iNEXT-Discovery Annual Meeting 2023

Experimental and computational aspects of Structural Chemistry and Biology

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Book of Abstracts

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HORIZON 2020
FRAMEWORK PROGRAMME
Research and Innovation Action

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X-ray and cryo-EM approaches

Invited Lecture 1

Tobias Krojer: Towards comprehensive analysis of large crystallographic fragment screening campaigns

Lund University, MAX IV Laboratory, Lund, Sweden

The throughput of macromolecular X-ray crystallography experiments has surged over the last decade. Increases in the availability of high-intensity X-ray beams, fast detectors, and high levels of automation have permitted this extraordinary improvement in productivity. These advancements have allowed for the establishment of the *FragMAX* facility and several other specialized centres for crystal-based fragment screening, which enable preparation and collection of hundreds of single-crystal diffraction datasets per day. In addition, crystal structure determination has become significantly easier due to the availability of user-friendly software packages, which support users with different levels of experience from data processing to model building and structure refinement. However, simultaneous analysis of hundreds of related crystal structures, such as those present in fragment screening or structure-based drug design programs, remains a formidable problem because all major software suites adhere to the prevalent idea of "*one project equals one structure*". Moreover, fragment screening generates an abundance of meta-data that must be tracked for subsequent analysis and PDB deposition, but such functionality is currently not integrated in the available software packages.
FragMAXdb and FragMAXapp are two applications developed at *MAX IV Laboratory* to overcome this issue by facilitating comprehensive project management and parallel processing of hundreds of datasets from crystallographic screening campaigns. This presentation will cover their implementation, current functionalities, and highlight recent advancements. It will also outline potential future developments because there is an unmet need for more generic systems that can also support newer approaches such as (time-resolved) serial crystallographic studies. Such advancements will be essential if we are to realize the full potential of the enormous throughput of modern synchrotron beamlines and allow structural biologists to devote their valuable time to structure analysis rather than data management and processing. Expansion of existing and creation of new tools will not be simple but will greatly boost protein crystallographers' output.

Invited Lecture 2

Darren Fearon: Accelerating structure-enabled drug discovery with high-throughput crystallographic fragment screening

Diamond House, Harwell Science and Innovation Campus, Oxfordshire, United Kingdom

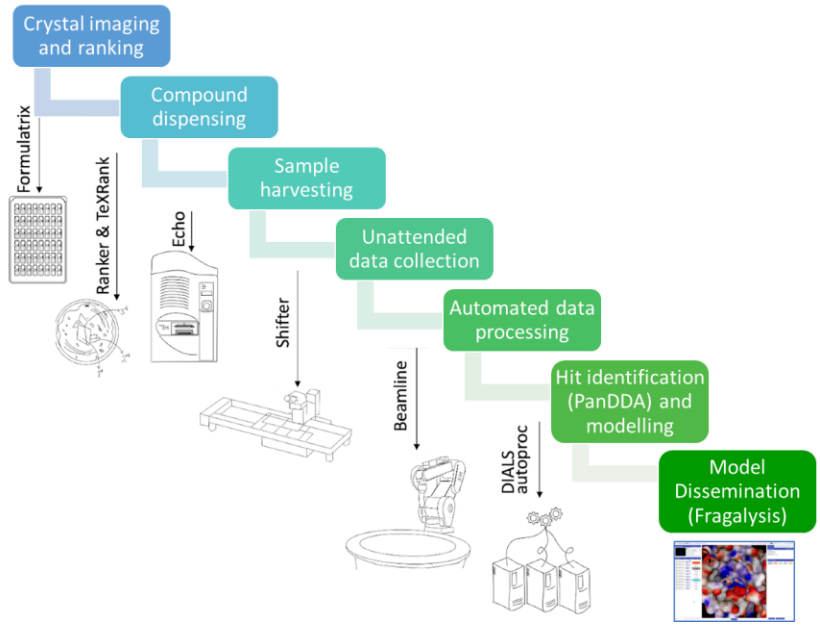

Figure 1. Overview of the XChem fragment screening platform at Diamond Light Source.

Fragment-based drug discovery is a well-established method for the identification of chemical starting points which can be developed into clinical drugs. Historically, crystallographic fragment screening using traditional methods was low-throughput and time consuming. However, thanks to advances in synchrotron capabilities and the introduction of dedicated facilities, such as the XChem platform at Diamond, there has been substantial improvements in throughput and integration between sample preparation, data collection and hit identification.

The XChem team and collaborators have identified numerous fragments which bind to various antiviral drug discovery targets and leveraged our high-throughput platform to rapidly drive the hit-to-lead process to deliver pre-clinical candidates for SARS-CoV-2 in under 2 years.

Invited Lecture 3

Jonathan Grimes: How does flu virus replicate? Mapping inhibitory sites on the RNA polymerase of the 1918 pandemic influenza virus using nanobodies

University of Oxford, *Oxford Particle Imaging Centre, United Kingdom*

Zihan Zhu, Jeremy Keown, Loic Carrique, Haitian Fan, Ervin Fodor Jonathan Grimes

Influenza A virus is a single-stranded negative-sense RNA virus responsible for seasonal influenza epidemics and global pandemics, presenting a considerable burden to healthcare systems worldwide. The virus encodes an RNA-dependent RNA polymerase which transcribes and replicates the viral genome inside the host cell nucleus. The polymerase undergoes dramatic conformational rearrangements and interacts with host and viral proteins to perform its functions. We structurally and functionally characterised the binding of a panel of 24 nanobodies, derived from single-domain camelid antibodies, to the 1918 pandemic influenza virus polymerase. We determined the binding sites for these nanobodies on the polymerase using cryo-electron microscopy and X-ray crystallography. We showed that a subset of these nanobodies strongly inhibits polymerase activity *in vitro* and in cell culture. Using biochemical and cell-based assays, we found that five inhibitory nanobodies, binding to distinct sites on the polymerase, interfere with different polymerase functions, such as conformational rearrangements, polymerase dimerization, or binding to the C-terminal domain of host RNA polymerase II. These nanobodies were also shown to have conserved inhibitory effect on polymerase activity across influenza A virus subtypes and inhibit the replication of a reassortant influenza virus, encoding 1918 polymerase, in cell culture. In summary, we have identified five sites on the polymerase that can be targeted for inhibition of the viral polymerase activity via various mechanisms when bound by a nanobody. This study provides targets for future antiviral development and demonstrates the power of nanobodies as a tool in studying protein functions.

Invited Lecture 4

Bruno Klaholz: High-resolution cryo-EM analysis of macromolecular complexes: when chemistry and biology meet.

Centre for Integrative Biology, Department of Integrated Structural Biology, IGBMC, Illkirch, France.

Cryo electron microscopy (cryo-EM) is currently moving forward at high pace towards the high-resolution analysis of macromolecular complexes. Two key factors contributing towards that are (i) new-generation cryo electron microscopes that include improved energy filters and direct electron detectors (Fréchin et al., 2022), and (ii) new tools for advanced image processing including 3D classification methods to sort different structural states and refine sub-regions of the complexes of interest by focused refinements (Barchet et al., 2023). Using latest-generation instrumentation and focused classification and refinement methods we have now succeeded in crossing the 2 Å resolution barrier on a particularly difficult complex to analyse, the human ribosome, which is much less stable than its bacterial counterpart, with many flexible regions such as the 40S ribosomal subunit difficult to visualize at high resolution. Focused refinements and multibody refinements allowed obtaining a 1.9 Å resolution structure revealing numerous chemical modifications of the ribosomal RNA (rRNA), ions such as Zn^{2+}, K^{+} and Mg^{2+} including water molecules of the Mg-octahedral coordination. Chemical modifications of the rRNA of the human ribosome comprise hundreds of nucleotides with 2'-O-methylations, pseudo-uridines and various base-specific modifications. They are involved in human protein synthesis dysregulations such as cancer (*NAR Cancer*, 2020) and other diseases but their role therein is unknown. The structure now reveals their precise mode of interaction, including series of 2'-O-Methylation and pseudo-uridine sites that could not be visualized in our previous study at 2.9 Å resolution (Natchiar et al., 2017). The integrated analysis of chemical modification, which also includes cutting-edge mass spectrometry of enzyme-induced rRNA fragments, provides unprecedented mechanistic insights into the translation mechanism in humans and paves the way to understanding the role of rRNA chemical modifications.
We will also present some highlights on the high-resolution structural analysis of a DNA-dependent bacteriophage and on new technological developments in super-resolution imaging including spectral demixing, which will offer new synergies in the context of integrated structural biology projects.
This project was run at the CBI, which hosts the national and European infrastructures FRISBI, Instruct-ERIC and iNEXT-Discovery. Within their framework, access can be provided to users to run experiments on protein production, single particle cryo-EM (sample optimization and high-resolution data collection) and FIB & tomography for the analysis of cellular samples: https://frisbi.eu http://instruct-eric.com https://inext-discovery.eu

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Invited Lecture 5

Corinna Brockhaus: The role of Instruct-ERIC in Europe

Corinna Brockhaus, Pauline Audergon, Claudia Alen Amaro, Natalie Haley, Harald Schwalbe

Instruct-ERIC, Oxford House, Parkway Court, John Smith Drive, Oxford, OX4 2JY, UK

Instruct-ERIC is a distributed infrastructure providing open access to high-end structural biology techniques to researchers from all countries to promote innovation in biomedical science in Europe. In addition to being an active partner in iNEXT-Discovery in which many Instruct centres provide access to their services, Instruct is a partner in two Horizon Europe projects: ISIDORe and canSERV. These projects offer funded access to cutting-edge services, from basic biology to clinical trials, to scientists in the field of infectious disease (ISIDORe) and cancer research (canSERV). Instruct also offers funding support towards access costs for researchers from its 16 member countries.
Moreover, Instruct offers a range of training opportunities for European scientists, enabling researchers to expand their expertise in structural biology and implement new techniques in their research.
Instruct aims to sustain and further extend funded access to structural biology techniques for European researchers through continuous participation in Horizon Europe projects as well as expansion of Instruct member countries.

Studying amyloids by structural biology approaches

Invited Lecture 6

Chao Qi: Cryo-EM structures of tau filaments from human brain

MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

The assembly of microtubule-associated protein tau into abundant filamentous inclusions underlies many neurodegenerative diseases called tauopathies. The groups of Sjors Scheres & Michel Goedert established atomic structure determination of amyloid filaments by electron cryo-microscopy (cryo-EM), and used this method to determine the structures of tau filaments extracted from the brains of individuals with Alzheimer's disease, Pick's disease, chronic traumatic encephalopathy (CTE), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and other tauopathies. Specific molecular conformations of tau characterize different diseases, providing the basis for a structure-based classification of tauopathies. Using cryo-EM, I show that tau filaments from Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia complex (ALS/PDC) and Subacute Sclerosing Panencephalitis (SSPE) adopt the same structures as observed in CTE, suggesting that neurodegenerative diseases that are caused by environmental factors share similar molecular mechanisms of ilament formation, with neuroinflammation possibly playing an important role.

