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Instruct Biennial Structural Biology Conference

Alcalá de Henares, Madrid, Spain
22 – 24 May, 2019

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Membrane disruption by malaria parasites during blood stage proliferation

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In the clinical phase of malaria infection, the parasite cells invade their host erythrocytes and create an intracellular vacuole, inside which they replicate. When the daughter parasites are mature, after about 48 h, they need to break through both vacuole and erythrocyte membranes in order to invade new erythrocytes. The process by which they escape (“egress”) is a highly organised sequence of secretion, activation and proteolytic events, culminating in the explosive release of the new parasites. Plasmodium species have several perforin-like proteins, two of which are expressed at the blood stage of their life cycle, but these do not appear to be required for the membrane breakage steps. In the related apicomplexan parasite Toxoplasma gondii, the perforin like protein TgPLP1 is essential for vacuole breakage and parasite egress.

We have used video microscopy, electron and X-ray tomography along with mutants and pharmacological blockers of different steps in egress, to study the membrane disruption and breakage during the process of egress. We found an early step of vacuole membrane permeabilization preceding the secretion of protein kinase G and activation of the protease cascade in P. falciparum, but the effector of this permeabilization remains unknown.

Subsequent to vacuole permeabilization by P. falciparum, the vacuole membrane is completely disrupted, followed by erythrocyte membrane permeabilisation and finally disruption to allow parasite escape. The major merozoite surface protein of P. falciparum, MSP1, is proteolytically processed during egress, and an MSP1 mutant shows a substantial defect in egress. We are examining the membrane structures and rearrangements in wild type and mutant parasites.

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Molecular Views into Cells by *in situ* Cryo-Electron Tomography

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Most structural biology focuses on the structure and function of individual macromolecular complexes, but falls short of revealing how they come together to give rise to cellular functions. As a consequence, structural and cell biology have traditionally been separate disciplines and employed techniques that were well defined within the realm of either one or the other. Here, cryo-electron tomography (cryo-ET) provides a unique opportunity for obtaining in situ structural information across a wide range of scales - from whole cells to individual macromolecules. There has been a major leap forward in cryo-ET of biological specimens thanks to the introduction of direct detection cameras, with their unsurpassed signal-to-noise ratio, contrast enhancing phase plates and computational image processing. These developments allow assignment of molecular structures directly from three-dimensional stills of intact cells. To image the dense, crowded interior of mammalian cells is furthermore restricted by the ‘immense’ sample thickness as seen from a nanoscopic perspective. Cryo-focused ion beam (FIB) micromachining literally opened ‘electron-transparent windows’ into cells, making large areas of unperturbed cells accessible for cryo-ET at molecular resolution. We demonstrate that the synergistic application of these recent methodological developments provide insight into the nanoscale architecture of mammalian photoreceptor cells and reveals the structural framework giving rise to the functional properties of highly specialized light sensing cellular compartments. These findings, as well as recent studies, highlight the enormous discovery potential of structural cell biology in elucidating cellular functions.

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Unravelling cancer cell nanoarchitecture through multiscale imaging; Coupling molecular activation, biosensors and their functional output

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There are many times when our cells need to move. Mobile cells guide our body's formation (embryonic development); immune cells roam to capture unwanted intruders; and healing cells (fibroblasts) migrate to mend wounds. But not all movement is desirable. Tumors are most dangerous when cancer cells gain the ability to travel throughout the body (metastasis); certain bacteria and viruses can harness the cell's motility machinery to invade our bodies. Understanding how cells move—and the rod-like actin filaments that drive the process—is key to learning how to halt or promote motility to improve human health.

Here, jointly with scientists from SBP and UNC-Chapel Hill, we identified, within the crowded environment of eukaryotic cells, a unique nanoscale architecture of a flexible, signal-dependent actin structure. We have correlated the molecular signaling behaviors reported by biosensors with nanometer-scale cell imaging using tailor-made computational approaches in order to associate nanometer-resolution three-dimensional images of mouse fibroblasts with time-stamped images of fluorescent Rac1 biosensors that report their state of activation. Rac1 activation is at the core of signaling pathways regulating polarized cell migration.

This technically complex workflow—which bridged five orders of magnitude in scale (tens of microns to nanometers), revealed a structural scaffold that spans from the ventral membrane up to a height of ~60 nm above that membrane, composed of directionally unaligned, densely packed actin filaments, most shorter than 150 nm and resembling a haystack. These structures sprung into view in defined regions where Rac1 was activated and quickly dissipated when Rac1 signaling stopped in as little as two and a half minutes. This unique Rac1-induced morphology contrasted sharply from the dendritic network architecture in which relatively short filaments emanate from existing, longer actin filaments. Also, volumes comprising the haystacks scaffold were devoid of common cellular structures, such as ribosomes, microtubules, vesicles or others, which are

abundant at the periphery of the haystack scaffolds and within the remainder of the imaged volumes. It is tempting to speculate that the highly dynamic Rac1 switching mechanism generates a nanoscaffold system that can propagate to

highly complex actin filament architectures mandated by biological processes and triggered by local spatial cues such as those driving, for example, epithelial to mesenchymal transition, a hallmark for cancer progression.

The study was published in the Proceedings of the National Academy of Sciences of the United States of America (PNAS) and supported by National Institutes of Health (NIH) funding.

Deadly Spiders & Scary Zombies - NOT a Halloween Story - A near atomic resolution glance into the CNS

Moran Shalev-Benami. Weizmann Institute of Science.

Synapses are specialized junctions between neurons that transmit and compute information in the central nervous system (CNS). The establishment, properties, and dynamics of synapses are governed by diverse trans-synaptic signaling molecules that communicate their signal via multifarious interactions with their synaptic partners. Mutations in the genes encoding these molecules have been associated with diverse neuropsychiatric and neurodegenerative disorders thus highlighting their crucial importance for normal brain function. Over the past few decades, tremendous efforts have been made to structurally characterize the trans-synaptic signaling molecules as well as their interacting partners. Nevertheless, their low expression levels and high structural complexity has posed a great challenge to traditional structural methods, such as NMR and X-ray crystallography. Advances in single particle electron cryo-microscopy (cryo-EM) now allow the capture of such complexed macromolecular assemblies in great details, providing snapshots of these fascinating molecules in action. Here we present the near atomic resolution structures of two such synaptic components, the cannabinoid receptor 1 (CB1R), and teneurin, two transmembrane receptors that are primarily expressed in neurons and are considered to mediate various functions in synapse formation and maintenance. The structures provide a high-resolution glance into the receptors' architectures and present structural insights into the interaction with their inter- and intra- cellular partners. Our results highlight cryo-EM as a highly effective alternative approach for studying challenging macromolecular machineries while providing a framework for elucidating the mechanisms of action of trans-synaptic signaling molecules that could in turn be used for design of future novel therapeutics.

GEMINI: an integrated structural approach for vaccine identification and pathogenesis insight

Ilaria Ferlenghi, GSK Vaccines

Vaccination is historically one of the most important medical interventions for the prevention of infectious disease. Traditionally vaccines have been developed by cultivating infectious agents and isolating the inactivated whole pathogen or some of its attenuated components. 20 years ago, reverse vaccinology enabled vaccine discovery and design based on information deriving from the sequence of microbial genomes rather than via the growth of pathogens. However, over the last years, several important technological and computational advances have enabled major progress in the discovery and design of potently immunogenic recombinant protein vaccine antigens. Today the increasing structural characterization of protective antigens and epitopes provide the molecular and mechanistic understanding to drive the discovery of novel vaccines that were previously impossible.

THE MOLECULAR MACHINERY OF PROTEIN DEGRADATION - STRUCTURAL STUDIES EX SITU AND IN SITU

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The 26S proteasome operates at the executive end of the ubiquitin-proteasome pathway for the controlled degradation of intracellular proteins. The 2.5 MDa complex comprises two subcomplexes: the 20S core where proteolysis takes place and one or two regulatory particles which prepare substrates for degradation. Whereas the structure of its 20S core particle has been determined by X-ray crystallography more than two decades ago, the structure of the regulatory particle, which recruits substrates, unfolds them, and assists in their translocation into the core particle remained elusive for a long time. Only in recent years has its structure been determined to high resolution using cryo-electron microscopy single particle analysis.

Cryo-electron tomography allows to perform structural studies of macromolecular and supramolecular structures in situ, i.e. in their functional cellular environments. We used this method to study the 26S proteasome in a number of cellular settings revealing their precise location, assembly and activity status as well as their interactions with other molecular players of the cellular degradation machinery.

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Structural insights into the allosteric control of DPCR activity

Andy Dore, Soseil Heptares

Many of the world's top selling drugs target G protein-coupled receptors (GPCRs). The past ten years have seen an almost exponential increase in structural knowledge for this important and clinically relevant superfamily of membrane proteins as a result of pioneering protein engineering techniques from multiple groups globally. This, coupled to advances in crystallization methodogolies *per se*, serial crystallographic techniques such as XFEL, cryo-electron microscopy and microfocus collection techniques using conventional synchrotron radiation, has not only driven structure based drug design (SBDD) at the orthosteric site(s) of clinically relevant targets, but also uncovered a myriad of allosteric sites that may also be targeted to modulate GCPR activity across all subclasses of this superfamily encompassing a wide range of indications.

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The atomic structures of Tau filaments in neurodegenerative disease

Sjors Scheres, Francis Crick Avenue, Cambridge Biomedical Campus

The assembly of microtubule-associated protein tau into abundant filamentous inclusions underlies many neurodegenerative diseases called tauopathies. Tau inclusions display distinct neuroanatomical and cellular distributions between different tauopathies. Morphological and biochemical differences suggest that tau filaments adopt disease-specific molecular conformations. Molecular conformers of filamentous tau may give rise to different neuropathological phenotypes, similar to prion strains, but the underlying structures are not known. Using electron cryo-microscopy (cryo-EM), we determined the structures of tau filaments that were extracted from the brain of an individual that died of Alzheimer's disease, which is the most common tauopathy. We also determined the structures of tau filaments from Pick's disease, from chronic traumatic encephalopathy (CTE), and from in-vitro aggregation experiments with over-expressed tau. I will discuss how these structures explain differences in isoform incorporation and phosphorylation patterns observed between the different diseases, and how they provide exciting avenues for further research to unravel the role of molecular tau conformers in disease.

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CryoEM analysis unveils the conformational activation of CRISPR-Cas12a and the endonuclease activity resetting

Guillermo Montoya. NNF-CPR

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Cas12a, also known as Cpf1, is a type V-A CRISPR-Cas RNA-guided endonuclease that is used for genome editing based on its ability to generate specific dsDNA breaks. Here, we show cryoEM structures of intermediates of the cleavage reaction, thus visualizing three protein regions that sense the crRNA-DNA hybrid assembly triggering the catalytic activation of Cas12a. Single molecule FRET provides the thermodynamics and kinetics of the conformational activation leading to phosphodiester bond hydrolysis. These findings illustrate why Cas12a cuts its target DNA and unleashes unspecific cleavage activity degrading ssDNA molecules after activation. In addition, we show that other crRNAs are able to displace the R-loop inside the protein after target DNA cleavage terminating indiscriminate ssDNA degradation. We propose a model whereby the conformational activation of the enzyme results in indiscriminate ssDNA cleavage. The displacement of the R-loop by a new crRNA molecule will recycle Cas12a specificity targeting new DNAs.

Stella, Mesa et al., Cell Nov 22 2018

Structure and Dynamics of Membrane Transport Proteins

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Using primarily membrane protein crystallography and cryo-EM, and a range of biochemical and biophysical methods such as electrophysiology, single-molecule FRET, and molecular dynamics simulations, we have obtained deep insight into the functional cycle of primary active transporters of the P-type ATPase family.

The transmembrane gradients for the key cations Na+, K+, and Ca2+ are generated by Na,K-ATPase and Ca2+-ATPases of the P-type ATPase family. In brain, Na,K-ATPase activity accounts for an estimated 40-70% of total ATP hydrolysis and potentiates e.g. Na+ and K+ channels for their activity in action potentials, membrane potential, Na+ coupled transport of e.g. glucose, metabolite, neurotransmitters, Ca2+ efflux, pH and Cl- control. Ca2+-ATPases of the same P-type ATPase family maintain steep calcium gradients, internal Ca2+ stores, and cytoplasmic free calcium at accurate levels that define and potentiate calcium signalling pathways. These activities are fundamental to physiology, and malfunctions are linked to diseases such as neurological and cardiovascular disorders.

Lipid flippases, also of the P-type ATPase family maintain asymmetric lipid distributions in biomembranes. Their activity potentiate membrane dynamics, but the structure and function of lipid flippases have so far remained enigmatic. We have determined the first structures using cryo-EM

The lecture will cover both methodological approaches and rationales as well the structural and mechanistic concepts that define these important transporters in the cell.

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Snapshots of T7 viral connector and tail machinery structures suggest a model for DNA retention inside the capsid.

Cuervo Ana. CNB-CSIC

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Viral assembly requires orchestrating a sequential and specific order of interactions among different proteins to build a mature infective particle. During the first steps of this process, bacteriophages package their genome inside an empty capsid using the energy provided by a viral ATPase (terminase). The DNA is translocated inside the capsid through the portal pore formed by the connector protein, an oligomeric ring that locates at a specific vertex of the procapsid. This protein serves also as a docking point for the rest of the tail components, which assemble after viral DNA packaging finishes. The genome can be temporarily hold inside the head in the absence of the tail components, but it remained unclear how bacteriophage proteins manage to retain highly pressured DNA after terminase departure. In mature viruses, the DNA is retained inside the ejection channel by the tail proteins, so different conformational changes need to take place to allow DNA ejection. In this work, we describe different structures of T7 bacteriophage connector and tail complex by combining cryo-EM and X-ray crystallography, which allow us to define a precise picture at the molecular level of the T7 genome retention mechanism during the assembly pathway.

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Unravelling the structure of toxic protein aggregates in situ
Rubén Fernández-Busnadiego. Max Planck Institute of Biochemistry.

Protein aggregation is a hallmark of many neurodegenerative diseases, including Huntington's, Parkinson's and amyotrophic lateral sclerosis. However, the mechanisms linking aggregation to neurotoxicity remain poorly understood, partly because only limited information is available on the native structure of protein aggregates inside cells. We address this pressing issue utilizing the latest developments in cryo-electron tomography (cryo-ET). We prepare thin lamellas of vitrified cells containing protein aggregates using cryo-focused ion beam, and subsequently image them in three dimensions by cryo-ET. This allows us to analyse aggregate structure within pristinely preserved cellular environments and at molecular resolution [1, 2]. Here, I will discuss how our latest results shed new light into the cellular mechanisms of neurodegeneration.

[1] Bäuerlein et al. and Fernández-Busnadiego, Cell (2017) 171 (1), 179-187
[2] Guo et al. and Fernández-Busnadiego, Cell (2018) 172 (4), 696-705

Integrative modeling of biomolecular assembly structures and pathways

Andrej Sali, California Institute for Quantitative Biosciences, University of California

The networks and spatial structures of biomolecular interactions provide insights into their function and thus help us to understand the workings of living cells. Detailed structural characterization of large and often dynamic assemblies and their networks is generally impossible by any single existing experimental or computational method. This challenge can be overcome by hybrid approaches that integrate data from diverse biophysical experiments (eg, X-ray crystallography, NMR spectroscopy, electron microscopy, chemical cross-linking, yeast-two hybrid system, and various chemical genetics and proteomics approaches). We formulate the hybrid approach to structure and/or network determination as an optimization problem, the solution of which requires three main components: the representation of the assembly or network, the scoring function, and the optimization method. The ensemble of solutions to the optimization problem embodies the most accurate characterization given the available information. The key challenges remain translating experimental data into restraints on the structure and/or network, combining these spatial and/or network restraints into a single scoring function, optimizing the scoring function, and analyzing the resulting ensemble of solutions. The approach will be illustrated by several applications to specific biological systems, including the structure determination of the nuclear pore complex and the mapping of the gulonate pathway in *Haemophilus influenzae*.

Visualizing the invisible genome: Fleeting structures of DNA in gene expression and genome stability

Hashim M. Al-Hashimi

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The high degree of functional heterogeneity observed across genomes *in vivo* stands in apparent contraction with the textbook description of the DNA double helix as a structure that is homogenously composed of G-C and A-T Watson-Crick base-pairs. This talk will challenge this cornerstone in molecular biology by providing evidence that in the DNA double helix, Watson-Crick base pairs are constantly dynamically morphing into lternative G-C^+ and A-T Hoogsteen base pairs. Relative to Watson-Crick base pairs, A-T or G-C^+ Hoogsteen base pairs form by flipping the adenine or guanine nucleotide base 180°. The resulting Hoogsteen base pairs are chemically and structurally different from Watson-Crick base pairs. The role of Hoogsteen base pairs in DNA-protein recognition, damage induction and repair will be discussed. Finally, a new sequencing technology will be presented for mapping Hoogsteen base pairs genome wide *in vivo*.

Correlative cryo soft X-ray imaging of cells

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Cryo soft X-ray tomography (SXT) of whole cells in the water window energy range can provide relevant structural information of complex cellular phenomena with chemical sensitivity at spatial resolutions of 40 nm [1, 2]. Functional studies can be achieved by correlating this information with visible light fluorescence microscopy data on the same cell [2] and with super resolution [3, 4]. Cryo-SXT can also be combined with cryo hard X-ray nano-fluorescence [5, 6] to precisely locate, for instance, specific organometallic drugs in the cellular environment [7]. Examples of such correlative studies will be presented.

[1] Schneider et al. Nat. Methods 7: 985-987 (2010)

[2] Pérez-Berná AJ et al. ACS Nano 10, 6597-6611 (2016)

[3] Varsano N et al. JACS 138: 14931-14940 (2016)

[4] Varsano N. et al. PNAS 115 (30): 7662-7669 (2018)

[5] Kapishnikov S et al. Sc. Rep. 7: 802 (2017)

[6] Kapishnikov S et al. Sc. Rep. 7: 7610 (2017)

[7] Conesa J.J. et al., submitted.

3D-Bioinfo: ELIXIR Community of Structural bioinformatics

Bohdan Schneider
Institute of Biotechnology of the Czech Academy of Sciences

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The ELIXIR structural bioinformatics community, 3D-BioInfo, was established with the aim of coordinating research activities in European structural bioinformatics and thereby maximizing their impact on the scientific community at large. 3D-BioInfo links scientists from most Elixir member countries, and plans a range of activities integrating existing tools and knowledge from structural biology to drug discovery and personalized medicine. Its initial focus will be on the prediction of protein 3D structures and complexes, annotation and prediction of protein functional sites, prediction of protein ligand docking and on characterizing and validating nucleic acid structures. In addition to facilitating access to structural bioinformatics tools and resources, 3D-BioInfo will organize courses to inform and train researchers in their use.

3D-Bioinfo remains highly inclusive and welcomes new ideas and research topics. Many of its stated goals are complementary to those of integrative structural biology, pursued by Instruct-ERIC. A close collaboration with Instruct-ERIC is therefore high on 3D BioInfo's agenda. Joining forces to improve key tools for archiving, analyzing, annotating, and predicting structures of biological molecules, should benefit both communities and also the wider research community.

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Molecular-scale biophysics methods for sample quality control and quantitative characterization: a perspective from the ARBRE-MOBIEU European network

Patrick ENGLAND (Institut Pasteur, Paris, France)

ARBRE-MOBIEU (www.arbre-mobieu.eu) is a large European Network that brings together more than 150 academic core facilities and research laboratories from 30 European countries. All of them focus on the optimization of molecular-scale biophysical methodologies to solve questions of biological and biomedical interest.
In this presentation, I will summarize how, in our view, a rational and integrated use of molecular-scale biophysical approaches (including stringent quality control assessment tools) can help improve structural biology workflows. I will also show how ARBRE-MOBIEU could synergize closely in the next few years with the INSTRUCT community.

High molecular-weight complexes in the regulation of gene expression:

a view by integrative structural biology

Nataliya Danilenko1, Lukas Lercher1, Frank Gabel3, John Kirkpatrick1,2, Teresa Carlomagno1,2,

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3. Universite´ Grenoble Alpes, Institut de Biologie Structurale, 38027 Grenoble, France

ABSTRACT

The Regulator of Ty1 Transposition protein 106 (Rtt109) is a fungal histone acetyltransferase required for histone H3 K9, K27 and K56 acetylation. These acetylation sites have been linked to processing and folding of nascent H3 and play an integral role in replication- and repair-coupled nucleosome assembly. Rtt109 is unique in its activation, performed by two structurally unrelated histone chaperones, Asf1 and Vps75. These proteins stimulate Rtt109 activity via different mechanisms1. Rtt109 - Asf1 association has been proposed to be responsible for K56 acetylation, while the Rtt109-Vps75 interaction is required for K9 acetylation2,3.

In our work we find that Rtt109, Vps75 and Asf1 are capable of assembling as a previously uncharacterized complex onto the substrate H3-H4 dimer. Using an integrative structural biology approach based on a powerful combination of solution state NMR and small angle neutron scattering (SANS) we solve the structure of this complex and provide a structural basis for the efficiency and selectivity of acetylation at the at the H3 K9, K27 and K56 sites.

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1. D'Arcy S., Luger K. Understanding Histone Acetyltransferase Rtt109 Structure and Function: how many chaperones does it take? Curr Opin Struct Biol, 21(6), 728–734 (2011).

2. Fillingham J., Recht J., Greenblatt JF. Chaperone control of the activity and specificity of the histone H3 acetyltransferase Rtt109. Mol Cell Biol. 28, 4342–4353 (2008).

3. Driscoll R., Hudson A., Jackson SP. Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. Science, 315, 649–652 (2007).

Structure determination by microcrystal electron diffraction

Brent L. Nannenga, Arizona State University

A common barrier to high-resolution structure determination is the growth of large well-ordered crystals. Electron diffraction is capable of producing high-quality diffraction data from crystals that are orders of magnitude smaller than those needed for conventional X-ray crystallographic experiments, and 3D electron diffraction methods have recently begun to yield high-resolution structures from extremely small microcrystals. In this presentation, the cryo-electron microscopy technique of microcrystal electron diffraction, or MicroED, will be described in detail along with representative structures determined by the method. Additionally, current methods development focused on improving the MicroED technique will be presented

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Increasing the Throughput cryo EM sample preparation

Alex de Marco, Dept. of Biochemistry and Molecular Biology, Monash University, Australia

Today, cryo-electron microscopy (cryo-EM) can deliver incredible results in both structural and cell biology. Despite great potential that this technique has widely demonstrated the major bottleneck, across the entire range of applications, is currently at the level of sample preparation. Our work focuses on increasing the throughput and the reproducibility in sample preparation for both single particle and cellular cryo-EM.

Regarding the sample preparation for single particle cryo-EM, we developed a sample delivery for blotless cryo-grid preparation. This method uses a surface acoustic wave to deliver the sample to the EM-grid in form of an aerosol. Considering the size of the droplets (~6 µm) there is no need for mechanical blotting nor the use of self-blotting grids, therefore the time between sample delivery and freezing can be reduced to a few milliseconds.

When it comes to cellular cryo-EM, the use of Focused Ion Beam (FIB) milling is key to obtaining samples of suitable thickness. The utility of FIB today ranges from large volume imaging of resin embedded samples to cryo-lamella preparation of frozen-hydrated samples. Our work demonstrates the advantages of working with non-conventional plasma FIB sources for fast FIB/SEM tomography and cryo-lamella preparation, demonstrating a throughput increase up to 30% and superior sample compatibility. In association with the use of optimised beams we developed an integrated correlative light and FIB/SEM setup which removes the need of cryo-transfers between cryo-light microscopy and cryo-lamella preparation, reducing the ice contamination typically cursing cryo-samples.

