emilia A.[1,2], Liwo Adam[2],

[1] Institute of Informatics, Faculty of Mathematics, Physic and Informatics, University of Gdańsk, Wita Stwosza 57, 80-308 Gdańsk, Poland

[2] Faculty of Chemistry, University of Gdańsk, ul. Wita Stwosza 63, Gdańsk 80-308, Poland

In this work, we have analysed restrained replica exchange molecular dynamics (REMD) simulations with the coarse-grained UNRES force field [1,2,3], with Lorentzian restraint function. We have used various weights of distance restraints. Additionally, the effects of non full restraint sets and false restraints occurring were tested. First, we have studied four simple proteins: 1A6S, 1BK2, 1STU and protein A. As reference structures to compute restraints we have used the experimental structures of these proteins. The next test set contained CASP12 targets: T0866, T0869, T0872, T0882, T0892 and T0900, for which restraints are provided by collaborators within the WEFOLD initiative directed by Silvia Crivelli (National Institutes of Health), as part of the wfCPUNK and wf-BAKER-UNRES groups [4]. Our results have shown, that restrained REMD simulations with UNRES can be useful also when contradictory restraints are present (which often occur for example in restraints from NMR spectroscopy of flexible peptides).

References

[1] Liwo A, Czaplewski C, Ołdziej S, Rojas AV, Kaźmierkiewicz R, Makowski M, Murarka RK, Scheraga HA (2008) Simulation of protein structure and dynamics with the coarse-grained UNRES force field. In: Voth G (ed.) Coarse-Graining of Condensed Phase and Biomolecular Systems. CRC Press, Boca Raton, chap. 8, pp. 1391-1411

[2] Czaplewski C, Kalinowski S, Liwo A, Scheraga HA (2009) Application of Multiplexed Replica Exchange Molecular Dynamics to the UNRES Force Field: Tests with alpha and alpha+beta Proteins. J Chem Theory Comput 5:627-640

[3] Liwo A, Baranowski M, Czaplewski C, Gołaś E, et al. (2014) A unified coarse-grained model of biological macromolecules based on mean-field multipole-multipole interactions. J Mol Model 20:2306

[4] Keasar C, McGuffin LJ, Wallner B, Chopra BG, et al. (2018) An analysis and evaluation of the WeFold collaborative for protein structure prediction and its pipelines in CASP11 and CASP12. Sci Rep 8:9939

Acknowledgments. Supported by grant UMO-2017/25/B/ST4/01026 from the National Science Centre of Poland (Narodowe Centrum Nauki). Computational resources were provided by (a) the Interdisciplinary Center of Mathematical and Computer Modeling (ICM) at the University of Warsaw under Grant No. GA71-23, (b) the Informatics Center of the Metropolitan Academic Network (TASK) in Gdańsk, and (c) our 796-processor Beowulf cluster at the Faculty of Chemistry, University of Gdańsk.

P18

Accurate Protein Side-Chain Modeling using Conformational Space Annealing Method

Seungryong Heo[1], Keehyoung Joo[2], Jooyoung Lee[1,2]

[1]School of Computational Sciences, Korea Institute for Advanced Study, Korea

[2]Center for Advanced Computation, Korea Institute for Advanced Study, Korea

The side-chain prediction problem is an important issue in the various protein structure research fields, especially protein structure prediction, protein-protein docking, and protein design. There are many methods which have been proposed, and most methods involve rotamer library-based method [1], which uses a statistically clustered side-chain conformations from known structures. Rotamer libraries can be classified into backbone-independent and backbone-dependent. Backbone-dependent rotamer libraries are strongly influenced by the backbone dihedral angles ϕ and ψ. Given a rotamer library, the side-chain prediction problem can be considered as a combinatorial search problem.

In this study, we employed backbone-dependent rotamer library and log probabilities of these rotamers as an energy function. As an additional energy function, a simple repulsive steric energy term, distance-scaled finite ideal gas reference (DFIRE) term [2], and hydrogen bond term are also included. For a search method, we used conformational space annealing (CSA) method, which has been quite successfully applied to various hard combinatorial optimization problems [3,4]. Then, we compared the performance of our side-chain prediction method with a widely used side-chain prediction software program, SCWRL4 [5]

References

[1] Dunbrack Jr., R.L. and Karplus, M. Backbone-dependent rotamer library for proteins: Application to side-chain prediction. J. Mol. Biol. 1993, 230: 543– 574.

