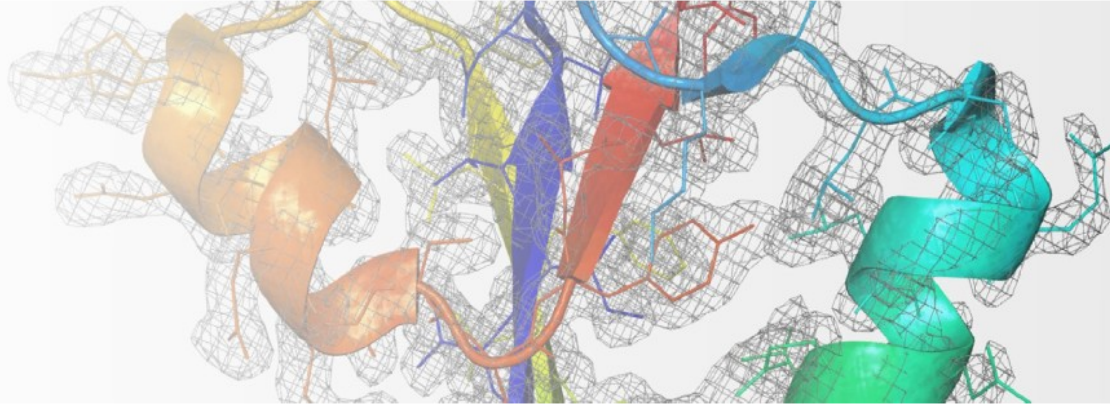

3rd EMBO Workshop on Computational and Structural Biology

Serock, Poland, March 3-5, 2017

Organizers:

Aleksandra Gierut (CENT, UW)
Aleksandra Jarmolińska (CENT, UW)
Gaja Klaudel (CENT, UW)
Piotr Setny (CENT, UW)
Joanna Sułkowska (CENT, UW)
Marta Wiśniewska (CENT, UW)

Funding:

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Schedule

Friday, 3rd of March

10:00	*bus from Warsaw (CeNT, Banacha 2c) to Serock (Nicola Conference Centre)*
1:30 – 11:35	*Opening Speech*
11:35 – 12:20	**Eric Rawdon, Introduction to the study of entanglement in nature**
12:25 – 12:40	Paweł Dąbrowski-Tumański, Two pathways of proteins with Gordian (52) knot folding
12:40 – 13:00	Wanda Niemyska, Gauss Linking Number reveals new looks in entangled proteins!
13:00 – 14:00	*Lunch*
14:00 – 14:45	**Adam Kubas, Theoretical approaches for understanding gas-processing metalloenzymes: the case of [FeFe]-hydrogenases**
14:45 – 15:05	Agata Perlińska, Role of the magnesium in a knotted methyltransferase
15:05 – 15:25	Vasilina Zayats, Modeling of yeast ion translocation protein Trk1 using combined theoretical and experimental approaches
15:25 – 15:45	Aleksandra Jarmolińska, Would evolution stop membrane proteins from knotting?
15:45 – 16:00	*Coffee break*
16:00 – 16:45	**Maria Górna, How do IFITs fit together in defense against viruses?**
16:45 – 17:05	Matthew Merski, The RAP domain: structure, fufnction, evolution and bioinformatics
17:05 – 17:25	Marcin Ziemniak, Structural basis of mRNA-cap recognition by Dcp1/Dcp2 complex
17:25 – 17:55	**Gregor Weiss, The influence of hydrophobicity on kinetics of ligand-receptor association**
17:55 – 18:10	*Coffee break*
18:10 – 18:30	Gaja Klaudel, Generic trace in proteins
18:30 – 18:50	Aleksandra Gierut, PyLasso - plugin to detect and analyze lasso type of entanglement in (bio) polymers
19:00 – 20:00	*Dinner*

Saturday, 4th of March

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09:00 – 09:45	**Sebastian Glatt, Lost in translation – revisiting the tRNA wobble base**
09:45 – 10:05	Monika Gaik, Uncovering molecular mechanisms of translational control with structural biology
10:05 – 10:25	Marta Smejda, The role of regulatory proteins in tRNA modifications in human cells
10:25 – 10:45	Anna Salerno-Kochan, Structural insights into the role of RNA-binding proteins in stem cell maintenance and differentiation
10:45 – 11:05	Karol Zakrzewski, Structural insights into the role of binding regulatory factors to Elongator subunit in the context of tRNA modification process
11:05 – 11:20	*Coffee break*
11:20 – 12:05	**Bartosz Trzaskowski, Computer-aided drug design, a tale of two successful (?) stories**
12:05 – 12:25	Joanna Macnar, The influence of the QM- calculated ligands' charge distribution on the docking results
12:25 – 12:45	Rafał Jakubowski, Toward a better life: in silico study of transthyretin related amyloidosis
12:45 – 13:05	Jan Kutner, Structure and function studies of RNA-binding proteins with FAST motifs and a RAP domain
13:10 – 14:10	*Lunch*
14:30 – 19:00	*Paintball*
20:00	*Dinner*

Sunday, 5th of March

08:30 – 09:45	*Breakfast*
10:00	*bus from Serock (Nicole Conference Centre) to Warsaw (CeNT, Banacha 2c)*

Abstracts

Introduction to the study of entanglement in nature

Rawdon Eric[1]
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Introduction to the study of entanglement in nature Long chains tend to get entangled. On the human scale, we see this inngarden hoses, headphone cables, computer cables, etc. Biologicalnchains get entangled as well, for example DNA and proteins. The study of entanglement has a rich history in the sciences, perhaps most famously in Lord Kelvin's proposal that atoms are knots made of ether. Modern advancements in imaging have created new opportunities for researchers to detect and analyze entanglement in physical systems. This talk will be a gentle introduction into the study of knotting and linking, focusing on issues relevant in biology and physics.

