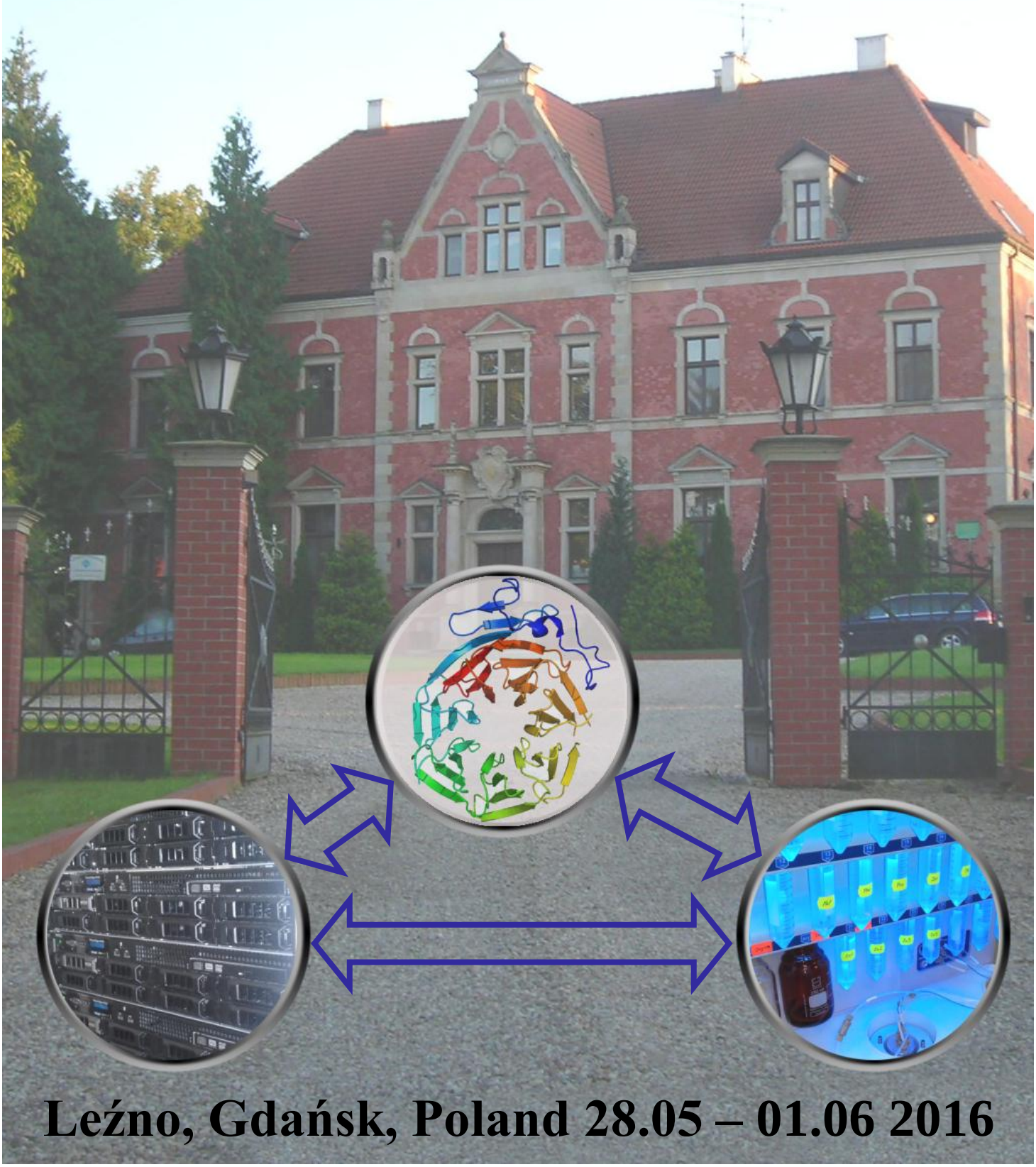
Second Polish-Korean Conference on Protein Folding

Theoretical and Experimental Approaches



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Investigating the folding and misfolding of proteins and other biological molecules is of utmost importance in the post-Genome era because of 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. 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.

The first conference of the series was held from May 24 to May 28, 2015 in Seoul.

Organizing committee

- Cezary Czaplewski (U of Gdansk, Poland)
- Jooyoung Lee (KIAS, Korea)
- Adam Liwo (U of Gdansk, Poland)
- Mariusz Makowski (U of Gdansk, Poland)
- Rafał Ślusarz (U of Gdansk, Poland)
- Bogdan Mierzejewski (PERSPEKTYWA, Poland)
- Artur Giełdoń (U of Gdansk, Poland)

IL – Invited Lecture

CL – Contributed Lecture

ST – Short Talk

Conference program

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

28.05 – SATURDAY

16:00 – 18:00 – Registration Desk

17:00 – 20:00 – Conference Welcome

28.05 – SUNDAY

Session I, chair: Masaki Sasai

28.05 – SUNDAY

Session II, chair: Andrzej Koliński

28.05 – SUNDAY

Session III, chair: Jooyoung Lee

18:30 – 19:30 POSTER SESION, chair: Cezary Czaplewski

28.05 – MONDAY

Session IV, chair: Sułkowska Joanna

28.05 – MONDAY

Session V, chair: Weontae Lee

28.05 – TUESDAY
Session VI, chair: Yi He

28.05 –WEDNESDAY
Session VII, chair: Igor Konieczny

Poster List

Abstracts

Non-Thermal Plasma and Protein Folding.

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Most of the biological function of proteins results from their three-dimensional structures to communicate genetic information. Human diseases related proteins in signal networking process have been recently studied in the point of protein structure and folding process. NMR spectroscopy and X-ray crystallography together with biophysical techniques have been used as powerful methods in probing protein structure and folding in atomic scale. Very recently, it has been proposed that non-thermal plasma could be applied to cancer cells and tissues for clinical purpose. However, a detailed molecular mechanism related to plasma effect has not been understood yet. NMR spectroscopy would be used as a powerful technique to uncover non-thermal plasma effect on proteins during cellular signaling. Our data suggest that plasma irradiation induces structural fluctuation as well as functional change of proteins, which are important aspects in understanding molecular function of proteins modified by cold plasma. In this presentation, I will also present plasma effect on protein folding which is of essence in understanding molecular function of the living cells during plasma irradiation.

IL102

Efficient Modeling of Protein Structure and Dynamics.

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The traditional computational modeling of protein structure, dynamics and interactions remains difficult for many protein systems. It is mostly due to the size of protein conformational spaces and required simulation timescales that are still too large to be studied in atomistic detail. Lowering the level of protein representation from all-atom to coarse-grained opens up new possibilities for studying protein systems [1]. Most recent applications of CABS modeling tools are briefly discuses. CABS (C-Alpha, Beta and Side-chain) is a medium resolution model. In comparison with UNRES, CABS provides similar resolution but it is based on qualitatively different interaction and sampling concepts. The choice of united atoms for modeling single amino acids is similar to that of UNRES except for the side chains which are represented by two spherical pseudo-atoms, one centered on Cβ and the other placed in the center of mass of the remaining portion of the side chain, where applicable. The main chain Cα positions are restricted to knots of a cubic lattice of small spacing, equal to 0.61 Å. This lattice Cα trace is used as the only independent variable that defines positions of other united atoms. Recently, we provided several easy to use web servers based on the CABS based modeling techniques (available at: http://biocomp.chem.uw.edu.pl/tools). The servers are dedicated to *de novo* and comparative modeling of structure prediction [2], studies of protein dynamics [3], and unrestrained, fully flexible, docking of peptides to protein receptors [4]-[5]. Multiscale modeling strategies combining CABS and related coarse-grained tools with all-atom Molecular Dynamics are briefly discussed.

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Acknowledgments

Support from the National Science Center (Poland) grant MAESTRO 2014/14/A/ST6/00088 is kindly acknowledged.

ST103

Molecular dynamics of protein A and a WW domain with a united-residue model including hydrodynamic interaction.

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Simulations of protein-folding pathways and folding kinetics are nowadays widely used in computational biology. Coarse-grained force fields with implicit solvent have become very popular because they are capable of simulating biological systems at a time scale unreachable for all-atom simulations. However, for the simulations to give reliable results, all major factors that influence the folding process have to be included in the energy function and equations of motion. One of these factors are the hydrodynamic interactions (HIs), which are manifested as apparent forces that drive two objects moving through liquid towards each other. In this work, to model HIs, we introduced the Rotne-Prager tensor of friction coefficients into the Langevin equations of motion for the coarse-grained United RESidue (UNRES) model of polypeptide chains developed in our laboratory. The effect of HIs was assessed by running UNRES/Langevin MD simulations of staphylococcal protein A (PDB ID: 1BDD) and the FBP 28 WW domain (PDB ID: 1E0L).

It was found that introducing HIs slows down both the formation of an intermediate state and the transition from the collapsed structures to the final native-like structures by creating multiple kinetic traps. Therefore, introducing HIs considerably slows the folding, as opposed to the results obtained from earlier studies with the use of Gō -like model.

Acknowledgments

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Protein Structure Prediction/Determination by Global Optimization.

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First, I will discuss our recent progresses on the protein structure prediction using the methodology of global optimization as illustrated in the CASP11 competition held in 2014. 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.

IL105

Correlations of the structure - dynamics – function of structurally homologous CheY-like proteins.

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The bacterial chemotaxis protein CheY [1], the N-terminal receiver domain of nitrogen regulation protein NtrC [2], and the sporulation response regulator Spo0F [3], are response regulators involved in various signal transduction pathways [4-7]. These three proteins have similar biological functions and are structurally homologous, but have low sequence identity [8]. The primary goal of this work is to identify factors in their amino acid sequences which lead to known similarities and differences in structural motions, related to biological function and thermostability. Previous all-atom molecular dynamic simulations of one of the three proteins reported by different groups [9-11] are mostly at a time scale of tens of nanoseconds. We obtained 33 one - μs all-atom molecular dynamic simulations with explicit solvents and counter ions, at 303.15K (nine trajectories) and 400K (24 trajectories). Common fluctuation regions among all three proteins are the β3-α3 loop, β4-α4 loop, α4 and α4-β5 loop. The stabilization cores of each protein were identified at different temperatures, providing an indication of the importance of specific secondary structural fragments at the N- and C- termini to the stability of each protein. The simulations also indicate that temperature change results in alteration of the stabilization core in CheY. The Property-Factor Method (PFM) was used to identify underlying sequence similarities between the three possible pairs of proteins, a result which is not accessible using conventional sequence comparison methods. Although all three CheY-like proteins have almost identical tertiary structure, NtrC and Spo0F have higher thermostability than CheY, especially in the C-terminus region, as observed at 400K, an observation which is in agreement with our PFM sequence-comparison results.