[2] Zhang, C.; Liu, S.; Zhou, Y. Accurate and Efficient Loop Selections by the DFIRE-based All-Atom Statistical Potential. Protein Sci. 2004, 13, 391 −399.

[3] Lee, J.; Gross, S. P.; Lee, J. Modularity Optimization by Conformational Space Annealing. Phys. Rev. E 2012, 85, 056702−1−5.

[4] Okur; Miller, B. T.; Joo, K.; Lee, J.; Brooks, B. R. Generating Reservoir Conformations for Replica Exchange through the Use of the Conformational Space Annealing Method. J. Chem. Theory Comput. 2013, 9, 1115−1124.

[5] G. G. Krivov, M. V. Shapovalov, and R. L. Dunbrack, Jr. Improved prediction of protein side-chain conformations with SCWRL4. *Proteins,* 2009.

P19

SAXS-assisted protein-protein docking

Hyunjin Kim, Keehyoung Joo, Jooyoung Lee

Center for In Silico Protein Science & School of Computational Sciences,
Korea Institute for Advanced Study, Seoul, 130-722, Republic of Korea

Protein-protein docking programs have been developed in order to predict biologically-relevant protein-protein complexed structures. Lately, the accuracy of docking methods tends to be improved with an aid of experimental data. In particular, small-angle X-ray scattering (SAXS)-assisted protein-protein docking methods [1, 2] have been implemented, because SAXS data provide low resolution but essential information on size and shape of complexes. However, these methods still reveal lack of accuracy. Thus, we are developing a new SAXS-assisted protein-protein docking method, taking advantage of well-established tools, ZDOCK [3], DockQ [4], and Pepsi-SAXS [5]. ZDOCK [3] is a rigid-body docking program to predict structures of protein-protein complexes and symmetric multimers. DockQ [4] is a continuous quality measure for protein-protein docking model, derived by combining Fnat, LRMS, and iRMS to a single score in the range [0, 1]. Pepsi-SAXS[5] is a recently developed program to calculate SAXS intensity profiles for atomic models with high accuracy and high speed. Details of our protocol are as follows: (1) Via ZDOCK, 2000 top-scored complexes are selected. (2) Via DockQ, all-to-all DockQ scores of the complexes are calculated and used for their clustering. (3) Via Pepsi-SAXS, scattering intensity profiles are evaluated. (4) Via computing pair-distribution functions vs. atom-pair distances between proteins, distance ranges representing protein-protein interface and outer edges of complexes are determined. (5) For the corresponding momentum ranges, so-called "partial" χ values, defined as difference between experimental and calculated intensities, are measured and used for sorting the complex clusters. Our results for test cases indicate that contrast to conventional χ values that show no correlation with the DockQ scores, the partial χ values are more or less anti-correlated with the scores, successfully classifying group including complexes with high DockQ scores and low χ values as a native-like form. This indicates that well-characterized information obtained from SAXS data plays a key role to predict the most probable protein-protein complexed structure.

References

[1] Schneidman-Duhovny D, Hammel M, Sali A. (2010) Macromolecular docking restrained by a small angle X-ray scattering profile. J Struc Biol.

[2] Duhovny D, Nussinov R, Wolfson HJ. (2002) Efficient Unbound Docking of Rigid Molecules. In Gusfield et al., Ed. Proceedings of the 2'nd Workshop on Algorithms in Bioinformatics(WABI) Rome, Italy, Lecture Notes in Computer Science 2452, pp. 185-200, Springer Verlag,

[3] Pierce BG, Wiehe K, Hwang H, Kim BH, Vreven T, Weng Z. (2014) ZDOCK Server: Interactive Docking Prediction of Protein-Protein Complexes and Symmetric Multimers. Bioinformatics 30(12): 1771-3.

[4] Sankar Basu and Björn Wallner (2016) DockQ: A Quality Measure for Protein-Protein Docking Models PLoS One. 11(8):e0161879.

[5] S. Grudinin, M. Garkavenko, A. Kazennov. (2017) Pepsi-SAXS : an adaptive method for rapid and accurate computation of small-angle X-ray scattering profiles. Acta Cryst., D73, 449 – 464.