Invited Lecture 7

Roland Riek: Phase Transition from Liquid to Solid (amyloids): from the origin to the end of life ssNMR and amyloid

ETH Zürich, Department of Chemistry and Applied Biosciences

Proteins may aggregate into amyloid fibrils. This phase transition is associated with neurodegenerative diseases. However also functional amyloids exist. The structure activity relationship studies of both Alzheimer' and Parkinson-related amyloids as well as functional amyloids will be presented. While the latter amyloids are reversible, the disease-associated ones comprise the so called mechanism of secondary nucleation which is a positive feed back loop accelerating the aggregation beyond control. In addition, since the structures have not been evolved they are highly environment-dependent yielding many distinct structural polymorphs like a chameleon. These concepts are illustrated based on physico chemical experiments and high resolution structural work including NMR and cryo EM. Finally, hypothesis that peptide amyloids may have been played an important role in the origin of life is investigated.

Invited Lecture 8

Matthias Schmidt: Common structural features of wild type and variant ATTR amyloid fibrils extracted from different patients

Ulm University, Institute of Protein Biochemistry, Ulm, Germany

Wild type transthyretin-derived amyloid is the major component of non-hereditary transthyretin amyloidosis. Its accumulation in the heart of elderly patients is life threatening. A variety of genetic variants of transthyretin can lead to hereditary transthyretin amyloidosis, which shows different clinical symptoms, like age of onset and pattern of organ involvement. However, in the case of non-hereditary and in several cases of hereditary ATTR amyloidosis fibril deposits are located primarily in heart tissue and cause cardiac failures.
Objectives:
The goal of this project is the analysis of wild type and variant ATTR amyloid fibril structures from the heart of different patients with non-hereditary or hereditary transthyretin amyloidosis and comparison with previous published ATTR fibril structures.
Materials & methods:
Fibrils were extracted from the amyloidotic tissue of an explanted heart of wild type and variant ATTR patient. Cryo electron microscopy with subsequent structure modelling and mass spectrometry was used to study these fibrils.
Results:
A density map of ex vivo ATTR fibrils with resolutions of 2.78 Å (wild type), 3.18 Å (V20I), 2.37 Å (G47E) and 2.99 Å (V122I) from different patients were reconstructed. The structures are formed by stacked N- and C-terminal fragments of transthyretin forming an amyloid fibril which was confirmed with mass spectrometry. All structures show a similar spearhead like shape in their cross-sectional view, formed by the same N- and C-terminal fragments with some minor differences. The N-terminal fragment has an extensive hydrophobic core. The C-terminal fragment on the other hand contains a big cavity surrounded by polar and charged amino acids and is probably filled with water.
Conclusion:
We compare the structure of ATTRwt fibrils with variant ATTR fibril structures (V30M, G47E, V122I and V20I) extracted from hearts and eye of different patients (Steinebrei, M. et al. (2022) Nat. Commun.; Schmidt, M. et al. (2019) Nat. Commun.; Iakovleva, I. et al. (2021) Nat. Commun.). All studied structures show a remarkably similar cross-sectional shape of the N- and C-terminal fragments with only some minor differences. This demonstrates common features for ATTR fibrils despite differences in mutations and patients. Additionally, the N- and C-terminal fragments found in all cases points to that amyloid formation starts with complete or partly unfolded native TTR peptides, followed by fibril formation of the full-length ATTR peptides and subsequent proteolytic cleavage at the accessible region between amino acid 44 and 58.

Invited Lecture 9

Phillipp Neudecker: solid state NMR with respect to amyloids

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Protein folding, misfolding and aggregation are tightly interconnected processes involving a variety of conformational states with very different stability and lifetimes. NMR spectroscopy is particularly well suited to characterize the structure and dynamics of proteins on the relevant time scale (milliseconds to real time). We used CPMG relaxation dispersion NMR spectroscopy to elucidate the kinetics, thermodynamics and structural changes of the folding pathway of the Fyn SH3 A39V/N53P/V55L domain. The atomic-resolution three-dimensional solution structure of the 2% populated, on-pathway folding intermediate provides a detailed characterization of the non-native interactions stabilizing an aggregation-prone intermediate under native conditions and insight into how such an intermediate can derail folding and initiate fibril formation [1].

Many of the proteins forming amyloid fibrils in neurodegenerative diseases such as Amyloid-beta (Abeta) or the human Prion Protein (huPrP) are partially or fully disordered in their monomeric state and therefore readily characterized by NMR spectroscopy in solution. The truncation variant huPrP(23-144), which has been reported to cause a Gerstmann-Sträussler-Scheinker-like disease with amyloid deposits in the brain, is devoid of any stable secondary structure in solution [2,3]. In addition to causing Transmissible Spongiform Encephalopathies, huPrP is also a high-affinity receptor for Abeta oligomers that has been suggested to contribute to Abeta toxicity in Alzheimer's Disease, but may also play a protective role by sequestration of Abeta oligomers. Comparison of the solution NMR spectra of the monomers and the solid-state NMR spectra of the large Abeta-huPrP heteroaggregates reveals that the N-terminus of huPrP becomes immobilized in the complex without adopting a regular secondary structure. By contrast, the Abeta oligomer preparation represents a heterogeneous mixture of beta-strand-rich assemblies, of which some have the potential to evolve and elongate into different fibril polymorphs [3].

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Towards Structural Cell Biology

Invited Lecture 10

Harald Schwalbe: RNA structural biology by NMR spectroscopy

Institut für Organische Chemie und Chemische Biologie, Goethe University Frankfurt, Frankfurt am Main, Germany

Structural determination of RNA is difficult due to the intrinsic structural dynamics of RNA. In this contribution, we will present work of joint MD/NMR methods to generate structural models describing the conformational heterogeneity of RNA (Oxenfarth et al, under evaluation).

The structural dynamics allows targeting RNAs by low molecular weight ligands. Examples derived from the viral RNAs of SARS-CoV-2 will be discussed.

Invited Lecture 11

Philipp Selenko: In-Cell NMR as a tool in Cellular Structural Biology

Weizmann Institute of Science, Department of Biological Regulation, Rehovot, Israel

Remarkable developments in cellular in situ methods, including cryo-electron tomography (cryoET), cross-linking mass spectrometry (CLMS), Förster resonance energy transfer (FRET), nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) spectroscopies have clearly shown that the future of Structural Biology is in the cell. Together, these methods delivered unprecedented insights into the structure & function of proteins, membranes and metabolites directly in intact specimens, with current efforts geared towards improving their robustness, sensitivity, and overall ease-of-use. In my talk, I discuss the role of in-cell NMR spectroscopy in the era of Cellular Structural Biology and how integrated approaches with other methods offer enticing possibilities for future applications. In particular, I highlight recent discoveries that in-cell NMR enabled us to make and how they (re)shaped our understanding of fundamental processes in cellular biology.

Invited Lecture 12

Maria Harkiolaki: Correlative imaging using soft X-ray tomography to investigate cell structure and function

Diamond House, Harwell Science and Innovation Campus, Oxfordshire, United Kingdom

High-content high-resolution imaging of the cellular world is currently brimming with potential due to a revolution in cellular imaging technology resulting in a better understanding of cell organization and behaviour. Amongst the highlights of microscopy developments in cellular imaging has been the emergence of new correlative imaging synergies that include Soft X-ray Tomography (SXT) of cryopreserved cells and tissue. At the correlative cryo-imaging beamline B24 of the UK synchrotron, we have implemented and refined such a correlative imaging platform to a high level of automation and throughput[1] by focusing not only on technique development but also on accessibility and ease of use.

SXT is a high-resolution non-destructive 3D mesoscale imaging technique for cells and tissues in the fully hydrated state without the need for chemical fixation, sectioning, or milling, or the use of contrast-enhancing agents. Under cryogenic conditions, vitrified samples are irradiated with 'water-window' X-rays at defined angular intervals and the projections collected can deliver a lateral resolution of structures within cells to 25 nm depending on the optical set-up used. At B24, a 3D super-resolution fluorescence microscope based on structured illumination microscopy (SIM) principles was developed as a complementary tool to match molecular localisation with high-resolution structural data acquired in SXT under cryogenic conditions leading to the development of a unique same-sample correlative light and X-ray tomography (CLXT) platform. Same-instrument same-samples additional modes of operation include elemental near-edge X-ray absorption tomography and phase-contrast soft X-ray imaging.

Here we will highlight the use of SXT within the current technology landscape and the partnerships that it supports across other imaging capabilities. Representative data from current biomedical areas will be presented.

[1] Kounatidis I, Stanifer ML, Phillips MA, Paul-Gilloteaux P, Heiligenstein X, Wang H, Okolo CA, Fish TM, Spink MC, Stuart DI, Davis I, Boulant S, Grimes JM, Dobbie IM, Harkiolaki M. 2020. 3D Correlative Cryo-Structured Illumination Fluorescence and Soft X-ray Microscopy Elucidates Reovirus Intracellular Release Pathway. Cell 182:515-530 e17.

Computational approaches for experimental data

Invited Lecture 13

Peter Güntert: Accelerating protein chemical shift assignment by deep learning for visual spectra analysis, structure and shift prediction

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Chemical shift assignments are required for most protein NMR studies and often demand most of the measurement and analysis time. Here, we present a hybrid automated approach for protein chemical shift assignment that allows to reduce the number of spectra to be measured and the time to analyze them. It is based on machine learning for visual spectra analysis with ARTINA (1,2), structure prediction with AlphaFold2 (3), chemical shift prediction with UCBShift (4), and automated assignment with FLYA (5). Results from more than 10'000 assignment calculations with 100 proteins show that a small number of spectra suffices to establish the backbone and side-chain assignments. In conjunction with AlphaFold2 structures, the five 3D spectra 15N-NOESY, 13C-NOESY, CBCAcoNH, HCCH-TOCSY, and CCH-TOCSY yield on average better assignments than if ARTINA is run with all available spectra but without AlphaFold2 structures. NOESY spectra are particularly valuable for automated assignment. To be beneficial, structures should have an accuracy better than 2 Å.

This new version of ARTINA, which is available for use at the open NMRtist web server (2), offers users the general possibility to load, in addition to NMR spectra, 3D structures, manually or otherwise prepared peak lists, chemical shifts, distance restraints and torsion angle restraints as additional input for the ARTINA shift assignment and ARTINA structure determination applications. The new version of NMRtist also provides quality scores for peak picking, shift assignments, and structure calculations that are computed purely on the basis of the input data without recourse to manually obtained reference results.

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Invited Lecture 14

Dóra Karancsiné Menyhárd: The inner dealings of a tetrameric serine protesase: calculations based on cryo-EM structures

A. J. Kiss-Szemán, I. Jákli, A. Perczel, V. Harmat, D. K. Menyhárd
Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Lórand University, Budapest, Hungary

Acyl-aminoacyl peptidase (AAP) is a homotetrameric serine-protease enzyme. Its primary function is the removal of N-acylated amino acids from the N-terminus of proteins in preparation of their proteosomal degradation. Through this, it plays a key role in the maturation and degradation processes of various proteins and peptides, functioning as a key enzyme of the protein quality control apparatus. As such, AAP has been indicated as a possible pharmacological target in various forms of cancer. AAP is the site of an unexpected drug-drug interaction too: carbapenem antibiotics were shown to interfere with a widely used anticonvulsant, valproate, via both forming crucial interactions with the enzyme in a competitive manner.
Recently we have determined the first structure of a mammalian AAP using cryo-EM, that of porcine liver AAP (pAAP). The structure is unique in the sense that the bacterial orthologues of AAP and other members of the S9 oligopeptidase family are monomeric, dimeric or hexameric in their active form. The tetrameric association seen in case of pAAP – confirmed as the functional form of the human variant too – is guided by amyloidogenic β-edges and mammalian-specific inserts and creates a toroid-shaped quaternary structure. The secluded nature of the substrate binding site, buried and protected by tetramerization, and the structural heterogeneity of the catalytic triad seems to be the key features potentiating the enzyme toward carbapenem antibiotics. In a subsequent work we have shown that binding of antibiotics requires the reorganization of the serine protease active site of pAAP which also leads to the irreversible inhibition of the enzyme. The cryo-EM structure of the pAAP-meropenem complex provides the first glimpse at the mechanisms by which antibiotics might produce side effects in human physiology – it is the first structure showing complex formation between a carbapenem antibiotic and (a very close homologue of) a human enzyme.