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Poster 1

Nanobodies4Instruct
Els Pardon, Vrije Universiteit Brussel

The Nanobodies4Instruct centre generates conformational Nanobodies to facilitate the structural analysis of proteins that are notoriously difficult to purify, to crystallize or to study by other methods. Nanobodies are the small (15 kDa) and stable single-domain fragments harboring the full antigen-binding capacity of camelid heavy chain–only antibodies. Collective efforts of several laboratories have demonstrated that Nanobodies are exquisite chaperones for crystallizing complex biological systems such as membrane proteins, transient multiprotein assemblies, transient conformational states and intrinsically disordered proteins. Further, they can be used as structural probes of protein misfolding and fibril formation. Domain-specific Nanobodies have been used in single-particle electron microscopy (EM) to track these domains in particle projections. As Nbs are too small to be commonly used in single particle cryo-EM, we rigidly grafted Nbs onto larger scaffold proteins to build Megabodies. These designed antibody chimeras are instrumental in producing larger and more homogeneous particles that vitrify with less preferential orientations than the Nb complexes. In combination with improvements in hardware and software, these Megabodies enable the structural characterization of ‘intractable’ targets at increasingly high resolution, including complexes with small molecules.

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Poster 2

Instruct Czech Republic

Instruct Centre CZ is coordinated within the **Czech Infrastructure for Integrative Structural Biology (CIISB)** formed by two Centers of Excellence for Structural Biology: CEITEC – Central European Institute of Technology, Brno and BIOCEV - Biotechnology and Biomedicine Centre, Vestec, Prague-West. CIISB offers open-access and assisted expertise to 10 high-end core facilities in NMR, X-ray crystallography and crystallization, cryo-electron microscopy and tomography, biophysical characterization of biomolecular interaction, nanobiotechnology, proteomics, and structural mass spectrometry. Four flagship technologies – high-field NMR, cryo-EM, X-ray diffraction, and structural mass spectrometry - are equipped with high-end instrumentation and provide top-level assistance with the measurements and analysis of the obtained data.

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Poster 3

The Integrated Structural Biology Platform at Instruct-Centre France-1
Birck Catherine. CBI-IGBMC

The Instruct-Centre France-1 at Strasbourg hosted in the Center of Integrative Biology (CBI), located on the IGBMC site at Illkirch/ Strasbourg, which is also the coordinating Centre for FRISBI (French Infrastructure for Integrated Structural Biology), provides an integrated environment for structural studies of protein and macromolecular complexes.
Our Integrated Structural Biology Platform offers project-based access to all advanced tools from sample preparation and biophysical characterization to structural analysis including in particular cryo-EM, X-ray crystallography, and small angle X-ray scattering of proteins and macromolecular complexes including nucleoprotein complexes. Taken together, this allows integrating functional data and various multi-resolution structural data. These activities are driven by experienced engineers and technicians with the support of the strong scientific environment and know-how provided by the Department of Integrated Structural Biology at IGBMC.
Many Instruct users have already benefited from our facilities and expertise through access visits, internships and several trainings organized by the Instruct-Centre France-1.

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Poster 4

The Integrated Structural Biology Platform at Instruct Centre France 2

Instruct Centre France 2 (Grenoble) provides user access to some of the highest level structural biology instrumentation in France. Our platforms are located at the Institut de Biologie Structurale (IBS) and the EMBL with user access managed by the Integrated Structural Biology Grenoble (ISBG) service unit.
Sample preparation includes mass spectrometry, cell-free expression, ESPRIT construct library screening, isotopic labelling, N-ter sequencing and Robiomol for automated molecular biology. The Molecular Biophysics platforms provides AUC, SEC-MALLS, MST, BLI, ITC, CD, DLS and SPR. Cellular imaging is available using cellular EM, confocal, video, PALM and STORM microscopy. Membrane protein crystallisation is available, as is structural analysis by CryoEM and NMR platforms. All our platforms follow a Quality Assurance programme, managed by a full-time quality engineer, and are certified ISO 9001 NFX 50-900.
Our flagship technology is the Electron Microscopy platform with its T12, F20 and Glacios microscopes.

PROSS "Protein Repair One Stop Shop" to produce much more stable proteins

Low stability and low (or no) heterologous expression yields are major practical bottlenecks in research, let alone in applying proteins as therapeutics. The solutions to this challenge have so far been protein-specific, laborious, and time consuming (*e.g.*, expression in eukaryotic systems such as HEK293 and insect cells). To address this challenge, Dr. Sarel Fleishman and his student Adi Goldenzweig (in the Dept. Biomolecular Science at the Weizmann) developed a novel and general algorithm that combines phylogenetic analysis with energy design to identify dozens of mutations that improve stability and enable high-yield *E. coli* expression without affecting function. It is called Protein Repair One Stop Shop (PROSS) [**http://pross.weizmann.ac.il**].

To do an initial test of this method, the Israel Structural Proteomics Center collaborated with the Fleishman group to try to express human AChE in *E. coli*. AChE is large (60kDa), membrane-associated, disulfide-linked, and glycosylated. These four hallmarks are often found in proteins that are challenging for bacterial over-expression. Indeed, AChE had not been expressed in prokaryotic systems, despite 20 years of intensive attempts due to its fundamental interest and its therapeutic importance as the target of organophosphate (OP) poisoning. The designs generated automatically by PROSS, in contrast, yielded 2 mg active enzyme per liter *E. coli* culture, and are ~20ºC more resistant to thermal denaturation, while maintaining the catalytic efficiency of the WT enzyme[1] and is virtually identical in 3D structure (Fig. 1). In the designed human AChE variant displayed in the figure, 51 mutations are introduced throughout the structure, both internal and on the surface. These designs will now serve as robust OP-detoxification reagents, and as potential countermeasures against poisoning by pesticides and nerve agents, such as VX, soman, and sarin.

In order to aid in seeing the overall structure in 3D as well as the position of the mutated individual amino acids, an Interactive 3D Complement of the *Molecular Cell* paper was created to complement the published paper (Fig. 2).

Fig. 1. Designed human AChE showing 51 mutations, which are distributed throughout the structure. The enzyme is ~20ºC more thermally stable than the WT enzyme, but maintains its catalytic activity. Its structure is virtually identical to that of the WT mammalian expressed enzyme.

[1] Goldenzweig, A., Goldsmith, M., Hill, S. E., Gertman, O., Laurino, P., Ashani, Y., Dym, O., Unger, T., Albeck, S., Prilusky, J., Lieberman, R. L., Aharoni, A., Silman, I., Sussman, J. L., Tawfik, D. S. & Fleishman, S. J. Automated structure- and sequence-based design of proteins for high bacterial expression and stability. *Mol. Cell* **63**, 337–346 (2016).

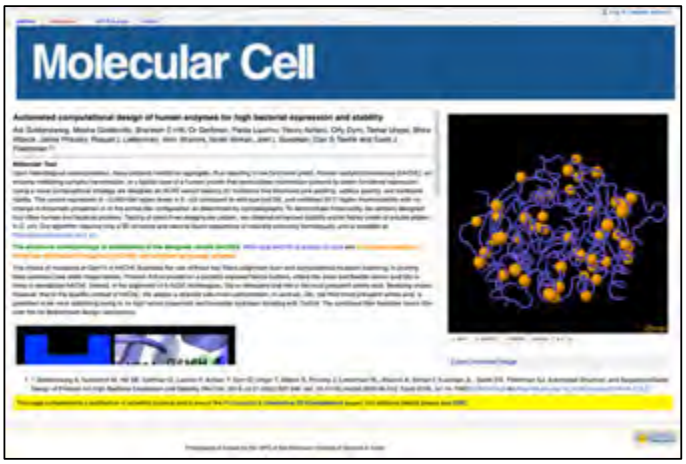


Fig. 2. An Interactive 3D Complement (I3DC) in Proteopedia for the recent paper in *Molecular Cell*[1] showing a 3D interactive view (right side of the page) of the 51 amino changes (shown as orange spheres) in the structure of the human AChE automatically designed by PROSS. It is clear from the image that the mutations are spread all over the structure of AChE, and not grouped in one particular region. This I3DC can be viewed at: https://proteopedia.org/w/Journal:Molecular_Cell:1.

The ISPC has used the PROSS algorithm for improved expression of multiple protein targets, including:

- Bacterial rhodopsin
- MMP14
- Glucocerebrosidase (GCase)
- Human stem cell factor (hSCF)
- IL24

Cryo-EM structure of Type-I Mycobacterium tuberculosis fatty acid synthase (FAS1) at 3.3 Å resolution

Mtb fatty acid synthase type-I (FAS-I) is an essential ~2 MDa enzymatic complex that contributes to the virulence of Mtb, and thus a prime target for anti-TB drugs. The enzyme was cloned and co-expressed in *E coli* with its activator AcpS and isolated using an engineered strep tag producing various oriented single particles in cryo-EM (Fig. 3)[2].

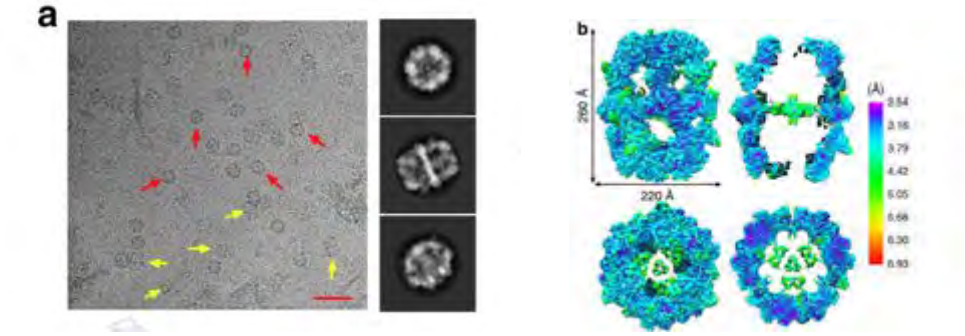


Fig. 3. FAS1 3D structure (a) A 3.3 Å resolution structure (PDB; 6GJC) was determined. (b) One of the distinct features that differentiate this structure from previously determined fungal FAS-1 systems is its larger catalytic cleft which provide its unique ability to produce C26 fatty acids.

[2] Elad, N., Baron, S., Peleg, Y., Albeck, S., Grunwald, J., Raviv, G., Shakked, Z., Zimhony, O. & Diskin, R. Structure of Type-I Mycobacterium tuberculosis fatty acid synthase at 3.3 Å resolution. *Nat. Commun.* **9**, 3886 (2018).

CERM/CIRMMP Italian Instruct Centre

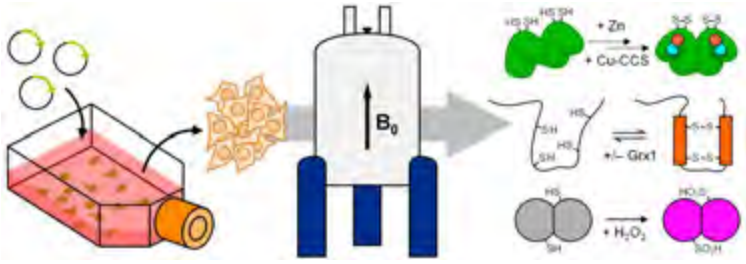
Lucia Banci, CERM, University of Florence, Italy

The Italian Centre of Instruct CERM/CIRMMP is an infrastructure for Life Sciences providing a unique environment for research in the field of Structural Biology offering unique research capabilities in the field of NMR. The Centre has a wide range of high-resolution spectrometers, for solution and solid-state NMR, ranging from 400 MHz to 950 MHz equipped with several probes to meet all conceivable experimental conditions. This allows the users: i) to determine the structure and dynamics of macromolecules with dedicated hardware; ii) to study protein-protein and protein-DNA interactions in a broad range from highly transient to stable; iii) to characterize biomolecules and functional processes directly in living cells.

Italy is one of the founding members of Instruct and Instruct-ERIC. CERM/CIRMMP also coordinates Instruct-ITALIA, the Italian Infrastructure for Structural Biology, consisting in a network of Italian research institutions and large centres of excellence covering all main areas of structural biology within Italy and providing access at the national level.

Among the various structural techniques, CERM has developed a unique strategy for in-cell NMR, based on protein expression and labelling in human cells that provides good expression levels and high labelling efficiency[1] and is ideally implemented on cutting-edge NMR hardware, as the high magnetic field 950 MHz spectrometer with latest generation of electronics available at CERM.

Challenging questions in cellular biology can be addressed: protein folding and maturation; protein-protein interactions; redox-dependent folding[2] Structural changes in response to external stimuli such as oxidizing agents [3], toxic metals[4] or potential drugs[5], can also be investigated.



Direct protein expression in human cells allows studying protein functional processes by in-cell NMR

This innovative cellular structural biology approach is being implemented as a service made available to non-expert users in the scientific communities of cellular and structural biology.

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[1] Barbieri L. et al. Nature Protocols, 2016
[2] Luchinat et al., Acc Chem Res, 2018
[3] Barbieri et al. JBIC, 2017
[4] Polykretis et al. Redox Biol, 2019
[5] Capper et al. Nat Commun, 2018

Instruct-NL

Instruct-NL, Bijvoet Center for Biomolecular Research, Padualaan 8, 3584 CH Utrecht, The Netherlands

INSTRUCT-NL provides three internationally operating research infrastructures with facilities that have a broad expertise in biochemistry, cell and molecular biology besides their structural biology technology.

The Bijvoet Center for Biomolecular Research of Utrecht University has facilities for high-throughput mass spectrometry on proteins and peptides and high-field solid and liquid-state NMR, as well as computational structural biology resources. In Instruct-ERIC, UU offers native protein mass spectrometry, which allows determination of protein mass, complex stoichiometry, and - in combination with ion mobility separation - characterization of the overall structure of protein complexes. The solid-state and solution NMR technology that is offered enables the determination of structure and dynamics of biomolecules. UU is well equipped for the atomic characterization of macromolecular complexes using a variety of techniques.

The NKI Protein Facility at the Division of Biochemistry of The Netherlands Cancer Institute in Amsterdam allows production, purification, biophysical characterization and crystallization of proteins. A particular strength of the facility is the characterization of macromolecular interactions using a range of biophysical methods. Researchers without experience or local means to produce and analyze proteins can request assistance with their experiments. Protein fragments can be designed with structural and bio-informatics tools to increase the chance for success. Platforms for protein expression in *E. coli*, insect cells and mammalian systems are offered as well as protein purification using different methodologies. Various biophysics equipment for the characterization of macromolecular properties and interactions is available. Finally, the facility offers automated high-throughput crystallization screening, in 96-well format in 100-200 nanoliter droplets.

The Netherlands Centre for Electron Nanoscopy at Leiden University provides access to cryo-transmission electron microscopy (FEI Titan Krios cryo-TEM). NeCEN offers two major aspects of cryo-TEM; one microscope is for visualizing cellular structures in 3D with nanometer precision (tomography), the other is equipped for analyzing single particles with highest resolution possible. In addition to data collection, NeCEN offers a wide range or cryo-EM related services, including sample preparation, sample screening, image processing and customer tailored trainings and courses.

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Poster 8

The Instruct Image Processing center (I2PC): support to structural biologists

Roberto Melero. CSIC

The Instruct Image Processing Center (I2PC) at the CNB-CSIC is the European Reference Center for infrastructure provision in Image Processing in Transmission Electron Microscopy and X-ray Microscopy. We provide support to structural biologists, helping them to maximize the extraction of biological knowledge from their electron microscopy images in three different platforms: Instruct, iNEXT and Corbel. In iNEXT we give support at sample level, with analysis of EM grids and acquisition of EM images using a FEI Talos Arctica and a Falcon III direct detector.

In Instruct we give support for full EM image processing using SCIPION package, including movie alignment, particle picking, classification, volume reconstruction and atomic structure determination. In Corbel we link the structural data with genomics and proteomics databases, with annotations of 3D protein structures at residue level using proteomic and genomic sources including UniProt and ENSEMBL databases, diseases and genomic variants, protein domain families, disordered regions, short linear motifs and immunological epitopes.

The Membrane Protein Laboratory (MPL). A dedicated facility for membrane protein structural biology.

Andrew Quigley. Diamond Light Source

The MPL is focused on the efficient determination of relevant membrane protein structures by combining high throughput technologies for protein production and crystallisation with the X-ray diffraction data collection system at Diamond. Recently the MPL has started to support scientists with sample preparation for cryo-EM. Based within the Research Complex at Harwell and funded by the Wellcome Trust, the MPL has supported well over 100 applications to use its facilities and has contributed to the solving at least one membrane protein structure a year since 2012. We have access to state-of-the-art equipment necessary for high-throughput cloning, expression, detergent screening, purification, grid preparation and crystallisation of membrane proteins. All the equipment and methodologies are available to MPL staff, visitors and collaborators and we have dedicated research scientists available to support all users of the facility.

RI-VIS: Expanding the visibility of European research infrastructures
Natalie Haley. Instruct-ERIC Hub.

European research infrastructures (RIs) provide fantastic resources and support to their specific communities and have a direct impact on research and innovation. The value of RIs is far-reaching as they help to advance the forefront of knowledge, train individuals, develop new technologies and promote the exploitation of these technologies, and more. Despite the clear benefits of research infrastructures, many key target communities have little knowledge of the vast array of benefits that the infrastructures can provide to them. RI-VIS will increase the visibility of European RIs to new and broader scientific communities, industrial communities, policy-makers and the general public. RI-VIS will provide information to these communities to help develop new collaboration opportunities, promote access to RI services, disseminate funding opportunities, transfer knowledge, and offer training.

Beyond Europe, RI-VIS will work to form strategic partnerships with communities and RIs globally through partnering and international outreach events and an international staff mobility program. Relationships established with third countries will be formalised to ensure their longevity beyond the project lifetime. RI-VIS will provide and develop tools and resources to enable efficient communication and collaboration with target groups.

The RI-VIS consortium is composed of 13 partners from 12 research infrastructures mostly in the biomedical sciences but with representation from the social sciences and the environmental sciences. The consortium itself will be supported by an expert advisory board composed of members of RIs in other fields such as physical sciences, e-infrastructure and humanities. The breadth of the consortium and advisory board will enable us to ensure that the results of the RI-VIS project are relevant to all.

Though improved visibility, RI sustainability will be enhanced and the benefits of research infrastructures will be enjoyed by a far greater audience. Whoever you are (academic researcher, infrastructure manager, industrial researcher, funding agency, member of the public) you can get involved with our project and our mission, and learn what benefits research infrastructures have for you. Find out more at ri-vis.eu

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Poster 11

New Insights into Glucocorticoid Receptor Quaternary Structure

Alba Jiménez Jiménez-Panizo. Institute of Biomedicine of the University of Barcelona

Nuclear Receptors (NR) form homo- and/or heterodimers as central scaffolds of multiprotein complexes, which activate or repress gene transcription to regulate development, homeostasis, and metabolism. Recent studies on NR quaternary structure reveal novel mechanisms of receptor dimerization, the existence of tetrameric chromatin-bound NRs, and previously unanticipated protein-protein/protein-DNA interactions.

In particular, Glucocorticoid Receptor (GR) has been shown to act not only as a monomer or dimer, but also as a tetramer when binding chromatin, bringing new questions about this steroid receptor functions in both physiological and pathophysiological states. We have solved two different crystal structures of the GR ligand binding domain, revealing new arrangements in its dimerization and oligomer formation. The deep study of these new conformations will shed light upon the significance of these NR quaternary complexes.

High-Resolution 2D NMR Spectroscopy of Patient-Derived Glycoproteins at Natural Isotopic Abundance

Alistair Jagger. University College London

Alpha-1 antitrypsin (α1AT) is a 52 kDa serine protease inhibitor (serpin) found at high concentrations in the human plasma. The most well characterised member of the serpin superfamily, α1AT has a metastable native fold (i.e. kinetically but not thermodynamically stable) that undergoes a large-scale conformational change to inhibit substrate proteases. A number of genetic mutations exist that perturb this process, promoting spontaneous conformational changes in the absence of the substrate protease that leads to misfolding or aggregation in to long, ordered polymers. The most common disease-causing mutation is the Z mutation (E342K), with the Z allele occurring in approximately 1 in 25 individuals in the UK. The resulting accumulation of toxic polymers at the site of synthesis in the liver endoplasmic reticulum is associated with liver cirrhosis while the reduction in circulating protease inhibitor leads to destruction of lung tissue predisposing chronic obstructive pulmonary disease (COPD) [1].

The propensity of the Z variant to aggregate has precluded recombinant expression and isotopic labelling in E.coli, severely limiting the number of structural biology studies on the disease relevant protein and thus the understanding of its mechanism in promoting polymerisation. Recently, the first published X-ray crystal structure of Z-α1AT (produced in Drosophila) was found to be essentially identical to the WT protein [2]. Therefore, we propose that it is the solution structure and dynamics of α1AT variants that is key to understanding the molecular basis of α1AT misfolding, polymerisation and disease.

To this end, we have used high-resolution 2D NMR spectroscopy of patient-derived WT and mutant α1AT at natural isotopic abundance to investigate the structural and dynamic consequences of disease-causing variants. Optimised pulse sequences and sample conditions give extremely high quality 1H-13C methyl SOFAST-HMQC spectra that show long-range chemical shift perturbations and altered linewidths induced by the Z (E342K) mutation. We will report the assignment of ILVMA methyl resonances in recombinant α1AT and the transfer of these assignments to spectra observed at natural abundance, and discuss the consequences of glycosylation and inter-patient glycan polydispersity. Lastly, we will describe the chemical shift perturbations induced by the Z mutation, and discuss these changes in relation to dynamics observed in recombinant wild type α1AT.

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Cryo-EM of Fully Recombinant Human Proteasomes – A New Tool for Functional and Structural Studies

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The 20S proteasome is the catalytical core of the 26S proteasome, a key player in the ubiquitin proteasome pathway (UPS). Because the proteasome is involved in many key cellular processes, it is a very well established drug target. Despite that, our understanding of its mechanisms of function and regulation is still significantly elusive. The major limitations in studying the human proteasome are its structural complexity, its physiological variants, the limited availability of purified homogeneous complexes and the impairment of relevant mutational studies due to lethality. These limitations could be overcome with the preparation of recombinant complexes, which has so far been unsuccessful. This can be due to the complexity of the human 20S proteasome assembly pathway, which requires the expression and chaperone assisted step assembly of two copies of 14 closely related subunits, α1-7 and β1-7.

Here we show an effective approach for the preparation of recombinant human proteasomes and demonstrate the structural and functional integrity of the recombinant complexes. For this purpose, we solve the cryo-EM structure of the recombinant human 20S proteasome in the apo form (at 2.6 Å resolution) and in complex with PA200 (at 3 Å resolution), a still poorly characterised proteasome activator involved in acetylation dependent histone degradation. These structures reveal new insights into the PA200 function and the proteasome activity allosteric modulation, which has so far been elusive. We have now the tools to further characterise proteasome variants and unveil many proteasome mechanistic aspects that have remained obscure. We also provide an important new tool for the design and development of novel therapeutic drugs.

Structural analysis of SAS-6 reveals the molecular mechanism of centriolar cartwheel assembly

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Centrioles are cylindrical organelles which are essential for forming cilia, flagella and centrosomes in animal cells. In this capacity centrioles are important for mitotic spindle formation, correct chromosome segregation and cell polarity during division. The wide-reaching contributions of these organelles are best appreciated when errors in centriole assembly occur leading to a broad range of human diseases such as primary microcephaly, male sterility and cancer.
Structurally, the best-studied region of centrioles is the cartwheel, which is the first region forming during centriole and basal body assembly. The cartwheel is 9-fold symmetric and, thus, it is seen as critical for imparting 9-fold symmetry to the entire organelle. The evolutionary conserved protein SAS-6 is essential for cartwheel formation and it is recruited to the site of centriole assembly at the onset of their duplication. The cartwheel consists of a circular hub from which nine spokes emanate and radiate towards the cartwheel periphery, where spokes originating from two superimposed rings merge. The joined spokes then connect to a pinhead that bridges them with peripheral-most microtubules. However, we currently do not understand the molecular basis of cartwheels stacking and how they connect to each other and assist centriole elongation.
Here we present our progress combining biophysical and structural approaches to elucidate the oligomerisation properties of SAS-6 and reveal how SAS-6 ring oligomers stack along the length of centrioles, thereby providing an initial scaffold for subsequent recruitment of further centriole components.