P20

Structural investigation of the amyloid aggregates in the presence of human serum albumin and human cystatin C

Adriana Żyła[12], Augustyn Moliński[12], Michał Taube[3], Agnieszka Belczyk Ciesielska[4], Maciej Kozak[123], Wojciech Bal[4]

[1] Department of Macromolecular Physics, Faculty of Physics, Adam Mickiewicz University in Poznań
[2] NanoBioMedical Centre, Adam Mickiewicz University in Poznań
[3] Joint Laboratory for SAXS Studies, Faculty of Physics, Adam Mickiewicz University in Poznań
[4] Department of Biophysics, Institute of Biochemistry and Biophysics, Polish Academy of Science

Neurodegenerative disorders (Alzheimer's, Parkinson's diseases) correlate with the extended life time in our population and these diseases are a serious challenge for science. Still we have no effective drugs that are able to combat these diseases. The discovery of proteins or peptides [1] responsible for development of these diseases was milestone in understanding them at molecular level and determination of three-dimensional structures of these macromolecules have allowed to understand some of the mechanisms of protein aggregation and the formation of their neurotoxic oligomers or amyloid deposits. Moreover, there are promising studies show that proteins like human serum albumin (HSA) [2] or human cystatin C (HCC) [3] could be involved in the inhibition of Aβ peptides aggregation into toxic amyloids.

In the first step of our study, we are optimizing a protocol of production and purification of HSA and HCC proteins. Purified proteins will be used for investigation of Aβ peptides aggregation by biophysical methods like nuclear magnetic resonance (NMR), circular dichroism (CD) or small angle X-ray scattering (SAXS). We will also model *in silico* binding of the Aβ peptides to the HSA molecule.

References:

[1] Castello MA, Soriano S (2014) On the origin of Alzheimer's disease. Trials and tribulations of the amyloid hypothesis. Ageing Res Rev 13:10-2. doi: 10.1016/j.arr.2013.10.001

[2] Choi TS et al (2017) Molecular Insights into Human Serum Albumin as a Receptor of Amyloid-β in the Extracellular Region. J Am Chem Soc. 139(43):15437-15445. doi: 10.1021/jacs.7b08584

[3] Spodzieja et al (2017) Characteristics of C-terminal, β-amyloid peptide binding fragment of neuroprotective protease inhibitor, cystatin C. J Mol Recognit. 30(2). doi: 10.1002/jmr.2581.

Acknowledgments:

The studies were supported by research grant UMO-2017/27/B/ST4/00485 from National Science Centre and international grant POLTUR2/3/2017 from National Center of Research and Development.

P21

Protein Structure Determination from Cryo-EM map using the CSA method

Park Jimin[1], Joo Keehyoung[1] and Lee Jooyoung[1]

[1] Center for *in silico* Protein Science, School of Computational Sciences, Korea Institute for Advanced Study, Republic of Korea

Knowing atomic structure of proteins is important to understand the biological function and molecular mechanism of proteins. To determine the atomic structure of proteins, various experimental and computational methodologies have been developed and performed. Recently, single particle electron cryomicroscopy (cryo-EM) became one of the most significant methods for investigating the protein structures. However, determination of the accurate atomic structure from cryo-EM map is one of the remaining challenging problems due to its relatively low resolution. One strategy is fitting a known atomic structure or homology modelling structure into a cryo-EM map and refining the fitted model using the cryo-EM map [1]. The conformational space annealing (CSA) method which is a powerful global optimization algorithm has been successfully applied for the experimental data-assisted protein structure modeling such as NMR data- [2] and SAXS data-based modeling [3]. Therefore, the cryo-EM structure determination could be carried out by using the CSA method. To assess the goodness of fit between CSA-generated model and cryo-EM map, cross-correlation between the cryo-EM map and the map calculated from the CSA-generated model was measured and used as a fitting score. This fitting score was implemented into previously developed multi-objective energy functions for protein modeling [4]. The CSA-generated models were assessed by the multi-objective functions in terms of their consistencies with the cryo-EM map and the requirements of protein-like stereochemistry. Optimizing the energy functionss will be progressed to obtain better structures from cryo-EM maps.