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Acknowledgments

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ST106

Support –vector-machine-based protein single-model quality assessment.

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Quality assessment (QA) for protein structure models is essential for successful protein structure prediction. QA methods can be grouped into two approaches: consensus methods and single-model methods. Consensus methods perform better than single-model methods, especially when there is a good consensus in the model pool. However, these methods fail when the pool contains a large portion of low-quality models similar to each other. Consensus methods are unable to generate proper quality scores for native-like structures distinct from the rest of the pool. Moreover, the computational cost of consensus methods increases as the square of the number of models, which makes it slow to apply them to a large number of models. Therefore, single-model QA methods are developed to address these problems. In this study, we have developed a support-vector-machine-based protein single-model global quality assessment (SVMQA) method. For a given protein model, SVMQA predicts its global quality scores such as TM-score and GDT-TS score based on the feature vector which contains statistical potential energy terms and consistency terms between actual structural features (extracted from the three-dimensional coordinates) and predicted values (from its primary sequence). We trained SVMQA using CASP8, CASP9, and CASP10 single-domain targets, and determined machine parameters by 10-fold cross-validation. We evaluated the performance of our SVMQA method on independent test sets including CASP11 targets, I-TASSER dataset and our in-house models. Results show that SVMQA outperforms existing top performing methods in selecting top models.

IL107

All-atom and coarse-grained simulations for protein structure prediction and protein aggregation dynamics and thermodynamics.

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A very large number of small monomeric proteins adopt rather well defined 3D structures in aqueous solution. Accurate and fast structural prediction of wild-type sequences and variants is of high interest in protein engineering.

There is, however, a class of peptides that can self-assembly and eventually form amyloid plaques. We would like by experimental and computational means to characterize all the early events preceeding nucleation at an atomic level of detail, as some of these intermediates are among the main culprits in Alzheimer's disease. Thus far, this has not been possible.

In this talk, I present our recent contribution to the field of amyloid and non-amyloid peptides using both all-atom and coarse-grained protein force fields [1,2,3].

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ST108

Study of conformational diversity of GC-repeating ds-DNA.

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GC-repeating ds-DNA is known to have multiple native conformations. B-DNA undergoes a conformational change to Z-DNA on high salt concentration. This transition is reversible but the transitional pathways have not been studied extensively. Conformational space annealing (CSA) is a technique to sample diverse conformations of a molecule. We used CSA to sample various conformations of (GC)4 ds-DNA and found various conformations including its native forms.

ST109

Role of disulfide bonds on folding and structures of proteins.

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Despite the years of studies on proteins, only the fraction of protein structures has been solved and even lesser number of transition states and folding mechanism is known. Presence of S-S bonds in proteins make folding studies more difficult for two major reasons: only few tools allow to study the folding process with possibility to form and break the disulfide bonds, as well as the folding process itself is more complicated. We used molecular dynamics simulations in physics-based UNRES (UNited RESidue) force field,[1,2] in which dynamic disulfide bond formation and breaking potentials have been introduced, to study the dynamics, structures and folding pathways of 12 lipid transfer proteins (LTP) with and without presence of dynamic disulfide bonds.[3] Disulfide bonds are the main reason why LTP proteins can perform their functions in plants and their highly allergic properties[4,5]. Our studies show that disulfide bonds significantly change the dynamics of folding process and are crucial to obtain native structures of 12 LTP proteins and that the presence of disulfide bonds in LTP proteins is the main reason of their similar fold type.

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ST110

A fragment search method for Dynamic Fragment Assembly.

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The template based modeling (TBM) is a powerful method for protein structure prediction when appropriate templates can be identified. However, this method highly depends on the quality and quantity of available templates. In many cases, parts of a target sequence are covered by good-quality templates, while many parts are not covered. For the non-covered parts, one can try to find local fragments to fill up the gap missing in the whole protein alignment. Dynamic fragment assembly (DFA)[1] is one way to incorporate generated fragments into the modeling of uncovered parts of a target sequence. In the previous work of our laboratory[2], we used the correlation coefficient between target and template profiles to select fragments for DFA. In this work, we use a new scoring function[3] that includes additional sequence and structural features for the fragment search. These features include the consistency between predicted and actual values of secondary structure (from PSI-PRED[4]), solvent accessibility (from SANN[5]), and phi and psi torsion angles (from TASVR). On average, better-quality fragments are generated by using the new scoring function than using only the profile correlation. For the parameter optimization, we used the global optimization of conformational space annealing (CSA)[6]. We have investigated the dependency of benchmarking results on the selection of both the objective function and the database with a different sequence identity cutoff value.

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The problem of protein folding.

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The problem of protein folding is an interdisciplinary problem between biological and physicochemical sciences, and hence fundamental in biophysics. Anfinsen and his colleagues first raised the protein folding problem in the early 1960s. Since then a variety of experimental, theoretical and simulation studies on the protein folding problem have been carried out for more than 50 years. In this talk, we would like to introduce these studies, mainly focusing on the kinetic studies on protein folding reactions, in which we have been involved. One of us characterized an equilibrium unfolding intermediate, later called the molten globule (MG) state, in a number of non-two-state proteins by a variety of spectroscopic techniques, and established the identity of the MG state with an early transient intermediate in kinetic refolding of the proteins, studied by stopped-flow spectroscopic techniques [1]. We also found that in non-two-state proteins, the rate constant (k_f) of refolding from the fully unfolded state was well correlated with a structure-based parameter, the number of nonlocal contact clusters (N_c), but that two-state proteins did not show a significant correlation between k_f and N_c. Because N_c approximately represents the number of the contacting pairs of substructures, obtained from the residue–residue contact map, it has been suggested that the arrangement of the substructures may be important for the folding kinetics in non-two-state protein [2, 3]. During the last decade, the number of literatures on the folding kinetics of non-two-state as well as two-state proteins has increased dramatically, and now we have 46 non-two-state proteins and 77 two-state proteins, which are useful for the analyses of correlations between k_f and structure-based parameters. The results of these new analyses will also be reported and discussed.

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IL112

Pathway Model of Mini-Protein BBA5.

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Via computer simulations we provide an explanation for experimental protein folding observations in which formations of α-helix and β-hairpin secondary structures had been observed for mini-protein BBA5. By using action-derived molecular dynamics simulations we present folding pathway of the protein BBA5, a bundle of secondary structures, α-helix and β-hairpin. Sequential formations of α-helix and β-hairpin after a significant chain compaction are a notable prerequisite event for the BBA5 folding. Calculation data represent the first formation of the α-helix against the β-hairpin during the folding process of BBA5. The most flexible region for BBA5 in the present calculation is the N-terminal loop. Our results are in good agreement with related experimental observations and provide significant insight into the general mechanisms of protein folding. Finally, a description of the folding pathway in terms of principal component analysis is presented to characterize the folding dynamics in reduced dimensions. With only three principal components, we were able to describe 83.4% of the pathway.

Mechanism of Fe/S cluster transfer.

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The Iron-sulfur clusters (ISCs) are highly spread among the organisms from prokaryote to eukaryote. They are the crucial part of many cellular processes like electron transfer, redox reactions, and catalysis of chemical reactions. Most of the proteins required presence of chaperons to achieve and stabilize their structures. In yeast *Saccharomyces cerevisiae* the Fe-S cluster biogenesis cycle is one of the mechanisms, which are necessary for the organisms, but not well known. Especially the last stages involving transfer of Fe-S cluster, which require the presence of multiple proteins: Isu1 (scaffold protein), Jac1 (co-chaperon), Ssq1 (chaperon) and Grx5, makes the system difficult to study (Figure 1).[1,2] To investigate such intricate system we used coarse-grained UNRES force [3] to obtain the structure and dynamics of the Isu1-Jac1 and Isu1-Jac1-Ssq1 complexes. Our studies reveal that the structure of Isu1-Jac1 complex is quite flexible, however in all observed conformations, the interface of interactions is conserved.[2] We found out that in presence of chaperon Ssq1 interactions between Isu1 and Jac1 weaken, and proteins begin to dissolve from each other what cause the transition of the Ssq1 structure.

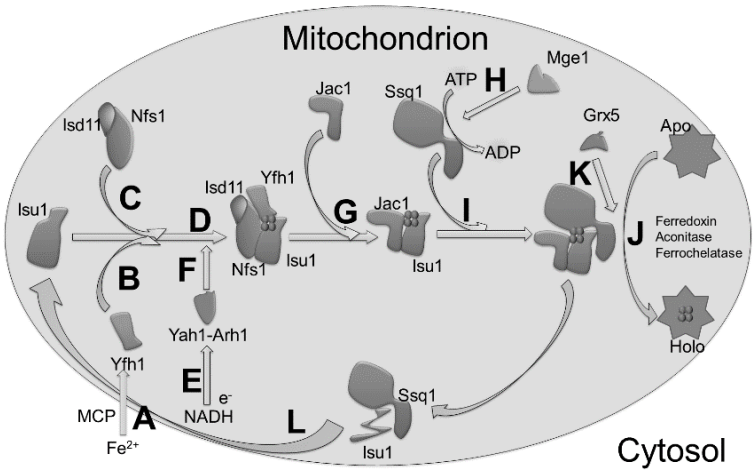

FIGURE 1 SCHEMATIC REPRESENTATION OF THE FE-S CLUSTER BIOGENESIS CYCLE.

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IL201

Experimental Studies of Fast-Folding Proteins: Lessons about Free Energy Barriers and Pre-Exponential Factors in Protein Folding.

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Protein folding research stalled for decades because conventional experiments indicated that proteins fold slowly and in single strokes, which impeded deriving any information about mechanisms, whereas theory predicted a complex interplay between dynamics and energetics resulting in myriads of microscopic pathways. A major turning point came from the development of ultrafast kinetic methods, which provided the means to experimentally probe fundamental aspects of folding, test theoretical predictions, and benchmark simulations. Using these techniques we could measure the timescales for all the relevant folding motions, estimate the folding speed limit, and confirm that folding barriers are entropic bottlenecks and relatively small. Moreover, a catalogue of proteins that fold extremely fast (microseconds) could be identified. Such fast folding proteins cross shallow free energy barriers or fold downhill, and thus unfold with minimal cooperativity (gradually). We are now taking advantage of the special properties of fast folding proteins to further investigate the pre-exponential factor in folding kinetics and its connection with the molecular mechanisms for folding. The pre-exponential factor is the inverse of the mean folding transition path time (the time it takes a protein to traverse the barrier) and is predicted by simulations to strongly depend on the size, fold topology and aminoacid sequence of the protein. In this talk I will review the main general findings that have come from investigating fast folding proteins and will also discuss new results from my group that specifically address how the pre-exponential factor depends on the aminoacid sequence.