Invited Lecture 15

Jose Maria Carazo: Novel tools to analyze macromolecularheterogeneity and increase resolution by cryo-EM

CryoEM conformational landscapes: Directly accessing macromolecular flexibility

Spanish National Center for Biotechnology, CNB-CSIC, Darwin 3, Universidad Autonoma de Madrid, Madrid, Spain

Electron Microscopy at cryogenic temperatures is currently a very well-known approach to solve the structure of biological macromolecules with sufficiently high resolution to obtain good structural models. Part of this success was our proposal of Maximum Likelihood approaches to disentangle macromolecular structural flexibility (first implemente in the software XMIPP and then in Relion) (1). However, we then assumed that flexibility was discrete and that the user had to estimate the number of these discrete states, which clearly was not optimum. Nowadays, almost 15 years after this initial approach, we have developed new methods that allow us to consider continuous flexibility, opening the possibility to obtain conformational landscapes (2). In this context I will present recent advances in our "Zernike3D " approach, showing how we effectively access the whole range of macromolecular flexibility present in the cryoEM images without needing further estimations from the user.

(1) Scheres et al., Nat. Met., 2007
(2) Herreros et al., Nat. Comm., 2023

Computational learning methods for structural biology and beyond

Invited Lecture 16

Robbie Joosten: AlphaFill: Enriching AlphaFold models with co-factors, small molecules and metal ions

Netherlands Cancer Institute, Amsterdam, Netherlands

Artificial Intelligence algorithms implemented in AlphaFold and the like are having a transformative effect on structural biology research: accurate predictions of 3D protein sequences can now be generated from the corresponding amino acid sequence only. The AlphaFold protein structure prediction database (AFDB) has millions of such predictions publicly available, and more are likely to come. However, biochemical interpretation of these predictions is limited to amino acids only. That is, co-factors, small molecules and/or metal ions, which are required for protein function or structural integrity are lacking.

To address this limitation we developed AlphaFill: an algorithm that enriches AlphaFold models with co-factors, small molecules and (metal) ions. Such compounds are transplanted from homologous experimental structures available in the PDB-REDO databank. Application of the AlphaFill algorithm to the core AFDB data has created some 586 thousands enriched models with over 12 million fitted compounds. The algorithm was validated against transplants obtained with 100% sequence identity.

All AlphaFill models are freely available through alphafill.eu: a new resource to support scientists interpreting the AlphaFold models while creating new hypotheses and designing experiments. AlphaFill also allows users to 'fill' there own structure models no matter if they are computational or experimental.

We will present the 'What and how' of AlphaFill, including judging the quality of enriched models, and will give a sneak preview of new developments to come.

Invited Lecture 17

Danny Sahtoe: Computational design of de novo protein-protein interactions

Baker Lab, Institute for Protein Design, University of Washington, USA

The design of new protein-protein interactions (PPIs) has many applications in synthetic biology and biomedicine. Here we present the development of a new approach to design beta sheet mediated PPIs from first principles. We demonstrate the utility of this approach to the design of ***1)*** small binders to the human Transferrin Receptor intended to cross the blood-brain-barrier for the delivery of therapeutics into the brain ***2)*** the design of fast exchanging heterodimers that can be used as protein “lego” blocks to reversibly assembly hundreds of new synthetic multi-protein complexes including 36 experimentally validated complexes and ***3)*** the design of alpha-beta scaffolds that can bind otherwise conformationally flexible peptides into beta strand conformations that can be used to sequester amyloid forming proteins to inhibit fibril formation.

Invited Lecture 18

Jonas Teuwen: Deep learning for image reconstruction

Netherlands Cancer Institute, Amsterdam, Netherlands

The use of deep learning techniques for reconstruction problems has gained significant attention due to their ability to provide high-quality and efficient solutions to challenging inverse problems. In this talk, we will explore supervised reconstruction, a prominent approach in deep learning, by showcasing examples from the medical domain. We will discuss the advantages and disadvantages of this approach, highlighting the requirement for ground truth data, which can be impractical for many applications.

To overcome the limitations of supervised reconstruction, we will delve into the current approaches to unsupervised reconstruction. By utilizing unlabelled data, these techniques aim to alleviate the need for ground truth information. We will also address the challenges associated with unsupervised reconstruction, including the availability of limited data. Furthermore, we will conclude the talk by discussing examples of deep learning reconstruction in CryoEM.

Overall, this talk will provide insights into the advancements, challenges, and potential solutions in deep learning-based reconstruction. By examining both supervised and unsupervised approaches, we aim to showcase the versatility of deep learning techniques in addressing complex reconstruction problems.

Structural Biology

Invited Lecture 19

Márton Gadanecz and Zsolt Fazekas Zsolt: Structure determination of the magnesium ion free and bound KRas G12C+GDP complex using NMR data driven molecular dynamics simulations

Márton Gadanecz [1,2], Zsolt Fazekas [1,2], Gyula Pálfy, Dóra K. Menyhárd [1] and András Perczel [1]

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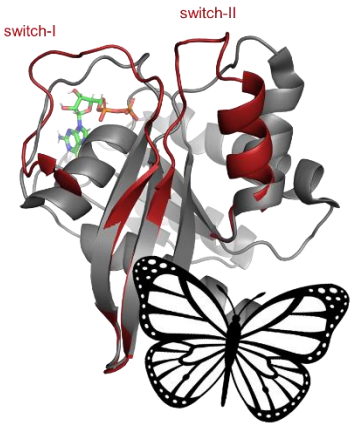

In this work, catalytically significant states of the G12C oncogenic variant of K-Ras, those of Mg^{2+}-free and Mg^{2+}-bound GDP-loaded forms, have been determined using NMR data driven molecular dynamics (MD) simulations. There are multiple Mg^{2+}-bound G12C K-Ras-GDP structures deposited in the Protein Data Bank (PDB) so this system was used as a reference, while the structure of the Mg^{2+}-free, but GDP-bound state of the RAS-cycle has not been determined previously. Due to the high flexibility of the switch-I and switch-II regions – which happen to be the catalytically most significant segments also - only chemical shift information can be collected for the most important regions of both systems. Chemical Shift-Rosetta software was applied for to derive an "NMR ensemble" based on the measured chemical shift values, which however does not contain any non-protein components of the complex, such as ligands, cofactors or ions. To overcome this limitation, we tested different methodologies, where we aimed to retain the structural information from the CS-Rosetta ensemble while re-introducing missing, but structurally crucial non-protein components into the models. We have carried out Monte Carlo Multiple Minimum (MCMM) simulations, constrained according to the best CS-Rosetta models, but have encountered unexpected difficulties. We then developed torsional restraint set for all backbone torsions of the studied systems based on the CS-Rosetta ensembles for GROMACS MD simulations, overriding force-field-based parametrization with knowledge-based steering in the presence of the reinserted cofactors. This protocol resulted in chemically meaningful, complete models for both systems that also retained the structural features and heterogeneity defined by the measured chemical shift values, and allowed the detailed comparison of Mg^{2+}-bound and Mg^{2+}-free states of G12C K-Ras-GDP.

Invited Lecture 20

Vineeta Kaushik*:* Structural elucidation of Retinol binding protein 3: One step closer to unfolding the effect of ligands on the conformation of the protein.

Vineeta Kaushik*, Luca Gessa, Nelam Kumar, Humberto Fernandes[#]
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RBP3 is a protein secreted in the interphotoreceptor matrix by photoreceptors cells, involved in the visual cycle, shuttles ligands between them and retinal pigment epithelium (RPE) cells. So far, there is no high-resolution, full-length structure available for this protein. Mammalian RBP3 is a single polypeptide with four modules, each containing ~300 amino acids. At least two conformational forms of the RBP3 exits, the elongated and bent ones, and each is favoured upon specific ligand binding. Even though decades of ligand bind assays and protein characterization went by, the details of the conformation changes after binding with ligands are still unresolved and need to be explored structurally.

In this study, the RBP3 protein was isolated from a native biological source and purified using different chromatography techniques. The most emerging technique CryoEm was used to investigate the structure of RBP3 protein at a high resolution to better understand the role of the cooperativity mechanism of RBP3 and open the possibility of structure-based drug design. We have also used the Small Angle X-Ray Scattering (SAXS) method to get more structural information about the protein upon titration of natural ligands. We gained iNEXT-discovery access to CEITEC, Brno and EMBL, Dessy, Germany, to achieve the objectives mentioned above.

This work provides an enlighted structure of RBP3 protein at a much higher resolution structure than previously available, around 4.3 Å, and where you can easily observe the flexibility of the protein among all four modules. Apart from the structural aspects, the data obtained from SAXS using protein in solution form with different retinoids shows the gradual conformational changes in the protein. With the SAXS data, it is clearly visible that the retinoids and fatty acids have different impacts on the conformational changes of the protein.

The obtained results allow exploring more about RBP3 in a correlation of structure-based drug design to minimize the impact of Diabetic retinopathy (DR). RBP3 has an anti-correlation property with DR, and the potential monitorization of RBP3 in DR at an early stage could be a ray of hope in developing new therapeutic approaches for DR.

Funding: iNEXT discovery (This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 871037) The International Centre for Translational Eye Research (MAB/2019/12) project is carried out within the International Research Agendas programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund. PASIFIC, Maria Sklodowska-Curie sponsored fellowship (This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Maria sklodowska-Curie grant agreement No 847639)

Invited Lecture 21

Kinga Nyíri: Antirepressor specificity is shaped by highly efficient dimerization of the repressors in regulation of staphylococcal pathogenicity islands

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Pathogenicity islands in Staphylococci are regulated by Stl repressors that form strong dimers. It has been recently shown that SaPIbov1-Stl dimers are separated during the activation of the *Staphylococcus aureus* pathogenicity island (SaPI) transcription via helper phage proteins. To understand the mechanism of this regulation, a quantitative analysis of the dimerization characteristics is required. Due to the highly efficient dimerization process, such an analysis has to involve specific solutions that permit relevant experiments to be performed.

In the present work, we focused on two staphylococcal Stls associated with high biomedical interest, namely Stl proteins of *Staphylococcus aureus* bov1 and *Staphylococcus hominis* ShoCI794_SEPI pathogenicity islands. Exploiting the interactions of these two Stl proteins with their antirepressor-mimicking interaction partners allowed precise determination of the Stl dimerization constant in the subnanomolar range.