Instruct Biennial Structural Biology Conference

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Structural characterization of the PHD5-C5HCH tandem domains of NSD family as epigenetic readers of H3K27me3 and interactors of Nizp1-C2HR

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The NSD transcription protein members (NSD1, NSD2, NSD3) contain several chromatin-related modules (a catalytic SET domain, two PWWP and six PHD domains), all implicated in developmental diseases and cancer [1,2]. As matter of the fact, the PHD tandem domains (PHDv-C5HCH) of these proteins have a prominent role in the transcriptional and tumorigenic activities of this protein family [2]. It is required for the recruitment of the NUP98-NSD1 fusion protein to the HoxA gene promoter in Acute Myeloid Leukaemia (AML) [3], it is essential for tumour cell proliferation induced by NSD2[4], and in NSD3 it is supposed to contribute to protein recruitment to chromatin through histone H3 interactions [5]. Here we present a systematic structural/functional investigation of the PHDv-C5HCH tandem domain of the three NSD family members. Our study shows that despite high sequence identity (~60%) [6], the NSD PHDv-C5HCH tandem domains have a divergent role in histones recognition and in protein-protein interactions. On one hand, the PHDv-C5HCH domain of NSD1 does not interact with histone H3 peptides, whereas it binds specifically with micromolar affinity to the C2HR domain of Nizp1 (Nizp1-C2HR), a co-repressor regulating NSD1 transcriptional activity in AML. On one hand, the PHDv-C5HCH domain of NSD1 does not interact with histone H3 peptides [6], whereas it binds specifically with micromolar affinity to the C2HR domain of Nizp1 (Nizp1-C2HR) [6,7], a co-repressor regulating NSD1 transcriptional activity in AML. On the other hand, the NSD2 and NSD3 tandem domains act as classical histone readers [5] but interact with low affinity with Nizp1-C2HR. We attribute these differences to small but crucial differences in aminoacidic sequence located on the interaction surfaces. Intriguingly, we demonstrate that NSD2 and NSD3 PHDv-C5HCH tandem domains specifically recognize H3K27me3, via the interdomain interface.

Methylation of H3K27 is usually associated to repressive chromatin, we thus hypothesize that PHDv-C5HCH of both NSD2 and NSD3 contribute to recruitment of NSD2/3 to repressed chromatin, to facilitate then activation through methylation of H3K36 via the catalytic NSD-SET domain.
In conclusion, our data propose a regulative scenario in which the same NSD tandem domain can differently regulate the recruitment of cofactors/epigenetic modifications necessary for gene transcription.

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Poster 16

Design of an *In Silico* workflow to discover new Influenza A NS1 inhibitors and Experimental Validation using NMR

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Influenza viruses are major human pathogens responsible for respiratory diseases affecting millions of people worldwide and characterized by high morbidity and significant mortality. The rapid emergence of influenza virus strains resistant to current antivirals accentuate the need for the development of new classes of antivirals. With this end in mind, several structural and functional studies of influenza non-structural protein 1 (NS1) have proposed this protein as a potential therapeutic target[1].

NS1 has two distinctive structural domains, the dsRNA-binding domain (RBD) and the C-terminal effector domain (ED). To allow experimental validation of potential NS1 inhibitors, we have devised a computational workflow supported in fragment-based approaches followed by experimental validation using nuclear magnetic resonance (NMR).

Said computational protocol begins with the prediction of druggable *hot spots* in RBD-NS1, i.e. putative pockets on the protein surface showing propensity for binding drug-like molecules. Fragment screening is then performed via search of fragment-bound binding sites holding high Molecular Interaction Field (MIF) similarity to the predicted NS1 binding sites, followed by hit confirmation via fragment docking. Finally, experimental validation is performed using chemical shift perturbation analysis of [^{1}H-^{15}N]-HSQC NMR spectra.

Complementary to this computational protocol we are studying molecules that resulted of bioassays, which the main goal was the search of molecules that may interact with NS1. We start to characterize these molecules using several fingerprints and ultimately, we perform clustering based on these fingerprints.

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Cryo-EM analysis of the role of RUVBL1-RUVBL2 ATPases during Nonsense-mediated mRNA decay

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Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism that recognizes and promotes the degradation of aberrant mRNA containing premature stop codons (PTC) but also targets for degradation a subset of naturally occurring transcripts. This process involves a dynamic network of transient protein-protein interactions between key factors, including canonical NMD factors, such as SMG1, UPF1, UPF2, UPF3, the ribosome release factors eRF1, eRF3 and the Exon Junction complex (EJC). Canonical NMD is coupled to translation initiation, where EJC marks exon-exon junctions thus indicating to the NMD machinery if a stop codon is premature or not. A translating ribosome stalled at a PTC in complex with eRF1-eRF3, leads to the recruitment of SMG1 kinase and UPF1 to assemble the surveillance complex (SURF, for SMG1, UPF1, eRF1, 3). Remodeling of this complex by association with EJC-UPF2-UPF3 results in the formation of the Decay-Inducing (DECID) complex, which promotes SMG1-mediated phosphorylation of UPF1. This is the key event that allows the degradation machinery to be recruited and promotes mRNA decay.

Although key steps are well stablished, increasing evidence suggests a more intricated regulatory mechanism including additional proteins. The DHX34 helicase has been recently described as a regulatory protein that interacts with SMG1 and UPF1 to active NMD by promoting UPF1 phosphorylation (1). A similar function has also been described for the RUVBL1-RUVBL2 ATPases that are found to be essential for NMD, associating with several NMD factors to promote remodeling of the SURF complex to activate NMD (2). Here, we explore more in detail the potential role of RUVBL1-RUVBL2 in NMD by dissecting direct protein-protein interactions between these ATPases and several NMD factors using pulldown experiments in vivo and in vitro. The complexes discovered are analyzed by cryo-electron microscopy to understand the structural basis of the interaction and the potential functional implications. Initial results show a heterohexameric ring of RUVBL1-2 interacting with a DHX34 monomer through the DII domains of the ATPases. Interestingly, assembly of the RUVBL1-2-DHX34 complex retains the ability of DHX34 to recruit UPF1 in in vitro assays, opening the possibility of higher order complexes to be assembled for the activation of NMD. Higher resolution will allow us to dissect the residues involved in this interaction to further explore its relevance in in vivo experiments. This will allow to understand how these NMD regulators could interplay with other NMD proteins to activate the decay response.

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Poster 18

Portugal at Instruct

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Portugal has set up the **Portuguese Centre for Integrated Structural Biology, PCISBIO,** which was approved as an Affiliate Centre of Instruct. This Centre integrates research groups from six Portuguese Universities, one private non-profit Institution and also the Portuguese networks on NMR and MS. From 2018 onwards ITQB-UNLwill represent Portugal at Instruct-ERIC and the financial support to the Portuguese membership has been endorsed by those Institutions, namely ITQB-UNL, FCT-UNL, FC-UL, UC, UA, UP, UM and IBET.

Portuguese expertise in Structural Biology spans over methodologies such as **mass spectrometry** and **proteomics, metabolomics, spectroscopy, NMR** and **X-ray crystallography**, as well as **cell- and bioimaging systems**. Additionally, in the BioPilot Plant of IBET, expertise is available in large-scale biomass production.

Two networks related with Instruct-ERIC activities have been approved and are coordinated by researchers from ITQB. One is an EU H2020 Widening – Twinning Project that aims to build the knowledge of Cryo-EM to life sciences research at ITQB NOVA. This project involves collaborative network with international renowned experts in this field, in which the Instruct Centres from Israel and Spain will be involved.

The other is a Cyted network involving Ibero-American groups and the aim is knowledge/technology transfer among researchers. Instruct-ERIC will mediate access to European facilities, in line with the goal of expanding access to non-EU communities, reinforced by activities/workpackages within Instruct-ULTRA, with the participation of Spanish Centre and Strasbourg.

Portuguese researchers have greatly benefited from Instruct through all the activities provided and available to country members. Highlights of relevant results of this participation in the last years will be presented. Portugal is one of the founding members of Instruct and is also a founding member of Instruct-ERIC.

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Poster 19

Structural basis of archaeal RNA polymerase elongation

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Multi-subunit RNA polymerases (RNAPs), present in all the living cells, evolved from a common ancestor. They transcribe DNA into an RNA molecule in a cycle of three stages: initiation, elongation and termination; and each phase is modulated by diverse transcription factors (TFs) (Cheung & Cramer, 2012; Werner et al., 2013).

The archaeal RNA polymerase (*a*RNAP) and the eukaryotic RNA Pol II are structurally and evolutionary related in terms of subunit composition and architecture, promoter elements and basic transcription factors required for Initiation and Elongation. The archaeal and eukaryotic RNAPs are divided in 3 main domains: the core, the mobile clamp, and the stalk. However, the archaeal transcription apparatus is a simplified form of the eukaryal machinery.

After loading of the DNA template, formation of the transcription bubble and several cycles of abortive nucleotide addition in the active site, the elongation starts with a 9 nucleotide longer DNA-RNA hybrid, establishing a stable transcription elongation complex (TEC). Interaction of RNAP with nucleic acids is tightly controlled for precise and processive RNA synthesis. While a wealth of structural information has been gathered on the eukaryotic Pol II in complex with DNA/RNA and more recently on Pol I and Pol III (Cramer et al., 2001; Gnatt et al., 2001; Fernandez-Tornero et al., 2013; Bernecky et al., 2016; Ehara et al., 2017; Abascal-Palacios et al., 2018), the molecular interactions governing archaeal elongation as way to decipher the evolutionary relationships with the eukaryotic counterpart are poorly understood.

To address this, we generated an archaeal RNAP/DNA-RNA complex *in cristallo* (Wojtas et al., 2012) mimicking a transcribing elongation complex (TEC). The resulting crystal diffracted to ~4.2 Å resolution and the derived electron density unequivocally showed the two 13-multisubunit *Sulfolobus shibatae* RNAP molecules composing the asymmetric unit of the crystal loaded with the corresponding nucleic acid in the DNA entry and RNA exit channels. Our study shows the molecular interactions governing transcription elongation in Archaea, providing the rationale for the mechanistic evolution from archaeal to eukaryal elongation.

ANTIBODY-DERIVED APTAMERS AS LIGANDS OF AB(1–42) AMYLOID PEPTIDE

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ANTIBODY-DERIVED APTAMERS AS LIGANDS OF AB(1–42) AMYLOID PEPTIDE
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Alzheimer's disease (AD), is the most common neurodegenerative disorder of the aging population resulting in progressive cognitive and functional decline. Senile plaques, consequence of the overproduction and/or impaired clearance of β-peptides (Aβ) are considered hallmark of AD.1,2 The main components of amyloid plaques are Aβ(1–40) and Aβ(1–42), soluble peptides that, in response to environmental factors, aggregate forming soluble oligomers, protofibrillar oligomers and finally, insoluble fibrils. Thousands of substances have been screened to search for anti-Alzheimer therapeutics that are able to control the Aβ conformation.3 Moreover neutralization of toxic Aβ peptides (in its multiple forms) using anti-Aβ monoclonal antibodies has receiving increasing interest as therapies for AD. Growing evidence, indeed, suggests that passive immunization against Aβ can provide clinical benefit, and perhaps AD prevention.4 Several monoclonal antibodies have been experimented to target different epitopes of the Aβ peptide, among them solanezumab5 and crenezumab6, that bind the fragments 16-26 and 13-23 respectively, of Aβ(1-42) peptide. In the present contribution we report design and synthesis of short peptides derived from the binding sites of Aβ(1-42)/solanezumab, Aβ(1-42)/crenezumab complexes. The newly synthesized peptides were preliminarily studied for their interaction with Aβ(1-42) using circular dichroism (CD) and saturation transfer difference (STD). In deep analysis of the peptides in complex with Aβ(1-42) was carried out using chemical shift mapping on 15N-labelled β-amyloid peptide.

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Structural basis for Acinetobacter baumannii biofilm formation

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Acinetobacter baumannii—a leading cause of nosocomial infections—has a remarkable capacity to persist in hospital environments and medical devices due to its ability to form biofilms. Biofilm formation is mediated by Csu pili, assembled via the 'archaic' chaperone-usher pathway. The X-ray structure of the CsuC-CsuE chaperone-adhesin pre-assembly complex reveals the basis for bacterial attachment to abiotic surfaces. CsuE exposes three hydrophobic finger-like loops at the tip of the pilus. Decreasing the hydrophobicity of these abolishes bacterial attachment, suggesting that archaic pili use tip-fingers to detect and bind to hydrophobic cavities in substrates. Anti-tip antibody completely blocks biofilm formation, presenting a means to prevent the spread of the pathogen. The use of hydrophilic materials instead of hydrophobic plastics in medical devices may represent another simple and cheap solution to reduce pathogen spread. Phylogenetic analysis suggests that the tip-fingers binding mechanism is shared by all archaic pili carrying two-domain adhesins. The use of flexible fingers instead of classical receptor-binding cavities is presumably more advantageous for attachment to structurally variable substrates, such as abiotic surfaces.

Structural characterization of the adaptor protein Nck1

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The family of Nck (non-catalytic region tyrosine kinase) proteins, Nck1 and Nck2 in humans, are adapter proteins that participate in signalling events that involve tyrosine phosphorylation. Nck1 and Nck2 have a very similar size (47 kDa) and domain structure. They contain three Src homology 3 domains (SH3-1, SH3-2, SH3-3) and a C-terminal SH2 domain that mediate the interaction with a large repertoire of proteins to create signalling complexes. The SH3 domains bind to Pro-rich motifs and each SH3 domain exhibit very distinct ligand specificity. The SH2 domain binds to phospho-Tyr. These globular domains are connected by linkers between 25 and 45 residues long. An intramolecular interaction has been described in Nck2 between the SH3-2 domain and a basic sequence in the linker between the SH3-1 and SH3-2 domains. This contact blocks the Pro-rich motif binding site of the SH3-2 [1]. The domain architecture of Nck1 remained largely uncharacterized. We have combined x-ray crystallography and small angle x-ray scattering (SAXS) to analyze the structure of human Nck1. We have solved the crystal structure of the SH3-2 domain to 1.2 Å resolution. SAXS analysis of full length Nck1 indicates that this protein is monomeric in solution, with a radius of gyration of ~34 Å and a maximum dimension of ~124 Å. Analysis of the scattering data using the Ensemble Optimization Method [2] indicates the presence of conformational heterogeneity. Analysis of shorter multi-domain fragments of Nck1 by SAXS indicates that the first pair of SH3 domains has low inter-domain flexibility and suggests the presence of an intramolecular interaction similar to that described in Nck2.

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Structural studies on carbonic anhydrases: the power of crystals.

Areej Abuhammad. School of Pharmacy, University of Jordan

There are sixteen human carbonic anhydrase (CA) isozymes that display varieties in their tissue distribution, and catalytic activities. These enzymes are involved in many essential physiological processes related to respiration, homeostasis, cell proliferation and bone resorption and calcification. The diversity of CAs functions in the body made them attractive drug targets for many diseases such as glaucoma, epilepsy, osteoporosis, and obesity.
However, selectivity of CA inhibitors is considered a major obstacle towards targeting these enzymes. None of the available CAIs selectively inhibits a particular CA isoform. Therefore, structure-based drug design is considered a method of choice for the design of selective and potent CA inhibitors. Protein crystallography is the most important component of this process. Structural information obtained from the crystals is pivotal in accelerating the identification and optimization of novel drug molecules.
As the case in many developing countries, protein crystallography research never existed in Jordan. I will give a brief summary of the establishment of the first protein crystallography laboratory in Jordan and the progress of the CAs project. I will also discuss the impact of transferring this cutting-edge technology to Jordan on enhancing regional and international scientific collaboration and on extending a new opportunity for young students in the developing countries.

Structural basis of RNA polymerase I stalling at UV light-induced DNA damage

Carlos Fernandez-Tornero. CIB-CSIC

DNA lesions threaten cell life and must be repaired to maintain genome integrity. During transcription, RNA polymerases actively scan DNA to find bulky lesions and trigger their repair. In growing eukaryotic cells, about 60% of the total transcriptional activity involves the synthesis of ribosomal RNA (rRNA) by RNA polymerase I (Pol I), a 14-subunit macromolecular machine with unique regulatory features [1]. Accordingly, Pol I monitors rDNA integrity and influences cell survival, but little is known about how this macromolecular machine processes UV light-induced lesions. We determined the cryo-EM structure of Pol I stalled by a UV-induced lesion, cyclobutane pyrimidine dimer (CPD), at 3.6 Å resolution to unveil how the enzyme manages this important DNA damage [2]. The structure shows that the lesion induces an early translocation intermediate with a peculiar arrangement in the vicinity of the active site. In particular, the bridge helix residue Arg1015 contributes to CPD-induced Pol I stalling, as confirmed by mutational analysis. These results, together with biochemical data, reveal how Pol I stalls at CPD lesions, which is distinct from Pol II arrest by this DNA damage. Our findings open the avenue to unravel the molecular mechanisms underlying cell endurance to lesions on ribosomal DNA.

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Structural insights into the regulation of human phenylalanine hydroxylase

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Phenylalanine hydroxylase deficiency is the most prevalent disorder of the amino acid metabolism. In non-pathological conditions, L-phenylalanine (L-Phe) from dietary intake is metabolized by phenylalanine hydroxylase (PAH) to L-tyrosine, a precursor for neurotransmitter biosynthetic pathways. Mutations that impair PAH function lead to toxic accumulation of L-Phe in the body and to the development of neurological problems in patients. Most PAH mutations are missense mutations that affect folding, catalysis and/or regulation. Understanding the structural and regulatory properties of PAH is essential to outline pathophysiological mechanisms in phenylalanine hydroxylase deficiency. However, the difficult manipulation of recombinant human PAH has hindered further structural characterization – the available data derive from truncated forms or from a rat homologue. Functional PAH is a homotetramer, each subunit consisting of an N-terminal regulatory domain, a central catalytic domain and a C-terminal oligomerization domain. In order to maintain physiological levels of L-Phe (preserving enough to sustain protein synthesis and removing excess to avoid neurotoxicity), PAH displays a complex net of mechanisms that involve transition between oligomeric states, conformational changes, substrate activation and cofactor inhibition. Crystal structures of the full-length rat PAH show an auto-inhibited conformation, where regulatory domains block access to the active site. Allosteric activation by L-Phe is posited to induce a repositioning of the regulatory domains – however, a structure of activated PAH is still lacking. We have addressed L-Phe-mediated conformational changes of human PAH and report the first structure of the allosterically activated state. Our solution structures determined by small-angle X-ray scattering show a tetrameric protein with a distorted P222 symmetry, where catalytic and oligomerization domains form a central core from which regulatory domains protrude. Alike the available crystal structures, in the absence of L-Phe, regulatory domains are positioned close to the active site entrance. L-Phe induces a large-scale movement with formation of dimers that expose the active site. The activated protein is more resistant to proteolytic cleavage, suggesting that the association of regulatory domains stabilizes PAH.

Dimeric structures of active quinol-dependent Nitric Oxide Reductases (qNOR) revealed by cryo-Electron Microscopy

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Quinol dependent Nitric Oxide Reductases (qNOR) are membrane integrated, iron containing enzymes of the denitrification pathway, catalysing the reduction of nitric oxide (NO) to the major ozone destroying gas nitrous oxide (N2O). qNORs' are more commonly found in pathogenic microorganisms than in denitrifiers, most likely to detoxify host cell produced NO. Efforts to elucidate the structure of an active qNOR has been limited, since the 2.7 Å Geobacillus stearothermophilus crystal structure incorporated a foreign Zinc into the active site, rendering the enzyme inactive and a highly active qNOR from Neisseria meningitidis had its structure resolved to only 4.5 Å. The combination of these results has hampered a real structure-function relationship for qNORs. Here we present Cryo-electron microscopy structures of active qNOR from Alcaligenes xylosoxidans, and an activity-enhancing mutant at global resolutions of 3.9 and 3.3 Å, respectively. They unexpectedly reveal a dimeric conformation (also confirmed for qNOR from Neisseria meningitidis) and define the true active site configuration, with a water channel from the cytoplasm. Structure-based mutagenesis has identified several novel and vital residues involved in proton transport and substrate delivery to the active site of qNORs. The proton supply direction differs from cytochrome c dependent NOR (cNOR), with water molecules from the cytoplasm serving as a proton source, similar to the evolutionarily related cytochrome c oxidase. Our work has addressed several areas of ambiguity in the qNOR field and help to inform more about an enzyme of agricultural and medical importance.

Structural insights into the PBX1-PREP1 and PBX1-MEIS1 interactions obtained by cross-linking mass-spectrometry approach

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PREP1 (Pbx-regulating protein 1, aka PKNOX1), and MEIS1 (Myeloid ecotropic insertion site 1) are three amino acids loop extension (TALE) homeodomain transcription factor, playing essential roles in pattern formation during embryonic development, as pre-B-cell leukemia (PBX) cofactor. PREP1 is essential in embryonic development; in the adult it functions as a tumor-suppressor. The tumor-suppressor role of PREP1 is associated with the maintenance of genomic stability, control of DNA replication timing, and protection of the nuclear envelope. PREP1 and MEIS1 bind PBX1 in the absence of DNA; such interaction regulates the subcellular localization and stability of PBX1.
Structural knowledge of TALE transcription factors is currently limited to the crystallographic and NMR structures of their homeodomains (the 60 amino acids DNA-binding motif of TALE, located at their C-termini).

Both the tumorigenic activity of MEIS1 and the anti-tumorigenic activity of PREP1 require a 100 amino acids N-terminal portion (HR1 and HR2 domains), through which they dimerize with their common cofactor PBX. In the HR1 and HR2 domains, both PREP and MEIS present highly conserved heptad leucine/isoleucine-rich motifs, and they have been previously described as a leucine zipper. As PREP1 and MEIS1 use the same surface of PBX, association with PBX is mutually exclusive. Therefore, PREP1 complete with MEIS in the oncogenic function, and PBX acts as an oncogene or as a tumor-suppressor, depending on its partner, MEIS1 or PREP1. In addition, although it is usually referred to the 200 amino acids N-terminal PBC domain of PBX (that include PBC-A and PBC-B domains) as essential for interaction with MEIS and PREP, so far only the direct involvement of the PBC-A domain has been demonstrated.

Critical to elucidating mechanisms of leukemogenesis is the identification of the specific PBX-MEIS interaction surface, which is crucial to leukemic activity. However, because of the high similarity of PREP1 and MEIS1 HR1 and HR2 regions, an in-depth comparative analysis of these regions is a prerequisite of the specificity of target drugs.

In this work we have highlighted the differences between the PBX-PREP1 and MEIS1-PBX interaction surfaces, and defined experimentally the minimalist block interface of PREP1 and MEIS1 needed to form a complex with PBX1 by mean of cross-linking mass-spectrometry approach, mutational analysis and immunofluorescence experiments in cell-lines.
Our cross-linking mass-spectrometry data provide a consistent footprint of PBX1-exposed regions, and highlight a direct involvement of both PBC-A and PBC-B domains as interaction surfaces. In addition, despite the sequence homology between PREP1 and MEIS1 in their HR1 and HR2 regions, we observe

a predominant role of HR2 in PBX1 interaction with MEIS1. PREP1 instead displays two distinct binding interfaces to PBX1, one between PBC-A and HR1 and the other between PBC-B and HR2. Dimerization with PBX1 allows PREP1 and MEIS1 to reach the nucleus. Therefore, we have tested the nuclear versus cytoplasm localization of PREP1, when mutated in critical regions identified by cross-linking mass-spectrometry. To this goal, we have used lung carcinoma A549 cells, in which PREP1 is endogenously down-regulated, and there we overexpressed GFP-tagged wild-type or mutants PREP1. Our immunofluorescence results suggest that both HR1 and HR2 domains of PREP1, are both indispensable for dimerization with PBX1 not only in vitro but also in vivo, as when mutated PREP1 is not able to reach the nucleus.