Theoretical approaches for understanding gas-processing metalloenzymes: the case of [FeFe]-hydrogenases

Kubas Adam[1]
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[FeFe]-hydrogenases are the best natural hydrogen producing enzymes[1] but their biotechnological exploitation is hampered by the extreme oxygen sensitivity of these proteins. Most of the previous theoretical studies put emphasis on explanation of the irreversibility of this process as they were related to experiments carried out under constant O_2 concentration. However, we have recently shown experimentally that such aerobic inactivation is partially reversible.[2,3] Here we combined the molecular dynamic (MD) simulations of the the dioxygen diffusion in the [FeFe] hydrogenase, extracted from *Clostridium Pasteurianum,* with the high level *ab initio* calculations of the O_2 binding and its transformations at the active site of the protein.[3,4]
We found that the partial reversibility results from the four-electron reduction of O_2 to water.[3] The third electron/proton transfer step is the bottleneck for water production, competing with formation of the highly reactive hydroxyl radical (·OH) and cysteine sulfenic acid (Cys299-SOH), consistent with the recent crystallographic evidence.[5] The rapid delivery of electrons and protons to the active site is therefore crucial to prevent the accumulation of these harmful species upon exposure to oxygen. Moreover, our Markov state modelling[6] of the diffusion process identified key residues that should be the target of the future mutational studies aiming to restrict O_2 access to the active site of the [FeFe] hydrogenases.

We will also present some general remarks about combining various theoretical approaches, such as MD or *ab initio* computations, in the construction of a successful kinetic model for gas-processing metalloenzyme.

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Acknowledgements

Support from the National Science Centre, Poland grant 2015/17/D/ST4/00112 is gratefully acknowledged.

How do IFITs fit together in defense against viruses?

Górna Maria W.[1,2], Boros-Majewska Joanna[1], Kowalska Monika[1], Młynarczyk Krzysztof [1], Vladimer Gregory I.[2], Abbas Yazan M.[3], Gebhardt Anna[4], Bennett Keiryn L.[2], Pichlmair Andreas[4], Nagar Bhushan[3], Giulio Superti-Furga[2]

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[2] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

[3] Department of Biochemistry, McGill University, Montreal, Canada

[4] Max-Planck Institute of Biochemistry, Martinsried, Germany

The interferon-induced proteins with tetratricopeptide repeats (IFITs) are potent innate immune effectors that bind non-self RNA, which results in the inhibition of translation of viral transcripts. The structure of IFIT5 [1] reveals the mode of recognition of the 5' triphosphate (PPP) group on RNA, whereas IFIT1 preferentially binds cap 0 groups [2]. IFIT1 interacts with IFIT3, which has no known RNA binding capability on its own, and for which the role in the larger multi-IFIT complex is elusive. We undertake the dissection of the role of the higher-order IFIT complexes and demonstrate that the IFIT1-IFIT3 complex binds RNA with a higher affinity than IFIT1 alone. The IFIT1-IFIT3 interaction is mediated by the last tetratricopeptide repeat motifs in both proteins, and results in reorganization of the RNA-binding site in IFIT1. In cells, IFIT1 and IFIT3 associated together, and re-distributed and co-localized together with PPP-RNA. We propose a role for IFIT3 as an enhancer of IFIT1 activity. Regulation of the IFIT1-IFIT3 complex may provide additional possibility for signal integration in the antiviral response.

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Acknowledgements

M.W.G. is supported by Marie Skłodowska-Curie Individual Fellowship (H2020-MSCA-EF-IF-2014 FAST-RAP #655075) and this work in M.W.G. laboratory is supported by the National Centre for Research and Development (LIDER/039/L-6/14/NCBR/2015) and EMBO Installation Grant to M.W.G. Work of M.W.G and G.I.V. in G.S.F laboratory was supported by ERC-2009-AdG I-FIVE #250179 to G.S.F.

The Influence of Hydrophobicity on Kinetics of Ligand-Receptor Association

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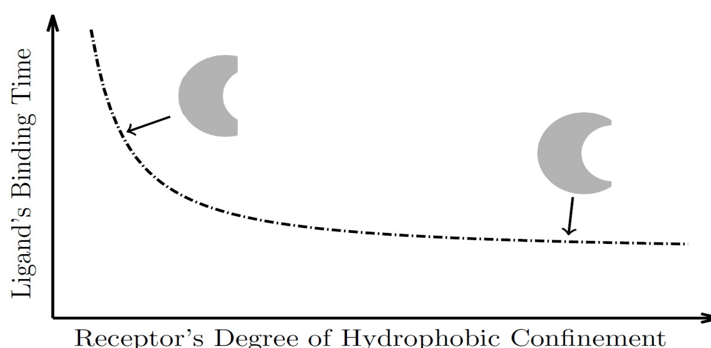
[1] Institut für Physik, Humboldt-Universität zu Berlin, Berlin, Germany
[2] Institut für Weiche Materie und Funktionale Materialien, Helmholtz-Zentrum Berlin, Berlin, Germany
[3] Centre of New Technologies, University of Warsaw, Warsaw, Poland