References

[1] Zhu J, Cheng L, Fang Q, Zhou ZH, Honig B (2010) Building and refining protein models within cryo-electron microscopy density maps based on homology modeling and multi-scale structure refinement. J Mol Med 397:835-851

[2] Joo K, Joung I, Lee J, Lee J, Lee W7 Brooks B, Lee SJ, Lee J (2015) Protein structure determination by conformational space annealing using NMR geometric restraints. Proteins 83:2251–2262

[3] Joo K, Heo S, Joung I, Hong SH, Lee SJ, Lee J (2018) Data-assisted protein structure modeling by global optimization in CASP12. Proteins 86:240–246

[4] Hong SH, Joung IS, Flores-Canales JC, Manavalan B, Cheng Q, Heo S, Kim JY, Lee SY, Nam M, Joo K, Lee IH, Lee SJ, Lee J (2018) Protein structure modeling and refinement by global optimization in CASP12. Proteins 86:122-135

P22

Mg_3F_7: a superhalogen with potential for new nanomaterials design

Sikorska Celina[1]

[1] University of Gdansk, Poland

One of the principal factors which imply molecular reactivity in chemistry is electron affinity (EA). Explicitly, species with high EA typically act as strong oxidizing agents in chemical processes and capture an excess electron to become strongly bound negative ions. As it is well known, among the chemical elements halogen atoms exhibit the largest EAs. The unique characteristic of halogen atoms has allowed for the creation of hypervalent clusters containing extra electronegative atoms, which, due to collective effects, have higher EA energies than that of the halogen atoms. These hypervalent structures were given the name "superhalogens" by Gutsev and Boldyrev who have introduced a simple MX_{k+1} formula, where M is the main group or the transition metal atom, X is a halogen atom, and *k* is the maximal formal valence of the atom M.[1] Due to their strong oxidizing capability, superhalogens can be used to access the high oxidation states otherwise unreachable in conventional chemistry. [2-5]

In this contribution, the stability of the Mg_3F_7 cluster and its ability to ionize nanoparticles has been investigated theoretically. At the CCSD(T) level of theory, the Mg_3F_7 cluster has been confirmed to be superhalogen due to its high adiabatic electron affinity (7.9 eV). [6] The corresponding daughter anionic species ($Mg_3F_7^-$) displays a highly symmetric (C_{3v}) umbrella-like structure and magic cluster stability. When interacting with the fullerene nanoparticle (C_{60}), the radical neutral Mg_3F_7 superhalogen captures an electron and forms stable and strongly bound "binary salts" consisted of $Mg_3F_7^-$ anion and $C_{60}^{\bullet+}$ radical cation. [6] Thus Mg_3F_7 can be used as an effective oxidizing agent to construct new ionized nanomaterials.

References

[1] Gutsev G, Boldyrev A (1981) Dvm-X-Alpha Calculations on the Ionization-Potentials of MX_{k+1}^- Complex Anions and the Electron-Affinities of MX_{k+1} Superhalogens. Chem Phys 56: 277-283.
[2] Sikorska C (2016) Are noble gas molecules able to exhibit a superhalogen nature? Rsc Adv 6: 103418-103427.
[3] Sikorska C (2015) Utilizing fluoroxyl groups as ligands in superhalogen anions: An *ab initio* study of the $M(OF)_{k+1}^-$ systems (M=Li, Na, K, Be, Mg, Ca, B, Al). Chem Phys Lett 638: 179-186.
[4] Sikorska C, Freza S, Skurski P (2011) The Reason Why $HAlCl_4$ Acid Does Not Exist. J Phys Chem A 114: 2235-2239.
[5] Sikorska C (2016) When a nanoparticle meets a superhalogen: a case study with C_{60} fullerene. Phys Chem Chem Phys 18: 18739-18749.
[6] Sikorska C (2018) Mg_3F_7: a superhalogen with potential for new nanomaterials design. Int J Quantum Chem, DOI: 10.1002/qua.25728.

Acknowledgments

The support from the National Science Centre (NCN) Grant No. UMO-2017/25/B/ST4/01026 is gratefully acknowledged.

P23

Computer simulations of hydrophobic effect on an example Xe atoms and water model: SPC/E, TIP3P and TIP4P-2005

Karolina Zięba[1], Adam Liwo[2], Cezary Czaplewski[1]

[1] University of Gdansk, Faculty of Chemistry, Laboratory of Simulations of Polymers, Poland
[2] University of Gdansk, Faculty of Chemistry, Laboratory of Molecular Modelling, Poland

Hydrophobic effect describes many physicochemistry processes in aqueous environment, such as protein folding, formation double-layers of molecular membranes and many others [1,2]. Results of these interactions is association molecules of non-polar substances. Experimentally study these effects is very difficult, due to the low solubility these substances in water. We can study hydrophobic association by using computer simulations methods. In theoretical approach a hydrophobic effect is described by *Potential Mean Force* (PMF) as a distance function between two interactions molecules. In approximate value of PMF respond to a contact minimum configuration two interacting molecules.