IL202

The order-disorder processes in solution SAXS studies of the protein folding.

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Dynamics of protein conformation is crucial for many biological processes. On the other hand, the structure of of proteins containing unstructured elements or intrinsically disordered proteins (IDPs) is difficult to investigate using techniques of protein crystallography or NMR. During the lecture will be presented the possibility of using SAXS technique in the study of low resolution structure of proteins containing disordered fragments and intrinsically disordered proteins. As practical examples of the use of SAXS techniques will be presented: the low resolution structure in solution of HOPQ1 protein (a type III secretion effector) from *Pseudomonas syringae*, its complex with 14-3-3a protein from *Nicotiana tabacum*, results of structural studies of selected proteins IDPs and also oligomerization processes of the human cystatin C.

Acknowledgments
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ST203

Folding Mechanisms of Small Proteins GB1 and LB1.

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The B1 domains of protein G (GB1) and protein L (LB1) are two small proteins that binds to antibody immunoglobulin G (IgG). GB1 and LB1 are similar in size (about 60 residues), and also have an overall similar structure (β-hairpin--α-helix--β-hairpin). However their sequences are very different, possessing only 15% identity in a structure-based alignment.[1,2] Therefore, there are interesting similarity and differences in their folding mechanisms. Experimental evidence indicated that LB1 folds in a two-state manner; while GB1 folds in a more complex way -- an early stage intermediate may exist in the folding path. Till now, the folding mechanisms are still under extensive experimental and computational study. Structure-based modeling is one of the less costly computational methods. It has a simple formulated potential energy function summing over various geometrical restraints from one or more targeted structures. Here, we used a new all-atom structure-based method to investigate the folding mechanisms of GB1 and LB1. In this approach, folded structures of the two proteins were used to construct the restraints and they are stabilized by Lorentzian attractive term instead of conventional harmonic term.[3] We presume that our model will be able to identify two-state and non-two-state proteins, and give us more insights of the their folding pathways.

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Dynamical Energy Landscape Perspective of Protein Functioning.

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In a classical view of structure biology, it has been often assumed that protein exerts its function through a sequential structure change along a unique pathway between two end structures determined by X-ray analyses. However, structure fluctuation expected during the macroscopic timescale (msec-sec) of protein functioning should be more diverse and we need to quantify it from energy landscape perspective. Because energy landscape itself is dynamically changed upon ligand binding/unbinding or chemical reactions, dynamical energy landscape method is the necessary method for describing protein functioning [1-3]. Here, we discuss mechanism of energy transduction in actomyosin motors with the dynamical energy landscape method [4,5]. Recent time-resolved fluorescence resonance energy transfer (TR-FRET) measurement showed that the lever-arm stroke of myosin takes place before the release of Pi from myosin [6], which showed not a sequential pathway as usually assumed by the lever-arm hypothesis but a kinetic network of structure fluctuation is necessary for describing motions and reactions of actomyosin motors. We discuss that the dynamical energy landscape method is consistent with the TR-FRET results and the recent electron-microscopic report on the actomyosin complex [7].

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IL205

Foldons, protein folding nucleation sites.

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Protein folding mechanism has been extensively studied over the past few decades and still is far from full understanding. One widely accepted model of protein folding mechanism proposes that folding proceeds by the stepwise assembly of the small units called foldons. Protein folding process starts by formation of one or more foldons, which stabilize formation of subsequent foldons and finally guide whole process to the native protein structure [1]. Despite the attractiveness of the foldons theory, no clear definition of a foldon is provided, even the size of a foldon unit is still a part of the scientific debate [2,3]. The aim of our work is to provide as precise as possible description of foldons (protein folding nucleation sites). To achieve our goal we are using both experimental and *in silico* methods. We are studying (using NMR spectroscopy) conformational properties of protein sequences fragments (peptides) which are suspected to play important role in the protein folding mechanism. Our structural studies allowed to identify a few sequential motifs (6-10 amino acid residues long, folding nucleation sites) which seem to be important on early stages of secondary structure elements formation. Our *in silico* studies are focused on identification in native protein structures small subdomains (up to 30-40 amino acid residues long) which possess the ability to quasi-independent folding.

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ST206

Buried Water Molecules in Protein Kinases.

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Protein kinases are one of the largest families of proteins found in the living cells. Their main function, *i.e.* controlling multiple cellular processes, is strictly regulated by precise activation process, during which the protein undergoes a structural transformation from inactive to an active form. Such conversion enables kinases to play the role of molecular switches. Malfunctioning of the regulatory mechanism leads to a number of serious diseases. Despite little sequence homology, all kinases share the same preserved bilobal shape. Interestingly, kinases structural analysis reveals several preserved hydration sites. Buried water molecules turn out to be localized in specific regions involved in the activation process. A question arises whether their presence plays any functional role.

The experimental approaches to characterise water molecules buried in proteins face significant limitations, therefore computational methods are a great tool for investigating their properties.

We are investigating the role of selected, universally preserved water molecules for kinase function by computational approaches. We focus on answering for the following questions:

1. Where are conserved hydration sites in kinases structure depending on different states (active/inactive)?
2. How tightly are the localized water molecules bound?
3. What is the influence of the considered hydration sites on conformational mobility of kinase catalytic subunits?

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IL207

Energy landscape of Supercoiled Protein Folding.

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We identify new entangled motifs in proteins that we call complex lassos [1]. Lassos arise in proteins with disulphide bridges or with amide linkages, when termini of a protein backbone pierce through an auxiliary surface of minimal area, spanned on a covalent loop. To analyze such configurations we develop tools based on graph theory and topology. We found that as much as 18% of all proteins with disulphide bridges in a non-redundant subset of PDB form complex lassos, and classify them into five distinct geometric classes, one of which resembles supercoiling in DNA.

Having presented the structure of proteins with lassos, I will discuss kinetics and thermodynamics of the folding process of a supercoiled protein, based on molecular dynamics simulations. We analyze the influence of the disulfide bond by comparing this protein in reduced and oxidized conditions. Our results reveal that self-supercoiling is possible, show that the folding rate is determined by the length of the-N terminal, and the topological barrier is overcome via slipknot configuration.

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Unique Features of Telomers.

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Since the discovery of telomeres and their loop structures scientist wonder why such repeating sequence has been chosen by nature. What is common feature of different telomere sequences among different classes or kingdoms? Why their sequences are so similar, despite the tremendous differences in their genetic codes and evolution? We studied human-like (TTAGGG), plant (TTTAGG) and insect (TTAGG) telomeric sequences and used three non-telomeric sequences (CG, AT, ATGC) as control sequences. In this study we present how mechanical properties of telomere sequences differ from other non-telomeric sequences and what makes them unique. We used steered molecular dynamics with all-atom AMBER14 force field and Nucleic Acid united RESidue (NARES) coarse-grained force field [1]. Our results reveal distinct features of all telomeric sequences, show their exceptional high mechanical resistance and stability to untangling and stretching.

Fig 1 $(GC)_{30}$ conformational changes during stretching.

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CL209

Evolutionary dynamics of protein:protein interactions, a case study of proteins involved in iron-sulfur cluster biogenesis.

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Protein interaction networks drive the operation and function of cells. These networks expand through gene duplication and divergence, but little is know how protein interactions become specific after gene duplication without passing through a non-functional or non-interacting state. One possibility is that the specificity of a given protein:protein interaction could change, and become insulated from the other paralog system, if one of the interacting partners passes through a promiscuous intermediate. We postulate that such a mechanism could explain the expansion of the mitochondrial (mt)Hsp70 chaperone network involved in an essential process of iron-sulfur cluster (FeS) biogenesis.

In most eukaryotes, including humans, a single, multi-functional mtHsp70 is involved in FeS biogenesis. However, a subset of fungi, including *Saccharomyces cerevisiae*, also contains a highly specialized Hsp70 paralog, Ssq1, that functions exclusively in FeS cluster biogenesis. All Hsp70s, including Ssq1, require for their function a J-protein co-chaperone, which stimulates their ATPase activity through the conserved J-domain consisting of two antiparallel helices (II and III) connected by a flexible loop. Using both experimental and computational approaches, we demonstrated that emergence of the specialized Ssq1 correlates with functional and structural changes in the J-domain of its J-protein partner Jac1. The loop region and the helix III become significantly shorter, while the helix II became rich in positively charged residues. These changes resulted in Jac1's higher specificity and higher affinity toward Ssq1. In contrast, Jac1 from pre-duplication species (*Yarrowia lipolytica* and *Schizosaccharomyces pombe*) interacts efficiently with mtHsp70 but not with Ssq1. Most importantly, a hybrid Jac1 that contains the loop region and part of the helix III from *Y. lipolytica* and the helix II and the remaining of the protein from *S. cerevisiae* behaves like the promiscuous intermediate discussed above as it interacts efficiently with both mtHsp70 and Ssq1. Taken together, these results suggest that the interaction with multifunctional mtHsp70 involves helix III and the loop region, while the interaction with specialized Ssq1 engages helix II. Thus upon emergence of Ssq1, Jac1's transition toward the new partner could have involved the shift of the J-domain regions engaged in the Hsp70 interaction. A promiscuous intermediate, similar to our hybrid Jac1, could explain how the transition took place without passing through a non-functional state.

IL301

Protein Structure Determination by Global Optimization Using Sparse and Ambiguous NMR restraints.

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Protein structure determination for large protein (> 20kDa) using NMR experiments has been challenging problem in the last decades. Slow tumbling of large molecules in solution leads to fast relaxation of NMR signals, causing severe line broadening, which is responsible for poor spectral resolution and low signal-to-noise ratios. To solve this problem, perdeuteration of the sample molecule, in which most ^{1}H nuclei are replaced with ^{2}H, is suggested. This generally increases the sensitivity and feasibility of NMR studies of larger proteins by reducing the fast decay of magnetization due to T2 (transverse) relaxation. However, the NMR data obtained by using perdeuterated proteins are generally "sparse and highly ambiguous". With sparse data, existing protein structure determination protocols such as CYANA often fail to provide reliable protein structures. Therefore, advanced methods incorporating additional experimental data and/or information are currently being developed to determine large-size protein structures using NMR methods.