In order to understand how to tune the rate of ligand-receptor association we investigate how water fluctuations impact binding kinetics by means of molecular dynamics (MD) simulations and the theory of stochastic processes. Recent studies presented [1,2] that fluctuations in the water occupancy of hydrophobic receptors are intimately coupled to the diffusion of associating ligands in all-atom MD simulations. We expand these findings whilst we systematically modify the receptor's physicochemical properties in terms of hydrophobicity and geometry. In specific cases of minor receptor modifications the ligand binding drastically accelerates, e.g., the binding switches from comparably slow to fast if the receptor becomes only slightly deeper. In a more universal picture we measure the degree of hydrophobic confinement inside a receptor by its solvation fluctuations. We find that this degree of hydrophobicity clearly correlates with the accelerated binding times and links the sudden acceleration to an abrupt increase in hydrophobicity. In sum we rationalize how the physicochemical properties of a cavity slow the binding kinetics due to a tunable, precursing friction which possibly can be utilized in a goal-oriented rate control by solvophobicity.



Thus our study complements the profound understanding of the solvent's influence in key-lock binding which is essential for tailored solutions in catalysis and pharmaceutical applications.

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The authors thank the Deutsche Forschungsgemeinschaft (DFG) for financial support of this project. G.W. and J.D. acknowledge funding from the ERC (European Research Council) within the Consolidator Grant with Project No. 646659-NANOREACTOR. G.W. is grateful for funding from the EMBO workshop organizing committee during his stay in Serock.

Lost in Translation – Revisiting the tRNA Wobble Base

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Two copies of each of the highly conserved six subunits of the eukaryotic Elongator complex (Elp1-6) constitute a large macromolecular complex, which in combination with additional modification pathways conducts the formation of specific post-transcriptional modifications on uridines in the wobble base position of tRNAs [1]. Its modification activity is essential for maintaining accurate translational rates, protein folding dynamics and global protein homeostasis. Neither the detailed chemistry of the reaction itself, nor the specific mechanisms by which temporarily associated regulatory factors, namely Kti11, Kti12, Kti13 and Kti14 are modulating Elongator's activity are currently fully understood. I will present recent insights into the overall complex architecture by electron microscopy [2] and the catalytic cycle of the modification reaction based on the crystal structure of a bacterial Elp3 homologue at 2.15 Å resolution [3]. Our results reveal the unexpected arrangement of Elp3 lysine acetyl transferase (KAT) and radical S-adenosyl-methionine (SAM) domains that share a large interface to form a composite active site and tRNA binding pocket. Furthermore, I will present our studies based on the crystal structures of Saccharomyces cerevisiae Kti13 and the Kti11/Kti13 heterodimer [4], including in-depth analyses and validation of the interacting residues of Kti11 and Kti13 through mutational analysis *in vitro* and *in vivo*. Furthermore, we show that metal coordination by Kti11 and its heterodimerization with Kti13 are equally essential for two translational control mechanisms, namely the Elongator mediated tRNA modification reaction and the diphthamide modification pathway. In summary, our structural and functional analyses provide novel insight into the molecular mechanisms that allow the Kti11/Kti13 heterodimer to co-regulate two consecutive steps in ribosomal protein synthesis and deepen our understanding of Elongator's modification activity as well as its role in the onset of various neurodegenerative diseases and cancer in humans.

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EMBO IG 2015, NCN OPUS 10, FNP First Team 1

Computer-aided drug design, a tale of two successful (?) stories

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The C-C chemokine receptor type 5 (CCR5) G protein-coupled receptor (GPCR) is a prime target for preventing HIV invasion. A major difficulty in developing effective therapeutics is that the CCR5 exhibits an ensemble of ~10–20 distinct low-energy conformations, each of which might favor binding to different ligands and/or lead to different downstream functions. X-ray structures generally provide only one of these conformations. We applied the GEnSeMBLE methodology to predict this ensemble, and we designed and carried out 11 experiments to validate the ability of this ensemble to predict binding of an HIV therapeutic to CCR5. We found that each of the mutations changes the binding site. The predicted effects of mutations on binding are in excellent agreement with experiments, providing CCR5 structures for designing new ligands [1].

Interleukin 15 a(IL-15) is a proinflammatory cytokine exerting pleiotropic activity towards immune and non-immune cells. Increase in IL-15 concentration is considered a crucial event in the etiology of rheumatoid arthritis, psoriasis, multiple sclerosis, inflammatory bowel disease, sarcoidosis, type I diabetes and T leukemias. High IL-15 concentration was also shown to influence transplant rejection, including kidney, heart, lung, islet and skin allografts rejection. Interestingly there is only one class of small-molecule ligands known to inhibit this protein. We have *in-silico* designed a series of compounds based on a completely new scaffold, which experimentally showed high affinity towards IL-15.

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Two pathways of proteins with Gordian (5_2) knot folding

Dabrowski-Tumanski Pawel[1,2], Zhao Yani[1,3], Niewieczerzal Szymon[1], Sulkowska Joanna[1,2]
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Proteins are ubiquitous biological compounds with their function crucially dependent on their 3D structure. For some proteins however, obtaining their native, enzymatically active form may be especially complicated, as their linear backbone has to self-tie [1,2]. Despite many theoretical and experimental investigations folding of knotted proteins still remains elusive.