By mean of cross-linking mass-spectrometry analysis, structural considerations, and mutational characterizations, we have therefore mapped the PBX1-MEIS1 interaction interfaces which can be potentially be used as target for future drug design in leukemia treatment.

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MOLECULAR UNDERSTANDING OF METAL-PORPHYRINS EFFICACY IN THE TREATMENT OF PRION DISEASES

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Porphyrins are organic compounds consisting of four pyrrole rings linked to each other by methine groups and able to coordinate di- or tri-valent metal cations. The cellular prion protein (PrPC) is a highly conserved mammalian glycoprotein, GPI anchored to the extracellular layer of the cell membrane, expressed predominantly in the central nervous system, and possibly involved in copper metabolism. It folds into a C-terminal globular domain and a long, unstructured N-terminal region, in which four octapeptide repeats are responsible for pH-dependent copper binding.

Prion diseases are invariably fatal, currently untreatable neurodegenerative disorders of mammals. They arise from the conversion of PrPC into a β-sheet rich, pathogenic and infectious isoform (PrPSc or prion), which propagates by inducing misfolding of native

PrPC. PrPSc accumulates in the brain, causing neuronal dysfunction and degeneration. Promising therapeutic strategies include blocking the PrPC to PrPSc conversion or depleting the substrate for PrPSc formation by reducing endogenous PrPC level. We identified a metal-porphyrin (VA01) that is effective in the treatment of prion diseases through a new mechanism. We applied NMR spectroscopy and other biophysical techniques to clarify at the molecular level the VA01 mechanism of action:

i) we proved VA01 binding to the human prion protein (hPrPC) and we characterized the ligand epitope by STD experiments and the protein interaction surface by NMR chemical shift mapping;

ii) we investigated whether different VA01 functionalizations and coordinated metal cations affect hPrPC binding;

iii) we collected the atomic contacts (NOEs) between VA01 and hPrPC octapeptide repeat;

iv) we exploited circular dichroism melting experiments and analytical ultracentrifugation sedimentation velocity experiments to highlight changes in hPrPC stability, size and shape upon VA01 binding.

Our results suggest that engagement of the hPrPC unfolded N-terminal region in VA01 binding is responsible for VA01 biological activities, as it stabilizes the protein in a close conformation, which likely is less prone to PrPSc-induced conversion. Collectively our structural and cellular data allow for a molecular understanding of VA01 efficacy in the treatment of prion diseases and prelude for the design of optimized metal-porphyrins with increased affinity and efficacy.

The structure of human pyrroline-5-carboxylate synthetase, determined by cryoEM, explains channelling within this bifunctional enzyme that is associated with two genetic disorders presenting dominant or recessive inheritance.

Clara Marco-Marín. Instituto de Biomedicina (IBV-CSIC) and CIBERER-ISCIII. .

Mutations in the bifunctional enzyme catalyzing the first two steps of de novo synthesis of both ornithine and proline, Δ1-pyrroline-5-carboxylate syntethase (P5CS), cause an inborn error presenting as two different syndromes, each one of them with recessive or dominant inheritance, depending on the mutation: 1) A neurocutaneous syndrome with skin and joint laxity, neurodevelopmental delay, and early cataract; and 2) a complicated spastic paraplegia with or without cognitive impairment and cataracts. Our functional evidence with recombinant human P5CS supports that both syndromes result from loss or decrease of P5CS function. The facts that dominant phenotypes are constantly associated with missense mutations whereas obligatorily inactivating mutations (truncations, splicing errors) are found only in recessively inherited cases support our belief that dominance is due to a negative dominant effect. Thus, some missense mutations would have a dominant effect because they would disturb the architecture of the P5CS homooligomer and the proper channelling of the unstable glutamyl-5-phosphate (G5P) produced by the glutamate-5-kinase (G5K) domain of one subunit to the glutamyl-5-phosphate reductase (G5PR) domain of another subunit.
This proposal is supported here by our determination of the 3-D structure of P5CS, found intractable by crystallography, but achieved for the human enzyme at 3.4 Å overall resolution by cryoEM. P5CS is organized as a tetramer with point group 222 symmetry. In this tetramer the G5P produced by the G5K domains cannot be channelled to the G5PR domains, since the active sites of both domains within the tetramer are wide apart and misoriented for product transfer. Thus G5P would have to dissociate into the bulk solution before it could have access to a G5PR active site. However, three tetramers stack by alignment of one of the twofold axes, with a screw rotation along this axis of approximately 60°. In this supratetrameric architecture cavities are formed in which G5K and G5PR active centers from different tetramers are exposed, looking one towards the other. Preferential use of the G5P released in this cavity from the G5K active center by the facing G5PR active center would result in channelling. The location of the dominant mutations fits this view, since these mutations generally affect residues involved in intertetrameric interactions or residues that are found in structural elements that mediate contacts between different subunits. In contrast, most missense mutations associated with recessive inheritance do not appear to have important roles in intersubunit interactions. In summary, proper P5CS function requires association of the basic tetrameric unit into supratetrameric highly specific architectures. Grants BFU2017-84264-P and BFU2017-85814-P (MICINNU, Spain) & Fundación Inocente Inocente.

Protein cysteinylation as a hallmark of chronic kidney disease

Dalila Fernandes. ITQB-NOVA

Protein cysteinylation is a post-translational modification recently posited as an in vivo marker of oxidative stress and a hallmark of chronic kidney disease (1,2). Cysteinylation refers to formation of a disulfide bond through oxidation of the thiol moieties of a protein-bound cysteine (Cys) and a free Cys. This usually reversible modification (1,3) plays both a regulatory and an antioxidant role, because it protects protein cysteinyl thiols against irreversible oxidative or electrophilic modification and also has a role in protein redox regulation (4). The molecular mechanisms whereby protein cysteinylation occurs are still not fully understood.
In this study, we investigated the possible involvement of the three hydrogen sulfide (H2S)-synthesizing enzymes (cystathionine β-synthase, CBS; cystathionine γ-lyase, CSE; and 3-mercaptopyruvate sulfurtransferase, MST) in protein cysteinylation. CBS and CSE both participate in the methionine metabolism transsulfuration pathway, collectively synthesizing cysteine from homocysteine, and H2S from alternative reactions employing different substrates. MST uses 3-mercaptopyruvate generated by cysteine aminotransferase (5). Additionally to H2S, these three enzymes also contribute to endogenous generation of persulfides and polysulfides species, and to do so, CBS and CSE use cystine (CysS), while MST uses Cys as a co-substrate (3,5). These species possess markedly high antioxidant and nucleophilic properties and are critically involved not only in the detoxification of environmental electrophiles but also in the regulation of redox signaling (3).
To test this hypothesis, we used model protein targets like bovine serum albumin and egg white lysozyme (as negative control) to evaluate their possible cysteinylation mediated by the three H2S-synthesizing enzymes. Cysteinylation detection was measured by high-performance liquid chromatography (HPLC) with fluorescence detection (6) and by a cysteine-targeting gel shift assay. X-ray crystallography was employed to confirm the cysteinylation of the target cysteines.
The results will be discussed in a perspective of H2S-synthesizing enzymes contribution to cysteinylation in the context of chronic kidney disease, and also focusing on these enzymes as potential drug targets.

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The Structure-based Development of Novel Bile Acid-derived Agonists of FXR
Dannielle Kydd-Sinclair. University of Reading.

The Farnesoid X Receptor (FXR) is a nuclear receptor that is highly expressed in the liver and intestines. As with other classic nuclear receptors, FXR acts as a ligand-activated transcription factor, and comprises an N-terminal DNA binding domain, and a highly flexible, alpha helix bundle, ligand binding domain (LBD) at its C-terminus. The cognate ligands for FXR are bile acids, which, once bound, induce a conformational change in the LBD, causing the dissociation of corepressors and recruitment of coactivator complexes, resulting in epigenetic changes that ultimately lead to the transcription of its target genes. FXR has been shown to regulate bile acid homeostasis, lipoprotein and glucose metabolism, as well as liver regeneration, and as such is an attractive potential therapeutic target for metabolic liver diseases. To date, several structurally diverse agonists have been described for FXR, however, despite heightened pharmaceutical interest, very few have progressed to clinical trials due to poor pharmacokinetic properties.

The aim of our work is to use a combination of experimental and computational approaches in an attempt to identify structural modifications to the bile acid backbone which may enhance receptor activation, improve bioavailability and reduce adverse effects. To date, the use of Molecular Interaction Field (MIF) - based in silico approaches, has allowed us to characterise the LBD according to favourable interactions. Furthermore, molecular docking, whereby theoretical bile acid analogues are placed in the FXR LBD to determine potential ligand-receptor interactions, has streamlined the workflow in lead compound identification and has confirmed key residues and helices involved in ligand binding, as previously acknowledged in co-crystal structures. In anticipation of the discovery of novel candidate(s), work has been initiated on the expression and purification of the FXR LBD, so that, in due course, co-crystallization and structure determination with this lead compound, will evaluate our predicted binding interactions and will further our understanding of binding modes required for FXR activation.

While in silico docking allows us to predict the occupancy of novel compounds in the FXR LBD, other experimental techniques have been used to assess the biological response of FXR upon ligand binding. Biochemical assays and real time- quantitative PCR have been used to evaluate lead compounds, for their ability to induce an active conformation of the FXR LBD and their ability to exert changes in the expression of genes involved in FXR-mediated pathways. Together, both approaches contribute to the design of bile acid-derived FXR agonists with improved affinity and potency.

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Poster 32

Bringing together functional annotations related to structure
David Armstrong. PDBe

It is essential to take into consideration the biological context of macromolecular structures, in order to understand these molecules archived in the world-wide Protein Data Bank (wwPDB). Many of the specialised resources provide one or more aspects of the biological context, but it takes significant effort to collect and compare all the information that may be relevant to a specific structure.

PDBe-KB (Protein Data Bank in Europe - Knowledge Base) is a new, community driven resource under development by PDBe, that will provide functional annotations for structural data. These annotation aim to assist the scientific community in answering biological questions. PDBe-KB is a collaborative effort between PDBe and a diverse group of biological resource, structural bioinformatics research teams. This new resource will consolidate older services, such as SIFTS, which focuses on providing seamless mappings between PDB entries and other databases, and data from multiple novel data enrichment project, which aim to collect and distribute highly enriched, valuable annotations that create a comprehensive biological context for structural models, effectively bringing structure to biology.

Genome release of Echovirus 18
David Buchta. CEITEC MU

Viruses from the genus Enterovirus are important human pathogens. Receptor binding or exposure to acidic pH in endosomes converts enterovirus particles to an “activated” state that is required for genome release. However, the mechanism of enterovirus genome release is not well understood. Here, we used cryo-electron microscopy to visualize virions of human echovirus 18 in the process of genome release. We discovered that viral RNA exits the echovirus 18 particle through a hole that forms in the capsid upon loss of one, two, or three adjacent capsid-protein pentamers. The resulting hole, which is more than 120 Å in diameter, enables the release of the genome without the need to unwind its putative double-stranded RNA segments. Thus, our findings uncover a novel and conserved mechanism of enterovirus genome release that could become target for antiviral drugs.

Structural studies of multispecific Antibody/Antigen complexes by cryo-EM

David Fernandez Martinez. ESRF

Multispecific antibodies are artificially engineered antibodies that aim to bind different targets at once by using only one molecule. The potential advantages of generating viable multispecific antibodies range from being able to identify malignous cells and recruiting immune cells at the same time to blocking complex viral escape mechanisms. The cross-over dual-variable immunoglobulin (CODV-Ig) has been proposed as a universal bispecific therapeutic format. Its unique antigen-binding fragment (Fab) architecture provides pM affinities to ligands, no positional effect in target binding and a stable self-supporting truss. Nevertheless, disparity between in vitro and in vivo effects have evidenced an effect that is thought to be caused by the relative three-dimensional arrangement of the constant and antigen-binding fragments. To further understand the molecule and its functions, the high-resolution structure of the full antibody is required, and for this end, cryo-electron microscopy (cryo-EM) has been considered as a tool, as recent advances in the field may enable the study of this highly flexible, small molecule.
We purified CODV-Ig, with and without one of the antigens that the antibody is specific towards, and validated the sample presence, quality and dispersion by SDS-PAGE, Small Angle X-Ray Scattering (SAXS) and negative-stain electron microscope (NSEM) at the Tecnai T12 microscope (IBS, Grenoble). Preliminary data and a very low-resolution model obtention from NSEM suggested a preferential orientation of the molecule under negative-stain conditions. The optimal vitrification conditions for CODV-Ig have not yet been achieved due to the molecule's aggregation propensity and aversion to conventional cryo-EM supports, nonetheless data of sufficient quality for image analysis has already been collected at a Titan Krios (ESRF, Grenoble). Image processing of both CODV-Ig alone and in complex suggests high flexibility and conformational heterogeneity.

Matching evolutionary couplings and ambiguous NMR contacts to derive homo-oligomers structure

Davide Sala. University of Florence, CERM

The structure determination of large proteins by NMR is often a challenging task due to the high ambiguity coming from resonance overlap. Further challenge, ambiguous contacts list of homo-oligomeric complexes usually contains from hundreds to thousands of assignments potentially belonging to either monomeric structure (intra-monomeric contact) or to homodimerization interface (inter-monomeric contact). The correct identification of inter-monomeric contacts requires extensive user efforts and experience. However, we took advantage of the recent availability of web-servers performing coevolution analysis to predict residue-residue contacts from sequence information alone. The so-called evolutionary couplings (ECs) can be used as complementary structural information to automatically extract contacts at the homo-oligomeric interface from ambiguous NMR contacts list. Our protocol needs three inputs to derive the list of residues to use in protein-protein docking: the target protein sequence, the structure of the monomer and the experimental NMR-derived ambiguous list. The whole procedure can be divided in two main parts. First, false-positives and intra-monomeric contacts are removed from the evolutionary couplings list. To do so, the ECs associated probability of being actually in contact and the experimental structure of the monomer are exploited. Second, the list of ECs precited to be at the complex interface is compared with the ambiguous NMR contacts list to extract all residue pairs matching both the predicted and the experimental dataset. The matched interface residues not at the protein surface are removed by calculating the relative solvent accessibility in the monomer. The resulting residues are provided to the HADDOCK software as ambiguous interaction restraints (AIRs) in a monomer-monomer docking calculation.
The protocol was validated in the prediction of the tetrameric E. coli L-asparaginase II (PDB ID: 6EOK) in which two distinct dimeric conformations must be recognized to reconstruct the functional complex. Furthermore, the robustness of the protocol in the identification of residues belonging to small interface regions was tested in the prediction of dimeric human apo Sod1 (PDB ID: 3ECU).
For both the proteins we reconstruct the functional homo-oligomeric complex with an RMSD of about 1 Å form the experimental crystal structure. Importantly, our protocol has proved effective in the signal extraction from solution 3D 1H15N NOESY HSQC and solid state 2D13C-13C DARR data.

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Poster 36

Developing electron diffraction of 3D protein nanocrystals at the IBS
HOUSSET Dominique. Insitut de Biologie Structurale - Grenoble

Electrons are strongly scattered by light atoms such as carbon (4 to 5 order of magnitude more than X-rays) and the energy deposited on the sample by diffracting particle is 1000 times less. They thus constitute an ideal probe for the study of protein nanocrystals. Recent studies have demonstrated that electron diffraction of three-dimensional protein nanocrystals (3D-ED) can be used to determine peptide or protein structures at resolution higher than 2 Å. Reasonably complete data sets can be collected from 10 to 20 crystals which size do not exceed 300 nm in at least one dimension [1,2]. Up to now, about 30 protein structures have been deposited in the PDB, corresponding to 12 different proteins. The recent evolution of direct detectors dedicated to electron diffraction should be instrumental in making 3D-ED a new tool in structural biology, very complementary of single particle cryo-EM or X-ray diffraction. We wish to develop a 3D-ED platform at IBS (Grenoble) in order to further explore the structural information that can be obtained by 3D-ED on several biological projects. With this aim, we have initiated a collaboration with Jan Pieter Abrahams (University of Basel). We have collected diffraction data on peptide and protein crystals and have refined their structures. We will present our latest results and discuss the advantages and the difficulties of the 3D-ED approach.

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Preliminary cryo-electron microscopy 3D reconstruction of the eukaryotic 4F2hc/LAT1 amino acid transporter

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Preliminary cryo-electron microscopy 3D reconstruction of the eukaryotic 4F2hc/LAT1 amino acid transporter

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Heteromeric Amino acid Transporters (HATs) are composed of a heavy (ancillary, SLC3 family) and a light (SLC7 family, LAT subfamily) subunit, linked by a disulfide bridge. HATs play key roles in human physiology and are implicated in several inherited diseases such as cystinuria, lysinuric protein intolerance, autism and age-related hearing loss. Particularly, mutations in 4F2hc/LAT1 are associated with autism spectrum disorder. Additionally, 4F2hc/LAT1 and 4F2hc/xCT transporters are also frequently overexpressed in cancer. Despite the relevance of HATs, knowledge of their structure is limited to the atomic structure of the extracellular domain of the heavy subunit 4F2hc (CD98, SLC3A2) and to low-resolution models of human 4F2hc/LAT2. Additionally, the crystal structures of prokaryotic amino acid transporters with low sequence identity with human LATs (14–22%) have been solved. Vertebrate 4F2hc/LAT1 can be functionally overexpressed in Pichia pastoris and pure recombinant protein purified. [3H]Isoleucine uptake in 4F2hc/LAT1 expressing Pichia cells shows intracellular amino acid accumulation over the background (4F2hc expressing cells). In parallel, [3H]isoleucine uptake inhibition by increasing concentrations of the high-affinity LAT1 competitive inhibitor KY-0353 resulted in a Ki value compatible with those previously reported. Preliminary cryo-electron microscopy 3D reconstruction of the eukaryotic 4F2hc/LAT1 heterodimer revealed a complex made of two distinct regions. An ectodomain protrudes from a flat region surrounded by detergent thus interpreted as containing the elements of 4F2hc/LAT1 embedded in the membrane. The resolution of the structure was worse than 13-15 Angstroms. Image classification strategies suggest that the heterogeneity and flexibility of the eukaryotic 4F2hc/LAT1 heterodimer might contribute to the limitations in resolution, together with the inherent difficulty to align images of a small membrane protein. Alternative strategies are being evaluated to overcome these issues. 4Fhc/LAT1 structure will help to dissect the molecular mechanisms underlying substrate selectivity, transport mechanism, study the molecular defects associated with autism mutations and build structure-guided inhibitors with potential pharmacological applications.

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Poster 38

Molecular Mechanism of Bacterial Replicative Helicase loading
Ernesto Arias-Palomo. CIB-CSIC

Loading of hexameric ring-shaped helicases at the origin of replication requires dedicated AAA+ ATPases. However, the molecular mechanisms that control nucleotide turnover and helicase loading are not well understood. We have used cryo-electron microscopy, in combination with functional assays, to analyze the helicase/loader complex from Escherichia coli. Our work provides the first high-resolution view of how replicative helicase loading occurs in bacteria and explains how this mechanism both parallels and diverges from homologous hexameric helicase and DNA polymerase clamp loader systems.

Mechanistic insights into peptidase gating of the 26S proteasome
Eri Sakata. Max Planck Institute of Biochemistry.

The proteasome is the central protease for intracellular protein breakdown. It is composed of a catalytic 20S core particle (CP) and 19S regulatory particles (RPs) containing a hexameric AAA+ ATPase ring. Targeted substrates are processed through several reactions by the RPs, including recognition, deubiquitination, unfolding and translocation. Importantly, coordinated ATP-binding and -hydrolysis by the six proteasomal ATPase subunits play key role for substrate unfolding and translocation. Recent structural studies of the 26S proteasome advance our understanding of the arrangement of the different subunits and the conformational dynamics of the holocomplex. However, the relationship between nucleotide binding and proteasome conformation and the upregulation mechanism of the proteasome remain unclear.
To address these issues, we analyzed structures of the 26S proteasome by single-particle cryo-electron microscopy (cryo-EM) upon incubation with different nucleotide analogs. This allowed us to identify several novel conformations in which the CP gate is open. We showed that the insertion of the C-terminal motifs of the ATPases opens the CP gate by rearranging the N-termini of α subunits of the CP. We also revealed the impact of individual ATP-binding events on proteasome conformational dynamics. The expanded knowledge of the conformational landscape and of gating by the ATPase ring will serve as a valuable framework for dissecting how these key functional events are mechanistically connected.
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Structural studies of intrinsically disordered proteins towards the development of formulations for market-oriented pharmaceutical products.

Evangelia Chrysina. National Hellenic Research Foundation

Structural studies of intrinsically disordered proteins towards the development of formulations for market-oriented pharmaceutical products.

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The elucidation of structure-function relationships of intrinsically disordered milk proteins from natural sources is of great research interest in view of their biological relevance, as well as their broad commercial and pharmaceutical applications. Caseins represent about 80% of the well-studied bovine milk proteins and due to their inherent tendency to stabilise forming large colloidal particles with calcium phosphate the casein micelles have been extensively studied. Casein micelles are natural nano-capsules that effectively deliver nutrients and therefore there is plenty of current literature that aims at their utilization as nano-carriers of biologically active agents, such as drugs. In this work we use biophysical and biochemical methods to investigate new functionalised caseins, which are utilized as active pharmaceutical ingredients. In specific, novel trivalent iron complexes with caseins are developed and studied by X-ray diffraction and spectroscopy in combination with dynamic light scattering. This study aims to correlate the casein structure at molecular level with the morphological characteristics at the micellar level, as a function of solution conditions (e.g. pH and temperature), using commercially available caseins as reference materials. Preparation protocols are developed in order to establish the caseins characterization in solution and in bulk and to optimise the formulation of these market-oriented pharmaceutical nano-carriers. This study demonstrates the importance of the combination of a series of experimental techniques in order to control and fine-tune the stability and function of novel protein-based pharmaceutical formulations.

This project has been supported by “Instruct-ULTRA: Releasing the full potential of Instruct to expand and consolidate infrastructure services for integrated structural life science research”, Proposal No. 731005, Horizon2020-INFRADEV-2016-2017 under the programme, “Development and long-term sustainability of new pan-European research infrastructures, coordination and support action INFRADEV-03-2016-2017”.

Structural and functional insights into the inhibition of HMGB1/CXCL12 axis by small molecule Diflunisal

Federica De Leo. Vita-Salute San Raffaele University

Extracellular HMGB1 triggers inflammation following infection or injury, and supports tumorigenesis in inflammation-related malignancies. HMGB1 has several redox states: reduced HMGB1 recruits inflammatory cells to injured tissues forming a heterocomplex with CXCL12 and signaling via its receptor CXCR4; disulfide-containing HMGB1 binds to TLR4 and promotes inflammatory responses. Here we show that Diflunisal, an aspirin-like nonsteroidal anti-inflammatory drug (NSAID) that has been in clinical use for decades, specifically inhibits in vitro and in vivo the chemotactic activity of reduced HMGB1 at nanomolar concentrations and, by applying NMR and MST methods we characterize the structural aspects of the inhibition mechanism. Surprisingly, we found that DFL binds to both HMGB1 and CXCL12 and affects residues on both proteins that are at the complex interface. Both NMR and MST experiments nicely demonstrated the disruption of the complex upon DFL binding. Importantly, Diflunisal does not inhibit TLR4-dependent responses.
We herewith reveal an unusual inhibition mechanism of a ligand concomitantly binding to both the proteins forming an heterocomplex, correlated with remarkable structural/functional insights for the HMGB1 inhibition. Our findings open the way to the rational design of functionally specific anti-inflammatory drugs.