Consequently only an intricate, fine-tuned interaction network between repressor:antirepressor complexation can adeptly compete with the highly efficient dimerization of the Stl repressors. In addition we showed at the first time that the strong interaction within the Stl repressor dimer dramatically interferes with the measurement of repressor:antirepressor equilibrium binding constant. Our results thus propose a generally applicable experimental approach to study other similar complexes.

Invited Lecture 22

Viktor Viglasky and Lukas Trizna: Non-canonical structural motifs of nucleic acids

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Nucleic acids can adopt various secondary structures, depending mainly on their sequence. These structural forms play a key role in the regulation of gene expression in cells due to their unique properties. A major challenge is to identify such sequences in the genome in order to predict their formation and to find a specific target molecule to either facilitate or eliminate occurrence of such structures. An alternative approach to search for putative sequences has recently been developed in our laboratory [1,2]. In addition, non-canonical motifs are contained in many aptamers that are applied in biomedical research as sensing molecules and have recently been used to functionalize next-generation nanoparticles.
Our recent advances in the structural stability of tetrahelical non-canonical forms of nucleic acids will be presented, as well as an entirely new motif predicted by us. In addition, DNA nanocircles and three-way junctions consisting of noncanonical motifs will be described.

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Invited Lecture 23

Andreas Schlundt: Using integrated structural biology to determine the specificity in RNA-protein interactions

Biocentrum, Goethe University Frankfurt, Frankfurt am Main, Germany

The regulation of gene expression is majorly steered on the level of proteins interacting with nucleic acids. An essential contribution is found in the formation of regulatory complexes between proteins and RNAs. Seeing the unexpected large number of approximately 2000 human RNA-binding proteins (RBPs) interacting with the limited chemical space of nucleotides, we are still at the beginning of understanding the hidden rules of specificity between RNAs and proteins. As some of the most basic concepts RBPs by default use multiple specialized RNA-binding domains (RBDs) to increase specificity. Also, RNAs represent specificity as targets via complex folds and intrinsic flexibility, which are often impossible to predict from their sequences.
High-resolution structures of RNA-protein complexes (RNPs) have and will yield relevant insights into the molecular parameters for specificity. Cryo-EM has tremendously enlarged the space for RNP structure determination of macromolecular complexes. However, large RNAs remain challenging in light of their dynamic heterogeneity, and the same is true for intrinsically disordered proteins that have recently emerged as RBPs. Some of those bottlenecks may be overcome with the integration of multiple methods, including structure prediction via molecular modeling and simulations. Modern approaches may thus, on the longer run, also help in the rational design of proteins and RNAs to specifically interact with each other.
In this lecture I will give an overview of challenges in analyzing RNPs on a structural level as well as a short introduction into combined experimental approaches applicable to this end. I will use examples from our own lab's previous and current projects, including different types of RBP, RBDs, and RNA elements. A focus will be on the interaction of highly specialized proteins with regulatory regions in mRNAs, which we investigate using X-ray crystallography; but more extensively with the solution methods NMR spectroscopy and small-angle X-ray scattering (SAXS), supported by solid wet-lab biochemistry and biophysics. The lecture will provide insight into the complementary utilization of methods to comprehensively study RNPs starting from structural information.

Invited Lecture 24

José A. Brito: Structural and functional insights into hydrogen sulfide homeostasis in pathogenic bacteria

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Keywords: *hydrogen sulfide, sulfur-metabolising pathways, pathogenic bacteria, X-ray crystallography, cryo-EM*

Hydrogen sulfide is an ancient molecule present in Earth's primordial atmosphere and organisms from all Domains of Life soon evolved to utilize it in their physiology. However, H_2S can have either beneficial or toxic effects, depending on the concentration. Therefore, tight regulation of intracellular H_2S/H_2S-derived more oxidized reactive sulfur species (RSS) is paramount for survival of all organisms. In bacterial pathogens, H_2S/RSS is regarded as an important component in microbial defence mechanisms against oxidative and antibiotic stress.

The *cst* operon in *Staphylococcus aureus* encodes a nearly complete mitochondrial-like H_2S oxidation system. In addition, a *cst*-like operon has also been described in the human pathogen *E. faecalis*. Three enzymes encoded by these two operons include the coenzyme A persulfide reductase CoAPR, the multidomain persulfide dioxygenase-sulfurtransferase fusion protein CstB and the sulfide:quinone oxidoreductase SQR, which collectively protect the organism against H_2S and RSS toxicity.

Herein, we describe the X-ray crystallographic structures of full-length *Sa*CstB (native and single cysteine substitution mutants) and the CoA-bound crystal structure of *Ef*CoAPR. Companion cryo-EM data on these enzymes suggest a high mobility of the C-terminal rhodanese domains that may be important for catalysis. The structures of sulfite-bound mutant CstBs suggests a mechanism by which the C-terminal domain facilitates the concerted oxidation of a thiol persulfide (RSSH) to thiosulfate and thiol, without the release of the toxic sulfite intermediate.

These studies provide an enhanced understanding of the mechanisms of H_2S/RSS homeostasis encoded by the RSS-regulated *cst* operons in bacteria, and were possible through an iNext-Discovery funded access proposal (PID 16108).

Invited Lecture 25

Francois-Xavier Theillet: In-cell structural biology using NMR: overview and latest developments to depict IDPs at 310K

Institute for Integrative Biology of the Cell, Université Paris-Saclay, Gif-sur-Yvette, France

In-cell structural biology by NMR is appealing in many regards: It proposes, among others, to investigate conformational equilibria or ligand binding/processing in very relevant conditions, i.e in cells [1,2]. We will briefly describe the passed and present experimental conditions exploited in the field, and give an overview of the contributions and limits of in-cell NMR.
The approach comes with a number of challenges, among which i) the many difficulties in sample production, and ii) important signal losses due to promiscuous, transient interactions with cellular entities, which, in turn, urges to use (too) high concentrations of the studied proteins. We will show how we and others are trying to facilitate in-cell NMR studies, using new production methods in situ, new labeling schemes, and better adapted pulse sequences.

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Poster Presentations

P1. Stabilization of proteins with cyclodextrins

György Tibor Balogh[1,2], István Puskás[2], Zoltán Fülöp[2], Levente Szőcs[2], Lajos Szente[2]

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The efficient physical stabilization of aqueous protein solutions has long been a challenge in biotechnological process development as well as designing safe and effective biopharmaceutical formulations. Among the number of technical possibilities, the utility of stabilizing excipients, primarily of non-ionic surfactants, such as different grades of Tween® (polyoxyethylene sorbitan monooleate or polyoxyethylene sorbitan monolaurate), carbohydrate additives such as low molecular weight monosaccharides, sugar polymers, dextrans, cyclodextrins (CDs), heparinoids are the most frequently applied additives.
The stabilization of biologicals such as therapeutic peptides, proteins, monoclonal antibodies etc. is required to make these potential therapeutic agents druggable formulations with acceptable storage shelf-life and maintained therapeutic efficacy. Certain protein aggregates are thought to be responsible for a number of important pathological conditions such as ageing, dementia, Alzheimer's disease etc.

By the example of a number of cyclodextrin-based, industrially applicable solubilization strategies are discussed exploiting the inclusion complex forming ability of these carbohydrates resulting in improved peptide/protein stabilizing effect. Especially, the use of parenterally applicable cyclodextrins, such as non-ionic (2-hydroxypropyl)-beta-cyclodextrin (hydroxypropyl betadex) and polyanionic sulfobutylether-beta-cyclodextrin (betadex sulfobutylether sodium) are highlighted. A probable mechanism of the stabilizing effect is based on the complex formation between lipophilic domains of the peptides/proteins (inclusion of the aromatic amino acid residues) and cyclodextrins. A potential additional electrostatic effect may contribute to the stabilization, if high charge density, polyanionic cyclodextrin derivative is applied in the peptide/protein formulation.

P2 AlphaFold2 and NMR structures of antifugal disulfide proteins

Gai Jiawei[1], András Czajlik[1] and Gyula Batta[1]
[1]*Research Group of Structural Biology, Institute of Chemistry, University of Debrecen, Hungary*

The COVID-19 pandemic caused more than 5 million victims worldwide from the 2019 outbreak. We have recently applied the STD-NMR method disclosing the binding site of SARS-COV-2 spike protein to the anti-cancer drug Rucaparib [1]. Some of our antifungal disulfide proteins also showed anti-corona virus activities [2] and their in-vitro and biological tests are now in progress. We continue intensive structure, dynamics, function studies of the three clusters [3] of small antifungal proteins. Now, we started the application of AI methods, e.g. AF2 [4] to speed up protein structure determination in line with experimental NMR data. Earlier we have submitted ca. ten solution NMR structures to Protein Data Bank that are examined now using AF2. According to our experience the AF2 prediction of the antifungal protein folds from sequence only (50-60 residues) are highly accurate and reliable including disulfide patterns[5], in agreement with recent reports[6]. Even in the case of the highly dynamic NFAP2, the application AF2 may be of help.

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P4. Molecular interactions underlying amyloid formation and stability

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Understanding the cause and mechanism of how amyloid-like protein aggregates form is still challenging. By studying the formation of oligomers and characterising protofibrils, a more accurate picture of the drivers of protein aggregation can be obtained. Due to the difficulties of growing single crystals and the aggregation that causes signal broadening in NMR, a more widely applicable technique was needed to provide accurate information about the backbone conformation of proteins. The elongation phase, which is important for amyloid formation, was studied using ECD, VCD, IR spectroscopy and AFM. Examining APR (Aggregation Prone Regions) sequences, which are thought to be the drivers of protein aggregation, we studied the oligomer formation of the GNNQQNY model.

We discovered two complex and intriguing interactions involving the fourth and fifth glutamines after analysing the crystal structure. The amino acids mentioned above stabilise the assembled system not only by intermolecular but also intramolecular bonds, in addition to the hydrogen bond formed by the peptide backbone. The hydrogen bonds were broken by glutamine -norleucine substitution (Gln**X**Nle), and the resulting structure confirmed our expectations.

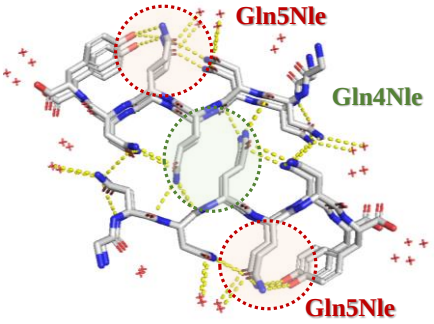

Figure 1.: Crystal structure of GNNQQNY heptapeptide (1YJP)

The glutamine in the fourth position acts as a kind of spring spacer, making it more hydrophobic. This results in closer contact at the dry zip interface, creating a more rigid structure. The additional immobility caused by the aromatic interaction prevails through the immobility of the tyrosine side chains located at the terminals. When the aromatic side chain stabilisation is removed by Gln5Nle substitution, a more flexible, less rigid structure is obtained.

Based on this, if a protein sequence contains sequences that are prone to aggregation, and a mutation occurs within it that makes this section hydrophobic, this will lead to very rigid, probably irreversible amyloid formation, the mutation can have a pathological effect. On the other hand, if the mutation disrupts structure-stabilising interactions and also retains its pathological nature, it may lead to a polymorphism that further complicates the understanding of amyloid formation processes. This makes it more difficult to develop drugs for targeted treatment.