UCH-L1 is a neuronal ubitiquinase with 5_2-knotted topology [3], constituting app. 2% of human brain proteins. The misfolding of this protein was associated with Parkinson's disease. In this work we involve the power of coarse grained simulations to study UCH-L1 folding pathway. In particular we show two topologically distinct folding routes (Fig. 1). We compare our results with the experimental works identifying the intermediate states prone to oligomerization, which may lead to neurodegenerative diseases [4,5]. Finally, we come up with new experiments which may confirm our proposed model of UCH-L1 folding, solving finally the mystery of self-tying of 5_2-knotted proteins.

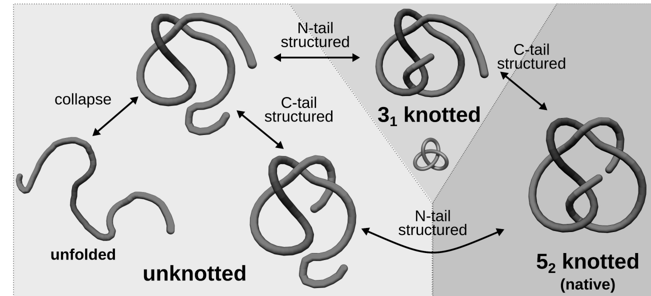

Fig. 1: Proposed pathways of UCH-L1 folding.

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Gauss Linking Number reveals new nooks in entangled proteins!

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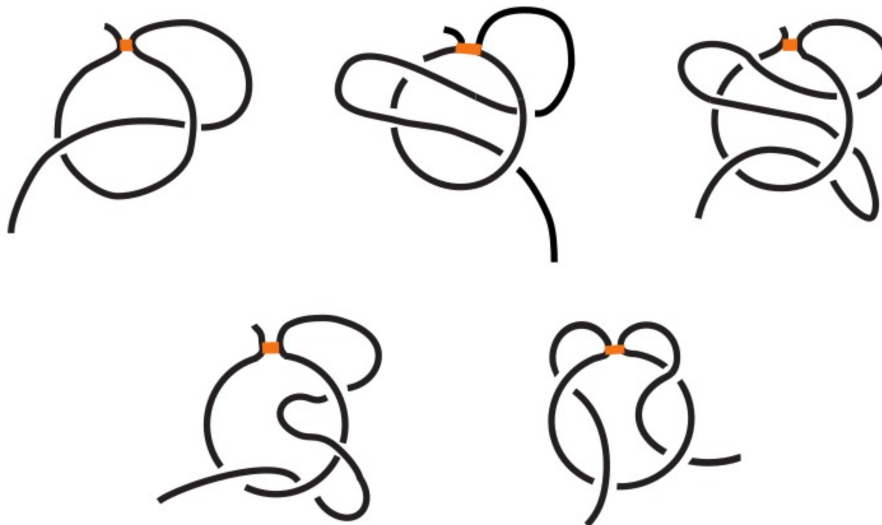
We've been studying entanglement of proteins for couple years and found it really interesting. Beside knots and links we defined new topological structures called lassos [1, 2], which appear i.e. in leptine, responsible for regulation of appetite, and in miniproteins used for projecting drugs.



For studying lasso structure we proposed technic based on minimal surfaces which are approximated by bubble soap. In the presentation I want to discuss new approach based on Gauss Linking Number which measures entanglement between any two open curves [3]. I would like to enlighten the differences in both technics and strong sides of them as well.

Reference

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Role of the magnesium in a knotted methyltransferase

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Magnesium is an important part of many protein complexes. It was shown that it is necessary for proper functioning of one of the knotted methyltransferases – TrmD protein [1]. This deeply knotted protein was recently discovered to be also a part of the magnesium sensing mechanism [2]. In this protein Mg^{2+} is responsible for catalysis and should be present in the active site, near the base that is methylated. Since the active site is a part of the knotted region, it is important to establish the exact location for the Mg^{2+} binding within the active site and connect it to the topology of the protein.

With theoretical approach we are able to study behavior of the protein and steps of the reaction it is performing. Using molecular dynamics simulations we identified potential places for the Mg^{2+} to bind. Next, using quantum calculations and DFT (Density Functional Theory) method we can verify whether the established location of Mg^{2+} lowers the barrier for the reaction and is a catalytic site. We have found that TrmD has two distinct sites for the magnesium, where one could act as a catalytic site and the other as a sensor for the Mg^{2+} concentration in the cell. These results motivate a question whether the transition pathway between those sites is possible or whether they are occupied both at once. During the talk I will summarise the most important results in this topic.