In the recent 11th CASP (Critical Assessment of Protein Structure Prediction) experiment, a new challenge, the contact assisted category (called Ts target) using simulated sparse NMR contacts was tested. A total of 19 Ts targets with the chain length in the range 108 to 462 residues were tested. By applying the global optimization method of conformational space annealing to the two-level optimization problem (assignment of the ambiguous data and subsequent determination of protein structure), we have generated 3D protein models. In most of the cases, rather accurate 3D models (1.0 – 3.0 Å RMSD away from native structures) were obtained. The proposed method is the first kind of a direct method solving the NMR structure with sparse and highly ambiguous data.

IL302

Homology modeling, molecular dynamics, biochemistry and mass spectrometry for description of nucleoprotein complex of DNA replication initiator

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During replication initiation origin binding proteins (OBPs) interact with the site where DNA replication is originated (*origin*). This interaction results in the formation of initial nucleoprotein complex causing local destabilization of the origin DNA unwinding element region (DUE). That exposes single-stranded DNA indispensable for subsequent steps leading to replisome assembly. Since replication initiation proteins (Reps) are intrinsically disordered, only limited number of crystal structures are available. By using broad-host-range plasmid system, bioinformatics analysis and biochemistry we demonstrate the molecular mechanisms and structural requirements underlying the replication initiation protein activity during replication initiation. Our data bring new insights into understanding of replication initiators' activities and describe structural relations of OBPs from different domains of life.

IL303

Structural studies of the HVEM protein and its fragments interacting with BTLA and CD160 proteins.

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Metastatic melanoma is responsible for 90% of deaths by skin cancers, because of poor prognosis with less than 10% of patients surviving after 10 years. Recently, immunotherapies against melanoma have demonstrated promising results, but further progress is still necessary. It was shown that BTLA and CD160 proteins are involved in the negative regulation of T cell responses in cancer patients and can be targeted by immunotherapy. Both of them bind to a member of the TNF receptor superfamily, herpes virus entry mediator (HVEM) [1]. BTLA and CD160 proteins are both expressed on the surface of immune cells, including T, B and NK cells. The HVEM-BTLA and HVEM-CD160 complexes inhibit $CD4^+$ T cell activation.

The main goal of the project is to design efficient and selective BTLA or CD160 blockers that prevent their interactions with the HVEM protein. In our research we determined whether a fragments (14-39) of the HVEM protein and its variants can inhibit the interaction between BTLA and HVEM. We determined NMR structures of peptides and their complexes with BTLA protein. In addition the structure of CD160 protein was predicted by the Multiplexed Replica Exchange Molecular Dynamics (MREMD) [2]. The HVEM-CD160 complexes were built from the HVEM crystal structure and CD160 (UNRES structure) by using ClusPro [3] online docking program.

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CL304

Regulation of the proteolytic activity of the HtrA (DegP) protease from *Escherichia coli;* role of the regulatory loops.

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HtrA (DegP) from *E. coli*, an important element of the extracytoplasmic protein quality control system, is a member of the evolutionarily conserved HtrA family of serine proteases. The characteristic feature of this protein is an allosteric mode of activation. The crucial role in the transmission of the allosteric signal is played by the regulatory loops, termed L3, L2, L1 and LD. An additional element of the regulatory system provides the inhibitory loop LA which stabilizes the inactive conformation of the protease. The HtrA proteases are important virulence factors in numerous pathogenic bacterial species and are regarded as attractive therapeutic targets [1], thus understanding their regulation is of special importance.

Although intensively studied, the precise mechanism of regulation of the proteolytic activity of HtrA has not been determined, yet. In particular, the exact modes of action of the inhibitory loop LA and activation loop LD were not deciphered.

As the LA loop was not visualised in the crystal structure, we obtained a theoretical model of the 3D structure of LA and verified its correctness experimentally. This allowed us to propose intra- and intersubunit contacts that formed with the LA loops involved in maintaining the inactive conformation of HtrA. Disturbance of these interactions caused the stimulation of the proteolytic activity [2]. The 3D structure of LA is stabilized by a disulphide bond. We demonstrated that the reduction of this S-S bond stimulated the HtrA's activity [3].

The LD loop plays an important role in transmission of the allosteric signal. Although its structure in HtrA does not change upon activation, it acts as a kind of scaffold to assemble the activation cluster comprising the remaining activation loops, L1, L2 and L3. The role of the particular residues of the LD loop will be discussed.

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Revealing structures and dynamics of proteins using the X-ray lasers: from a computational perspective.

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X-ray free electron lasers (XFELs) provide new opportunities for structural biology. I will give an overview on XFEL applications in structural determinations, covering the following topics: (1) the special features of XFEL experiments, (2) the typical setups, and (3) data analysis flow. As examples, I will show a few successes in nanocrystallography [1,2] exploiting diffraction signals from very tiny crystals, then discuss the potentials and challenges in structure determination from single molecules. Both approaches utilize the ultra-short, super-intense X-ray pulses to realize 'diffract-before-destroy' mode and to '**image ultrasmall**'. Coupling with pump-probe methods, the ultrashort X-ray pulses can be used to study protein conformation changes in very short time intervals, i.e., **capture the superfast** [3]. At last, I will present some challenges posed to the computational modeling community [4]. The take-home message is that the X-ray lasers provide very powerful tools that can be widely used in structure and dynamics studies, and there are lots of opportunities for computational structural biologists in this field.

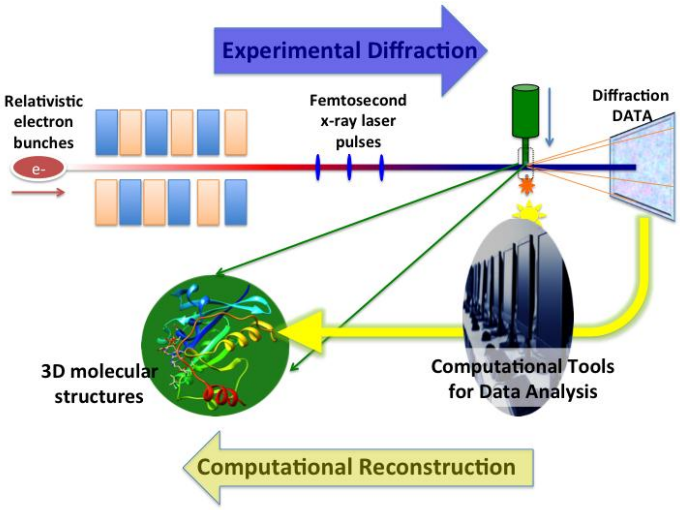

Figure 1. X-rays can generate lots of diffraction data, which will be used to convert to 3D structural models using computational methods.

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IL402

Exploring biomolecular dynamics and interactions using enhanced sampling approaches.

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Investigating the dynamics and stability of proteins and protein-protein complexes is of critical importance to better understand its biological function. Still, currently accessible time scales of molecular dynamics simulations are often too short to sufficiently sample relevant conformational states. We employ enhanced sampling molecular dynamics (MD) simulations based on Hamiltonian replica exchange (H-REMD) and using specific biasing potentials to study the dynamics of proteins and nucleic acids.

The biasing potentials can specifically lower the barrier for backbone dihedral transitions and promote enhanced backbone transitions along the replica coordinate. In another approach a coarse-grained elastic network model (ENM) of a protein is used to construct a biasing potential that controls the motion along soft directions compatible with the ENM model. Applications of the REMD methods to simulate structural transitions will be presented. In addition to the study of folding similar approaches can also be used to efficiently extract free energy changes from simulations and to study protein-ligand association processes. A second part will focus on using enhanced sampling techniques to improve and refine predicted protein-protein and protein-ligand complexes.

IL403

Contact statistics highlight distinct organizing principles of RNA and proteins.

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Although both RNA and proteins have densely packed native structures, chain organizations of these two biopolymers are fundamentally different. Motivated by the recent discoveries in chromatin folding that interphase chromosomes have territorial organization with signatures pointing to metastability, we analyzed the biomolecular structures deposited in the Protein Data Bank and found that the intrachain contact probabilities, P(s) as a function of the arc length s, decay in power-law $\sim s^{-\gamma}$ over the intermediate range of s, $10 \lesssim s \lesssim 110$. We found that the contact probability scaling exponent is $\gamma \approx 1.11$ for large RNA ($N>110$), $\gamma \approx 1.41$ for small sized RNA ($N<110$), and $\gamma \approx 1.65$ for proteins. Given that Gaussian statistics is expected for a fully equilibrated chain in polymer melts, the deviation of γ value from $\gamma=1.5$ for the subchains of large RNA in the native state suggests that the chain configuration of RNA is not fully equilibrated. It is visually clear that folded structures of large sized RNA ($N \gtrsim 110$) adopt crumpled structures, partitioned into modular multi-domains assembled by proximal sequences along the chain, whereas the polypeptide chain of folded proteins looks better mixed with the rest of the structure. Our finding of $\gamma \approx 1$ for large RNA might be an ineluctable consequence of the hierarchical ordering of the secondary to tertiary elements in the folding process.

IL404

Solution X-ray scattering (S/WAXS) and the formation of large scale structure in protein dynamics.

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We propose to develop the methods of non-equilibrium statistical field theory into a computationally efficient tool, to model and interpret protein dynamics in Small Angle X-ray Scattering (SAXS) experiments. As an example we analyse an Engrailed protein, and demonstrate that experimental observations are reproduced with a good precision and over an extended temperature range.

In addition, we propose how to interpret the observed phenomena in terms of protein phase structure. We compare the predictions with results from all-atom molecular dynamics simulation, which confirms that an effective Landau model approach has the potential to describe experimental data in a highly accurate and computationally effective manner.

P01

Protein folding in water surrounding.

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The water environment for protein folding traditionally expressed as oil drop [1] order has been extended to the form of fuzzy oil drop model [2]. It assumes the idealized hydrophobicity distribution in protein molecule to be expressed by 3D Gauss function with highest hydrophobicity concentration in a central part of ellipsoid with zero hydrophobicity on the surface – it means in the distance of 3 sigma in each direction. Divergence entropy is applied to measure differences between idealized and observed distribution [4]. It is assumed that this form of external force field (minimization of the hydrophobity difference) together with internal force field (optimization non-bonding interaction in protein molecule) may be the factor directing hydrophobic residues toward the center of folding molecule with the exposure of hydrophilic residues on the surface. The generation of specific local discrepancies very often related to the biological function of protein molecule [5] is the puzzle: How to ensure the local irregularity of highly specific character. It is suggested that ligand shall be present during folding to ensure the expected high specificity of ligand binding [6].