Arsenite oxidase: Structural and functional insides on the electron transfer pathway

Filipa Engrola. FCT-NOVA

According to the WHO, Arsenic is one of the top 10 chemical contaminants in drinking-water worldwide and affects more than 140 million people. Two arsenite oxidizing enzymes – Aio - and their final electron acceptors - cytochrome c552 and azurin - from Rhizobium sp. NT-26 and Alcaligenes faecalis, respectively, are currently being studied for their use as biosensors and bioremediation. Both share high structural similarity (r.m.s.d (Cα) = 1.84 Å) and are composed of a subunit (AioA) which contains a molybdenum centre and a 3Fe-4S cluster and a Rieske like subunit (AioB) that possess a 2Fe-2S cluster.
The heterologous expression of Aio in Escherichia coli was optimized and a 20-fold increase, from the initial expression and purification conditions, was obtained (0.3 mg.L-1 vs 7.2 mg.L-1); accompanied by a 5 degree increase in the melting temperature, determined by Thermal Shift Assays. We are currently using biophysical techniques to characterize the interaction between the enzyme and the electron acceptor: SEC-SAXS data shows that the protein in solution presents several oligomerization states; Microscale Thermophoresis assays, show very strong binding between Aio and Cytochrome c552 (KD=0.2 µM), with a 1:1 stoichiometry. To better understand the catalysis mechanism of the enzyme from Rhizobium sp. NT-26, we mutated Asp169A and Glu453 from the active centre to Ala using site-directed mutagenesis; using UV-vis, we observe that the protein is still active; X-ray crystallography and STD-NMR are currently under way in order to obtain molecular details of the catalytic mechanism and electron transfer pathway.

Characterization of the novel type rotary ATPase as an essential component of the Chlamydial Na+ coupled energetics.

Ganna Krasnoselska. Oxford University.

Based on the structure, function and underwent evolution there are 3 known types of rotary ATPases: F-, V/A- and V-types. While eukaryotes keep both, F-type and V-type enzymes in cells, the majority of prokaryotes gave preference to only one type – F or V/A. Surprisingly, non-canonical V/A-type ATPase (smaller overall number of subunits, one unique subunit, novel motifs and low sequence similarity to other orthologs) is produced in Chlamydia sp., a group of obligate intracellular parasites of human epithelial cells, which for a long period of time were considered to be strict energy parasites. In our studies, we have found that this type of operon is not an outlier of Chlamydiales genome reduction mode of evolution but this operon is found in some other strict or opportunistic parasites as well as in some free-living eubacteria with full-size genomes. Indeed, phylogenetic analysis of catalytic subunit A limits distribution of this unique type of operon to a small group of Spirochetes, Bacteroidetes, Chlamydiales and Proteobacterial species. Based on the presence of conserved sodium ion binding motif in the membrane part, the plausible role of this enzyme is a generation/consumption of sodium ion gradient. However, low sequence similarity with other orthologous genes and the presence of unique motifs can't exclude this enzyme acquired a new function in the infection. For the Chlamydia sp, the role of sodium gradient in infection is actively discussed nowadays and lead to the reconsideration of energy parasite hypothesis. Three main membrane systems are considered as potent producers of sodium gradient (Na+-NQR, Na+/H+ antiporter NhaD, and Na+-V/A-ATPase) and few systems are considered to be potent consumers of sodium gradient (Na+-V/A-ATPase and Na+-dependent nutrients uptake systems). In general low sequence similarity with human ATPase subunits and plausible role in infection makes this enzyme a good drug candidate. Finding a new drug target is of great importance for Chlamydia research field as annually millions of people are newly infected and face the problem of persistent and chronic infections.

In our study, we have initiated recombinant analysis of the structure and function of V/A-ATPase of Chlamydia trachomatis serovar D. Preliminary analysis of this enzyme shows interesting features of its assembly (smallest number of subunits among all known rotary ATPases), presence of unique subunit (no homologs outside Chlamydiales are found) and its activity is strictly regulated by both, catalytic and ion translocating substrate, what makes its activity potentially very stage-specific and responding to both, ATP/ADP levels and Na+ levels in the cells.

Cross-linking and mass spectrometry as a tool for structural biology

Gianluca Degliesposti. MRC - Laboratory of Molecular Biology

Cross-linking and Mass Spectrometry (XL-MS) have proved to be a powerful combination for the identification of protein-protein interactions in both functional and structural investigations. XL-MS provides data that, used as spatial restraints, are useful for interpreting the three-dimensional arrangement of protein complexes and for the fitting of subunits into structural models. Interest in XL-MS has increased with the growing popularity of cryo-electron microscopy (cryo-EM). In recent years, the structures of a number of complexes differing in subunit complexity and solubility (e.g. cytosolic or membrane bound proteins) have been solved by cryo-EM aided by XL-MS.
Here it is described how XL-MS provided key information for the structural characterisation of two different complexes: the membrane bound mitochondrial NADH:ubiquinone oxidoreductase (Complex I)(1)and the soluble eukaryotic mRNA 3'-end processing machinery CPF (Cleavage and Polyadenylation Factor)(2, 3).
Mitochondrial complex I is the largest protein assembly of the respiratory chain with a total mass of 970 kDa. It transfers electrons from NADH to ubiquinone concomitant with the translocation of protons across the membrane. The combined approach of cryo-EM and XL-MS solved a nearly complete atomic structure to 3.9 Å resolution. All 14 conserved core subunits and 31 mitochondria-specific supernumerary subunits were unambiguously assigned using the data from XL-MS investigation and resolved within the characteristic L-shaped assembly with a hydrophilic matrix arm involved in electron transfer and a membrane arm that contains 78 transmembrane helices, mostly associated with the antiporter-like subunits involved in proton translocation. Supernumerary subunits form an interlinked, stabilizing shell around the conserved core. The structure provides insight into the mechanism, assembly, maturation and dysfunction of mitochondrial complex I, and allows detailed molecular analysis of disease-causing mutations.
The cleavage and polyadenylation factor (CPF) is a ~1 MDa multiprotein complex responsible of the 3' ends processing of newly transcribed eukaryotic precursor messenger RNAs (pre-mRNAs). CPF cleaves pre-mRNAs, adds a polyadenylate tail and triggers transcription termination. How the various enzymes are assembled and coordinated was unknown. The support of XL-MS to cryo-EM and X-ray investigation helped to the elucidation of nuclease and polymerase activities of yeast CPF complex. A partial structure of the CPF polymerase module (~200 kDa) was determined and the XL-MS data were used to validate the structural model and to probe the binding of the missing subunits Pap1 and Fip1. Combined with in vitro reconstitution experiments, these data show that the polymerase module brings together factors required for the specific and efficient mRNA 3'-end processing.

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Encapsulation mechanisms and structural studies of GRM2 bacterial microcompartment particles

Gints Kalnins. Latvian Biomedical Research and Study Centre.

Bacterial microcompartments (BMC) are prokaryotic organelles consisting of an icosahedral or quasi-icosahedral protein shell and an encapsulated enzymatic core. BMC are involved several biochemical processes such as choline, glycerine and ethanolamine degradation and carbon fixation. In all cases described so far the encapsulation is ensured by encapsulation peptides (EP), short amphipathic N- or C-terminal sequences interacting with the shell proteins. The shell of BMC generally consists of three types of structural proteins: hexameric BMC-H, pentameric BMC-P and trimeric BMC-T. The encapsulation of enzymes has the benefit of increasing the efficiency of multistep catalytic reactions, protecting the cell from toxic intermediary products and protecting oxygen-sensitive enzymes from oxygen. Understanding BMC shell assembly and encapsulation processes could therefore be useful for synthetic biology applications, since non-native enzymes can also be encapsulated in BMC.

We have isolated and expressed BMC structural genes from Klebsiella pneumoniae GRM2 locus and developed a system for recombinant expression of empty BMC particles. We have also developed a two plasmid system for encapsulation experiments and discovered that the native enzymatic core is encapsulated in a hierarchical manner with CutC choline lyase playing a central role as an adaptor protein and CutF aldehyde dehydrogenase having additional cross-linking role. Interestingly, it seems that the GRM2 encapsulation mechanisms are independent of EP, a feature not demonstrated previously.

We characterized our BMC particles with cryo-EM. We have solved the cryo-EM structure of a T=4 quasi-symmetric BMC particle structure at 3.28 Å resolution. We have also solved cryo-EM structure of T4Q7 elongated particles at 9.64 Å resolution and asymmetric structure of T=4 particles with one missing pentameric unit at 8.75 Å resolution. Several other 2D classes were identified representing yet other minor types of BMC particles such as T4Q16 and T7 or T9 particles and triangular-looking particles most likely formed by fusion of three T=4 particles. This diversity of BMC particles demonstrates the multiple shell growing modes and the flexibility of BMC-H proteins.

Succinimide-based conjugates improve isoDGR cyclopeptide affinity to aVb3 without promoting integrin allosteric activation

Giovanna Musco. Ospedale San Raffaele

The isoDGR sequence is an integrin-binding motif that has been successfully employed as tumor vasculature-homing molecule or for the targeted delivery of drugs and diagnostic agents to tumors. In this context we have previously demonstrated that the product of the conjugation of c(CGisoDGRG) (1) to 4-[N-maleimidomethyl] cyclohexane-1-carboxamide, (cyclopeptide 2) can be successfully used as a tumor-homing ligand for nanodrug delivery to neoplastic tissues. Here, combining NMR, computational and biochemical methods we show that the succinimide ring contained in 2 contributes to stabilizing interactions with aVb3, an integrin overexpressed in the tumor vasculature. Furthermore, we demonstrate that various cyclopeptides containing the isoDGR sequence embedded in different molecular scaffolds, do not induce aVb3 allosteric activation and work as pure integrin antagonists. These results could be profitably exploited for the rational design of novel isoDGR-based ligands and tumor targeting molecules with improved aVb3-binding properties and devoid of adverse integrin activating effects (1).

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Structural insight into the lipi raft scaffold protein by EM

Ilaria Peschiera. National Center of Biotechnology (CNB)

Bacteria organize a number of cellular processes in functional membrane microdomains that in certain structural and functional aspects resemble lipid rafts of eukaryotic cells. These microdomains assemble by aggregation of unusual membrane lipids and colocalize with the membrane scaffold protein flotillin, which provides structural consistency to the rafts. These membrane platforms accumulate specific membrane proteins, whose localization in the lipid raft is essential for its functionality. We are using the human pathogen S.aureus as model system to investigate the structure of lipid rafts in bacteria. One of the aims of this project is to structurally characterized flotillin, as it is a key protein on rafts organization in both eukaryotes and prokaryotes. Different constructs of flotillin were expressed, purified and used for a biochemical and preliminary structure characterization confirming the oligomeric nature of the protein.

Arrythmia-Associated Mutations to the Human Cardiac Ryanodine Receptor N-Terminal Domain Alter its Dynamics

Jacob Bauer. Institute of Molecular Biology, Slovak Academy of Sciences

The primary role of the cardiac Ryanodine receptor (RyR2), one of the largest known ion channels, is to release Ca2+ ions from the sarcoplasmic reticulum of cardiomyocytes into the cytoplasm, thereby triggering heart cell contraction [1]. Cryo-EM studies of the whole channel have shown that these channels are roughly mushroom-shaped, with a large, cytosol-facing cap and a much smaller transmembrane stalk embedded in the SR membrane [2]. Higher-resolution crystal structures of the first 544 residues of the N-terminal domain (NTD) have also been determined, showing that this fragment can be divided into three domains, A, B, and C, surrounding a central helix [3, 4]. Mutations to the RyR2 NTD may disrupt the ability of the channel to close and open correctly. We used molecular dynamics simulation (MD) to study how the I419F and R420W mutations affect the structure and dynamics of the human RyR2 NTD. We selected likely solution structures from the MD simulation and carried out principal components analysis (PCA) on the trajectories to infer how the mutations might change the dynamics of the NTD. The mutations do not appear to greatly change the overall structure of the fragment, but do alter the position of a loop-and-helix motif in domain C (Cα3; residues 463–487). PCA showed that the largest characteristic motion of the wild-type protein (23% of the total) is a rotation of domains A and C towards and away from each other while domain B remains relatively stationary. This motion remains in the I419F mutant, but at a greatly reduced amplitude, comprising only 7% of the total motion. While this mutation appears to make the NTD more rigid overall, the R420W mutation, in contrast, becomes more flexible. The wild-type characteristic motion rises to 38% of the total and has changed in character: domain B has now become flexible and moves together with domain A. By altering the motion of domains A and C with respect to one another, the changes wrought by both mutants would disrupt a proposed gating mechanism [5] suggested to strengthen the closed conformation of the receptor.

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NMR Feasibility Assessment Road Map

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NMR is a powerful tool for protein structure characterization (PSC). However, the process requires expert knowledge of carefully selecting, acquiring and analyzing NMR experiments and experience in judging the feasibility for PSC for a given sample. Here we present an NMR Feasibility Assessment Road Map (NMR-FARM), which is an objective procedure for judging the feasibility of PSC and providing a road map of required NMR experiments for full structure determination. The software, takes two inputs: peak lists (or number of peaks) from simple 2D 1H-15N-HSQC and/or 1H-13C-HSQC spectra (the latter possible with natural abundance) and the protein sequence.
This input is passed on to a server that uses machine learning (neural network) based on chemical shifts, protein sequence, and reported sets of performed experiments from the BioMagResBank (BMRB), and 2D HSQC spectra simulated using VirtualSpectrum to identify relevant experiments to use from this system, The NMR-FARM provides an objective assessment of the requirements for NMR PSC of a given system. We envisage the use of NMR-FARM in a service-based pipeline of INSTRUCT and other NMR centers, that allows scientists with limited experience in NMR to obtain a qualified estimate of the required time and resources required for the given PSC.

Structural characterisation of α1-antitrypsin polymers isolated from patient tissue

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α1-Antitrypsin is an abundant plasma inhibitor of neutrophil elastase, expressed at high levels by hepatocytes, and one of the causative agents of a class of conformational diseases termed serpinopathies. In its active state, α1-antitrypsin is in a kinetically stable, but thermodynamically unstable, configuration, rendering it susceptible to inappropriate conformational change. In individuals homozygous for the Z (E342K) mutation, α1-antitrypsin accumulates in the liver as dense intracellular deposits, leading to a reduced level in circulation. These deposits are the consequence of an 'ordered aggregation' that yields linear, unbranched protein chains, termed polymers, that are both extremely stable and functionally inactive. The circulating deficiency results in a protease-antiprotease imbalance in the lung, predisposing affected individuals to emphysema and COPD, whilst the hepatic accumulation can lead to liver disease, including cirrhosis and hepatocellular carcinoma.

Our aim is to define the molecular details of the polymerisation pathway, in which α1-antitrypsin passes through different conformational states as it transitions from the active monomer via one or more structural intermediates to a hyperstable polymeric form. Different models have been proposed for the terminal structure adopted by the pathological polymer; these are are largely based on characterisation of polymers produced under conditions mechanistically or biologically distinct from those existing in vivo, and as such their relevance to the pathological context has not been established. To probe the structural and energetic aspects of the polymerisation pathway, we have generated a molecular toolkit of conformation-specific monoclonal antibodies (mAbs), and mapped their epitopes. We have utilised these mAbs and applied single-particle reconstruction techniques to negative stain and cryo-EM images of polymers extracted from patient explant liver tissue. The resulting maps, in conjunction with molecular modelling, have allowed us to critically evaluate the proposed mechanisms of polymer formation.

Centre of Molecular Structure in BIOCEV – State of art structural biology facility

Jan Stránský. Centre of Molecular Structure, Institute of Biotechnology, Academy of Sciences of the Czech Republic

The Centre of Molecular Structure offers wide range of methods of structural biology. It operates in BIOCEV as part of Institute of Biotechnology, AS CR. CMS consists of facilities devoted to crystallization of macromolecules, X-ray diffraction and scattering, biophysical characterization, advanced mass spectrometry, and infrared and fluorescence spectroscopy. The open access services are provided via the Czech Infrastructure for Integrative Structural Biology (CIISB) and Instruct-ERIC. The essential core equipment consists of 15T-Solarix XR FT-ICR (Bruker Daltonics) for mass spectrometry, D8 Venture (Bruker) diffractometer with MetalJet X-ray source (Excillum), crystallization hotel RI-1000 (Formulatrix), Prometheus and two Monoliths (Nanotemper) for protein characterization and affinity measurements, and Chirascan for circular dichroism measurements. Recently, this instrument portfolio was extended by SAXSPoint 2.0 (Anton Paar) with MetalJet X-ray source (Excillum) for small angle X-ray scattering studies of biomolecules in solution, MALDI TOF mass spectrometer, excimer laser for induced protein modification, and newly equipped room for spectroscopy with Fourier-transformed Infrared (FTIR) spectrometer and a FLS1000 spectrofluorometer. The Centre of Molecular Structure also participate in active development of the instruments and methods.

The Centre of Molecular Structure is supported by: MEYS CR (LM2015043 CIISB); project Czech Infrastructure for Integrative Structural Biology for Human Health (CZ.02.1.01/0.0/0.0/16_013/0001776) from the ERDF; project Structural dynamics of biomolecular systems (CZ.02.1.01/0.0/0.0/15_003/0000447) from the ERDF.

Three-dimensional structure of a prolate ssRNA bacteriophage virus-like particle

Janis Rumnieks. Latvian Biomedical Research and Study Center

Bacteriophages of the Leviviridae family are small viruses with single-stranded RNA (ssRNA) genomes. Most of the known ssRNA phage isolates infect E.coli and related Enterobacteria, and a few phages are known that utilize Pseudomonas, Acinetobacter or Caulobacter as their hosts. While the range of isolated ssRNA phages remains small, recent metagenomic studies (PMID: 27010970, 27880757) have revealed many more novel ssRNA phage sequences that have opened a window into the true ubiquity and diversity of these viruses in nature.

Analysis of the metagenomic data reveals a cluster of more than 30 sequences that represent a previously unknown lineage within the Leviviridae family with notably long genomes and negligible sequence similarity to the isolated phages; the coat proteins (CPs) of these phages are so different that they can be identified solely based on their location in the genome. While the host bacteria of these phages remain unknown and it is not possible to resurrect live viruses from the metagenomic data, ssRNA phage CPs have shown the ability to assemble into virus-like particles (VLPs) in absence of other phage components. We therefore obtained a number of the metagenomic CP sequences using gene synthesis and expressed them in E.coli to obtain VLPs for functional and structural studies. EM data revealed that these CPs indeed assemble into apparent T=3 VLPs, although in some cases different morphologies were also observed. Interestingly, one of the CPs produced a mixture of spherical and prolate icosahedral particles, which we crystallized and determined their three-dimensional structure using x-ray crystallography.

The elongated particle is composed of 210 CP monomers, or 105 CP dimers, and is approximately 270 Å wide and 345 Å long with a T=3, Q=4 icosahedral symmetry. While the core fold of the CP resembles that of the previously studied ssRNA phages with a capsid interior-facing β-sheet and two surface-exposed C-terminal α-helices, the protein has a range of unique features. The CP dimer is of a rather elongated diamond shape, and besides extended loops between β-strands E and F that are bent back towards the interior of the particle and a long loop between the C-terminal α-helices, the whole N-terminal region of the protein is entirely different, with an additional α-helix and, remarkably, with the N-termini of the two monomers intertwined over the dimer in a β-structure. The very N-termini complement an additional strand to the central β-sheet, which is another

unique feature never observed before in ssRNA phage CPs.
A number of the large tailed dsDNA phages have prolate icosahedral heads, but such structures have not been previously observed among the ssRNA phages. While it cannot be excluded that the elongated particles are an assembly artefact of the recombinant CP, such well-defined aberrant structures are uncommon and might indicate biological relevance. The structure presented here together with further structures that we have determined for other novel ssRNA phage CPs provide better insight into ssRNA phage and protein evolution and are of importance for applications such as development of new vaccines based on novel ssRNA phage VLPs.

Structural and functional analysis of the role of the chaperonin CCT in mTOR complex assembly

Jorge Cuéllar. CENTRO NACIONAL DE BIOTECNOLOGÍA-CSIC

The mechanistic target of rapamycin (mTOR) kinase forms two multi-protein signaling complexes, mTORC1 and mTORC2, which are master regulators of cell growth, metabolism, survival and autophagy. Two of the main subunits of these complexes are mLST8 and Raptor, b-propeller proteins that stabilize the mTOR kinase domain and recruit substrates, respectively. We have found that the eukaryotic chaperonin CCT plays a key role in mTORC assembly and signaling by folding both mLST8 and Raptor. A high resolution (3.97 Å) cryo-EM structure of the human mLST8-CCT intermediate isolated directly from cells shows an almost native mLST8 bound to CCT deep within the folding chamber between the two CCT rings, and interacting mainly with the disordered N- and C-termini of specific CCT subunits of both rings. This structure reveals a unique binding site in CCT for mLST8, far from those found for similar b-propeller proteins, which are near the top of the folding cavity. Moreover, mLST8 associates on the side of CCT that harbors subunits with poor nucleotide release activity (the CCT6 hemisphere), suggesting a correlation between substrate release and ATP utilization. These observations are consistent with an asymmetrical and sequential mechanism for protein folding by CCT, driven by ATP binding, hydrolysis and release, which is radically different from the rest of the chaperonins.

Mechanism of action of pyruvate carboxylase

Jorge Pedro López-Alonso. CIC bioGUNE

Mechanism of action of pyruvate carboxylase
Jorge P. López-Alonso (a), Liang Tong (b) and Mikel Valle (a)

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Pyruvate carboxylase is a biotin-dependent enzyme that catalyses the carboxylation of pyruvate into oxalacetate, an essential metabolite in the tricarboxylic acid cycle which is the substrate in several anabolic biosynthetic reactions as gluconeogenesis and lipogenesis. The reaction takes place in two different steps, first the ATP-mediated carboxylation of biotin followed by the transfer of the carboxyl to the pyruvate molecule. PC is a homotetramer organized in two layers, with two opposite monomers in each layer. As other biotin-dependent carboxylases it contains biotin carboxylase (BC) and carboxyltransferase (CT) domains which catalyze each consecutive reaction. In addition, PC contains a biotin-carboxyl carrier protein (BCCP) domain that transfers the product of the BC active site to the CT active site where it acts as substrate. The mechanism of action of PC requires large conformational changes with the BCCP domain traveling long distances between catalytic sites located at opposite subunits. These large movements of domains are subjected to allosteric regulation by acetyl co-enzyme A. The structure of this symmetric tetramer has been largely studied by X-ray crystallography. However, due to the flexibility of the domains that couple the two consecutive reactions, the exact mechanism of action of PC has not yet been fully resolved.

In the present study we compare the structure of Lactococcus lactis PC and Listeria monocytogenes PC. Both structures were obtained by cryoelectron microscopy at 3.5 Å resolution. This resolution allowed us to build an atomic model of the enzyme and get insights into the mechanism of action. Different classes of each protein show the BC domain opening to allow the entry and release of reaction intermediates; and the BCCP domain movement from the BC domain to the CT domain.

Structural basis of the interaction between integrin α6β4 and the bullous pemphigoid antigen BP230 in hemidesmosomes

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Hemidesmosomes are junctional complexes that mediate the stable adhesion of basal epithelial cells to the basement membrane by linking the extracellular matrix to the intermediate filament system of the cytoskeleton. Disorders that target hemidesmosomal proteins cause severe skin blistering diseases. Type I hemidesmosomes, present in the epidermis, contain three transmembrane proteins integrin α6β4, bullous pemphigoid antigen 180 (BP180 or BPAG2), and tetraspanin CD151, and the cytoplasmic proteins plectin, and BP230 (BPAG1e). Integrin α6β4 is connected to intermediate filaments via plectin and BP230, which bind to the cyto-domain of the β4 subunit. To unravel the molecular basis of the BP230-β4 interaction, we first mapped their mutual binding sites and subsequently solved the crystal structure of a human BP230-β4 complex, which was refined to 2.05 Å resolution. A ~25-residues long segment of the N-terminal region of BP230 binds to the third and fourth fibronectin type III domains (FnIII-3,4) of β4. The initial part of the BP230 site contacts the FnIII-4 domain, while the final part is inserted in a cleft between the FnIII-3 and FnIII-4 domains, which in turn form an inter-domain ionic clasp required for binding. Using double electron-electron resonance spectroscopy (DEER), we show that BP230-binding induces closure of the two FnIII domains of β4. Disruption of the BP230-β4 interface prevents the recruitment of BP230 to hemidesmosomes in keratinocytes in culture, revealing a key role of the BP230-β4 interaction for the assembly of hemidesmosomes. Phosphomimetic substitutions of T1663 of β4, and T39 and S46 of BP230, disrupt binding, suggesting that the BP230-β4 interaction might be regulated by phosphorylation during hemidesmosome disassembly. In summary, our study provides insights into the molecular mechanisms of hemidesmosome architecture and regulation.