Understanding the interactions that stabilise the amyloid structure may open up new path for the development of therapies aimed at inhibiting amyloid plaque formation by reducing stability or disrupting the structure to make the surface more accessible to targeted drug treatment.

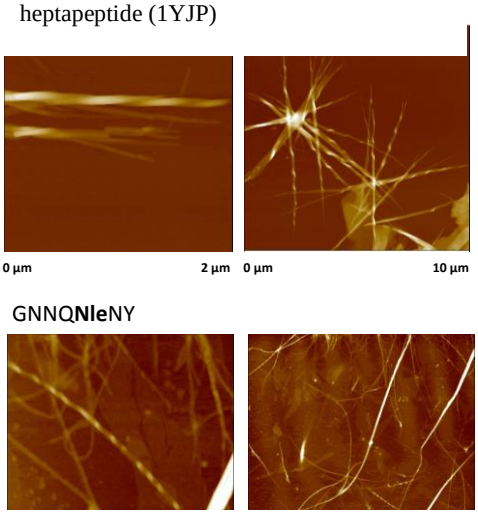

Figure 2.: AFM images of GNNQQNY variants. Gln5Nle (top panel), Gln4Nle (bottom panel).
c=10mg/ml, pH=3.6, t=24 h mixing at 37°C

P5 How Euro-BioImaging can support your research with access to the best imaging tools

Johanna Bischof

Euro-BioImaging ERIC, Turku, Finland

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P6 Discrimination of anomeric chimera oligopeptides using cím-MS and NMR

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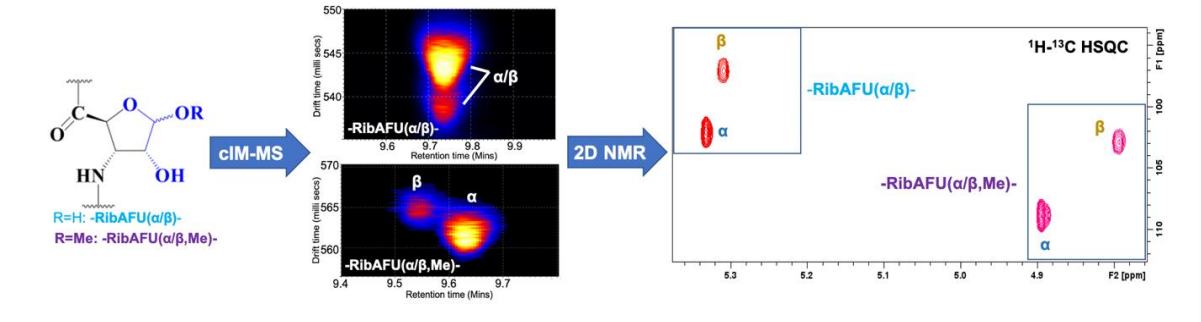
β-Sugar Amino Acids (βSAAs) have been developed as building blocks of chimeric peptides. The furanoid βSAA building block was a good alternative to α-amino acid to construct biologically relevant oligopeptides. This had tunable hydrophilicity as 1,2-*O*-isopropylidene protecting group was selectively removable. The free OH-1 in furanoid βSAA-containing peptides was a mixture of the α- and β- anomers in equilibrium. In our research, cyclic ion mobility-mass spectrometry (cIM-MS) and nuclear magnetic resonance spectrometry (NMR) techniques were used to discriminate the α/β-anomers. To block the mutarotation in solution of -RibAFU(α/β)-chimeras, the formation of methyl glycosides was performed to achieve corresponding -RibAFU(α/β,Me)-peptides. We explored the combination of cIM-MS and NMR was efficient to separate and identify the anomers of -RibAFU(α/β)-/-RibAFU(α/β,Me)-containing chimeric peptides.



P7 Structures of calmodulin-melittin complexes show multiple binding modes lacking classical anchoring interactions

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Interaction of calmodulin (CaM) and melittin is widely used as a test system for method development, and as a reference in studies of calmodulin interactions. However, experimental results on the interaction pattern of this complex were non-conclusive so far. Our aim was to explore the fine details of this interaction. Our crystallographic study revealed multiple different binding modes: we solved the structures of CaM from two species complexed with melittin - presenting three variants of melittin conformation, anchoring residues as well as CaM domain orientation. Our succeeding MD simulations revealed that these binding modes could transform to each other. These are not species-specific binding modes, but instead representations of a highly dynamic complex where melittin has adjacent alternative residues to form the key secondary interactions. We concluded that the nanomolar affinity binding of this complex is realized by a ‘slippery’ binding way and interchanging interactions [1].

The project was supported by project no. VEKOP-2.3.2-16-2017-00014, and VEKOP-2.3.3-15-2017-00018 by the European Union and the State of Hungary, co-financed by the European Regional Development Fund.

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P8. CryoET analysis of rod outer segments: is PDE6 the molecular driver of the light-induced photoreceptor morphological changes?

Vineeta Kaushik[a], Luca Gessa[b], Sławomir Tomczewski[ab], Sathi Goswami[b], Łukasz Olejnik[b], Nelam Kumar[b], Humberto Fernandes[#b]

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Sight is perhaps the most spectacular human sense, and understanding the molecular mechanisms underlying vision is of great importance. The conversion of photons to electrical pulses relies on the phototransduction cascade, which relies on many proteins at the outer segments of photoreceptors and on the stacking of discs. In addition to the phototransduction activation, light stimulus also evokes morphological changes in the photoreceptors [1], but the molecular determinates of this phenomenon have remained elusive. Recent evidence suggests a phosphodiesterase enzyme, PDE6, which is at the core of the phototransduction signal pathway and is attached to the photoreceptor's discs, may bridge consecutive discs [2]. PDE6 can adopt different conformations that render it with different protein lengths [3,4], opening the possibility that it is the molecular driver of the morphological changes observed.

In this study, to shed light on the molecular mechanisms underlying the observations, we employed cryoET to test our hypothesis. We isolated rod PDE6 and full outer segments (ROS) to test our theory in the cellular environment. Despite the resolution limitations of tomography, we calculate ROS averaging disc spacing under different conditions to gain inside into the vision mechanisms and add to the "big picture" of the observed morphological changes. Notably, the measurements are done with PDE6 in its natural environment and in the presence of relevant membranes, with such knowledge may be useful for therapeutics, diagnostics and functional imaging of photoreceptor physiology.

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Funding: iNEXT discovery (This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 871037) The International Centre for Translational Eye Research (MAB/2019/12) project is carried out within the International Research Agendas programme of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund. PASIFIC, Maria Sklodowska-Curie sponsored fellowship (This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Maria Sklodowska-Curie grant agreement No 847639)

P9. Polymorphic Amyloid Nanostructures of Hormone Peptides Involved in Glucose Homeostasis: Designed for Reversible Amyloid Formation

Dániel Horváth, Zsolt Dürvanger, Dóra K. Menyhárd, Máté Sulyok-Eiler, Fruzsina Bencs, Gergő Gyulai, Péter Horváth, Nóra Taricska and András Perczel

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Laboratory of Structural Chemistry and Biology, Eötvös Loránd University, Institute of Chemistry, Budapest, Hungary
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A large group of hormones are stored as amyloid fibrils in acidic secretion vesicles before they are released into the bloodstream and readopt their functional state. We identified an evolutionarily conserved hexapeptide sequence as the major aggregation-prone region (APR) of gastrointestinal peptides of the glucagon family: xFxxWL. We determined nine polymorphic crystal structures of the APR segments of glucagon-like peptides 1 and 2, and exendin and its derivatives. We followed amyloid formation by ECD, FTIR, ThT assays, and AFM. We propose that the pH-dependent changes of the protonation states of glutamate/aspartate residues of APRs initiate switching between the amyloid and the folded, monomeric forms of the hormones. We found that pH sensitivity diminishes in the absence of acidic gatekeepers and amyloid formation progresses over a broad pH range. Our results highlight the dual role of short aggregation core motifs in reversible amyloid formation and receptor binding.

P10 Mapping of the reaction route of amide cis-trans interconversion

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[2]*Laboratory of Structural Chemistry and Biology, Eötvös Loránd University, Institute of Chemistry, Budapest, Hungary*
[3]*ELKH-ELTE Protein Modeling Research Group ELTE Eötvös Loránd University, Pázmány Péter sétány 1/A, H-1117 Budapest, Hungary.*

In spite of their rare frequency, non-proline *cis* peptide bonds unequivocally identified in protein structures occur at or near functionally important sites more often than if their spatial distribution was random. In case of peptidyl-prolyl *cis/trans* isomerase enzymes, one of the proposed reaction mechanism suggests an acid-base reaction step. The *pH* dependence of *cis/trans* isomerization of Xxx-nonPro amides was studied using *N*-methylacetamide, as a peptide bond model with DFT, CCSD and CCSD(T) calculations in water solvent model. The solvent model can be expanded with introducing at least 2 H_2O molecules to the system to study the possibly formed H-bonds. In this case a low-energy transition state can be found between the *O*- and *N*- protonated structures at (ω= -139.6°, α= +41.5°) and at (ω= -1.3°, α=+46.2°) (where α is a dihedral angle, measuring the extent of pyramidality) with net energy cost 11.6 kJ·mol^{-1} and 9.4 kJ·mol^{-1}, respectively, lower than the isomerization energy barrier of 85 kJ·mol^{-1} found in literature. From IRC calculations it was found, that the *N*-protonation of the peptide bond may occur through this key transition state started from an initial *O*-protonated state. Our calculations reveal a possible low-energy isomerization route which can be a possible mechanism for the secondary peptide isomerase enzymes.

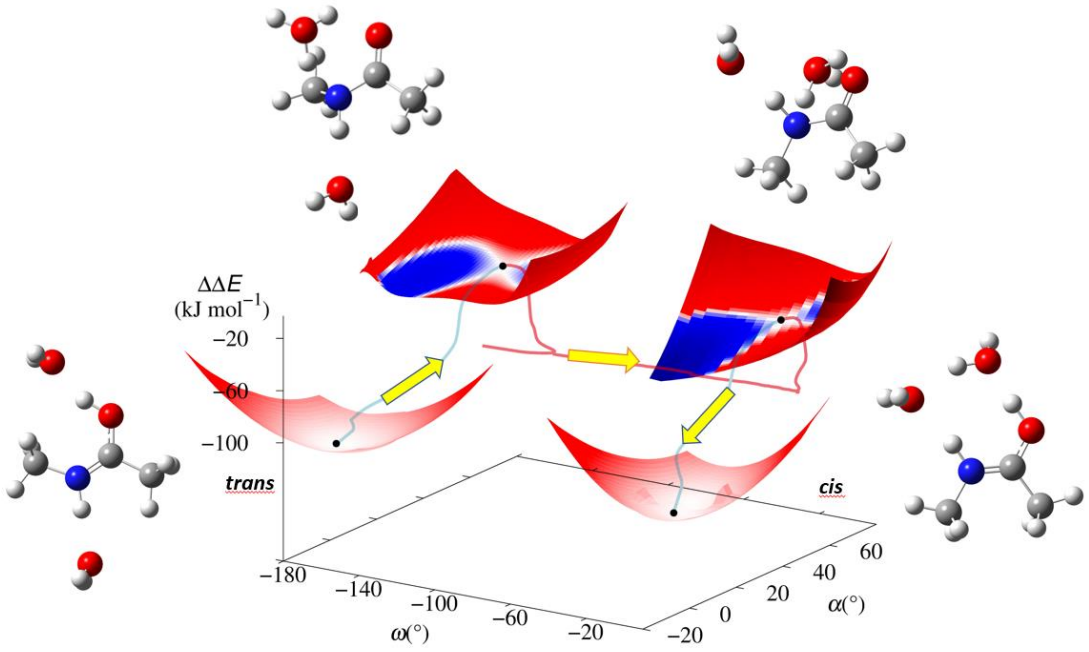

Figure 1: The O→N proton transfer of the protonated amide, as the critical step of the acid catalyzed amide *cis/trans* isomerization