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P02

Structure analysis of the plasmid replication initiation protein TrfA.

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The DNA binding proteins play an important role in all aspects of the genetic activity within an organism such as DNA replication, repair, transcription and DNA conformational rearrangements. It is therefore of great importance to understand the nature of the interactions between proteins and DNA. In this study molecular modelling approaches were carried out combined with the experimental data in order to better understand the function of the replication initiation protein TrfA of RK2 plasmid and to find the interactions with the double strand DNA structure (dsDNA). The crystal structures of the TrfA protein has not been yet determined. This protein exists in two conformational variants namely 33kDa and 44kDa and belongs to the group of proteins, which are intrinsically unstructured. Therefore homology and fold recognition methods combined with experimental data and extensive structure analysis were applied to predict the structure of the TrfA protein. The *in silico* approaches were employed to optimize and validate the TrfA-dsDNA complex. A set of molecular dynamic simulations (MD) and binding free energy calculations were applied to study the binding of DNA to TrfA protein and to the experimentally identified TrfA mutants. The described TrfA-DNA interactions were shown to be in agreement with those obtained during the MALDI mass spectrum analysis.

Our data demonstrated that TrfA-33kDa protein is composed of three wing helix domains (WH1, WH2 and WH3). Sequence analysis on the TrfA wing-helix domains showed that the WH3 domain poses a common binding motif similar as identified in eukaryotic binding proteins.

P03

Study on Conformational Transition of the Maltose Binding Protein using Lorentzian Structure-Based Potential.

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Maltose binding protein (MBP) is a part of the maltose/maltodextrin system of Escherichia coli, which is responsible for the uptake and efficient catabolism of maltodextrins. Crystallographic studies have revealed two stable conformations: a ligand-free open form and a ligand-bound closed form. NMR experiments have suggested that the ligand-free protein is flexible enough to visit partially closed states, which may display higher binding affinity for the ligand than the open state. A molecular dynamic study has recently characterized the flexibility of the MBP protein and confirm the existence of a hidden semi-closed state. Here, we investigate conformational transitions between open and semi-closed MBP states driven by structure-based Lorentzian attractive interactions. Results are compared with existing MD and experimental data.

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P04

To fold or not to fold: substrates of the Hsp70/40 foldase machinery.

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First identified as response and remedy to heat shock conditions, Hsp70 and Hsp40 proteins form together the Hsp70/40 machinery which is essential for nearly any living cell. Their classic function is to fold other proteins after, e.g., translation, heat shock, or trans-membrane transport. These functions require the ability to bind nearly any protein in the proteome - but only when those proteins are unfolded.

How do the chaperones adapt to this multitude of sequences they have to bind? How do they recognize folded and unfolded proteins? As far as we understand the Hsp70/40 system, it's the Hsp40 component which is responsible for initial substrate recognition and binding, so in order to better understand the mechanism by which chaperones bind their substrates, we decided to focus on the substrate binding domain of Hsp40. Our model was yeast cytosolic Hsp40 chaperone Sis1. We present results from evolutionary analysis and molecular dynamics simulations of the Sis1's substrate binding domain. Our simulations reveal a new conformational state of this domain, not visible in crystal structures. Not only this second conformational state allows us to propose how the substrate binding domain adapts to the shape of its substrate peptides, but taken together with evolutionary data it allows to roughly describe the sequence of those substrates.

It would seem that Hsp40 bind very similar, but not identical sequences to their Hsp70 partners. Given that Hsp40 is a dimer, so it has two substrate binding sites, and Hsp70 is thought to act as a monomer with one substrate binding site, it leads us to suspicion that polypeptides with three to five secondary structure elements are not only the absolute minimum to make a stable protein, but also the most basic unit of folding.

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P05

Conformational properties of peptides based on pyrophosphates binding motif.

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Pyrophosphates (for example ATP) are widely present in the living organisms and play a role mainly as chemical energy storage or carriers. Importance of pyrophosphates is manifested by a large number of pyrophosphate binding motifs (PBM) identified in protein sequences [1]. PBMs' amino acid sequences are usually very rich in glycine and charged residues and they form loop/hairpin structures [1]. It is also suspected that PBMs are the most primitive functional elements which evolved on the very early stages of protein evolution [2]. Taking into account the importance of PBMs in the living organisms as well as in evolution, we decided to study conformational properties of PBMs and their ability to bind pyrophosphates in *in vitro* experiments. For our study we selected two most frequently occuring PBMs: the P-loop (consensus sequence: GXXXXGK[T,S] where X is any amino acid); and ATP binding loop from nucleotide binding domains (consensus sequence: DXGGGSXD). Peptide conformations were studied using NMR spectroscopy, also in presence of phosphates and/or pyrophosphates. Preliminary results suggest that some of the studied peptides form in solution well organized three-dimensional structures which resemble a loop/hairpin shape. Despite of surprisingly well organized structure of such short peptides, they seem to be unable to form stable complexes with phosphates/pyrophosphates or facilitate pyrophosphates' hydrolysis.

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P06

The emergence of additional protein:protein binding site enhances the catalytically activity of mtHsp70.

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In most eukaryotes, including humans, the Jac1 protein stimulates the ATPase activity of the single multi-functional mtHsp70 chaperon (Ssc1). This process is a key step in iron–sulfur clusters (FeS) biogenesis, and is crucial for proper functioning of the mitochondria. However, in *Saccharomyces cerevisiae* and closely related species, Jac1 interacts with the specialized and hyperactive form of mtHsp70 (Ssq1), which emerged through the duplication of mtHsp70. Alanine substitutions within the conserved His–Pro–Asp (HPD) motif, which is critical for Jac1:mtHsp70 interaction, are lethal for species expressing only multi-functional mtHsp70, but not in species expressing specialized Ssq1, suggesting the emergence of an additional binding site.

We identified Jac1 protein sequences from 56 fungi, members of Ascomycota phylum, and using empirical Bayes method we inferred expected number of substitutions per position of Jac1 protein. Surprisingly, we found four positions located in Helix 2 of the J-domain, in close proximity of HPD motif, which were variable in Jac1 proteins cooperating with multi-functional Ssc1, but otherwise conserved in Jac1 proteins cooperating with specialized Ssq1. Furthermore, we found that this four residues are responsible for the formation of the uniform positive electrostatic potential ≥2 kT/e around J-domain, which stretches up to 1.4 nm from Helix 2, and was present around constantly over 200ns Molecular Dynamics simulation. Both *in silico* protein-protein docking and *in vitro* studies in a pull-down assay confirm that these residues are involved in electrostatic interaction between Jac1 and Ssq1, and suggest that the emergence of this additional binding site is responsible for the enhanced ATPase activity of Ssq1 in cooperation with Jac1.

P07

Asymmetry in a knotted homodimeric methyltransferase.

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Methyltransferases are enzymes that are crucial for organisms' life, because they are responsible for important post-translational modifications of proteins or nucleic acids. Surprisingly these proteins perform the same biological function possessing two different folds, with their backbone being knotted or unknotted. There are over 60 distinct proteins from various organisms that possess a non-trivial topology [1] of trefoil knot, which is responsible for binding the cofactor essential for the methylation. The second surprise comes form the fact that knotted methyltransferases exist as homodimeric structures capable of binding two ligands and two substrates, while unknotted ones perform their function as monomers. Moreover, despite the symmetry in a knotted dimer, it has been shown for TrmD protein [2], that only one substrate is bound, indicating that one active site is more favorable than the other. To address this issue, we established possible function mechanism of this protein by means of molecular dynamics simulations. We were able to detect motions that lead to the asymmetrical structure in which one of the active sites is distorted. Since the crevice for ligand in the active site is created by the knot, its existence may be related to the observed asymmetry. However the function of the knot, i.e. the impact of the complexity of its structure on the behavior of the protein or the methylation process, is still not fully understood.

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P08

Structure Based Modeling and Simulations of Kinesin Eg5: A Conformational Change Study.

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Kinesin Eg5 belongs to a family of molecular motors that move along microtubules by using the energy of ATP hydrolysis and has an essential role in mitotic cell division. Thus, disruption of Eg5's function in cancer cell division has a considerable interest for development of new drugs; however, its molecular mechanism is not understood. Truncated Eg5 dimer consists of two catalytic domains (microtubule-binding), each of them joined by a neck linker to one terminus of a coiled-coil domain. In general, Eg5 moves along microtubules using a hand over hand mechanism. This mechanism involves the binding of ATP to the catalytic domain, catalysis and release of ADP, which direct the motion of Eg5 over the positive direction of microtubules. X-ray structures of Eg5 in the ATP- and ADP-like bound states have suggested that conformational changes in the catalytic domain are coupled to the generation of force and motion along the micro-tubulin (MT). To gain insight into the conformational changes between different bound states of kinesin Eg5 motors in solution, we use a dual-basin structure-based model. This model consists of Lorentzian restrains, which provide a double-basin energy surface with a finite energy barrier between the two end states. Standard molecular dynamics (MD) simulations are carried out to generate trajectories that can sample the structural transitions. Detailed analysis of the conformational changes will be presented.

P09

Folding of proteins with topological links.

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Topology imposes several conditions on properties and dynamics of proteins. However, the cost of e.g. exceptional stability is hindered folding time. One of the most interesting cases in which topology plays a role is folding process of proteins with link topology.

Mathematically, links are generalization of knots which are nowadays well settled in proteins fold spectrum [1]. The recent discovery of complex lasso proteins [2,3] in which (at least) one of the tail threads the covalent loop (Fig. 1) formed by the main chain closed by cysteine bridge, opens a new chapter in description of complex topology proteins. In particular this results in existence of topological links in proteins (Fig. 1) [4].

In this work we study the folding mechanism of proteins with links. We show, that in oxidative conditions the folding probability depends on the order of the loop formation. We also identify topological traps in protein folding and we relate them with the experiment. This altogether shows, that topological analysis can be crucial in understanding the experimental data.

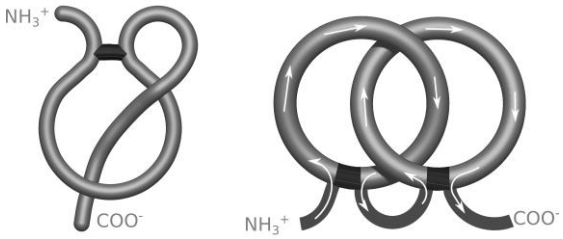

Fig.1 (left panel) The simplest complex lasso protein – the covalent loop is formed by the disulfide bridge with one of the tails piercing through the loop; (right panel) the realization of the simplest link (Hopf link) in protein. Two covalent loops, closed by cysteine bridges are piercing one another. The arrows indicate the chain orientation.