Iridium metallodrug intracellular localization by a correlative approach between cryo-SXT and cryo-XRF

José Javier Conesa. ALBA Synchrotron.

In recent years organometallic compounds with potent (nanomolar) cytotoxic activity have begun to emerge. Metallodrugs offer unprecedented versatility in medicinal chemistry because of the different building blocks from which they can be constructed, the variety of available interactions (H-bond, π-stacking, coordinative bond, spatial recognition), and their redox properties. The latter makes them extremely attractive as potential biocatalysts in cancer research. Understanding the intracellular fate of this class of drugs is crucial to further their development towards their clinical use. Previous work has confirmed intracellular accumulation and distribution inside the cells through cell fractionation and elemental quantification using Inductively Coupled Plasma Mass Spectrometry. However, cross-contamination during cell manipulation and fraction separation, metal efflux during sample handling, and sensitivity too close to the detection limit depending on the specific cell fraction, are major drawbacks of this type of experiments. Therefore, to elucidate the intracellular trafficking and the overall cellular accumulation of Ir compounds with potent cytotoxic activity we have chosen the following correlative approach. Cryo Soft X-ray tomography is used to obtain the 3D ultrastructural information of whole cells treated with an Ir metallodrug, at resolutions better than 50 nm. This 3D structural information is then correlated with elemental specific information obtained by cryo X-ray fluorescence. This novel strategy allow us to shed light on the cellular accumulation trafficking and localizing unambiguously which organelles are involved in the intracellular Ir metallodrug accumulation. Our data strikingly show a clear preference for the mitochondria, which can help design the next new generation of highly potent anticancer metallodrugs.

Structural basis for the inhibition of translation through eIF2α phosphorylation

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In translation initiation the small ribosomal subunit positions the initiator tRNA and the AUG start codon of mRNA at the P site with the help of multiple initiation factors. Translation initiation is a highly regulated process and for example one of the responses to stress by eukaryotic cells is the down-regulation of protein synthesis by phosphorylation of translation initiation factor eIF2. Phosphorylation results in low availability of the eIF2 ternary complex (eIF2-GTP-tRNAi) by affecting its interaction with its GTP-GDP exchange factor eIF2B. We have determined the cryo-EM structure of yeast eIF2B in complex with phosphorylated eIF2 at an overall resolution of 4.15 Å. Two eIF2 molecules bind opposite sides of an eIF2B hetero-decamer through eIF2α-D1, which contains the phosphorylated Ser51. eIF2α-D1 is mainly inserted between the N-terminal helix bundle domains of δ and α subunits of eIF2B. Phosphorylation of Ser51 enhances binding to eIF2B through direct interactions of phosphate groups with residues in eIF2Bα and indirectly by inducing contacts of eIF2α helix 58-63 with eIF2Bδ leading to a competition with Met-tRNAi

Multivalent interactions between Pub1, Pab1 and eIF4G drive the formation of protein condensates

Jose Manuel Perez Cañadillas. IQFR-CSIC

Protein translation, the ultimate step on gene regulation, is heavily controlled by different mechanism that allow living organism to adapt quickly to different environmentally situations; in particular physical and nutritional stresses. This response is known as posttranslational control and involves several regulatory proteins that has the ability to induce phase-phase separations that are named as: stress granules, liquid droplets or more generally ribonucleoprotein condensates. These discrete assembles in the cytoplasm form upon various types of environmental stress: temperature, nutrient deprivation, free radicals, and allow the cell to temporally disconnect translation machinery of housekeeping genes and concentrate resources on stress-response genes (chaperons and others). The composition of the condensates is relatively discrete: various RNA binding proteins, some eukaryotic initiation factors, mRNAs and small ribosome subunits. However, their structure, dynamics and mechanism of assemble are not fully understood at the biophysical level. Here we study by NMR the interplay between three of the main components of yeast stress granules: eIF4G, poly(A) binding protein (Pab1) and poly(U) binding protein (Pub1). We characterize the complex conformational organization of the N-terminal intrinsically unstructured region of eIF4G. We assigned the NMR spectra this essential regulatory region (residues 1-249) that contains several MoRFs (Molecular Recognition Fragments) involved in interactions with Pub 1 and Pab1. We describe various self-association processes in the three proteins eIF4G, Pub1 and Pab1 as well as their interactions in pairs, and all three together. We find that although some of these interactions are weak, under certain circumstances are able to trigger a strong and quick protein-protein interaction networks that we postulate might be an early event in stress granules nucleation. We studied this phenomenon by fluorescence microscopy at protein concentration and with agglomerates resembling the cell cytoplasm and present a model of how multiple interactions between these proteins could work as scaffolding for the condensates, that might resemble some of the features of the biological ones.

Cell wall repair and antibiotics resistance mediated by Lytic Transglycosylase Slt of Pseudomonas aeruginosa

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b-Lactam antibiotics inhibit cell-wall transpeptidases, preventing the peptidoglycan, the major constituent of the bacterial cell wall, from crosslinking. This causes accumulation of long non-crosslinked strands of peptidoglycan, which leads to bacterial death. Pseudomonas aeruginosa, a nefarious bacterial pathogen, attempts to repair this aberrantly formed peptidoglycan by the function of the lytic transglycosylase Slt. We document in this report that Slt turns over the peptidoglycan by both exolytic and endolytic reactions, which cause glycosidic bond scission from a terminus or in the middle of the peptidoglycan, respectively. These reactions were characterized with complex synthetic peptidoglycan fragments that ranged in size from tetrasaccharides to octasaccharides. The X-ray structure of the wild-type apo Slt revealed it to be a doughnut-shaped protein. In a series of six additional X-ray crystal structures, we provide the first insights with authentic substrates into how Slt is enabled for catalysis for both the endolytic and exolytic reactions. The substrate for the exolytic reaction binds Slt in a canonical arrangement and reveals how both the glycan chain and the peptide stems are recognized by the Slt. We document that the apo enzyme does not have a fully formed active site for the endolytic reaction. However, binding of the peptidoglycan at the existing subsites within the catalytic domain causes a conformational change in the protein that assembles the surface for binding of a more expansive peptidoglycan between the catalytic domain and an adjacent domain. The complexes of Slt with synthetic peptidoglycan substrates provide an unprecedented snapshot of the endolytic reaction.

Crystal structure of Borrelia burgdorferi outer surface protein BBA69

Kalvis Brangulis. Latvian Biomedical Research and Study Cente

Borrelia burgdorferi is the causative agent of Lyme disease. Once the tick starts the blood meal, the spirochetes from the tick's gut can escape to the new host organism. Taking into account that the environment in the Ixodes tick's gut is very different from that of the mammalian organism, the bacteria has managed to adapt to the encountered environment by adjusting the expression of several outer surface proteins. We have solved the crystal structure of B. burgdorferi outer surface protein BBA69 that belongs to the paralogous protein family 54 (PFam54) that shows the highest up-regulation rate once the Borrelia enters the mammalian organism. The overall structure of BBA69 reveals the same Bbcrasp-1 fold that is found also for the other PFam54 members. Structural data allows us to evaluate and characterize the differences at the molecular level with the other paralogous proteins and thus make it possible to explain the observed functional differences between the PFam54 members.

Acknowledgments
This work was supported by the ERDF grant Nr. 1.1.1.2/VIAA/1/16/144 "Structural and functional studies of Lyme disease agent Borrelia burgdorferi outer surface proteins to reveal the mechanisms of pathogenesis with the intention to create a new vaccine".

Structural effect of synthetic peptide incorporation in alpha-1 antitrypsin investigated by biomolecular NMR and X-ray crystallography

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Alpha-1 antitrypsin (AAT) is a 52 kDa protein from the serpin family of serine protease inhibitors. The protein is highly abundant in the human plasma, where it is secreted from the liver hepatocytes. Numerous mutations of AAT exist with many being aggregation-prone, the most common of which is the Z mutation (E342K). The Z mutation leads to the accumulation of toxic polymers at the site of synthesis and results in a liver cirrhosis as well as a deficiency of AAT in the plasma, which leads to the early onset emphysema.

Synthetic peptides were previously found to have an inhibitory effect on a formation of polymers for both wild type and mutant variant of AAT (1). In this study, we aim to investigate the conformational dynamics of AAT upon the binding and insertion of the peptide and determine binding parameters. For this purpose, we have expressed methyl 1H-13C ILV-labelled AAT for Nuclear Magnetic Resonance (NMR) studies. Incorporation of the peptide was monitored over time using 2D HMQC kinetics experiments. Moreover, to get a better understanding of the incorporation mechanism temperature and peptide concentration titration experiments were performed by NMR. Additionally, NMR data was analysed in the context of an X-ray crystal structure of the peptide-bound state of AAT.

Taken together, these data provide an insight into the conformational changes observed in AAT upon peptide binding and highlight the mechanism by which an extrinsic peptide can block polymerisation. We will discuss structural differences and similarities between the AAT peptide-bound state together with a Z-mutant and substrate-bound state of AAT. Detailed understanding of the molecular basis of a transition from the monomeric form to a polymer state can result in the development of the novel treatment targeting this pathogenic transition.

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Architecture of TAF11/TAF13/TBP complex suggests novel regulation state of basal transcription factor TFIID

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General transcription factor TFIID is a key component of RNA polymerase II transcription initiation. Human TFIID is a megadalton-sized complex comprising TATA-binding protein (TBP) and 13 TBP-associated factors (TAFs). TBP binds to core promoter DNA, recognizing the TATA-box. We identified a ternary complex formed by TBP and the histone fold (HF) domain-containing TFIID subunits TAF11 and TAF13. We demonstrate that TAF11/TAF13 competes for TBP binding with TATA-box DNA, and also with the N-terminal domain of TAF1 previously implicated in TATA-box mimicry. In an integrative approach combining crystal coordinates, biochemical analyses and data from cross-linking mass-spectrometry (CLMS), we determined the architecture of the TAF11/TAF13/TBP complex, revealing TAF11/TAF13 interaction with the DNA binding surface of TBP. We identified a novel highly conserved C-terminal TBP-interaction domain (CTID) in TAF13, which is essential for supporting cell growth. Our results thus have implications for cellular TFIID assembly and suggest a novel regulatory state for TFIID function. Our findings, and the tools we developed in the process, will be presented and discussed.

What can we learn from high pressure protein crystallography?

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Historically, crystallographic research on the influence of high pressure on protein structures was initiated in 1987 by Kundrot and Richards [1]. Since then number of studies regarding high pressure protein crystallography (HPPX) proved the potential importance of this method for current structural biology [2]. The remarkable adaptation of macromolecular crystals to high pressure perturbation can be exploited for the investigation of all aspects of pressure's influence on stability of macromolecules, including conformational changes, dissociation, folding, unfolding and denaturation [3]. The field of HPPX is now reaching towards its potential to becoming an integral part of biological science with applications in: studies of the high-energy conformers of proteins, investigation of interactions between molecules, studies of the structural principles of organization of oligomeric proteins, pressure induced disorder-order transition in the crystalline state, structure-based prediction of various thermodynamic parameters, studies of the mechanisms of enzymatic reactions and studies of proteins from piezophiles.

The influence of pressure on proteins, technical aspects of high pressure diffraction data collection and latest achievements for β-lactoglobulin investigated under non-ambient condition will be presented. Structural changes in high pressure β-lactoglobulin structure revealed by X-ray diffraction and correlated with the physicochemical properties of pressure-treated β-lactoglobulin examined by dynamic light scattering, electrophoretic mobility and quartz crystal microbalance with dissipation monitoring measurements proved to be a promising approach in understanding of functionally important β-lactoglobulin conformers.

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Access Models for EU-OPENSCREEN ERIC
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Katja HERZOG, EU-OPENSCREEN ERIC, Berlin, Germany

The European Research infrastructure EU-OPENSCREEN was founded in March 2018 with support of its member countries and the European Commission. Its distributed character offers complementary knowledge, expertise and instrumentation in the field of chemical biology from more than 20 European partner institutes while its open working model ensures that academia and industry can readily access EU-OPENSCREEN´s collection of chemical compounds, equipment and associated screening data. It will be outlined how biologists and chemists in Europe and beyond can use our screening library, high-throughput and medicinal chemistry technologies to identify novel chemical tool compounds.

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Poster 65

Candida parapsilosis Mgm101 in the maintenance of mitochondrial telomeres

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Mgm101 is yeast Rad52 homolog which appears to be involved in the homologous recombination of mitochondrial DNA. In *S. cerevisiae*, it is found in both mitochondria and nucleus, where it is involved either in the recombination-dependent repair of oxidatively damaged mtDNA molecules (1) or repair of inter-strand crosslinks of nuclear DNA (2). Structurally, *Sc*Mgm101 is divided into two functional domains: an unconserved, N-terminal segment and conserved, Rad52-like core. Our focus was turned into characterization of *Candida parapsilosis* Mgm101 homolog, which reveals many similarities as well as striking differences to *Sc*Mgm101. *C. parapsilosis* has a linear mitochondrial genome that needs to be replicated by homologous recombination. Here, *Cp*Mgm101 seems to play an important role by catalyzing single-strand annealing and D-loop formation, thus mediating the maintenance of its telomeric ends. In our study, we have found out that *Cp*Mgm101 is able to bind a broad range of DNA substrates resembling recombination-generated replication intermediates, with affinities comparable to or even higher than that for ssDNA. According to SAXS analysis, *Cp*Mgm101 forms trimers of homooligomers in solution with molecular weight of about 85-kDa that are roughly triangular (3). Further electron microscopy studies showed a complex of *Cp*Mgm101 with mitochondrial telomere forming homogenous, ring-shaped structures at the telomere′s single-stranded ends (3). Moreover, Mgm101 was determined as a substrate of mitochondrial ATP-dependent protease Lon which degrades oxidatively damaged, partially unfolded, and short-lived regulatory proteins *in vivo*. Closer analysis revealed that association of substrate proteins with nucleic acid proved to be an important determinant of Lon′s substrate recognition and activity (4). All these data might suggest a role for Mgm101 in the recombination-dependent replication of linear mtDNA and mitochondrial telomere maintenance.

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Development of Coherent Phasing Method for Macromolecular Electron Crystallography

Krishna Khakurel. ELI Beamlines

Ultrafast electrons sources are constantly evolving in the past decade. The short probe-pulse from such sources enables to follow the events in physical and biological sciences at unprecedented time scale. The only competent technique that allows following the changes in molecules at the length scales comparable to their atomic dimensions, is crystallography.

In crystallography, an array of molecule interacts with electrons and the corresponding diffraction photographs are recorded. Such diffraction photographs contains the information on the magnitude of scattered wave but the phase information is entirely lost. In this work, we propose to develop a novel phasing method for crystalline object, based on iterative phasing algorithm. The iterative phasing method we propose to use is called ptychography. Use of ptychographic phasing can relax the stringent requirement posed in existing phasing methods used in crystallography. A comprehensive numerical simulation with continuous electron sources has been used in verifying the proposed phasing method. The method will be further extended in biomedical sciences.

Spider silk: from NMR structural studies to mechanism of formation and artificial fibres

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Spider silk, one of the toughest biomaterials known, is produced through the assembly of large proteins (spidroins) that consist of three structural units: a central repetitive region which accounts for spider silk's exceptional mechanical properties and two terminal domains (NT and CT) implicated in the silk formation process. The spidroins are soluble up to concentrations of 30-50% (w/v), when stored in the sac of the spider silk gland but form solid fibres upon passage through an elongated and narrowing duct. During this transition spidroins experience changes in pH, ion composition and shear forces, that have been shown to be of importance for the silk fibre formation. Despite many efforts, the mechanical properties of current artificial spider silks lag behind their natural counterparts. The main reason for this is the inability to reproduce the complex molecular mechanisms of native silk spinning. We used NMR, CD and fluorescence spectroscopy to study how the structure of the different spidroin domains is affected by the changing environment conditions in the duct.
Our studies revealed the structural changes experienced by each of the spidroin domains upon fibre spinning[1-4]. Solution NMR spectroscopy allowed probing of inter-domain interactions and structural changes that occur before fibre formation in response to low pH and altered ion composition[1-3]. The extremely high solubility of spidroins is achieved by forming micellar structures, with hydrophobic poly-alanine segments of the repetitive domain in the micelle core and the terminal domains outlining the micelle shell[4]. This protein stabilization mechanism can be used for efficient production of other aggregation-prone proteins by fusion with an engineered spider silk N-terminal domain[5-6]. Solid-state NMR spectroscopy of artificial fibres revealed the degree of conversion to beta-sheet structure and the conformation of the terminal domains that relates to the mimicry of the mechanism of native silk spinning[4]. We think that this approach will be useful for guiding the optimization of artificial spider silk fibres.

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FAD-dependent oxidoreductase from Chaetomium thermophilum: Structural data-based identification of substrate specificity

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Chaetomium thermophilum is a thermophilic cellulose-degrading fungus occurring in soil, compost heaps and dung. Since it is most thriving at higher temperatures (between 45 – 60°C), it is of wide interest as potential source of thermostable enzymes exploitable in high-temperature industrial processes [1].

The novel thermostable FAD-dependent oxidoreductase from Chaetomium thermophilum var. thermophilum (CtAO) is a monomeric extracellular glycoprotein of molecular mass around 85 kDa. It is a member of glucose-methanol-choline oxidoreductase (GMC) family, whose enzymes have typically a conserved GxGxxG sequence motif in the N-terminal part and a highly conserved histidine residue in the C-terminal part. CtAO related enzymes catalyse the oxidation of primary and secondary alcohols yielding aldehydes or ketones. The reaction is accompanied by reduction of molecular oxygen to hydrogen peroxide [2]. The measurement of catalytic activity with variety of substrates showed that CtAO appears to be inactive with common substrates of GMC family oxidases. The substrate specificity of CtAO is a subject of the studies.

Our CtAO X-ray crystal structure at 1.31 Å resolution revealed new features both in the active site and the rest of the structure. The active site is far more open in comparison to related enzymes, therefore, it is likely accessible for larger molecules. Due to unsuccessful identification of the substrate by catalytic activity measurements, we used a reduced crystallographic fragment screening approach to get a better insight into the possible substrate moieties and their organization. Based on our recent structures of CtAO complexes, we are able to suggest possible substrates for CtAO.

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A fragment screening experience against ABA-receptors possibilities the definition of key residues that trigger ABA signaling

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Abscisic acid (ABA) is the main phytohormone involved in adaptive crop responses to salinity and drought. SlPYL1 is a *Solanum Lycopersicum* (tomato) ABA receptor of the PYR/PYL/RCAR family. It presents an oligomeric state of dimers in solution. Upon ABA binding, dimeric PYLs undergo a conformational change in two loops at ABA binding site named latch and gate. That leads to dimer dissociation and, to the interaction with the protein phosphatases 2C and their inactivation (Moreno-Alvero, 2017)

Crystallization of SlPYL1 in absence and presence of ABA produces isomorphs crystals that present the same space group with a rearrangement in the cell axes dimensions that leads to an increase of 400Å^3 in the unit cell volume of the ABA-SlPYL1 complex. The unit cell increase is a consequence of a twist between the molecules that form the homo-dimers due to the gate loop rearrangement. The same outcomes can be observed when the ABA-SlPYL1 complex is formed through the soaking of the apoenzyme crystals in an ABA solution.

Solutions of more than 10 hundred molecular fragments have been used for soaking SlPYL1 crystals and, X-ray data have been collected and analysed for all of them through the platform “High-Throughput automated ligand screening at EMBL-ESRF” funded by iNext project.[a] Results show that some of the fragments reproduce the effect observed for ABA-SlPYL1 complex and they have provided insight to establish the molecular bases for synchronized changes of the gate loop movement, the dimer twist, and the unit cell increase.

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The structure of cell wall binding domain of Corynephage BFK20 endolysin revealed a tetrameric arrangement

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Bacteriophage-encoded endolysins recognize and bind specific bacteria, and cleave peptidoglycan bacterial cell wall. Their utility as bacteriolytic agents could be exploited for human and veterinary medicine as enzybiotics to treat bacterial infections, as well as having numerous potential biotechnological applications.
Bacteriophage BFK20 is a lytic phage of non-pathogenic corynebacteria Brevibacterium flavum, an industrial producer of L-Lysine. In the genome of the phage BFK20, lytic enzymes holin and endolysin have been identified [1]. The endolysin (gp24´) is composed of two domains, the catalytic domain showing N-acetylmuramoyl-L-alanine amidase activity, and cell wall binding domain (gp24BD) connected by proline-rich linker [2]. The gp24BD was cloned, purified and crystallized. Needle shaped crystals belonging to hexagonal space group P622 were grown overnight. Later, the crystals had recrystallized directly in the drop. The newly obtained crystals belonged to tetragonal space group P4212. Two sets of data have been collected, to 3.2 and 1.4 Å resolution using hexagonal and tetragonal crystals, respectively. Protein sequencing revealed accidental truncation of the gp24BD at the N-terminus. Small size of the protein gp24BD (89 amino acid residues) and good resolution data allowed to solve the structure by the direct method using the program ARCIMBOLDO-LITE [3]. The overall structure revealed very loose bundle of three alpha-helices. Tetragonal crystal symmetry gives four molecules in the unit cell which form very compact tetramer. As the oligomeric assembly has been previously determined also in the solution, we assume that the tetramer represent biologically active unit. The gp24BD does not resemble any cell wall binding domain, either by the sequence or by tertiary structure. The specificity of gp24BD binding on peptidoglycan substrate has not been determined so far. Further work is necessary to elucidate how the cell wall binding domain facilitates its function and how it cooperates with catalytic domain of phage endolysin.

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Structural characterization of the human tyrosine hydroxylase

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The aromatic amino acid hydroxylases (AAAHs) constitute a family of enzymes that catalyse the hydroxylation of aromatic amino acids using tetrahydrobiopterin (BH4) as cofactor and di-oxygen as additional substrate. Tyrosine hydroxylase (TH) is an AAAH that catalyses the conversion of L-tyrosine to L-DOPA, the first and rate-limiting step in the biosynthesis of catecholamine neurotransmitters (dopamine, noradrenaline and adrenaline). TH is a highly controlled enzyme, and the regulatory mechanisms include feed-back inhibition by catecholamine end products and phosphorylation at four different Ser/Thr sites. Mutations in TH are associated with a neuropsychiatric disorder characterized by a large reduction in dopamine and noradrenaline levels, and a metabolic phenotype that is also observed in the non-motor and motor symptoms of the neurodegenerative disease Parkinson's disease (PD).
TH is a 224 kDa homotetramer built by two dimers with a D2 symmetry. Each subunit consists of a regulatory ACT domain with an unstructured N-terminal tail, a catalytic domain and a C-terminal tetramerization domain. To date, only structures of truncated forms of the protein are available, such as the crystal structure of the catalytic and oligomerization domains. Improvements in the purification process have allowed to obtain an active TH with an intact N-terminus. The importance of the N-terminal region lies in its phosphorylation sites and a separated Ala-rich helical motif. These features most likely display a leading role in the regulation of TH.
In this work we have obtained a structure of the full-length human TH at 3.8 Å resolution. The data was collected in a FEI Titan Krios electron microscope equipped with a Gatan K2 Summit direct electron detector. The 3D reconstruction of the homotetramer shows a resolution range from 2.24 Å to 10 Å, corresponding to the oligomerization domains and the regulatory N-terminal domains, respectively. The lowest resolution obtained in the N-terminal tails is consistent with their high flexibility and the disordered region found between residues 1-43. This structural study shows for the first time how these regulatory domains are arranged as a dimer perpendicular to the plane formed by the four catalytic domains. This information will help complete the understanding of the hydroxylation mechanism of TH and its regulatory properties.