P11 SAXS Mail in on B21

Nikul Khunti, Nathan Cowieson, Katsuaki Inoue, Jodie Lavender, Robert P. Rambo,

Diamond House, Harwell Science and Innovation Campus, Oxfordshire, United Kingdom

B21-BioSAXS
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Nikul Khunti, Nathan Cowieson, Katsuaki Inoue, Jodie Lavender, Robert P. Rambo, Diamond

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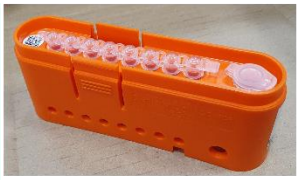
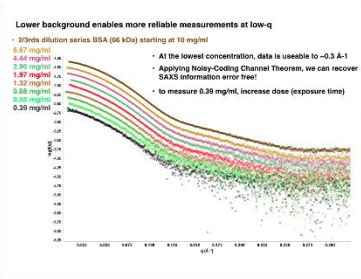


Mail-In SAXS Shipping Container



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- Require at least 500 µl matched buffer

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P12 Cryo-EM structure of acylpeptide hydrolase: substrate selection by a multi state serine-protease triad and inhibition by Meropenem

Anna J. Kiss-Szemán[1], LucaTakács[1], Zoltán Orgován[2], Pál Stráner[3], Imre Jákli[3], Naoki Hosogi[4], Simonas Masiulis[5], Gitta Schlosser[6], Veronika Harmat[1,3], Dóra K. Menyhárd[3], András Perczel[1,3]

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AAP is catalyzing the removal of *N*-acetylated amino acids from the *N*-terminus of oligopeptides and proteins[1] thus contributing to the upkeep of healthy protein-homeostasis,[2] exerting an influence over the functioning of the proteasome.[3] As such, AAP has been indicated as a possible pharmacological target in certain types of cancers.[4-6] AAP can also function as an endopeptidase, taking part in the recognition and disassembly of damaged or misfolded proteins[7] and has been proposed as being capable of cleaving amyloid aggregates[8] through which it was suggested as a modulator of cognitive enhancement processes.[9] It was shown to contribute to DNA damage repair mechanisms[10] and to the membrane localization of oncogenic protein K-Ras possibly through its interaction with phosphatidylserine.[11]
AAP is also the site of a drug-drug interaction (DDI) between a widely used antipsychotic, valproate (more precisely its glucuronated metabolite, VPA-G) and carbapenem antibiotics.[12] The inhibition of AAP is irreversible, so fast and effective that carbapenem antibiotic meropenem (MEPM) could be successfully applied in cases of VPA overdose in emergency medicinal practice, inducing its fast clearance.[13-15]

The determined structures[16-17] - which are, to the best of our knowledge, the first structural information concerning the association between a β-lactam antibiotic and a mammalian enzyme (that shares an over 90% sequence identity with its human counterpart) - can form the basis of a better understanding of AAP's function and possible new roles in therapy, as well as providing a glimpse at the mechanisms by which antibiotics might produce side effects in human physiology.

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P13. How much do we know about the secondary structural propensities of IDPs? What does the random coil chemical shift prediction show and what is hidden?

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Intrinsically disordered proteins IDPs – constituting a large portion of the protein world and having been associated with various types of diseases – lack the classical secondary structural motifs (helices, sheets). Instead, they have preferred conformational states – similar to the classical motifs – called structural propensities. Regions with structural propensities seem to hold the only hints which could make IDPs targets of rational drug design.
While the identification of secondary structural motifs by the so called secondary chemical shift (SCS) method is routinely preformed for folded proteins, it is a challenging task for IDPs. The main reason is the ambiguity in the random coil chemical shift (RCCS) values that are necessary to calculate SCSs. This ambiguity appears as a 'noise' in SCS calculation for IDPs and it might cover up real tendencies or create false ones.
We demonstrate that while using different RCCS predictors for the same experimentally determined chemical shifts has very little effect in the case of folded proteins; in the case of IDPs it can lead to contradictory conclusions. As an escape, we recommend statistical methods for the analysis of SCS data. The sum of ranking differences (SRD) is applied for finding the most consensual one(s) from among a set of RCCS predictors. We also introduce a novel metric and two related statistical tests based on Cα and Hα SCSs for characterizing the self-consistency of RCCS predictors. The presented results are expected to, facilitate the development of better RCCS predictors, deepen our understanding of IDPs and contribute to their pharmaceutical targetability.

Reference:

D. Kovács, A. Bodor, The influence of random-coil chemical shifts on the assessment of structural propensities in folded proteins and in IDPs, *RSC Advances,* 13 (2023) 10182-10203

P14. Structural elucidation of Retinol binding protein 3: One step closer to unfolding the effect of ligands on the conformation of the protein.

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RBP3 is a protein secreted in the interphotoreceptor matrix by photoreceptors cells, involved in the visual cycle, shuttles ligands between them and retinal pigment epithelium (RPE) cells. So far, there is no high-resolution, full-length structure available for this protein. Mammalian RBP3 is a single polypeptide with four modules, each containing ~300 amino acids. At least two conformational forms of the RBP3 exits, the elongated and bent ones, and each is favoured upon specific ligand binding. Even though decades of ligand bind assays and protein characterization went by, the details of the conformation changes after binding with ligands are still unresolved and need to be explored structurally.

In this study, the RBP3 protein was isolated from a native biological source and purified using different chromatography techniques. The most emerging technique CryoEm was used to investigate the structure of RBP3 protein at a high resolution to better understand the role of the cooperativity mechanism of RBP3 and open the possibility of structure-based drug design. We have also used the Small Angle X-Ray Scattering (SAXS) method to get more structural information about the protein upon titration of natural ligands. We gained iNEXT-discovery access to CEITEC, Brno and EMBL, Dessy, Germany, to achieve the objectives mentioned above.

This work provides an enlighted structure of RBP3 protein at a much higher resolution structure than previously available, around 4.3 Å, and where you can easily observe the flexibility of the protein among all four modules. Apart from the structural aspects, the data obtained from SAXS using protein in solution form with different retinoids shows the gradual conformational changes in the protein. With the SAXS data, it is clearly visible that the retinoids and fatty acids have different impacts on the conformational changes of the protein.

The obtained results allow exploring more about RBP3 in a correlation of structure-based drug design to minimize the impact of Diabetic retinopathy (DR). RBP3 has an anti-correlation property with DR, and the potential monitorization of RBP3 in DR at an early stage could be a ray of hope in developing new therapeutic approaches for DR.

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P15 Basic residues are associated to functional phosphorylation sites in the Unique domain of c-Src

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In contrast to the well-studied canonical regulatory mechanisms, the way by which the recently discovered Src N-terminal regulatory element (SNRE) modulates Src activity is not yet well understood. Phosphorylation of serine and threonine residues modulate the charge distribution along the disordered region of the SNRE and may affect a fuzzy complex with the SH3 domain that is believed to act as an information transduction element. The preexisting positively charged sites can interact with the newly introduced phosphate groups by modulating their acidity, introducing local conformational restrictions, or coupling various phosphosites into a functional unit. In this paper we use pH dependent NMR measurements combined with single point mutations to identify the interactions of basic residues with physiologically important phosphorylated residues and to characterize the effect of these interactions in neighbor residues, thus providing insight on the electrostatic network in the isolated disordered regions and in the entire SNRE. From a methodological point of view, the linear relationship observed between the mutation induced pKa changes of the phosphate groups of phosphoserine and phosphorthreonine and the pH induced chemical shifts of the NH groups of these residues provides a very convenient alternative to identify interacting phosphate groups without the need to introduce point mutations.

P16. A novel Semaphorin-5A fold variation enables bifunctional glycosaminoglycan specificity and regulates signalling strength.

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Semaphorin-5A (Sema5A) is a bifunctional guidance cue exerting attractive and inhibitory effects with heparan sulfate (HS) and chondroitin sulfate (CS) glycosaminoglycans (GAGs), respectively. Sema5A harbors seven thrombospondin type-1 repeats (TSR1-7) important for GAG binding, the underlying molecular basis and functions *in vivo* remain enigmatic. Using x-ray crystallography we reveal a novel dimeric fold variation for TSR4 that accommodates GAG interactions. TSR4 co-crystal structures identify GAG binding residues validated by site-directed mutagenesis. Using *in vitro* and cell-based assays we uncover specific GAG epitopes necessary for TSR association. We demonstrate that HS binding is preferred over CS and mediates Sema5A oligomerization. *In vivo*, Sema5A:GAG interactions are necessary for proper distribution of dentate progenitor cells in the hippocampus, and we provide evidence that the Sema5A:GAG interaction regulates signalling strength through Plexin-A2.

P17. A NEW SPIDER PEPTIDE THAT AFFECTS THE KV1.5 VOLTAGE-GATED POTASSIUM CHANNEL, MAKING IT A POTENTIAL ANTIARRHYTHMIC AGENT

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Spider venoms include various peptide toxins that modify the ion channel currents, mainly of excitable insect cells. Consequently, scientific research on spider venoms has revealed a broad range of peptide toxins with different pharmacological properties, even for mammalian species. The human voltage-gated potassium channel Kv1.5 is an important component of the atrial action potential. Its mutations are associated with hereditary forms of atrial fibrillation. It is therefore a potential target for atrial fibrillation therapy.
The effects of thirty different animal venoms were tested by patch-clamp measurements on the human Kv1.5 ion channel expressed in CHO cells. The complete venom of the *Ocolicosa supermirabilis* spider showed an inhibitory effect on the Kv1.5 channel. In order to identify the active component, reverse-phase HPLC fractionation was performed. One fraction showed an effect on Kv1.5 similar to that of the whole venom. The primary amino acid sequence of this isolated active peptide (designated Osu1) was determined. Prior to this work, recombinant Osu1 was produced using *E. coli*. In addition, the 3D structure was predicted using Modeller, based on the solved structure of OtTx1a (PDB: 2n86) and thus a disulfide bridge pattern was obtained: C28-C40, C10-C26, C19-C42, and C17-C56. Due to the low yield using *E. coli* as expression system and based on the 3D structure prediction, Osu1 was synthesized by a company. Unfortunately, the inhibitory effect on the Kv1.5 channel observed with the native and the recombinant Osu1 could not be reproduced with the synthetic Osu1, suggesting that the predicted disulfide bridges may be incorrect. To the best of our knowledge, this is the first study which examine the venom of the spider *Oculicosa supermirabilis*. It would be a big step forward if we could somehow produce the native structure of Osu1 for further experiments.