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P10

Nuclear magnetic resonance studies of the human cystatin C and its V57G mutant

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Human cystatin C (hCC), member of the superfamily of papain-like cysteine protease inhibitors, is the most widespread cystatin in mammalian body fluids. It is small protein, involved in various diseases, including cerebral amyloid angiopathy, cerebral hemorrhage, stroke, and dementia [1]. In pathological conditions human cystatin C participates in the formation of amyloid deposits together with the amyloid β peptide (Aβ), particularly in elderly individuals and in patients suffering from Alzheimer's disease or Down's syndrome [2]. Under physiological conditions hCC is a monomer but attempts to crystallize allowed receiving only the dimeric form [3], formed as a result of the three-dimensional exchange domains (3D domain swapping) [4]. While the experimental structure of wild-type hCC occurs as a dimer, the monomeric crystal structure is known for two hCC variants: V57N hCC and stab1 hCC. The first hCC mutant is stable in monomeric form and crystallizes as a monomer [5]. In our group we have designed and performed biophysical investigations for two groups of hCC mutants: one located in loop L1 [5,6] (residues 56-57) and second introduced to the hydrophobic core of the molecule (residue 68) [7]. In this studies we selected V57G mutant to our NMR investigations as the most promising to give a monomeric form of hCC in solution. We also conducted NMR studies for the wild type human cystatin C.

Human cystatin C and its V57G mutant were expressed in *E.coli* and purified in triple labeled form (wild type hCC) and double labeled form (V57G mutant). Analysis of acquired heteronuclear NMR data sets provide to assignments of ^{1}H, ^{13}C, and ^{15}N backbone resonances. Evaluated chemical shifts were used as input for TALOS+ and CS23D programs to extract information about 3D structure of hCC and V57G proteins. Further structural analysis will be presented on a poster.

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P11

A Role of the unique PD-PDZ interactions in the HtrA3 protease.

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HtrA3 protein belongs to the evolutionarily conserved HtrA family of serine endoproteases, responsible for maintenance of cellular homeostasis. Human HtrA proteins (HtrA1, HtrA2/Omi, HtrA3 and HtrA4) are involved in oncogenesis and neurodegenerative disorders. They are considered as promising targets in treatment of these disorders. The HtrA3 protease is located in mitochondria. Upon cellular stress (e.g. anticancer drugs) HtrA3 is released to cytoplasm and stimulates apoptosis. The release is accompanied by autocleavage of its N-terminal domain. The truncated HtrA3 protein (ΔN-HtrA3) is composed of the protease (PD) and PDZ domains, and is more active compared to the full-length protease [1]. In our previous paper we presented the crystal structure of the ΔN-HtrA3. In the ΔN-HtrA3 trimer the PDZ domains are placed in a position intermediate between that in flat saucer-like ΔN-HtrA1 SAXS structure and the compact pyramidal ΔN-HtrA2 X-ray structure. The PDZ domain interacts closely with the LB loop of the PD (PDZ-PD interactions) in a way not found in other HtrAs [2]. **The aim of this study was to investigate the role of the PDZ-PD interactions in sustenance of quaternary structure and proteolytic activity of ΔN-HtrA3 protease.** We constructed a set of ΔN-HtrA3 variants with substitutions of amino acid residues in the PDZ domain (R362A, E371A and Q389A) and a deletion of the unique six residues in the LB loop (Δ196-201). Analysis of the mutated proteins by size exclusion chromatography showed that the PDZ-PD interactions stabilize the trimeric form of ΔN-HtrA3 protease. Comparison of the kinetic parameters of the ΔN-HtrA3 variants with the fluorogenic peptide as a substrate revealed that the rupture of the PDZ-PD interactions caused a significant decrease of the ΔN-HtrA3 protease substrate affinity and catalytic efficiency. The results presented in this work indicate the importance of PDZ-PD interactions for maintaining quaternary structure and proper proteolytic activity of the ΔN-HtrA3 protein.

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P12

Use of Lorentzian and Gaussian restraint functions in molecular dynamics simulations.

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Prediction of protein structures is one of the most important problems of current computational biology and bioinformatics. One approach to solve this problem is use of molecular dynamics (MD) simulations with geometry restraints provided by template-based modeling tools. Relevant information is extracted from databases and is subsequently converted into specified restraints. There are several pseudo-energy functions to utilize these restraints by designing how the additional force depends on the degree of correct folding of the protein structure. In our previous work [1] we applied the log-Gaussian restraint function which enabled us to take into account the restraints from multiple knowledge-based models. In this study, two restraint functions, namely the log-Gaussian and Lorentzian type were applied in MD simulations with the coarse-grained UNRES (UNited RESidue) [2] force field with 4 proteins that have different types of secondary structure (α, β, and α+β) to compare the performance of these restraint functions. The results of these calculations showed that (i) use of both restraint functions results in the elimination of structures which are far away from the native structure from the population of final results, (ii) the Lorentzian restraint function, as opposed to the Gaussian function, is bounded which prevents us from over-weighting incompatible restraints that cannot be satisfied and (iii) UNRES simulations with restraints built using the most accurate models (experimental structures) can reproduce protein structures very precisely.

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P13

Conformational transition of LAO-binding protein by molecular dynamic simulation.

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Protein conformational transitions are often very important in biological systems. In this study we applied molecular dynamic simulation on Lysine, Arginine, Ornithine (LAO) binding protein, and we want to have a better understanding of the complex transition process between an open and a closed form. Of LAO binding protein has two domain and it is one of the bacterial periplasmic transport systems while its topological structure is different from other amino acid-binding proteins. We started from both the open (PDB ID : 2LAO)and closed (PDB ID : 1LAF) form. All MD simulations are based on experimental data about temperature. We performed 12 MD simulations, each 100 ns, of the LAO-binding protein. RMSD trajectories have been generated and numerous transition in both simulations have been observed. Our method can provide rich information to fully exam complex processes of protein conformational transition.

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P14

Retrieving the kinetic information from MREMD and MREHMC simulations.

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The aim of the study was to retrieve the kinetic information from the Multiplexed Replica Exchange Molecular Dynamics (MREMD) and Multiplexed Replica Hybrid Monte Carlo (MREHMC) protein folding simulations in UNRES force field [1,2]. For this purpose the original g_kinetics algorithm from Gromacs package was used [3]. For the test of the g_kinetics algorithm performance for UNRES results, the two different force field parameterizations were chosen: 'E0LL2Y' [4] and 'MAXLIK' [5], and the three types of performing simulations algorithms: MREMD, MREHMC and classic Molecular Dynamics (MD). The performance with regards to time of the protein folding of the three methods therefore was tested. The classic MD was used to compare the time of folding and to visualize the improvement in the Replica Exchange methods. Finally the method allowed to obtain the kinetic information from the simulations, and the comparison of the simulation methods regarding the folding times of the proteins was possible. The obtained results allowed to study the dependence of the folding time on the protein size. It also allowed to compare the simulation folding time with the experimental protein folding time values [6] and asses the approximate difference between the UNRES protein folding simulation times and the real time of protein folding.

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Acknowledgments

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P15

New UNRES package with Fortran 90.

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UNRES is a coarse-grained model of polypeptide chains. The UNRES force field that corresponds to this model is ha physics-based force field that has been derived as a restricted free energy (RFE) function, which corresponds to averaging the energy over the degrees of freedom that are neglected in the united-residue model [1]. The UNRES force field is capable of predicting protein-structure. Moreover, it can lead to exceptionally good results when correct domain packing is an issue, even for highly homologous targets [2]. Until now, each version of UNRES (UNRESPACK v. 3.2 and earlier ones) was *written in Fortran 77. Due to the fact, that Fortran 77 enables us to use only static arrays, problems, especially with memory, in UNRES program were often occur, and the UNRESPACK had to be split up into many subversions.*

Our recent work was focused on creating a new UNRES package in Fortran 90, based on the previous versions in Fortran 77. Fortran 90 provides most of all dynamic memory allocation, user defined data types, and structuring the code into modules which encompass subroutines, functions, and variables. Moreover, Fortran 90 adds internal function and subroutines, providing greater flexibility. The whole code of UNRES in Fortran 90 was restructured so that it now consists of modules that can be assembled to create the main simulation program. This restructuring was carried out using multiple modules but without any additional crucial function or subroutine. This approach enabled us to reduce to a great extent the *redundancy* in the code. The new UNRES package with Fortran 90 provides the all functionality of the previous UNRESPACK v. 3.2 in Fortran 77.

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P16

Potential of Mean Force surfaces of model glucosyl disaccharide systems.

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The Unified Coarse Grained model for biological macromolecules that is being developed in our laboratory is designed to treat proteins (UNRES), nucleic acids (NARES-2P), and polysaccharides (SUGRES-1P). The prototype of the effective energy function of a system is defined as a potential of mean force (PMF), also termed the restricted free energy function, with all degrees of freedom that are lost when passing from the all-atom to the coarse-grained model averaged out [1]. In the current work we treat the polysaccharide component of the model, which is a single-center model, in which the anchor points are the glycosidic oxygen atoms, with the sugar interaction site positioned halfway between the two consecutive glycosidic oxygen atoms. The major degrees of freedom that are averaged out are the rotation angles of the sugar rings about the O...O virtual bonds, usually the O(1)...O(4) virtual bonds. Our recent work was focused on the first step of the construction of the SUGRES-1P force field, namely the determination a PMF surfaces of model systems of glucosyl disaccharides as functions of the rotation angles $\lambda^{(1)}$ and $\lambda^{(2)}$ of the sugar rings about the O4...O4 virtual-bond axes. The L- and D-glucosyl disaccharides with different glycosidic 1-4 bond stereochemistry (*alpha*- or *beta*-) were analyzed. All-atom molecular dynamics (MD) simulations were carried out with the parm99 force field in AMBER 12 package [2]. Initial structure of each disaccharide was generated in a random conformation using Leap program. Next the energy of each system was minimized the system was heated and a short MD simulation was carried out to relax it before the production umbrella sampling simulation. The umbrella sampling calculations work by breaking the reaction coordinate up into a series of windows and then applying a restraint that acts to force the reaction coordinate to remain close to the center of the window [3]. The final stages of the umbrella sampling were used to calculate the PMF's by using the weighted-histogram analysis method.