Catching dynamics of ribosomal RNA for new antibiotic targets

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World health organization estimates that more than 700.000 people a year die because of otherwise preventable infections with antibiotics resistant bacteria. The discovery of new antibiotics, particularly those with novel mechanisms of action, is key to combating drug-resistant infections and represents one of the health problems of 21st century.1
We are investigating alternative antibiotic targets of bacterial ribosomes with emphasis on the subunit interface, where several rRNA-rRNA and protein-rRNA contacts between subunits termed bridges break and re-adjust during protein synthesis cycle.2 These large structural rearrangements arise from short-lived transient structural fluctuations – or excited states – that are coded in the RNA sequence. By identifying and trapping excited states ribosomal motions can be blocked and ribosomes rendered ineffective. To explore this option, we have focused on the dynamic properties of helix 44 that is positioned at the subunit interface and forms several intersubunit bridges. Our study simultaneously addresses and compares excited states of helix 44 in E. coli, human cytosolic and human mitochondrial ribosomes, showing that its dynamic "hotspots" are potential antibiotic targets and at the same time dissimilar enough for the three different ribosomes to prevent cross-targeting.
Excited states of helix 44 in E. coli, human cytosolic and human mitochondrial ribosomes were investigated in RNA constructs of different sizes, designed to represent whole helix 44 and each specific bridge region of helix 44. Nuclear magnetic resonance (NMR) was used as the only technique equipped for the detection and identification of excited states in macromolecules.3,4 NMR characterization of RNA constructs representing helix 44 enabled determination of structural and dynamic details of each isolated bridge region. Comparison of excited states of the smaller RNA constructs with whole helix 44 confirmed that the small constructs properly reflect dynamics of the whole helix 44 and led to identification of positions in helix 44 that represent well-defined regions for ligand targeting.

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NMR structure of the membrane proximal external region of FIV gp36 envelope glycoprotein

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FIV is a lentivirus that resembles the human HIV.1 FIV (gp36) and HIV (gp41) envelope glycoproteins mediate virus entry thanks to a conformational arrangement involving special glycoprotein regions identified as fusion peptide (FP), N-terminal heptad repeat (NHR), C-terminal heptad repeat (CHR) and membrane proximal extracellular region (MPER).2 The formation of a low energy stable six-helical bundle including NHR and CHR and the correct positioning of MPER on the host cell membrane is pivotal for the fusion of the virus envelop with the cell membrane. The design of molecules interfering with the formation of the stable six-helical bundle or inhibiting the correct positioning of MPER on the membrane are strategies currently attempted to design new virus entry inhibitors.3-4
In the present contribution, we report the NMR structural analysis of gp36-MPER in mixed DPC/SDS micelle solution. gp36-MPER was studied for its positioning on phospholipid surface using NMR experiments in presence of spin labels. On a greater size scale, the effect of gp36-MPER on the size and shape of DOPC/DOPG vesicles was monitored using confocal microscopy.

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Fluorinated Ionic Liquids for Encapsulation of a Therapeutic Protein

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Fluorinated Ionic Liquids for Encapsulation of a Therapeutic Protein
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Abstract:
Fluorinated Ionic Liquids (FILs), a new class of environmentally benign and tailor-made solvents, have surfactant properties and spontaneously self-assemble at concentrations above their critical aggregation concentration (CAC) [1]. Since these FILs are biocompatible and non toxic, they are ideal candidates for protein drug delivery systems in order to overcome problems such as route of administration, dosage, protein instability and degradation.
Our previous studies showed that the addition of ionic liquids had no significant effect on the stability, structure and activity of lysozyme [2]. A distinct behaviour was observed in dynamic light scattering experiments for non-surfactant and surfactant ILs, with the latter encapsulating the protein at concentrations above the CAC [2].
Phenylketonuria (PKU), the most frequent disorder of the amino acid metabolism, is related with the deficient activity of the enzyme Phenylalanine Hydroxylase (PAH). PAH metabolizes L-Phenylalanine (L-Phe) to L-Tyrosine (L-Tyr) in the liver, and its malfunction leads to intolerance to the nutritional intake of L-Phe. PKU patients suffer from severe psycho-motor impairment due to the toxic accumulation of L-Phe in the central nervous system, and low levels of of L-Phe derived biosynthesized neurotransmitters also contribute to negative clinical outcomes. [3]
In this work, our aim is to analyze the effect of FILs on the stability, function and structure of human PAH, a potentially therapeutic protein. Different techniques were used for this purpose, such as differential scanning fluorimetry (DSF), circular dichroism (CD), dynamic light scattering (DLS) and enzymatic activity assays.

Keywords: Ionic Liquids; Drug Delivery Systems; Phenylalanine Hydroxylase; Interactions;

Therapeutic Proteins

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Cryo-EM Structures and Regulation of Arabinofuranosyltransferase AftD from Mycobacteria

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Tuberculosis (TB) is currently the leading cause of mortality from a single infectious agent, resulting in more than 1.5 million deaths annually (WHO, 2018). The unique cell wall structure of Mycobacterium tuberculosis, composed mainly of mycolic acids, arabinogalactan, peptidoglycan and lipoarabinomannan, accounts for its unusual low permeability and resistance towards common antibiotics (1,2). The importance of this unique structure in mycobacteria makes its biosynthetic enzymes attractive drug targets.
Arabinofuranosyltransferase D (AftD) is an essential enzyme involved in assembling arabinose containing glycolipids of mycobacteria (3). It is the largest predicted glycosyltransferase (~150 kDa) encoded in the mycobacterial genome. We present the 2.9 Å resolution structure of M. abscessus AftD determined by single particle cryo-electron microscopy. AftD consists of a membrane embedded portion with 16 TM helices and a large soluble portion that resides on the periplasmic side of the inner membrane. AftD has a conserved glycosyltransferase fold and three carbohydrate binding modules. Glycan array analysis showed that AftD binds complex arabinose glycans. Unexpectedly, analysis of the structure revealed that AftD is tightly bound to an acyl carrier protein (ACP), which is physiological relevant. We constructed a conditional aftD deletion mutant in M. smegmatis to interrogate the functionality of AftD mutants in vivo. Mutagenesis experiments confirmed the essentiality of putative active site residues and ACP binding for AftD function. Finally, we determined structures of an AftD mutant designed to abrogate ACP binding to 3.4 and 3.5 Å resolution. These structures revealed ordering of a loop at the putative active site, suggesting that the ACP may regulate AftD function.

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Poster 76

Structural model for differential cap maturation at growing microtubule ends

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Perturbation of microtubules' (MTs) function is an essential target in cancer chemotherapy. MTs are hollow cylinders made of tubulin, where GTP hydrolysis triggers lattice structural changes needed for their function (mainly movement, scaffolding and direction roads for other proteins). The development of drugs able to smoothly modulate MTs requires in depth understanding of the molecular mechanism underlying tubulin structural transitions switch inside these fibres and its dependence on the nucleotide-bound state. Although, many studies have used different GTP analogues and approaches to mimic the activation and transition states in the polymer, the link between MTs function and the GTPase cycle is still a matter of debate. We investigate the activation mechanism of tubulin using γ-phosphate analogues (beryllium and aluminium fluorides, BeF3-, AlF4-) and GTP and GDP analogues (GMPCPP and GMPCP). Crystal structure of BeF3--tubulin confirms that γ-phosphate supports tubulin activation through movements on T3 and T5 loops, affecting longitudinal contacts upon assembly. Unexpectedly, the BeF3- GTP-like lattice is not expanded as previously observed with the putative GTP mimetic GMPCPP. Instead, this axial breathing is a supporting assembly step related to the hydrolysed Pi release that we found is blocked by the presence of α-β methylene link in both, GMPCPP and GMPCP. Additionally, the AlF4- GDP•Pi-like lattice contributes on the description of the transition state. Together, these structures come to build a model of the MT cap, where lattice lateral and axial breathing converge with different nucleotide bound states along the tip in a non-uniform structure.

TGFβ- and BMP-activated Smad proteins adopt different monomer/dimer structures to interact with cis regulatory elements

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Receptor-activated Smads and Smad4 are effectors of the transforming growth factor β superfamily. These transcription factors form heterotrimeric complexes via the MH2 domain and bind specific DNA motifs through their MH1 domains. The complexes of Smad5 and Smad8 proteins determined here corroborate that these BMP-activated MH1 domains bind 5GC sites as dimers with each monomer covering a 7bp region and with their binding sites separated 60 Å. These complexes and the analysis of Smad3, Smad4 and Smad5/8 in solution reveal that the main functional difference between them resides in the capacity of BMP-activated Smads to dimerize, absent in the TGFβ-activated Smads and in Smad4 MH1 domains. The structural requirements for the monomer/dimer association of the MH1 domains were defined at the origin of animal multicellularity and subsequently conserved over metazoan evolution in all Smad proteins. Besides, these dimer/monomer propensities define the composition of MH2-mediated heterotrimeric Smad complexes and condition how these complexes associate with clusters of DNA sites. For efficient and simultaneous binding of the three Smad proteins in a given complex, optimal DNA motifs would have to be adequately distributed to satisfy the spatial requirements. This would explain how similar Smad complexes can recognize different regions genome-wide and also how similar regions in the genome can be bound to different Smad complexes.

New hit compounds for the therapeutic regulation of synapse dysfunction in neurodevelopmental disorders and neurodegeneration

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The protein complex formed by the Ca2+ sensor neuronal calcium sensor 1 (NCS-1) and the guanine exchange factor protein Ric8a co-regulates synapse number and probability of neurotransmitter release, emerging as a potential therapeutic target for diseases affecting synapses [1]. In neurodevelopmental disorders, such as Fragile X syndrome (FXS) or Autism, neurons show an abnormally high synapse number. On the contrary, in neurodegeneration, such as Alzheimer´s, Huntington´s or Parkinson´s diseases, patients show a low synapse number. In the recent years, we have been investigating the structural basis of the NCS-1/Ric8a interaction and found out that the formation of this complex is essential to increase synapse number [1,2]. Therefore, an inhibition of the NCS-1/Ric8a complex would constitute a potential strategy to regulate synapse function in FXS and related disorders. Conversely, the stabilization of this protein-protein interaction could be useful to regulate synapses in neurodegeneration. With this aim, virtual screenings and dynamic combinatorial chemistry approaches have been used to find out the PPI regulatory molecules. Further, a multidisciplinary approach including, biochemical, biophysical, crystallographic, cellular and in vivo studies have been performed to demonstrate the activity of the compounds, their therapeutic potential and their molecular mechanism of action [3,4].

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Zinc-dependent S1–P1 type nuclease from Legionella pneumophila

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Legionella pneumophila is a Gram-negative bacterium primarily inhabiting freshwater environment as a facultative intracellular parasite of amoebae [1]. L. pneumophila is also considered to be an opportunistic human pathogen which is able to infect human alveolar macrophages and cause the Legionnaire´s disease [2, 3, 4]. To ensure bacterial survival and replication in intracellular host environment, L. pneumophila is able to produce more than 320 effectors to alternate many of molecular and regulatory host processes [5].
The aim of this work was to produce and characterize recombinant secretory zinc-dependent S1-P1 nuclease (Lpn1) from L. pneumophila. Contrary to eukaryotic representatives, the group of bacterial zinc-dependent 3´- nucleases/nucleotidases is not well described and understood [6].

The successful expression of soluble and functional Lpn1 in E. coli was achieved by using a strategy of periplasmic targeting of the recombinant nuclease. This approach helped overcome potential Lpn1 toxicity for E. coli cells and also solved the need to preserve the authentic N-terminus of the protein in order to maintain the correct folding and function. The enzymatic and biophysical studies showed that Lpn1 is a highly thermostable, mainly α-helical nuclease with a strikingly high isoelectric point compared to the other members of this enzyme family. Lpn1 is a single-strand specific nuclease preferring RNA over ssDNA as substrate with pH optima around 7.0 and 6.0, respectively. The cleavage rate towards dsDNA is 13x lower then towards ssDNA.
The Kinetic profiles revealed remarkably strong inhibition of RNase and DNase activities at higher substrate/product concentrations [7].

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Molecular level investigation of ubiquitinated Tau

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Alzheimer's disease (AD) is an irreversible, progressive brain disorder, currently the most common cause of dementia in the elderly population. AD is characterized by intracellular neurofibrillary tangles formed by hyperphosphorylated Tau protein and extracellular senile plaques containing the amyloid β peptide. A number of post-translational modifications, including phosphorylation, acetylation, or glycosylation, have been shown to be crucial in Tau function and dysfunction, however very little is known about the impact of another important post-translational modification, ubiquitination. Due to the role of ubiquitin in the clearance of misfolded proteins by the proteasome, dysfunction of the ubiquitin-proteasome system was proposed to be one of the key mechanisms of neurodegeneration, however it remains still poorly understood. The present research aims at acquiring basic knowledge, to understand whether ubiquitination of Tau is a key factor in its transition to toxic species, relevant in the pathogenesis of AD. To this aim, we obtained and characterized ubiquitin-Tau conjugates in vitro using different methodologies. Our results show that several lysine residues can be modified by monoubiquitin. The next step was then to determine whether ubiquitination could have a differential effect on Tau aggregation. We therefore performed a series of in vitro aggregation assays, based on Thioflavin T fluorescence. Our preliminary results show that ubiquitination has a strong influence on Tau aggregation propensity, and modulates fibril formation.
Interestingly, ubiquitination is a reversible post-translational modification: a variety of deubiquitinating enzymes (DUBs) can hydrolyze ubiquitin from the target protein, thus remodeling or reversing its ubiquitin signal. The impairment of the fine balance between ubiquitinating enzymes and DUB activities can alter the level of ubiquitination of proteins and may lead to their pathological accumulation in cells.
We therefore aimed to provide novel insight into the interaction of Tau with a candidate modifier enzyme, the deubiquitinase OTUB1, using a variety of biophysical techniques including NMR spectroscopy.
We believe that altogether these results will contribute to increase significantly our knowledge about the onset of Tau pathological species, starting from findings at the molecular level.
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THE RUVBL1-RUVBL2 COMPLEX REGULATES ASSEMBLY OF RIBONUCLEOPROTEINS THROUGH THE ZNHIT ADAPTOR PROTEINS

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RUVBL1 and RUVBL2 belong to the AAA+ superfamily of proteins. They are ATPases that form ring-shaped macromolecular complexes (RuvBLs) dedicated to the assembly of large macromolecular complexes involved in a wide variety of cellular processes. Foremost among these, are the phosphatidylinositol-3-kinase-like kinases (PIKKs), RNA polymerase II and a number of ribonucleoproteins (snoRNPs, snRNPs). Recently, a family of zinc-finger proteins (znHIT) has been described with most of its members serving as specific RUVBL1-RUVBL2 interactors, and linking it to several RNPs biogenesis pathways such as chromatin remodelling (znHIT1 and znHIT4), spliceosomal U5 snRNP assembly (znHIT2) and box C/D snoRNP biogenesis (znHIT3, znHIT4).

Given the wide variety of other RUVBL1-RUVBL2 known partners, it has become clear that involvement of this protein complex in one or another cellular pathway must be tightly regulated. In this sense, znHITs might act as adaptor proteins that help direct the RUVBL1-RUVBL2 complex to an specific RNP biogenesis pathway. However, how these adaptor proteins interact with RuvBLs and how they determine the RUVBL1-RUVBL2 activity remains poorly understood. In general, biogenesis of noncoding RNPs requires dedicated cellular machinery, including RNP assembly factors. Those factors might perform multiple roles such as facilitate RNP core proteins preassembly in the absence of RNA, stabilizing labile intermediates and/or prevent nonspecific RNA binding. Moreover, the assembly of the RNPs constitutes a key regulation mechanism for the cellular process where the RNPs are involved to and they are considered as putative therapeutic targets. Here, we aim to provide part of a highly detailed molecular picture of the role of the RUVBL1-RUVBL2 complexes in the biogenesis of RNPs by integrating biochemical and structural information about the interaction between RUVBL1-RUVBL2 and the znHIT proteins.

Structural studies of flexuous Potato virus Y filaments

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Potato virus Y (PVY) is among top five economically important plant pathogens. The virus causes potato tuber necrotic ringspot disease, which can result in up to 70 % yield reduction, and also severely affects other economically important solanaceous crops. PVY belongs to the genus Potyvirus (family Potyviridae), with its 9.7 kb positive-sense single stranded RNA (ssRNA) genome coding for 11 viral proteins. The viral genome is encapsidated by approximately 2000 copies of a coat protein (CP), thereby forming flexuous filamentous virions. Besides capsid formation, CP is also involved in many other steps of the viral infectious cycle. To address the urgent need to better understand mechanisms of viral infectivity of this important pathogen, we determined the near-atomic structure of PVY's flexuous virions using cryo-electron microscopy (cryo-EM). The structure revealed a novel lumenal interplay between extended C-terminal regions of CP units and viral RNA. These RNA-protein interactions are crucial for the helical configuration and stability of the virion, as further confirmed by the unique structure of RNA-free virus-like particles (VLPs), also determined by cryo-EM at near-atomic resolution. Viral RNA does not only affect the overall assembly of the filaments, but also determines the structure of a single CP, which is different in virions compared to VLPs. These differences are particularly pronounced in the extended N- and C-terminal regions of CP, thereby revealing the first evidence for the intrinsic plasticity of CP, which may explain the ability of CP to perform multiple biological tasks. Structure-based mutational analysis and in planta experiments further revealed the crucial roles of both terminal extensions in PVY infectivity. These results are important contribution to understating of PVY biology, and its pathogenesis in particular. Moreover, modularity of PVY VLPs shown by this work suggests a new molecular scaffold for nanobiotechnological applications.

AAnchor: CNN guided detection of anchor amino acids in high resolution cryo-EM density maps

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Atomic accuracy models of protein structures are an invaluable tool for the elucidation of protein function. As cryo-EM microscopy is becoming an important method for revealing structure information, methods for obtaining atomic models from cryo-EM density maps are required.Traditionally, the modelling from cryo-EM was based on locating protein fragments with known structure, such solitude secondary structure elements (helices and beta-sheets) or their assemblies.The recent "resolution revolution" in cryo electron microscopy (cryoEM) has led to an ever-increasing number of near-atomic resolution density maps deposited in the EM databank EMDB. While in high resolution maps (roughly 3.5 Angstrom or better), sidechains become visible and individual rotamers may be distinguished, no automated method has been suggested to detect specific amino acids in a cryo-EM density map.
We present AAnchor (amino-acid anchor), a machine learning method for the detection and localisation of amino acids in a high resolution cryo-EM density map. The main goal is the detection of reliable amino acid anchors in the density map, namely having knowledge of even a relatively small number of amino acids, whose identity and location have been established with high confidence. The detected amino acids are used to guide the various de-novo modelling methods, as well as serve as a starting point for the development of novel methods. Given a 3D density map, obtained from single-particle cryo-EM reconstruction, AAnchor reports coordinates (of the centre of mass) and type of the detected amino acids. AAnchor achieves a confidence level of 80 percents, i.e. the reported results contain less than 20 percents of errors. AAnchor utilises 3D Convolutional Neural Networks combined with the sliding window approach to search the input density maps.
The preliminary results of our algorithm are quite encouraging. For example, on the 3.1 Angstrom cryo-EM map of lysenin pore, the algorithm localizes above one hundred amino acids of different types with confidence above 80%. These preliminary results also indicate that the quality of detection is best for amino acids, which have a small number of rotamers and a large enough training set. This last observation also explains, why contrary to expectation, the results for 2.9 and 3.1 Angstrom resolution maps are better than those for 2.2 Angstrom maps. This is due to the limited training data set existing for better resolution maps. Thus, with the rapid increase in high resolution cryoEM structures and the availability of larger training sets, the results of our machine learning based methodology are expected to improve significantly.

Online implementation of the algorithm is available at http://bioinfo3d.cs.tau.ac.il/AAnchor/

Crystal structures of bacteriophage receptor binding proteins

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Bacteriophages have specialised receptor binding proteins (RBPs) for initial, reversible, host cell wall recognition. Once a suitable host is found, the phage commits to infection by irreversible attachment via a secondary receptor interaction. The crystal structures of several of these receptor-binding proteins have been solved and have been shown to be mainly beta-structured, but structurally highly diverse and containing several new protein folds. Here we present structures of the receptor-binding proteins of the Escherichia coli phages T4, T5 and T7, and of Staphylococcus phages S24-1 and K. Bacteriophage receptor-recognising proteins may be used for bacterial detection, while modification by natural or experimental mutation of bacteriophage receptor-binding domains may allow retargeting of phages to alternative host bacteria. Their shape and stability may also allow their use in nano-technological applications.

Crystal structure of Bacillus subtilis transcription repressor DeoR in complex with its operator DNA

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DeoR is involved in B. subtilis carbon catabolism as the local repressor for transcription of enzymes digesting deoxyribose and deoxyribonucleosides. This repressor binds an operator DNA by its N terminal DNA binding domain (DBD) and blocks transcription of catabolic enzymes in the absence of an effector molecule. If the effector is present it binds to the C terminal effector binding domain (EBD) of the repressor and causes its release from the DNA binding site. The structures of the C terminal EBD of DeoR in free form and in the complex with the effector deoxyribose-5'-phosphate have already been reported [1]. Nevertheless, for the understanding of the allosteric effects of the repressor during the metabolic regulatory process, it is necessary to know the 3D structure of the full length protein in complex with its operator DNA. To achieve this, we initiated structural studies DeoR in complex with DNA operator.
Recombinant DeoR was prepared by heterologous expression in E. coli BL21 (DE3) and purified with yield of 3.4 mg per 1 L of bacterial culture. Crystallization of DeoR in complex with DNA duplex derived from the operator sequence was carried out using the vapor diffusion techniques. Needle shaped crystals were obtained and diffraction data were collected to maximal resolution of 3.6 Å. Crystal structure was determined by molecular replacement and confirmed the presence of DeoR-DNA complex. For achieving of a higher resolution, we chose to perform alternative structural study on DeoR DBD in complex with DNA operator derivatives.
DeoR DBD was prepared analogously to DeoR and purified with a yield of 5.8 mg per 1 L of bacterial culture. Crystallization of DBD in complex with DNA derivative yielded crystals diffracting to 2.3 Å resolution. Crystal structure of DBD-DNA complex was solved by molecular replacement using the preliminary model from full-length DeoR structure. Refinement of crystallographic model is currently in progress.

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Structural characterization of thaumatin-like proteins from various species and analysis of their putative allergenic potential

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Thaumatin-like proteins (TLPs) exist in many plants, fungi, animals and bacteria and play important roles in host defense, developmental processes and stress response. A lot is known about their function in plants but only little about their tasks in other organisms. In general, TLPs seem to participate in binding or degradation of β-1-3-glucans. TLPs consist of 200 to 250 amino acid residues including up to 16 conserved cysteine residues. The TLP fold is composed of three domains. Domain 1 is a highly conserved β-sandwich, which is the core structure of the TLP molecule, followed by a less conserved α-helical part (Domain 2) and a small β-sheet (Domain 3).

We are working on thaumatin-like proteins from different organisms such as Schistocerca gregaria (desert locust), Amycolatopsis rifamycinica (soil bacterium), Puccinia graminis (fungus), Tyrophagus putrescentiae (mite) and the pollen allergen from Juniperus ashei (cedar tree). Our aim was the expression of properly folded proteins and their structural characterization. To date the vast majority of structurally characterized TLPs are plant food allergens. In this project we want to elucidate the putative role of TLPs from organisms other than plants as allergens or aero-allergens.
Since TLPs contain several disulfide bonds, expression is not trivial and therefore His-tagged TLPs were expressed in different organisms ranging from E. Coli, K. phaffii to the mammalian cell expression system Expi293. The recombinant TLPs were purified by IMAC chromatography, followed by size exclusion chromatography, subsequent characterization by CD spectroscopy and finally by crystallography.
Crystallization enabled us to get an insight into the structures of an insect, a bacterial and a fungal TLP for the first time. All structures were solved at a resolution ranging from 1.6 Å - 1.9 Å and gave us important insights into the structural differences and similarities between the structures.
The recombinant TLPs will allow us to investigate their role as allergens as well as possible cross-reactivities and their role as putative allergens. This knowledge will be beneficial to improve our structure based IgE-Epitope prediction software “SPADE”. Furthermore, we are investigating their potential catalytic and anti-fungal activity.