P18 Initial insight into the proline-rich region of the postsynaptic Shank3 protein

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Shank3 is a postsynaptic scaffolding protein involved in the organization and function of excitatory synapses. Its mutations have been implicated in various neurological disorders, including autism spectrum disorder (ASD) and schizophrenia. Shank3 is a modular protein containing globular domains and a long intrinsically disordered segment. The function of its proline-rich segment is undoubtedly important as judged by the presence of ASD-linked mutations in the region, however, we do not have any detailed information about its exact mode of action, structural preferences or internal dynamics. This region contains a binding motif for the EVH1 domain of a partner scaffold protein, Homer.

The aim of this study was the production of the Homer EVH1 binding segment of the Shank3 proline-rich region (S3-PRO) for *in vitro* functional and structural investigation, including NMR measurements.

After codon optimization, BL21(DE3) cells were able to express the protein constructs, which had excellent solubility, but were susceptible for fragmentation. Gel chromatography analysis revealed that S3-PRO constructs have a large hydrodynamic radius, and low mobility on PAGE gel. According to CD spectroscopy analysis and thermal shift assay, S3-PRO construct can be characterized by approximately 50% disordered, 20% turn and 25% beta sheet structural elements, and is largely temperature resistant. Preliminary interaction studies have shown that the strength and kinetics of Homer binding is affected by the length of the S3-PRO constructs used.

Our results demonstrate that recombinant bacterial expression of functional S3 PRO protein constructs is feasible. The expressed regions exhibit properties typical for disordered proteins. The length-dependence of the Homer interaction is largely unexpected and requires further investigations.

P19. Mechanism of asparagine deamidation – tunneling in tetrapeptides?

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It is well-known that NG-peptides transformed into α- and β-DG derivatives with ~1:4 ratio during deamidation and hydrolysis under physiological conditions, but the exact mechanism is yet unclear. Our scientific goal was the investigation of –Asn-Gly– (NG) motif containing peptides, in the interest of the isomerization mechanism.
In order to this Ac-NGXA-NH_2 (X: R(+), K(+), A, E(-)) modelpeptides were used for NMR spectroscopy experiments. With the help of integral values proportional to concentration of 1D NMR experiments, the effect of temperature and the charge of the amino acid following the NG sequence on the reaction rate of the peptide isomerization has been determined, by determining the value of the reaction rate coefficients (assuming a first order kinetics), half-lives and activation energies, in order to establish a more comprehensive kinetic model. The primary kinetic isotope effect was determined at different temperatures in buffer in order to distinguish whether reaction goes either by classical and/or quantum tunneling mechanism.
Furthermore, the step-by-step understanding of the reaction was investigated with QM calculations (Gaussian). For this, the full geometric optimization of the molecules (B3LYP/6-31+G(d) and B3LYP/6-311++G(d,p)) and the analysis of their natural bond orbitals (NBO) were performed.

P20. At the mitochondrial level we are electric living beings

How properties of electron and proton help the reaction rate in mitochondria?

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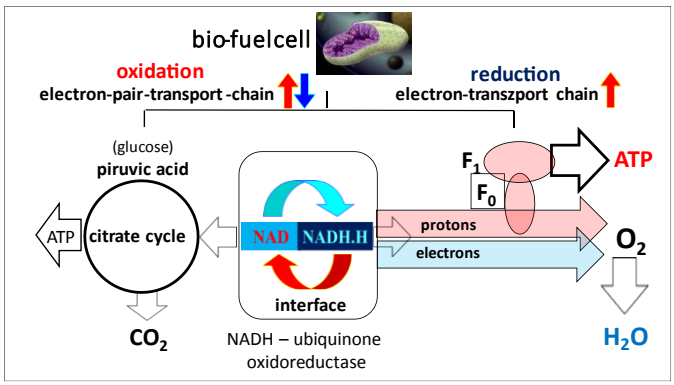
Due the space-separated oxidation-reduction and continuous operation, the mitochondrion is a biological fuel cell (*Fig.*)



In mitochondrion the electric conduction takes place by consecutive redox reactions on both sides. According to the law of electro-neutrality, the charge does not accumulate, therefore the electron and proton current are connected to each other, and determined by the rate of reactions. Consequently, the properties of electrons and protons play a key in the redox conduction. The reaction mechanisms help to find the mechanisms of electric conduction. The nucleophile reactions of the oxidation side take place with electron-pair rearrangement. The oxidation by NAD and reduction NADH.H happen with hydride ion transfer by two electrons - three centre bond. The proton is a "bridge" for moving electron-pair in the oxidation of iso-citric acid, which takes place by enzymatic synchronous rearrangement. The consequence of the group transfer charge separation is carbocation formation. During the reaction, the electron-pairs and the carbocations migrate in opposite directions, like in semi-conducting polymers. The group transfer reactions and the synchronous electron pair rearrangement of the consecutive redox reactions together form an electron pair transport chain to NAD.
On the reduction side, the electron transport chain starts active proton transport by the proton pumps. During the oxidation step in the cytochromes, the Lewis acidity of the Fe^{2+} ion increases, so the histidine ligand becomes a stronger Bronsted acid and lost its proton. The intermembrane space becomes acidic, which causes a hydrogen bonds network to form on the intermembrane space side of the inner membrane. If a driving force is present, the structure becomes a charge-transporter with the synchronous movement of protons towards proton-utilising processes. The synchronous movement of electrons and protons ensures the fastest possible mechanism which is essential for the speed of biological energy transformation.

P21. PROLINE *CIS/TRANS* ISOMERIZATION IN INTRINSICALLY DISORDERED PROTEINS AND PEPTIDES

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Intrinsically disordered proteins and protein regions (IDPs/IDRs) play important roles in diverse biological processes. Lacking a stable secondary structure, they display an ensemble of conformations. One factor contributing to this conformational heterogeneity is the proline *cis/trans* isomerization. As the different conformational states can be responsible for different biological function, it is of utmost importance the knowledge and the value of a given *cis/trans* proline ratio. NMR spectroscopy is the only method to characterize the two co-existing isomers on atomic level, and only few works report on these data.

Our previous results and literature data suggest that the amount of the *cis*-Pro isomer in IDPs varies between 4-20% in solution[1]. Based on solution state NMR data, we collected new data and updated our previous dataset, and a statistical analysis was performed to determine which type of amino acids in the proline neighbouring region have an impact the *cis*-Pro content in the *i*±4 region, where *i* is the proline. Based on this, several regularities were formulated[2].

An increased >10% cis-Pro isomer can be found if there is an aromatic residue in *i*±1 positions. This is in agreement with previous literature findings. Furthermore, the *cis*-isomer is content is increased if negatively charged aspartic and glutamic acid are more frequent in *i*-2, *i*-1 and *i*+3 positions. Not only negatively charged but Pro residues in the *i*+1 position increase the *cis*-Pro content. Furthermore, i±4 residue type occurrence is significantly different from the reference.

An indicator of a decreased *cis*-Pro content (less than 5 %) is the presence of positively charged residues in *i*-3 and *i*-1 positions, as the electrostatic interaction between the positively charged side chain and the proline carbonyl group in *trans* isomer is energetically favoured. Data also show that Arg and Lys residues are more common for < 5% *cis*-Pro content.

Further on, we designed peptides to bring experimental proof to our observations based on the statistical analysis, and NMR spectroscopy was used to define the *cis*-Pro content on these model peptides[3]. Analysis of NMR spectra prove the dependence of the *cis*-Pro content on the type of the neighboring amino acid – with special attention on aromatic and positively charged sidechains. Our results may help in the design of protein regions with a given *cis*-Pro content, and they also contribute to a better understanding of the roles and function IDPs.

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P22 Analysis of intertwined side chains of amyloidogenic oligopeptide crystals: revisiting of amyloid interface descriptors

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Amyloid fibrils are a type of protein aggregates that are associated with both diseases (Alzheimer's, Parkinson's and type 2 diabetes) and biological functions (folding and reservoir). Amyloids feature a common cross beta spine but they are very diverse in overall structure [1]. The dry interface has a particular interest; they are thought to be a main driving force behind the amyloid formation. These interfaces have been studied with smaller peptide sequence models (based on amyloid prone regions, APRs) that are capable of self-assembly. In the last 15 years since the description of the 8 main topological classes [2], 170 APR structures have been deposited in the PDB. We use a uniformized calculation strategy of the common descriptors of contact area and complementarity of beta-sheets, and complement them with a novel descriptor. Using these tools of structural characteristics of amyloid structures are evaluated. The project was supported grants VEKOP-2.3.2-16-2017-00014, and VEKOP-2.3.3-15-2017-00018 by the European Union and the State of Hungary, co-financed by the European Regional Development Fund.

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P23. Protein diffusion under denaturing conditions and in crowded environments

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The translational diffusion coefficient (*D*) is an important hydrodynamic parameter that carries information about the size of the diffusing entity. Numerical values can be obtained by different PFG-NMR approaches, and multiple applications are available, such as characterization of mixtures containing small molecules and/or macromolecules (proteins, synthetic polymers).

In our previous work we did a comprehensive analysis on protein diffusion in dilute aqueous solutions, at 288K. We unambiguously distinguished folded and unfolded proteins with similar molar masses based on the fact that folded proteins are compact and nearly spherical, while intrinsically disordered proteins (IDPs) are elongated and flexible, and we established empirical relations between the protein molar mass (*M*) and their *D* value.[1] Further on, we investigated how these relations can be successfully extended to other temperatures. Moreover, we prove that this tool can be used to monitor folding-unfolding. To do so we tested different chaotropic media (DMSO, 8M urea), as well as the behavior of various globular proteins. Special attention was taken to the effect of disulphide bonds.[2]

As a continuation of our work, we intended to evaluate what is happening in cellular media. As macromolecular concentration may reach 400 mg/ml in cells, this can alter protein structure and function compared to that in dilute solutions. This condition is called “crowding”, as the macromolecules occupy a large part of the solution volume.[3] In a crowded environment, diffusion becomes obstructed and this influences material transport.

As the cytoplasm would be too complex, as an initial step we use model crowding agents: Ficoll PM 70 (branched synthetic polysaccharide, M_W = 70 000 g/mol) and the linear polyethylene glycol (PEG) with M_W = 20 000 g/mol. Before testing the behavior of proteins, the physico-chemical characterization of the media has to be performed. In this respect the effect of self-crowding on the diffusion of the crowding agents and the solvent water was investigated. The diffusion of these components cannot be described with the Stokes-Einstein equation, as excluded volume effect and hydrodynamic interactions should be considered.[4]

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P24. Molecular Pathomechanisms in Lipoamide Dehydrogenase Deficiency

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(Dihydro)lipoamide dehydrogenase (LADH) is a homodimeric flavin-disulfide oxidoreductase that catalyzes the oxidation of the multienzyme complex cofactor (dihydro)lipoamide, using NAD^+ as a co-substrate, serving as a common third subunit (E3) to the mitochondrial alpha-keto acid dehydrogenase complexes for pyruvate, alpha-ketoglutarate, alpha-ketoadipate and branched-chain alpha-keto acids. Clinically relevant, disease-causing mutations in hLADH may result in compromised protein stability, folding and catalytic activity, enhanced reactive oxygen species (ROS) generation, and/or dissociation from the above multienzyme complexes that affect several central metabolic pathways simultaneously and lead to the often prematurely lethal hLADH deficiency. We determined the high-resolution crystal structures of the wild type hLADH and nine of its disease-causing variants at resolutions ranging from 1.44 to 2.89 Å to analyze the structural bases of hLADH deficiency. The investigated substitutions reside in either the active site (P453L), the cofactor-binding region (G194C, I318T, I358T), or the dimer interface (G426E, D444V, I445M, R447G, R460G). The P453L substitution triggered a significant remodeling of the active site that correlates well with the almost complete lack of residual activity and the severe clinical outcomes. The G426E and G194C substitutions altered the charge distribution and dynamics near the nicotinamide binding site, but did not induce significant structural changes, which is in accord with the respective residual LADH activities and relatively milder clinical manifestations. The D444V, I445M, R447G and R460G substitutions all reside farther away from the active site on the homodimer interface of hLADH, however, they are still associated with significant losses in catalytic activity. Our structural data revealed considerable perturbations in the so-called H^+/H_2O channel, leading to the active site, for the above four variants. In I318T-hLADH, a crucial interaction of the catalytic base His-452 (with Glu-457) was slightly modulated; the minor stuctural changes are in accord with the virtually unaltered catalytic activity. Finally, the I358T substitution primarily perturbed an FAD binding interaction (with His329).