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P17

Probing the binding of Cu^{2+} ions to the fragment of $A\beta_{(1-42)}$ protein using ITC measurements and MD simulations.

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It was shown that probably three residues: His6, His14 and His16 [1,2] in original sequence $A\beta_{(1-42)}$ serve as metal-binding sites for Cu^{2+} ions. On the other hand there is a possibility that only one of them plays a crucial role in the formation of the $\{A\beta_{(1-42)}\text{-}Cu^{2+}\}$ complex [1]. The isothermal titration calorimetry (ITC) measurements supported by molecular dynamic simulation (MD) with the NMR-derived restrains were used to investigate the interactions of Cu^{2+} with a fragment of $A\beta_{(1-42)}$ protein (HZ1). The conditional thermodynamic parameters suggest that, under the experimental conditions, the formation of the Cu^{2+}- HZ1 complex is both enthalpy and entropy driven process. Studies presented here (after comparison with our previous results [3]) show that the affinity of the peptides to copper metal ions depends on two factors: the primary structure (amino acid composition) and the shape of the peptide conformation adopted.

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Acknowledgments

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P18

Docking of trigonelline as potential anti-Alzheimer disease agent in β-amyloid (1–42) peptide.

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Trigonelline, which is known as a nicotine-related compound [1], was taken into consideration for the study as an anti-Alzheimer's disease drug candidate. According to the earlier proposed mechanism, nicotine [2] or cotinine [3] binds the segment of Aβ between amino acids 1–28 when folded in an α-helical conformation. Therefore, nicotine as well as cotinine inhibits the conformational change from α-helix to the β-sheet conformation (known as the amyloidogenic conformation). Preliminary considerations, presented here, have shown that trigonelline could have similar influence on the helical Aβ (1–42) structure. Therefore, trigonelline might appear to be an interesting anti-neurodegenerative drug candidate, providing that its ability to penetrate blood/brain barrier will be proved.

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P19

Application of predicted relative solvent accessibility to the development of surface area energy function.

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Solvent accessible surface area (SASA) of a protein is one of the most important structural features. SASA is often used as an analysis tool for describing protein-water interactions or calculating the transfer free energy of proteins [1,2]. The insights often gained through more accurate predictions of protein surface area are highly useful for also predicting that protein's structure as well as its function. In 2012, we had presented a method to predict the solvent accessibility of proteins, called SANN, which is based on a nearest neighbor method applied to the sequence profile [3]. The overall performance of SANN was shown to be superior to the currently available methods (*e.g.* FKNN, SABLE, PROF, ACCpro, NETASA).

In order to utilize the solvent accessibility for the protein structure prediction, a restraining energy term E_{SA} was designed and then implemented within the TINKER molecular modeling package. The performance of E_{SA} was measured using seven benchmark sets and five knowledge-based potential functions. And, to reduce the calculation time for surface area of each residue, we employed 'Neighbor Vector' algorithm which published by Jens Meiler et al [4]. In addition, E_{SA} was implemented into CASP11 energy function for the conformational sampling and the conformational space annealing (CSA) method was used, which has been successfully applied to various hard combinatorial optimization problems.

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P20

Human cystatin C / HCC3 antibody interactions. Overproduction, affinity chromatography and NMR.

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Human cystatn C (hCC) is a small protein belonging to the family of papain-like cysteine proteinases. It takes part in several different processes that lead to amyloid formation what is associated with a number of neurodegenerative diseases that affect the independence and quality of life of aging.

hCC is it is widely studied in both experimental and theoretical laboratories. Studies show that the occurrence of hCC (Fig. 1.) associated diseases correlates with its dimerization which leads to amyloid formation.

The aim of this project is to determine the sequence of epitope of hCC for monoclonal HCC3 antibodies. This should help with determination of specific sequences which could be incorporated not only into proteins and antibodies but possibly also short peptides. They can be potentially used as inhibitors of dimerization of hCC and amyloid formation.

Human cystatin C was expressed in *E. coli* bacterial cells. For the purpose of NMR studies we labeled hCC with isotopes of carbon (^{13}C) and nitrogen (^{15}N). We performed the expression of double labeled ($^{13}C/^{15}N$ hCC) and single labeled (^{13}C hCC and ^{15}N hCC) protein.

The initial screening of epitope mapping studies was performed by such techniques as affinity chromatography, etc.

The ^{1}H, ^{13}C and ^{15}N assignments of hCC have been determined using standard NMR protocols. From this assignment, we will attempt to follow titrations of hCC with the antibody using HSQC spectra of the protein to map the HCC3 antibody binding site.

Figure1. Ribbon representation of the hCC dimer.

Acknowledgments

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P21

Opioid receptors and their interactions with the enkephalins containing sugar residue at the C-terminus – molecular modeling study

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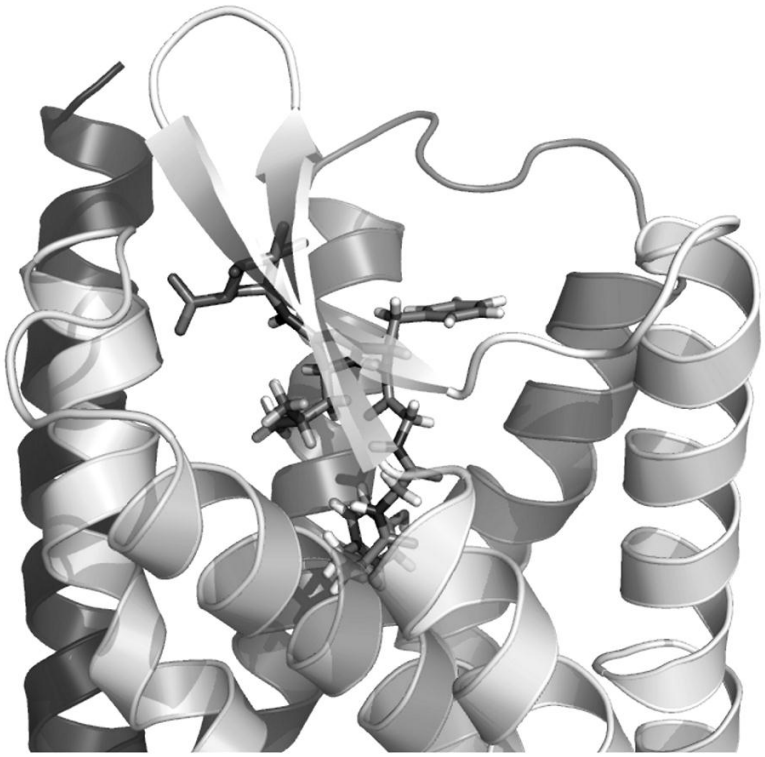
Opioid receptors (δ, μ and κ subtypes) belong to the G protein-coupled receptor (GPCR) superfamily. They are transmembrane proteins consisting of seven helices (TM1–TM7), connected by alternating extracellular and intracellular loops. They are responsible for pain perception being activated both by endogenous opioid peptides such as enkephalins or endorphins and by exogenous opiates, such as morphine. The enkephalins ([Leu]enkephalin and [Met]enkephalin) are endogenous pentapeptides with the amino acid sequence Tyr-Gly-Gly-Phe-Leu/Met. Both enkephalins are potent agonists of the δ-opioid receptor, and to a lesser extent the μ-opioid receptor, with little effect on the κ-opioid receptor.

It has been known, that the attachment of sugars to peptides increases their penetration of the blood-brain barrier and affect the interaction with receptors. In this study, both the unmodified enkephalins and the enkephalins with the glucuronic acid conjugated onto the C-terminal residue have been investigated to explain how the addition of the sugar moiety influences the receptor–ligand interaction. The ligands have been docked into the δ- and μ-opioid receptors and molecular dynamics has been conducted. The interaction with receptors has been analyzed in details and the receptor–ligand binding mode have been proposed.



Acknowledgments
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P22

Conformational flexibility and bound states of selected flavonols.

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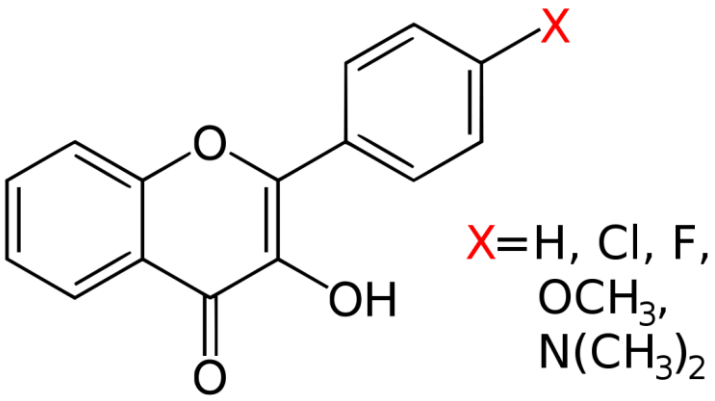
Flavonols (3-hydroxyflavones) are an important class of bioactive naturally occurring phenolic compounds of the flavonoid group. They can present antioxidant, anticancer, antiviral, anti-inflammatory and heart disease protective activities. These molecules undergo a photoinduced excited state intramolecular proton transfer reaction (via the internal hydrogen bond linking the C=O and 3-OH groups), resulting in the transformation of the excited state to the tautomer form. This reaction is extremely sensitive to external hydrogen bonding perturbation of the environment on the internal hydrogen bond of the molecules.

In this study we have prepared full-atom model of five 4'-substituted 3-hydroxy-2-phenylchromen-4-one compounds for the molecular dynamics and molecular docking to the bovine serum album (BSA; 4F5S model).



Flexibility studies, deprotonation and internal 3-OH to C=O proton transfer influence on conformational stability as well as the preliminary modified flavonols to BSA docking results are tabelarized and discussed.

Acknowledgments

The computational time in the Academic Computer Center in Gdansk CI TASK, Poland is acknowledged.

P23

Simple analytical formulas for the potentials of mean force of interactions between O-phosphorylated and hydrophobic amino-acid side-chain models in water.

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The potential of mean force is one of the free-energy properties with which to calculate the physicochemical properties of systems studied. The calculated potentials of mean force can subsequently be used to compute measurable physicochemical characteristics of the systems studied such as, e.g., association constants and, consequently, validate the use of the force field of choice.