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Modeling of yeast ion translocation protein Trk1 using combined theoretical and experimental approaches

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[2] University of South Bohemia, Ceske Budejovice, Czech Republic
[3] University of Bonn, Bonn, Germany

Trk1 is a K^+ - translocation system in yeasts which is required for cells to survive at low K^+ concentrations. Also, it was shown experimentally that Trk1 is able to conduct anions. The structure of Trk1 is not solved yet and it is not known whether Trk1 is an active or passive ion translocation system. We used molecular modeling and experimental approaches to predict the structure and understand the function of Trk1. The first insight into structure of Trk1 was given by studies of Durell and Guy (1999) who predicted an evolutionary relation of the family of TRK/HKT/Ktr to K^+ channels [1]. The crystal structures of TrkH and KtrB proteins [2,3] were used to model Trk1. Although, sequence similarity is very low, modeling combined with experimental verification allowed to predict a structure. Our results showed that putative selectivity filter (SF) glycines are located inside the SF and are also important for protein function and folding [4]. This finding is in agreement with an evolutionary connection of Trk1 to K^+ channels. Further we focused on the multimerization state of Trk1. Both structural templates were solved as dimers. However, it is not yet clear whether Trk1 can be functional as monomer, or exists as a dimer or multimer. Moreover, it was hypothesized that tetramerization of Trk1 could form an additional pore and thus explain the observed anion permeability. To study these questions bimolecular fluorescence complementation is used. Our results suggest that Trk1 most probably exists in multimeric state.

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Acknowledgments

The work was supported by BmBF (SysMO, 031 5768 B), GACR (1619221S) and MSMT CR (C4Sys, LM2015055).

Would evolution stop membrane proteins from knotting?

Aleksandra I Jarmolinska[1,2], Peter Virnau[3], Joanna I Sulkowska[1]
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[2] College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, Warsaw, Poland
[3] Johannes Gutenberg University Mainz, Department of Physics, Mainz, Germany

Study of knots in proteins is a relatively recent, yet rapidly developing field. Discovery of the first non-trivial protein in mid '90s has sparked a snowball effect with new entangled structures being found every couple of months – new, both in terms of the function of the protein and the organisms from which they hail.

Recently, the first examples of deep (impossible to form just due to thermal fluctuations) knots in membrane proteins have been found. All of them belong to the same family of calcium/cation antiporters, although supervise slightly different exchanges – and share well below 30% of their sequence. Our question was whether this gives a resonable basis to expect all the other proteins within this family to be also knotted.

Through molecular phylogenetics approach we have obtained a probable evolutionary tree of this family. Placement of known, knotted, structures within its branches supports the hypothesis that all of these antiporters should be knotted. Although not all relationships indicated by our tree are expected, they confrom with some previous suggestions [1,2] on the possible evolutionary pathway of this family.

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Acknowledgements

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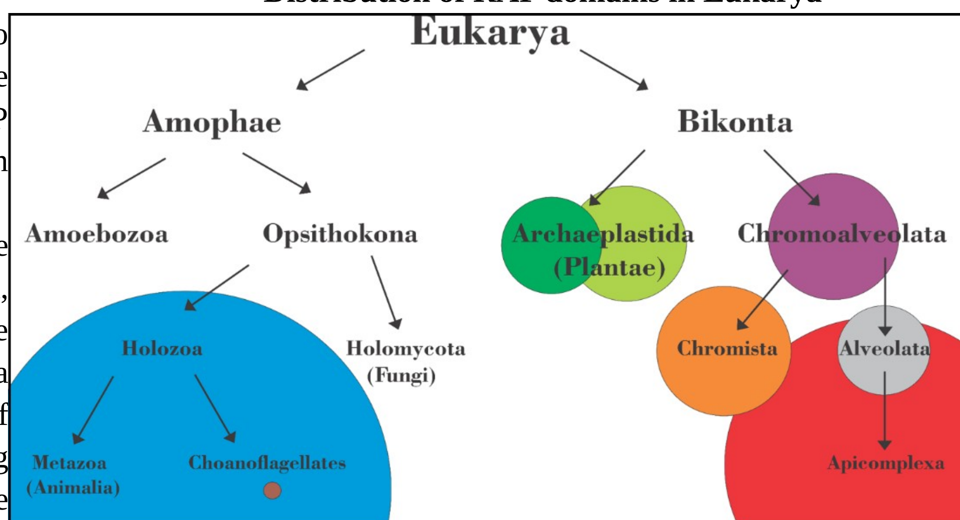
The RAP domain: Structure, Function, Evolution and Bioinformatics

Merski Matthew[1], Skrzeczkowski Jakub[1], Jurska Aleksandra[1], Gorna Maria[1]
[1] Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland

The RAP domain (**R**na-binding **A**bundant in a**P**icomplexans) is an approximately 60 residue long C-terminal protein domain that is widely conserved in eukaryotes, although curiously absent in fungi (see figure). Originally discovered by sequence analysis, the putative RNA-binding function was attributed due to the presence of the RAP domain in the small number of proteins originally used to identify the RAP domain, but without any experimental confirmation [1]. Typically, RAP domain containing proteins are large, with several functional domains, often including an organelle directing signal domain. More recently, a large-scale bioinformatic analysis revealed that the RAP domain corresponds to the C-terminal portion of one class of PD-(D/E)XK enzymes, however, the catalytic residues P, D, E, & K are all notably absent [2].

Distribution of RAP domains in Eukarya



We set out to elucidate the structure and function of the RAP domain. Using domain deconstruction techniques [3], we isolated, expressed, refolded and purified the RAP domains from a wide array of eukaryotes, including several of which have not currently have any publicly known protein structures. We obtained crystals of a RAP domain from the chocolate plant, T. cacao and others are underway. With this and other structures we will begin to unravel the role of this domain in the eukarya.