P25. ELECTROPHYSIOLOGICAL AND STRUCTURAL CHARACTERIZATION OF A RECENTLY DISCOVERED SCORPION TOXIN WITH SIGNIFICANT PHARMACOLOGICAL ACTIVITY

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Animal venoms are complex mixtures containing mainly enzymatic and non-enzymatic proteins or peptides. Understanding the structure and function of these biomolecules provides valuable knowledge for protein engineering since toxins might be used as templates for designing molecules with improved biological functions.[1] The recently discovered Cm39 peptide with 37 amino acid residues and a molecular weight of 3980.2 Da was identified in the venom of *Centruroides margaritatus* and was successfully synthesized by our collaboration partners. The synthetic Cm39 peptide blocks $K_V1.2$ and Ca^{2+}-activated potassium channels ($K_{Ca}2.2$ and $K_{Ca}3.1$) with nanomolar affinities while it does not affect several other K^+ and Na^+ channels.[2] To verify the primary structure of the peptide, different 2D homonuclear and heteronuclear NMR experiments were carried out. Namely, 2D ^{1}H-^{1}H CLIP-COSY, TOCSY, NOESY, ^{1}H-^{13}C HSQC, HSQC-TOCSY, HMBC and ^{1}H-^{15}N HSQC NMR spectra were recorded on the sample with natural isotopic abundance. After the analysis of these spectra, the suggested amino acid sequence of the molecule was confirmed by the complete assignment of ^{1}H, ^{13}C, ^{15}N resonances. In the next step, ^{1}H-^{1}H distance constraints extracted from the NOESY spectrum combined with torsional angle restraints from backbone ^{13}C chemical shifts were used for NMR-based 3D structural calculations. Until now, the disulfide bridge patterns cannot be determined unambiguously, thus the obtained Cm39 structural ensembles are being refined by molecular dynamics (MD) simulations.

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P26 Structural and dynamic determinants of site-selectivity in human ileal bile acid-binding protein

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Disorders in bile acid transport and metabolism have been related to a number of metabolic disease states, atherosclerosis, type-II diabetes, and cancer. Human ileal bile acid-binding protein (hI-BABP) has a key role in the cellular trafficking and metabolic targeting of bile salts. Its two internal binding sites exhibit positive cooperativity accompanied by a site-selectivity of glycocholate (GCA) and glycochenodeoxycholate (GCDA), the two most abundant bile salts in humans, differing in a single hydroxyl group in the 12α position of the steroid ring system. To improve our understanding of the structural and dynamic determinants of ligand binding, NMR chemical shift and spin relaxation analysis was carried out on wild-type and functionally impaired mutant hI-BABP-bile salt complexes. According to our analysis, while the homotypic GCDA (3α, 7α)-complex of wild-type hI-BABP shows a high degree of similarity to the thermodynamically most stable heterotypic hI-BABP:GCA:GCDA complex, GCA (3α, 7α, 12α) instead of GCDA at site 1 increases the flexibility of the protein backbone in both the E/F-portal region and the helical cap. Mutation Q51A, resulting in the loss of site preference of di- and trihydroxy bile salts, decreases the flexibility of the GCA-complex on both the ps-ns and μs-ms timescales. Regarding the GCDA-complex, while the contribution of slow exchange processes is prevalent in both the wild type form and in Q51A hI-BABP, local ps motions superimposed on slow fluctuations become dominant in the mutant. The increased local flexibility of key amino acids in GCDA-bound Q51A hI-BABP appears to interfere with the formation of stabilizing interactions in the binding pocket. Taken together, site-selectivity in hI-BABP is governed by a fine balance of enthalpic and entropic effects, where the affinity of bile salts is affected by long-range dynamic effects.

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P27. Investigating Carbohydrate-Galectin Interactions with Advanced Multinuclear NMR and Computational Methods

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Structural investigation of ligand-protein interactions is crucial for better understanding of biological processes at the molecular level. Among these binding interactions, the recognition of glycans by lectins is of particular importance in several diseases, such as cancer, therefore, inhibition of glycan-galectin interactions represents a promising perspective towards developing therapeutics controlling cancer development.

As a step forward, our goals were to design and probe multiple human Galectin-3 (*h*Gal-3) inhibitors including two selenium (Se) containing glycomimetics, specifically that of di(β-D-galactopyranosyl)selenide (SeDG), in which two galactose rings were linked by one Se atom and a di(β-D-galactopyranosyl)diselenide (DSeDG) analog with a diseleno bond between the two sugar units.

The binding affinities of these derivatives to *h*Gal-3 were determined by ^{1}H-^{15}N HSQC and competition STD NMR experiments in solution, indicating a slight decrease in the strength of interaction for SeDG compared to thiodigalactoside (TDG), a well-known inhibitor of *h*Gal-3, while DSeDG displayed much weaker interaction strength.[1] The recent introduction of ^{77}Se NMR spectroscopy for monitoring the binding of selenoglycosides to galectins triggered interest to optimize the sensitivity by increasing the ^{77}Se content from the natural 7.63% abundance to 99%. Thus, we have synthesized the ^{77}Se-enriched selenodigalactoside ([^{77}Se]DG) and have also demonstrated that the improved detection sensitivity inherent in our original ^{1}H-^{77}Se CPMG-HSQMBC sequence got a further significant boost by using ^{77}Se-enriched ligands, such as [^{77}Se]DG.[2]

Our work opens perspectives for applying isotopically enriched selenoglycosides for rapid monitoring of lectin-binding of selenated as well as non-selenated ligands and for ligand screening in competition experiments. The synthesis, NMR and computational study of novel glycomimetics targeting of *h*Gal-3 protein are on the way in our group.

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P28. Investigation of proteinaceous inhibition of M. tuberculosis dUTPase

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The dUTPase enzyme plays a key role in the maintenance of genomic integrity in various species by preventing the uracil incorporation into DNA. The decreased activity of the enzyme can indirectly lead to DNA double-strand brakes and to cell death (1). The design of species-specific dUTPase inhibitor could help in defence against human pathogens, like *Mycobacterium tuberculosis*, as the emergence of multidrug-resistant strains causes an increasing problem in the treatment of tuberculosis (2). A known dUTPase inhibitor, the staphylococcal protein Stl strongly inhibits the Mycobacterium tuberculosis dUTPase (MtDUT) (3,4). The understanding of their interaction may help in the design of efficient MtDUT inhibitors.

For this reason, we have crystallized the complex of MtDUT and a truncated Stl protein variant, Stl^{1-159} and obtained an X-ray dataset at Elettra Trieste with 3.4 Å resolution. Bio-layer interferometry measurements were performed in favour of the analysis of the protein-protein binding kinetics. In order to examine whether shorter, truncated versions of Stl possess a more effective inhibitory effect on MtDUT activity than the full-length protein, we tested the effect of three truncated versions of Stl by steady-state activity measurements.

According to the result of the Bio-layer interferometry and steady-state activity measurements, the full-length Stl protein possesses the highest affinity to MtDUT and has the most effective inhibitory effect on dUTPase activity. The structural model of the Stl^{1-159}-MtDUT complex reveals the main amino acid residues involved in the interaction. However, there may still be unknown interacting residues on the C-terminal region of Stl, which contribute to the formation of the strong complex explaining the most effective inhibition of MtDUT.

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P29.Lipid Binding by the Human Caskin1 SH3 Domain Suggests a Novel Regulatory Mechanism

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SH3 domains constitute an important class of protein modules involved in a variety of cellular functions. They participate in protein-protein interactions via their canonical ligand binding interfaces composed of several evolutionarily conserved aromatic residues forming binding grooves for typical (PxxP) and atypical (PxxxPR, RxxK, RKxxY) binding motifs. The calcium/calmodulin-dependent serine protein kinase (CASK)-interacting protein 1, or Caskin1, a multidomain scaffold protein regulating the cortical actin filaments, is enriched in neural synapses in mammals. Based on its known interaction partners and knock-out animal studies, Caskin1 may play various roles in neural function and it is thought to participate in several pathological processes of the brain. Caskin1 has a single, atypical SH3 domain in which key aromatic residues are missing from the canonical binding groove. No protein interacting partner for this SH3 domain has been identified yet. Here we provide evidence that the SH3 domain of human Caskin1 selectively binds lysophospatidic acid (LPA), a signalling born lipid mediator *in vitro*. The binding strength and stoichiometry depend on the association-state of the lipid, with nanomolar affinity to LPA-containing membraneous surfaces. According to the solution NMR structure of Caskin1 SH3, the canonical binding groove found in typical SH3 domains accommodating proline-rich motifs is missing in Caskin1 SH3, most likely excluding a bona fide protein target for the domain. The LPA binding site is distinct from the altered protein binding groove. We hypothesize that the SH3 domain of Caskin1 might mediate the association of Caskin1 with membrane surfaces with locally elevated LPA content.

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P30 Structural characterization of the postsynaptic Drebrin protein

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The postsynaptic density (PSD) of excitatory synapses is a complex network of nervous system proteins involved in postsynaptic signaling. It also modulates and regulates several functions of the nervous system, thereby responsible for a few molecular mechanisms involved in learning and memory [1]. Our research group focuses on the function of proteins in PSD organization.
The Drebrin protein is an essential component of the cytoskeleton, and its presence is required for actin polymerization of synapses and recruitment of CXCR4 chemokine receptors [2], as well as for the morphogenesis of the dendritic spike. Drebrin also plays an important role in synaptic plasticity associated with hippocampal memory and establishes several key interactions with other proteins present in PSD [3].
In this work we aim to characterize the structure of three different Drebrin domains, namely the ADFH (Actin-Depolymerizing Factor Homology) domain at the N-terminal, the SAH (Single Alpha Helix) domain which was earlier predicted with bioinformatic methods [4], and the HBMs (Homer Binding Motifs) near the C terminus. We have optimized the bacterial expression of the corresponding constructs and already performed initial structural analysis with CD (Circular Dichroism) and NMR (Nuclear Magnetic Resonance) spectroscopy. Molecular interactions with other PSD proteins and F-Actin will also be investigated with BLI (Biolayer Interferometry) and ITC (Isothermal Titration Calorimetry) measurements.

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