To determine the potentials of mean force of phosphorylated side chains, a series of umbrella-sampling molecular dynamic (MD) simulations with the AMBER force field were carried out for pairs of O-phosphorylated serine (pSer), threonine (pThr), and tyrosine, (pTyr) with natural hydrophobic amino acids in a TIP3P water model as a solvent at 298K. The weighted-histogram analysis method was used to calculate the four-dimensional potentials of mean force (dependent on the distance between side-chain centers and their relative orientation).

A general expression for the effective energy of interaction between O-phosphorylated and hydrophobic amino acid-chain models is given by the following equation:

$$W_{cn} = E_{vdW} + E_{pol} + E_{pol}^{GB} + F_{cav}$$

where: (W) is the potential function, (E_{pol}^{GB}) solvent-polarization terms represented by the Generalized Born Model, (E_{pol}) solute-polarization terms, (E_{vdW}) van der Waals terms, and (F_{cav}) is energy of cavity creation.

The positions and depths of the contact minima and the positions and heights of the desolvation maxima, including their dependence on the orientation of the molecules were well represented by analytical expressions for all systems. The values of the parameters of all the energy components are physically reasonable.

Acknowledgments
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P24

Determination of backbone-local potentials for the coarse-grained NARES-2P model of nucleic acids.

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Molecular dynamics (MD) simulations are nowadays a technique which is commonly used to study the structure, dynamics, and interactions of biological macromolecules, including nucleic acids. Reduced (coarse-grained models) are very important in this area because they enable us to increase the time and size of calculations by orders of magnitude. For this reason, we are developing the highly reduced NARES-2P [1] model of nucleic acids, in which a polynucleotide chain is represented as a sequence of sugar-centers (S) with phosphate groups (P) positioned halfway between the two consecutive centers and united sugarbase (B) groups attached to each center. The interaction sites are only the Ps and the Bs, while the sugar centers serve to define the chain geometry only.

The main goal was determination of backbone-local potentials for NARES-2P; these potentials include the S...S virtual-bond-stretching, the S...S...S virtual-bond-angle and the S...S...S...S virtual-bond-dihedral-angle potentials. For this purpose, we used replica exchange molecular dynamics (REMD) simulations of model systems, with the Amber 12 [2] force field with explicit water to determined the respective potentials of mean force. The di-, tri-, and tetrasugar-phosphate model compounds, based on deoxyribose (for DNA) and ribose (for RNA) were used as model compounds. The simulations were conducted with restraints on the distance between center of mass of terminal residues for the di- and tri-sugar-phosphate systems and on the virtual-bond-dihedral angle between for the 4-sugar-phosphate system. The geometry was analyzed with the PTRAJ [2] module of AMBER to compute the geometric parameters and to generate the respective histograms. Subsequently, the weighted histogram analysis method (WHAM) was used to compute the potentials of mean force corresponding to the potentials under consideration and the maximum likelihood method was used to determine the parameters of the analytical expressions for the potentials.

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Acknowledgments

This work was supported by grant from the Polish National Science Centre (grant UMO-2012/06/A/ST4/00376). Computational resources were provided by Beowulf cluster at the Faculty of Chemistry, University of Gdansk and by the Academic Computer Center in Gdansk, TASK.

RASMOL AB – a new functionalities in the program for structure analysis.

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For many years RasMol was one of the most used programs for molecular visualization. It was an excellent tool due to its simplicity and its low demand of computer power, today it is replaced by OpenGL programs.

The standard menu of RasMol possess a lot of useful functions, as is described by D.S. Goodsel [1]. However, many functions of the program are hidden, and are available only from the command line. In the work presented herein, we extended the standard menu of RasMol to get more of its useful features, as was implemented in the program from its point-and-click menu. This modification made the software more easy and effective to use.

The major modification is the addition of user predefined macros, which are now available as a separate option in the menu. This is one of the most useful options in the program, especially when multiple analyses are to be made. It allows the user to create user-specific button - macros with a set of commands as read from the separate files.

About 20 years ago, Roger Sayle wrote [2]: "In the future, RASMOL will have to work hard to provide the many extra facilities that are going to be needed for molecular graphics while maintaining the simple interface that makes the program attractive to new users." With this work, we tried to continue this spirit.

The program can be downloaded from the project webpage [3]:
http://etoh.chem.univ.gda.pl/rasmol/

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Index

Lezno Palace

History of the Palace

Lezno was mentioned for the first time in the 14th century documents. First, by the road leading from Gdansk to Kartuzy Lezno Wielkie was founded, then another estate - Lezenko. This very estate - subject to numerous changes during its history - survived till today. The first known owner of the Lezno estate was Knight Gottk, endowed by the Teutonic Knights Order in 1338. After the collapse of the Teutonic Order the Lezno estate became a property of the Polish king. In 1623 the estate got briefly into the hands of an eminent Gdansk burgher family, the Gieses, and then the site came once again at the disposal of the king.

Among the king's beneficiaries, in 1681 there appears the name of Jan Jerzy Przebendowski, an eminent figure in Polish public life at the turn of the 17th and the 18th centuries, a participant of the Battle of Chocim, and a Royal Treasurer since 1703. Thanks to his efforts a splendid palace, surrounded by a small landscape park, was erected in the years 1720 - 1722.

The three-storey U-shaped brick building with a span of 56 meters is known from description only. There exists an old description of its beauty: You could count its rooms and halls easily more than thirty, each in different style. Contemporary writers say with astonishment that such a building can be found nowhere in whole Prussia, and that can it be compared to the famous Teutonic castles.

In the years to follow, the estate was managed by the Przebendowski family, then by the Grabowski family who contributed to the fall of the estate. The destruction of the estate continued during the Napoleonic times, when the French troops were stationed in the Palace. Lezno at that time was in the hands of the Hellfenstein, who transferred it in 1832 into the hands of Friedrich Hoene, an owner of a thriving shop in Gdansk. Since then Lezno had been owned by the Hoenes until 1945. The highest changes of the estate were made by Georg Hoene who - in 1884 - started thorough refurbishment of the 18th century Palace. As a result, a building existing until today was created, and a landscape park, one of the most magnificent in the area, was created.

After the WW II, when the Palace and the Park vandalized by the Red Army, were nationalized, the estate was turned into a state-owned collective farm, which contributed to further destruction of this historical complex.

In 1994, the Palace, together with a part of the former estate became the property of the University of Gdansk. At that time, the park and the Palace itself were effectively devastated. Thanks to the University's efforts, within a few years, the palace was carefully renovated, and the interiors were cleaned and refurbished.

Gdańsk

A thousand-year history, location at the crossroads of important commercial and communication routes, a busy harbor and mercantile traditions - all this makes Gdańsk a meeting place of many cultures, nationalities and creed.

The first written mention of Gdańsk comes from 997. The fortification and urban complex as well as a port started to really form in the second half of the 10th century. The dynamic development of trade, fishery and craft guilds soon pushed the city to the leading position in Pomerania. The city kept this position despite being taken over by the Teutonic Knights in 1308. The city continued to develop dynamically. Joining the League of Hanseatic Cities (in 1361) and the fast development of the port are just some of the factors contributing to the strong position of Gdańsk in Europe. In 1457 King Kazimierz Jagiellończyk incorporated Gdańsk into the Crown and, in recognition of the merits of Gdańsk burghers, granted Gdańsk numerous privileges, thus starting a three-hundred-year period of prosperity.

The following years are traditionally called the "golden age." During this time Gdańsk was one of the wealthiest and most significant cities in Europe. The religious freedom gained in the 16th century turned the city into a true melting pot of nationalities and creed, giving it yet another stimulus for development, thanks to the specific "community of differences." It was one of the few such places in the world at the time. This prosperity was checked by the Swedish wars and partitions of Poland in the 18th century. The city was cut off from Poland and in 1793 it was annexed to Prussia. What followed was a period of slow decline, the gloomiest in its history, briefly interrupted by the Napoleonic wars.

In 1919, the Free City of Gdańsk was established under the Treaty of Versailles, which brought the city back to the elite of European ports. Unfortunately, in 1933 Nazis took power and the fascist terror started to escalate in the city. On September 1, 1939, at around 4.30 in the morning, it was here, in Gdańsk that the Second World War started with shots fired from the battleship Schleswig-Holstein. It was a time of bravery and martyrdom of its citizens. The war and the particularly fierce struggle for liberation left Gdańsk completely devastated. Its reconstruction, with the help of fully dedicated citizens, took several dozen years. Gdańsk once again became the biggest Baltic port and regained its former splendor.

The tragic December 1970, and then August 1980 and the martial law period are the successive dates symbolizing the fight of the citizens of Gdańsk against the prevailing communist regime. It was Gdańsk that became the cradle of "Solidarność" which was to transform the then map of Europe by bringing down the communist system. History has come full circle. Contemporary Gdańsk - a half-a-million, dynamically developing agglomeration - is vibrant with life as before and again deserves to be called "the Pearl of the North."

Castle of the Teutonic Order in Malbork

The Castle of the Teutonic Order in Malbork is the largest castle in the world by surface area. It was built in Marienburg, Prussia (now Malbork) by the Teutonic Knights, a Roman Catholic religious order of crusaders from Germany, in a form of an Ordensburg fortress. The Order named it Marienburg (Mary's Castle). The town which grew around it was also named Marienburg.

The castle is a classic example of a medieval fortress and, on its completion in 1406, was the world's largest brick castle. UNESCO designated the "Castle of the Teutonic Order in Malbork" and the Malbork Castle Museum a World Heritage Site in December 1997.

The castle was built by the Teutonic Order after the conquest of Old Prussia. Its main purpose was to strengthen their own control of the area following the Order's 1274 suppression of the Great Prussian Uprising of the Baltic tribes.

The castle was expanded several times to house the growing number of Knights. Soon, it became the largest fortified Gothic building in Europe, on a nearly 21-hectare (52-acre) site. The castle has several subdivisions and numerous layers of defensive walls. It consists of three separate castles - the High, Middle and Lower Castles, separated by multiple dry moats and towers. The castle once housed approximately 3,000 "brothers in arms". The outermost castle walls enclose 21 ha (52 acres), four times the enclosed area of Windsor Castle. The developed part of the property designated as a World Heritage Site is 18.038 ha (44.57 acres).

The favourable position of the castle by the river Nogat allowed easy access by barges and trading ships arriving from the Vistula and the Baltic Sea. During their governance, the Teutonic Knights collected river tolls from passing ships, as did other castles along the rivers. They controlled a monopoly on the trade of amber. When the city became a member of the Hanseatic League, many Hanseatic meetings were held there. Currently the Malbork Castle is a museum where many cultural events take place.