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Structural basis of mRNA-cap recognition by Dcp1/Dcp2 complex

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Dcp1/2 is the major eukaryotic RNA decapping complex composed of regulatory (Dcp1) and catalytic (Dcp2) subunit, which release m^7GDP molecule from m^7G capped transcripts. Dcp1/2 activity is crucial for RNA quality control and turnover hence deregulation of those processes may be detrimental. Since m^7GDP has low affinity to Dcp2 we screened a set of synthetic m^7G nucleotides (cap analogues) bearing modifications in the oligophosphate chain to find better Dcp1/2 binders. Using a radioactivity-based decapping assay we identified compounds binding Dcp2 much tighter than m^7GDP. These nucleotides are based on m^7Gppppm^{7}G structure and they contain either boranophosphate or phosphorothioate moiety in the phosphate chain. The most potent inhibitor, m^7Gp$_S$ppp$_S$m^{7}G is 20-times more potent than m^7GDP, thus it was subjected to kinetic and structural studies. NMR binding experiments revealed that both regulatory and catalytic domains of Dcp2 recognise that compound with submicromolar affinities. Single-turnover kinetics inhibition assay showed that mentioned compound is a mixed inhibitor with higher affinity for *apo* enzyme than for ES complex [1]. We also solved a crystal structure of fission yeast Dcp1/2 complex with the human NMD cofactor PNRC2 complexed with m^7Gp$_S$ppp$_S$m^{7}G. Cap binding is accompanied by the forming a composite nucleotide-binding site comprising evolutionary stable residues in the catalytic and regulatory domains. Additional kinetic analysis of PNRC2 revealed that a conserved short linear motif enhances both substrate affinity and the catalytic step of decapping [2]. It is the first known structure of a small molecule ligand bound to Dcp2, and these findings may be beneficial for further studies on RNA decapping and development of new Dcp2 binders.

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Generic trace in proteins

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For a long time a connection between biomolecules structures and their functions is known. For this reason, analysis and classification of biomolecules structures, like RNA and proteins, is an important field of research. Many types of structure classifications have been developed; amongst them is a classification based on genus. Genus can be used as a measure of entanglement of a folded biomolecule, and so far it has been applied to classify RNA structures with pseudoknots. We adopt a similar approach to analyze structures of protein chains, that are known to contain a complex system of interactions between amino acids. For this purpose, we analyze generic trace of protein chains, that gives information how genus increases and a complexity of bonds connecting amino acids in a given segment of a protein chain. Moreover, we presume that there exists a correlation between generic trace behavior and a secondary structure motif, which occurs in a given protein fragment. Furthermore, the domain division of a protein chain can also affect the form of its generic trace.

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PyLasso – plugin to detect and analyze lasso type of entanglement in (bio)polymers

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Entanglement in proteins is an important phenomenon and a subject of multidisciplinary researchers. Lasso is a new entanglement motif recently discovered in proteins [1]. The lasso occurs in structures with a closed loop, e.g. based on disulfide bridges in situation where at least one terminus of the protein backbone pierces through a surface spanned on such closed loop. A need to detect and analyze such entangled configurations with precisely specified geometric conditions resulted in creating PyLasso.

PyLasso is a multiplatform plugin for PyMOL. It detects any type of lasso entanglement in a given (bio) based on techniques developed in [1]. Generally, a type of lasso is detected based on surfaces of minimal are a spanned on closed loops, and number and direction of pierced segments through such surfaces. The plugin is equipped with various tools to detect different types of covalently closed loops, as well as to design a new closing. Throughout PyMOL's protein visualization, detected type of entanglement for a given (bio)polymer can be presented in 3D computer graphics with the minimal surface spanned on the chosen covalent loop as well as highlighted piercings if such are present. PyLasso is equipped with different tools to facilitate further visual detection and analysis of entanglement in proteins such as structure smoothing and minimal surface smoothing. Smoothing is helpful in understanding protein's topology and clarifying the perception of entanglement geometry. The smoothed structure is a schematic visualization of structure in which e.g. the topology has been exposed.

PyLasso is also equipment in advanced tools, such as manual parametrization of algorithm to detect lasso and an alternative method to detect entanglement by means of Gaussian Linking Number (GLN). It provides different type of information about lasso entanglement. The lasso can be analyzed based on a single structure or across trajectory (set of structures which depend on time). The trajectory analysis mode offers two charts describing the change in the topology along the consecutive frames (e.g. time evolution), and an option to visualize entanglement in chosen frames (configuration).

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Uncovering molecular mechanisms of translational control with structural biology.

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In eukaryotes post-transcriptional regulation of gene expression is key to all major physiological processes. Any defects in this regulation may lead to severe dysfunction in human such as particular neurodegenerative diseases and cancers. The precise translational regulation employs diverse RNA-binding proteins (RBPs) and chemical modifications of tRNA molecules, which have a crucial effect on codon-anticodon pairing and fine-tuning the translation and protein folding dynamics.

Through detailed structural characteristic of molecular machineries involved in translational control we aim to further our understanding about regulatory mechanisms of protein synthesis. Our main objective is to obtain structural details of macromolecular complexes involved in tRNA modification and gain insights into translational control by combining structural biology (x-ray crystallography and electron microscopy) with protein biochemistry and biophysics. Based on the resolved crystal structures we introduce structure guided mutations into proteins and study their influence on the macromolecular complex formation and protein-RNA interactions. In addition, based on available disease models we can specifically test role of individual components of these complexes. Thus laying foundation for further research hypothesis about their function during the translational control and facilitate the design of potential diagnostic strategies for several severe human diseases.