Main goal our studies was determine thermodynamic association process for SPC/E, TIP3P and TIP4P-2005 water models and two Xe atoms. We performed MD simulations by using LAMMPS software for hydrophobic interactions Xe-Xe in water. The simulations were performed for a cubic periodic box with a 37 Å side in the NpT ensemble. The time step in MD simulations was equal 2.0 fs, additionally we applied SHAKE algorithm to retain the water molecules rigid. The configurational space in MD simulations was sampling in both scheme umbrella sampling / WHAM and Particle Insertion methods. In the first of above methods, the simulations were carried out by using 25 windows, each with a harmonic potential for temperature range 273-373 K (every 5th K) with p = 1 atm. To calculate PMF from *umbrella sampling* simulations we used WHAM method. Another step in this method was sketched dependence PMF at the contact minimum configuration terms of the temperature and fitted to this quadratic and logarithmic function. In Particle Insertion method, we calculated the excess chemical potential for single Xe atom in water. In this method, the enthalpic and entropic contributions were calculated using quadratic fit as we did for contact minima of PMF Xe-Xe, but only for five temperatures, such as: 273, 298, 323, 348 and 373 K. These simulations were carried out for two independent potentials, such as Lennard-Jones and hard sphere Mie potentials. Results of MD simulations in scheme *umbrella sampling* / WHAM for Lennard-Jones potential shows that the free energy [ΔG] and enthalpy [ΔH] decrease, if the temperature increase. On the other hand, in the case of Mie potential we can observed much larger decrease above values for growing temperature. The changes of entropy [$-T\Delta S$] were small for SPC/E and TIP4P-2005 water models, but TIP3P shows much larger temperature dependency and in this case enthalpy was negative for higher temperatures, especially for Mie. Our study shows, that the results, which obtained for Mie potential are close to Scale Particle Theory. In the Particle Inserted method results for Lennard-Jones potential shows classical picture of hydrophobic hydration, with hard sphere Mie potential the hydration enthalpy is always positive and temperature dependence is a little bit smaller.

[1] W. Kauzmann, *Some factors in the interpretation of protein denaturation*, Adv. Protein Chem., *14*:1-63(1959)
[2] H. A. Scheraga, *Theory of hydrophobic interactions*, J. Biomol. Struct. Dyn., *16*:447-460 (1998)

Iława

First mentions of the lands where Iława is situated date back to 1226 when Duke Konrad I of Masovia issued a document heralding the Order of Teutonic Knights and after the conquest of Prussia in 1283, the territory was in the process of colonization. The town was established by the commander Sieghard von Schwarzburg in 1305. In the same year, Iława was granted a town charter.

Iława is located in the Iława Lake District, in the territory of the Green Lungs of Poland, in southern end of the longest lake in Poland – Jeziorak. In the administrative borders of the town there is Wielka Żuława situated – the largest inland island in Poland of 82.4 hectares. Iława is one of the largest towns in Warmińsko-Mazurskie Province. Well developed transport accessibility causes that tourists traveling both by motor vehicles as well as train may reach this place easily. The Iławka River goes through the town and it is surrounded by beautiful forests and lakes. Numerous monuments are witnesses of history. Thanks to perfect location of this corner of the country, in Iława and surroundings, hiking, bicycle touring and water tourism has been developing. Particularly the latter is accelerated by the Jeziorak Lake in a close neighborhood, where there are a lot of marinas and water equipment rentals. Admirers of this kind of active leisure, will take adventage of the goods offered by the natural environment of this territory. An additional attraction are numerous canoe trails which cover with their routes nearby basins and towns. A well-prepared tourists accommodation , including hotels of every standard, boarding houses, marinas, water equipment rentals, restaurants and cafes, is waiting for everyone. A well-developed sport, recreational and cultural infrastructure is a big advantage of the town. Modern sports fields complex, extreme sports center, sports and show hall as well as tourist and recreation center with a swimming pool, salt chamber and bowling alley are waiting for tourists. Children who have come to Iława, may try their hand at the Road Traffic Park or may use a recreational pump truck. One may also use a water tram around the lake. Nightlife in Iława pulsates in many places: discos, pubs and clubs. Sightseeing in Iława may be also combined with finding the culinary offer of the town.