The results obtained in collaboration allowed us to directly link a mutation of tRNA modifying complex to progressive onset of neurodegenerative condition in animal models. By conducting biochemical and biophysical assays we seek to unravel the molecular mechanisms underlying this severe disease.

In the near future we also plan to exploit our research on translational control by investigating novel macromolecular assemblies involved in translational repression during stem cells switch to differentiation pathway in early oogenesis of fruit flies as a model.

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The role of regulatory proteins in tRNA modifications in human cells

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In all organisms, transfer RNAs (tRNAs) undergo a great number of post-transcriptional modifications which, depending on the position, perform different functions. Modifications at the anticodon loop have mostly effects on the speed of decoding, folding of nascent polypeptide chains, and also prevent translational frameshifting [1]. A number of studies have described enzymes responsible for catalyzing tRNA modifications in yeast. As the human homologues for many of these enzymes have been identified, and specific mutations of genes encoding them have been linked to certain human diseases, it appears important also to study their functional role in human cells. Enzymatic pathways responsible for modifications of uridine at the wobble position 34 (U34) of the anticodon loop are particularly important, as alterations in their components lead to neurological diseases, cancer and respiratory diseases [2]. Those modifications involve the incorporation of methoxycarbonylmethyl group at the C5 position of U34 by the Elongator pathway lading to 5-methoxycarbonyl-methyluridine (mcm^5U34). Subsequent incorporation of sulfur at the C2 position by the Ubiquitin-related modifier-1 pathway leads to 5-methoxycarbonyl-methyl-2-thiouridine (mcm^5s^2U34) [3]. In addition, regulatory proteins (Kti11–14), which are associated with Elongator pathway, are very important for tRNA modifications [4]. So far, only human homologue of yeast Kti11 and Kti12 have been identified: DPH3 and KTI12 respectively.

I plan to study the role of those proteins in human cell lines, namely their influence on cell viability, proliferation, subsequently tRNA modifications and the interplay between them and other proteins taking part in U34 modification. To achieve my goals, I use lentiviral vectors with shRNA sequences silencing DPH3 and KTI12 proteins. After constitutive downregulation of those proteins with the optimal shRNA sequences, I will measure cell viability with Alamar Blue assay, cell proliferation with BrdU test, and the modifications of tRNA using mass spectrometry techniques. If the above mentioned parameters will differ between treated and untreated, I will perform rescue experiments with vectors encoding the open reading frame for DPH3 and KTI12, and specific structure guided point mutants. In the next step of my research I will generate stable inducible cell lines using tetracycline dependent expression of previously selected shRNAs. The dynamic down regulation of proteins will enable me to recapitulate results from previous experiments.

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Structural insights into the role of RNA-binding proteins in stem cell maintenance and differentiation

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Post-transcriptional gene regulation via RNA-binding proteins (RBPs) plays an important role during fundamental physiological processes in eukaryotes, thus receiving increased scientific attention in recent years. By forming ribonucleoproteins (RNPs) upon binding to specific motifs of mRNA transcripts, RBPs act as regulatory elements in variety of cellular processes. Dynamic assembly of these complexes determines the lifetime, rate and occurrence of the translation process of specific mRNA.

Recently, RNPs have been shown to play an essential role in the early development of different organisms, due to their abilities to control stem cell self-renewal and differentiation. Through the use of various mechanisms based on protein-protein and protein-RNA interactions, RBPs can tightly regulate crucial stem cell fate decision. Any impairments in regulation of these processes may cause deregulated and continuous proliferation of stem cells leading to carcinogenesis.

The main aim of our work is to obtain structural and functional insights into the repressive complex triggering stem cell differentiation. By determining its interactions with molecular partners (proteins and RNA) we plan to understand the mechanism by which RBPs regulate translational control during oogenesis. Our research is based on a *Drosophila melanogaster* germline development model system which is widely used for studying transcriptional and translational control during stem cells maintenance and differentiation. During our studies we are combining different biochemical and biophysical methods, as well as common techniques used in structural biology (crystallography and electron microscopy).

So far, we obtained first preliminary results, which allow us to test the interaction between RNA-binding domain and different RNA regulatory elements and determine specific motifs involved in these binding events.

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Structural insights into the role of binding regulatory factors to Elongator subunit in the context of tRNA modification process.

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Translation is a process crucial for the cell's survival and requires the involvement of many types of macromolecular complexes. Translation is based on the encoding of information about the protein sequence in messenger RNA (mRNA) molecules and converting it to an ordered chain of a polypeptides. In recent years more and more research shows the great importance of small chemical modifications in anticodons of tRNA for this process of protein synthesis. One of the key players responsible for tRNA modifications is a large multi-protein complex called Elongator. During our work we are trying to understand the molecular mechanisms that lead to the specific base modifications in tRNAs, which are important for the efficiency of translational rates and folding dynamics of protein synthesis.

We are focused on revealing and characterizing the molecular mechanisms that regulate the highly conserved subunit of the complex, which is temporarily contacted by specific regulatory factors. Although, it was previously shown that those regulatory elements are also crucial for proper function of the tRNA modification activity, their individual functions remain unclear. The main objective of my research project is to gain information about the exact interaction between these regulatory factor and the catalytically active subunit of Elongator and try to define the contribution of specific motifs and residues in these molecules for the interaction. Understanding these molecular mechanisms involved in the translation system is of prime importance and has broad meaning for many fields of molecular medicine to clarify the influence of this process for various human diseases.

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The influence of the QM- calculated ligands' charge distribution on the docking results

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Methylation is a process of great biological significance. tRNA methyltransferases are a group of enzymes that catalyze the methylation of tRNA. This modification provides a proper reading frame and prevent blocking of the translation. Both knotted and unknotted topologies can be found in this group of enzymes. Presence of a knot in the binding site forces a bent conformation of the ligand. The enzymes with trivial topology bind cofactor in an open conformation. This difference was used to create the knotted tRNA guanine methyltransferases-selective inhibitor [1] and methyltransferases inhibitor [2]. Unknotted structures - Trm5, occur mostly in eucaria, while knotted - TrmD, mostly in bacteria. Such designed chemical compound could be used as a new antimicrobial drug for proteins with non-trivial topology. Using numerical simulation, the efficacy of the ligand can be studied theoretically. We performed inhibitor and natural substrate docking to proteins from their co-crystal structures deposited at PDB [3]. We used ligands with different partial charges obtained via the QM-calculation with three types of functionals and three basis sets. Comparing the RMSD between docked pose and crystal structure, we chose the best pairs of functionals and basis set for Mulliken and ESP partial charges. In the future, we prepare TrmD's inhibitor to Trm5 docking to check selectivity of this chemical compounds and confirm the results of our calculation using *in vitro* methods.

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Toward a better life: in silico study of transthyretin related amyloidosis

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The growing expected average lifetime is an effect of many achievements in science. We live longer as diagnostic methods support early disease detection, new drugs facilitate treatment of previously incurable health issues. Also, the overall health-related awareness seems to be bigger than some decades ago. Unfortunately, there are still many issues which aging society has to face, for instance emerging number of diseases caused by creation of protein deposits – called amyloidosis, which usually in long time perspective are lethal or, at least, significantly decrease the joy of longer life.

While nearly 40 proteins with amyloidogenic potential were determined so far, here investigations of transthyretin (TTR) – a tetrameric T4 and retinol transport protein [1] are presented. The aggregation of TTR has been previously described as a sequence of stages, beginning with tetramer dissociation into two dimers, through decay to monomers, when finally misfolding and aggregation may take place [2]. The whole process may be accelerated by various cofactors, dominated by single point mutations. In this study three variants were considered: the wild type, one of the most dangerous mutants – L55P and one of the most common, mild in terms of pathogeny – V30M. Various configurations of molecular dynamics and steered molecular dynamics simulations were performed, followed by analysis. Changes of free energy upon single point mutations by means of FoldX forcefield were estimated [3], as well as differences in *per residuum* entropy in terms of quasi-harmonic approximation was performed [4].

Our investigations revealed interesting mechanism that stands behind significantly increased tendencies of the L55P variant to create dangerous fibrils. Obtained results shed new light on the TTR amyloidosis and may support design of new TTR related amyloidosis inhibitors.

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Structure and function studies of RNA-binding proteins with FAST motifs and a RAP domain

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The FASTK family (Fas-activated Serine/Threonine Kinase) contains six human proteins which localize to the mitochondria and have been functionally linked to cellular respiration and a rare mitochondrial disease. While human FASTK was initially annotated as an atypical Ser/Thr kinase later studies dispute this annotation [1]. Structurally, FASTKD proteins contain an N-terminal mitochondrial targeting signal, a pair of FAST motifs and a C-terminal RAP domain (Figure 1).The N-terminal part is predicted to be highly globular but with small disordered regions. The FAST motifs are putative RNA binding domains with a novel α-helical repeat fold that does not display sequence similarity to any other known helical repeat motifs. Interestingly, the RAP domain is found in many members of the recently identified class of octotricopeptide repeat (OPR) proteins, which are abundant in plants and green algae and is believed to play a role in chloroplast RNA biology [2]. The OPR proteins have been shown to bind RNA with preference for some substrates [3], but their structure or RNA binding specificity is unknown. The RAP domain is also overrepresented in *Plasmodium*, and hence structural information of this domain is relevant to the field of malaria.

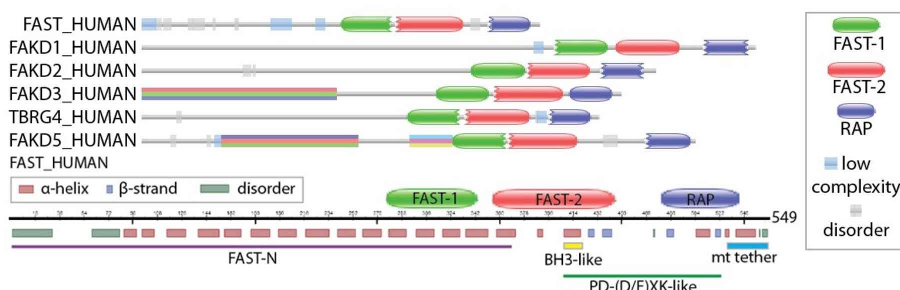

***Figure 1**. Domain composition and secondary structure prediction of the human FASTK family (Pfam: PF08373).*

Our project aims to provide for the first time structural and novel biochemical information about the relatively understudied FASTK family. This will have relevance to drug design therapeutic strategies, particularly of cancer and inflammation and will likely reveal new folds of RNA binding domains thus contributing to the general knowledge of the rules that govern RNA recognition.

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