Modeling atomic structures from cryo-EM maps using Scipion

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Technological improvements, increasing computing power and new software development are triggering a resolution revolution in the field of cryo-EM. As a result, processing workflows generate more frequently near-atomic resolution electron density maps. Atomic interpretation of these high resolution maps has thus become possible in cryo-EM. Reliable atomic models obtained serve as starting point to seek drug inhibitors or to accomplish exhaustive structure mechanistic studies. The potential relevance of these biomedical applications promotes the development of software tools focused on atom model building from cryo-EM maps, some of them slightly adapting mature protocols generally used in crystallography. However, research community complains about current resources of cryo-EM-based model building, highly heterogeneous and spread over different software platforms, and suggests centralization and interoperability of programs, together with clear help documentation (Cassidy et al., 2018). To meet this demand, we have addressed the integration of several software suites and model building programs by taking advantages of Scipion, a software framework that constitutes a reference tool in the field of image processing and reconstruction of cryo-EM density volumes (de la Rosa-Trevín et al., 2016). As main strength, Scipion integrates programs from several developing sources and supports interoperability among them, based on the object-oriented data organizing model, which facilitates linking compatible outputs and inputs of different programs by conversion routines. In addition, Scipion friendly graphical user interphase displays the protocol processing workflow, allowing reusability, traceability and reproducibility of resulting volumes and now, as next logical step in the processing workflow, of atomic structures inferred from those volumes. Modeling workflow starts in Scipion with protocols designed to import volumes, in case they were generated in a different platform or Scipion project, as well as atomic structures and sequences, both from public databases and user files. For large symmetrical volumes, the work can be initially simplified by extracting the volume of the unit cell, the smallest asymmetrical unit, by using the extract-unit-cell protocol. At the end of the workflow, the final structure of the whole volume may be restored by symmetry. After setting the volume, Chimera model-from-template protocol has been implemented to get initial structures from sequences based on Modeler web server and sequence homology. Other Chimera-derived protocols are available to handle structures and accomplish intermediate operations, such as Chimera operate and Chimera restore-session. Next, the rigid fitting of the initial structure to the volume can be performed using Powerfit program, followed by Chimera rigid-fit protocol. The following refinement or

flexible fitting of that coarse structure has been implemented with protocols derived from software suites CCP4 (programs Coot and Refmac5) and Phenix (program real-space-refine). The last step in the workflow, validation of generated structures, can be carried out by using EMRinger and MolProbity programs, included in Phenix. Another program of this software suite, superpose-pdbs, facilitates structure comparisons.

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Structural studies of the Penicilin Binding Protein 2a (Pbp2a) from Staphylococus aureus

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Infection with the methicilin resistance S.aureus (MRSA) is a global health problem and a cause of high morbidity and mortality. It is due to the high resistance of this bacterium to β-lactam antibiotics thanks to the presence of an additional penicilin binding protein (Pbp2a). Pbp2a, like other PBP proteins, catalyze the formation of peptide crosslinks between glycan chains of the cell wall. B-lactams act as substrate analogs, when bound to PBSs inhibit they function and as a result the cell wall is weakened and eventually the cell dies. Pbp2a shows very low affinity to β-lactams therefore remains active and allows the cell growth in the presence of the antibiotics. We are applying cryo-EM structural approach allongside with the biochemical characterization, to study the architecture of the Penicilin Binding Protein 2a (Pbp2a) from S.aureus MRSA strain. Current studies have been focused only on the soluable (lacking the transmembrane region) form of the protein. Our experiments with the full length version suggest that is forms a dimer in vivo and in vitro and the transmembrane region is responsible for the self- interaction. Mechanism of the antibiotic resistance has been proposed based only on the truncated, monomeric structure of the protein and the oligomerization might have a big structural impact on the general architecture and activity of the enzyme.

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Structural and functional studies of Salmonella virus epsilon15 tailspike

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With the rise of diseases caused by antibiotic-resistant bacteria, it is necessary to find alternative ways to fight pathogenic bacteria. Bacteriophages can be a natural one. They kill bacteria to produce their progeny. However, key aspects of the viral cycle are unknown. For instance, phage attachment, the first step of the infection, has been poorly studied. Bacteriophages use their tailfibers or tailspikes to bind to bacterial receptors in the cell wall. Hence, the host range of phages depends on these proteins. Our work is focused on the atomic interaction between epsilon15 and its host bacterium Salmonella enterica subsp. enterica serovar Anatum A1. Epsilon15 tailspikes both bind and digest O-Antigen repetitions of the bacterial lipopolisaccharide. Thanks to this function, the phage gets close enough to injects its genome into the bacterium. We have solved the structure of epsilon15 tailspike in complex with the bacterial O-Antigen. One O-Antigen repetition is a trisaccharide composed of a Rhamnose, a Mannose and a Galactose bound to an Acetyl group. It is bound in three different regions distributed throughout the tailspike. However, further experiments are needed to find the active site of the protein. Structural and functional information will allow us to unravel the mechanism of attachment of epsilon15.

Role of adenovirus core protein VII in capsid stabilization and maturations
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The adenovirus genome is organized within the capsid in a nucleoprotein core composed of 35 kbp of double stranded DNA and over 20 MDa of DNA-binding proteins: ~500 copies of VII, ~300 copies of μ, ~150 copies of V, the maturation protease, and the terminal protein [1] . Proteins V, VII and μ have a large amount of positive charges and are thought to regulate the electrostatic interactions between DNA strands, condensing the genome by a strategy similar to cell histones [2]. Previous studies showed that internal pressure generated upon adenovirus maturation, which entails proteolytic cleavage of proteins VII and μ, contributes to modulate the stability of the viral capsid. The pressure generated push out forces induce stress in the capsid, favoring penton dissociation for initiation of the stepwise uncoating cascade [3-5].
A recent study on an adenovirus variant lacking the major core protein, VII, shows that this protein is not needed for genome packaging. However, VII- particles are not infectious [6]. Surprisingly absence of protein VII hinders maturation of polypeptide VI, necessary for escape from the endosome.
Here we use extrinsic fluorescence assays and cryo-electron microscopy to determine the differences in adenovirus stability and architecture resulting from lack of VII.

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Structure-based design of carboranes and metallocarboranes inhibitors targeting cancer-associated carbonic anhydrase isoforms IX and XII

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Human carbonic anhydrases (CAs) are zinc metalloenzymes playing an important role in many physiological processes. Several CAs are also involved in various pathological processes, therefore, these isozymes are validated drug targets. Carbonic anhydrase IX (CA IX) and carbonic anhydrase XII (CA XII) are transmembrane isoforms overexpressed in several solid hypoxic tumors. Thus, both isoforms belong to recently identified targets for cancer therapy and diagnostics. Direct inhibition of CA IX leads to tumor reduction and a decrease in the formation of metastases. Moreover, specific inhibition of CA XII was shown to affect P-glycoprotein (Pgp) mediated tumor cell chemoresistance. Previously, we have identified carboranes as a promising class of potent inhibitors of human carbonic anhydrases. Here we report on recent advances in the structure-assisted design of carborane and metallacarborane inhibitors targeting specifically either CA IX or CA XII.

The challenge of finding the isoform-specific inhibitor is due to high sequence and structural homology between CAs isoforms. Lack of selectivity is the major issue of the currently used CA inhibitors. Furthermore, traditional CA inhibitors are mostly planar aromatic compounds that are not able to effectively fill pockets forming the entrance to the CAs active site. Carboranes cages, three-dimensional scaffolds, not only act as space-filling fragments but also increase interaction energy, in vivo stability and bioavailability. Thus, we site-directed modified boron cages to synthesize carboranes and metallacarboranes substituted by sulfamide, sulfonamide or sulfamate groups, i.e. moieties known to bind tightly to the zinc atom in the active site of CAs. Consequently, the small library of ca. 80 substituted carboranes and metallacarboranes emerged. Several compounds exhibit significantly enhanced in vitro activities with corresponding Ki values in low nanomolar or even picomolar range. In addition, we observed that the length of the linker between carborane clusters and zinc binding groups is crucial for CA selectivity.

Structure-activity relationship (SAR) observed within the library is complemented by high-resolution X-ray structures of enzyme-inhibitor complexes. The atomic resolution of structures further helped to tune inhibitors for the more selective inhibition. The panel of inhibitors was tested for pharmacological, toxicological and pharmacokinetic properties. Selected compounds exhibited a tumor-specific growth inhibitory effect in both two-dimensional and multicellular spheroid cultures

of CA IX-positive cell lines. In mouse models, the compounds showed a significant anti-cancer effect in colorectal tumors leading to a tumor size reduction. To follow inhibition of Pgp activity, chemoresistance derivatives of U-87 MG cells were generated. Exploration of indirect inhibition of Pgp in the presence of carborane and metallacarborane inhibitors of CA XII will follow.

Structure of Leishmania RNA virus 1 uncovers cap4 binding site

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Leishmania RNA virus 1 (LRV1, Totiviridae) infects human protozoan parasite, Leishmania. Leishmaniasis has symptoms ranging from self-healing ulcers to a lethal visceral form. Several million people annually are at risk of infection by leishmaniasis, predominantly in subtropical and tropical areas. South American Leishmania parasites L. guyanensis and L. braziliensis containing LRV1 are more likely to cause disfiguring mucocutaneous form of leishmaniasis and the treatment fails more often than in species that do not carry the virus. Even though the importance of LRV1 for severity of leishmaniasis was suggested decades ago, its structure remained unknown.

Number of virions in Leishmania cells is generally low and there is no evidence of stable horizontal transfer to naive parasite that would facilitate the purification. To circumvent the issue of low virus concentration, we took advantage of recombinant capsid protein tendency to form virus-like particles (VLPs). Here we present the cryo-electron microscopy reconstruction of LRV1 VLP determined to a resolution of 3.7 Å. The icosahedral capsid, which is 42 nm in diameter, is formed by sixty dimers of the capsid protein. Organization of LRV1 capsid is similar to that of the inner capsid layer of double-stranded RNA viruses, including L-A virus of Saccharomyces cerevisiae.

Capsid proteins of L-A virus cleave-off the 5’ caps from host mRNAs and transfer them to viral ssRNA to protect virus transcripts from degradation by the host cell. The de-capping activity is performed by an active site assembled from three capsid protein loops with hydrolytic His154. The features in L-A virus involved in de-capping are not preserved in LRV1. Instead, each LRV1 capsid protein contains a positively charged pocket, distinct from the active site of L-A virus, which may be involved in mRNA de-capping. This positively charged “furrow” is formed by four arginines with His410 at the end of 12 Å wide and 30 Å long groove, which can accommodate whole Leishmania cap4 structure.

Our results showed that LRV1 capsid protein harbours the RNA-binding activity. The LRV1 capsid protein bound to biotinylated L. guyanensis mRNA produces an electrophoretic mobility shift when compared to clear mRNA, and this signal is diminished when the unlabelled mRNA is added. Molecular modelling of cap4 structure binding to LRV1 capsid protein reveals the preference of cap4 towards the positively charged furrow. Furthermore, the immobilized LRV1 capsid protein interacts 1.3 time more strongly with coding sequences, as shown with sequencing of flow-through and elution fractions from a pulldown experiment.

The atomic resolution of LRV1 virion is a missing piece to a considerable body of knowledge which could lead to more efficient therapy of mucocutaneous

leishmaniasis. Here we present the 3.7 Å resolution model of VLP spontaneously formed during purification of the capsid protein. Although the VLP resembles the native virion, it lacks a copy of readthrough product, the capsid-RNA dependent RNA polymerase, present in a few copies as part of the native capsid. To show the position of capsid-polymerase, we will isolate native LRV1 from L. guyanensis LRV1 positive cell line and solve the structure with cryoEM.

Degron recognition by the 26S proteasome

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The ubiquitin proteasome system is essential for the bulk protein degradation in living cells. Its substrates are usually modified by K-48 linked ubiquitin chains and degraded by the 26S proteasome. Understanding the mechanisms of substrate recognition by the 26S proteasome is crucial to explain the inherent logic of proteasome function, and how it performs selective and efficient proteolysis. Recently, cryo-EM studies of substrate-engaged 26S proteasomes have shown conformational reorganization upon substrate engagement. These studies suggest a sequential rotary ATP hydrolysis mechanism for substrate unfolding and translocation. However, a dynamic state of substrate recognition that occurs just before substrate commitment, the pre-engagement state, is still portly understood. We investigated 26S proteasome model degrons and revealed novel details about the optimal architecture of the degradation signal. We determined two cryo-EM structures of the human 26S proteasome in complex with an optimised model degron that mimics the minimal ubiquitin signal required for proteasomal recognition, corresponding to a pre-engagement state. The structural and biochemical data suggest that the 26S proteasome interaction with the ubiquitin degradation signal induces structural rearrangements for optimal substrate engagement and processing.

Theoretical Analysis of Molecular Structure and NMR Spin-Spin Coupling Constants in Sulphated Oligosaccharides.
Milos Hricovini. Institute of Chemistry.

Theoretical Analysis of Molecular Structure and NMR Spin-Spin Coupling Constants in Sulphated Oligosaccharides.

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Heparan sulphate and heparin are negatively charged glycosaminoglycans participating in a number of biological processes. Methods of theoretical analysis essentially contributes to understanding of solution properties of heparin-oligosacchades and formation of their intermolecular complexes with proteins. In the present contribution, results of density functional theory (DFT) calculations of molecular geometry and indirect three–bond proton–proton coupling constants (3JH–C–C–H) are discussed. Theoretical data showed that formation of a complex hydrogen bond network and strong ionic interactions influence the first hydration shell and play an important role in shaping the 3D saccharide molecules.
Computed 3JH–C–C–H coupling constants indicated that the oxygen lone pairs of neighbouring oxygen atoms could significantly contribute to the Fermi-contact terms. The DFT analysis proved that the magnitude of spin-orbit contributions are large for some proton-proton coupling constants and significantly affect the 3JH–C–C–H values. These data highlight the need of appropriate quantum-chemical calculations for detailed understanding solution properties of negatively charged oligosaccharides.

Architecture of the membrane-assembled retromer coat by cryo-electron tomography

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One of the key features of eukaryotic cells is compartmentalization. The membrane-bound compartments or organelles rely on a complex system of transport vesicle and tubule movements to maintain connectivity between them. The vesicles/tubules bud from parent membranes and fuse with target membranes and are surrounded by protein coats recruited from cytosolic proteins. The coats' roles include transmembrane protein cargo selection and membrane deformation. To date, the most extensively structurally characterised coats have been Clathrin/AP2 (plasma membrane to endosomes), clathrin/AP1 (to and from Golgi) COPI and COPII (Golgi to ER and ER to Golgi). The retromer complex is a further essential, evolutionary-conserved vesicle/tubule coat, which is involved in the trafficking events from endosomes to a variety of destinations including the cell surface and the Golgi.

Recent improvements in cryo-electron tomography permitted structural studies of membrane-associated coats at high resolution despite their relative heterogeneity. We have reported the first structure and arrangement of the in vitro reconstituted BAR/retromer coat with subnanometer resolution. Sub-tomogram averaging allowed to resolve local arrangement of the coat, which would be hidden when using a helical reconstruction.

A Molecular Dynamics Insight to Non-Structural Protein 1 (NS1) – A Hub Protein Essential for Influenza Infection

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Influenza (flu) is a contagious viral disease, which attacks the respiratory tract spreading through the population in seasonal infections. In fact, every year around 10% of world population is infected and an estimated 650.000 people die from Influenza according to the World Health Organization (WHO)[1]. Since the vaccination's efficacy is limited, and due to high rates of mutation and recurrent genetic assortment, new preventive and therapeutic approaches and better understanding of the virus-host interactions are urgently needed[2].

NS1, one of the 11 proteins encoded by the virus, became a potential target as it promotes enhancement of viral replication, while negatively affects the host's innate immune response[3]. NS1 protein has indeed a plethora of functions by interacting with different host partners[4]. Structurally, NS1 is a 26 kDa multifunctional protein with around 230 residues and formed by 2 domains, a linker and a disordered C-terminal tail. The linker that connects the N-terminal RNA-Binding Domain (RBD) and the Effector Domain (ED) is a short, flexible region without a defined/fixed number of residues[5]. In host cells, NS1 is likely to be a homodimer that can shift between different quaternary conformational statuses possibly depending on its partners, location in cell, and linker size[6].

This biological system presents itself as a highly evolutionary-conserved protein, but there is lack of information about its structure and behavior [7]. To this end, we performed three replicas of 1 μs Molecular Dynamics (MD) simulation of each NS1 system in order to fully characterize the conformational space visited by both domains and how the presence of the flexible linker affects mobility and the binding surface. In particularly, we carried out MDs of 4 different full-length NS1 proteins: i) Wild-Type (WT) and a 15 amino-acids linker; ii) WT and a 10 amino-acids linker ; iii) W187Y NS1 mutant with a 15 amino-acid linker; and iv) W187Y NS1 mutant with a 10 amino-acid linker. This mutation is known to lower the prevalence of dimers in solution. Overall, our approach offers a new strategy for a better understanding of the NS1 homodimer dynamical behavior in human cells.

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Structure-function Relationships and Modulation of Biofilm-associated Amyloids

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Structure-function Relationships and Modulation of Biofilm-associated Amyloids
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Microbial functional amyloids are structured protein aggregates serving specific and highly diverse functions, mostly as key virulence factors involved in aggressive infections, including as protein fibrils structuring the microbial biofilms, thereby offering antimicrobial drug targets1. Nevertheless, amyloids are mostly known for their involvement in fatal human aggregation diseases2, and their structures have been studied in depth only in eukaryotes, while the study of microbial amyloids has proceeded in a relative vacuum of structural knowledge. By using dedicated methods of X-ray microcrystallography, we managed to determine atomic structures of amyloid-like segments of the major curli subunit, CsgA, secreted by Enterobacteriaceae. We found that these amyloid-like segments share structural similarity with segments of human pathological amyloids, forming ultra-stable cross-beta steric zipper fibrils. In accordance with the structural similarity of the biofilm-associated and pathological amyloids, we found two compounds, originally designed to interfere with Amyloid-β associated with Alzheimer's disease3, which prevented the fibrillation of the CsgA biofilm-associated bacterial amyloids and correspondingly reduced the biofilm biomass of Salmonella typhimurium (Perov et al., Submitted, BioRxiv). To expand the structural knowledge on biofilm-forming proteins, we focus attention on amyloids secreted by the human pathogen Pseudomonas aeruginosa. In earlier studies, two proteins, FapC and FapB, were found to be involved in the structuring of the P. aeruginosa biofilm, with one serving as the main amyloid subunit forming the fibril, and the second serving as a nucleator of fibrillation4,5. We aim to provide structural insights into these proteins, and to design modulators of fibrillation which might open new directions to design novel therapeutics against biofilms, one of the most resilient and aggressive forms of microbial infections.

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Candida parapsilosis Mgm101 in the maintenance of mitochondrial telomeres

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Mgm101 is yeast Rad52 homolog which appears to be involved in the homologous recombination of mitochondrial DNA. In S. cerevisiae, it is found in both mitochondria and nucleus, where it is involved either in the recombination-dependent repair of oxidatively damaged mtDNA molecules (1) or repair of inter-strand crosslinks of nuclear DNA (2). Structurally, ScMgm101 is divided into two functional domains: an unconserved, N-terminal segment and conserved, Rad52-like core. Our focus was turned into characterization of Candida parapsilosis Mgm101 homolog, which reveals many similarities as well as striking differences to ScMgm101. C. parapsilosis has a linear mitochondrial genome that needs to be replicated by homologous recombination. Here, CpMgm101 seems to play an important role by catalyzing single-strand annealing and D-loop formation, thus mediating the maintenance of its telomeric ends. In our study, we have found out that CpMgm101 is able to bind a broad range of DNA substrates resembling recombination-generated replication intermediates, with affinities comparable to or even higher than that for ssDNA. According to SAXS analysis, CpMgm101 forms trimers of homooligomers in solution with molecular weight of about 85-kDa that are roughly triangular (3). Further electron microscopy studies showed a complex of CpMgm101 with mitochondrial telomere forming homogenous, ring-shaped structures at the telomere′s single-stranded ends (3). Moreover, Mgm101 was determined as a substrate of mitochondrial ATP-dependent protease Lon which degrades oxidatively damaged, partially unfolded, and short-lived regulatory proteins in vivo. Closer analysis revealed that association of substrate proteins with nucleic acid proved to be an important determinant of Lon′s substrate recognition and activity (4). All these data might suggest a role for Mgm101 in the recombination-dependent replication of linear mtDNA and mitochondrial telomere maintenance.

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Extreme Amyloid Polymorphism in Staphylococcus aureus Virulent PSMα Peptides

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The mechanisms of amyloid protein assembly into fibrous structures have been studied for decades, particularly since amyloids are associated with neurodegenerative and systemic human diseases. In contrast, functional amyloids that participate in dedicated physiological activities in all kingdoms of life were poorly characterized and their importance to human health is only starting to emerge. Functional amyloids were discovered mostly in microbes, serving as key virulence factors and thus present novel targets for antimicrobial agents. The structural hallmarks of functional amyloids – if any – and how they can be distinguished from disease-associated amyloids remain unclear. We investigated the structure-function-fibrillation relationships of microbial functional amyloids, their interactions with host amyloids and receptors and explore routes to modulate their activities. By leveraging unique methodologies of X-ray microcrystallography, we were the first to obtain atomic structures of bacterial functional amyloids. We discovered that two peptides, PSMα1 and PSMα4, involved in biofilm structuring of the pathogenic bacterium Staphylococcus aureus, form cross-β amyloid fibrils linked with eukaryotic amyloid pathologies, shown here for the first time at atomic resolution in bacteria (Salinas et. al., Nature Communications 2018). These fibrils confer ultra-stability to the biofilm. We also revealed unique amyloid-like structures in the S. aureus PSM peptide family, including, to our surprise, a structure of a cross-alpha amyloid-like fibril exposing surprising departure from pathological amyloids in which β-rich structures are central. The fibrils, of the full-length PSMα3 peptide, are toxic to human cells, clarifying their involvement in pathogenicity (Tayeb-Fligelman et. al., Science 2017). Interestingly, a truncated PSMα3, which forms reversible fibrils and has antibacterial activity, revealed two polymorphic and atypical β-rich fibril architectures, both radically different from both the cross-α fibrils formed by full-length PSMα3, and from the cross-β fibrils formed by PSMα1 and PSMα4 (Salinas et. al., Nature Communications 2018). Our results point to structural plasticity being at the basis of functional diversity exhibited by S. aureus PSMαs.

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Cryo-EM Structure Determination of the Vault Particle from Dictyostelium discoideum

Pablo Guerra. IBMB-CSIC

With a mass of 11-MDa, vaults are large ribonucleoprotein particles found in a wide variety of eukaryotes. When imaged by electron microscopy vaults present a conserved barrel-shaped structure with an invaginated waist and two protruding caps. In mammals, vaults contain three major proteins: the 100 kDa major vault protein (MVP), the 193 kDa vault poly(ADP-ribosyl)ating polymerase (VPARP) and the 240 kDa telomerase-associated protein (TEP1). Additionally at least one small untranslated RNA is found as a constitutive component (vRNA). MVP, which is the main constituent of vaults (~70%) contains all the information necessary for the assembly of the vault shell (1).

The X-ray crystal structure of rat liver vaults reported by our laboratory (2) and others (3) have revealed that the vault shell is organized in two identical halves, each consisting of 39 copies of MVP, which assemble to form a barrel-like cage with an enormous interior volume. Given the high degree of structural conservation that these particles exhibit among species, it is remarkable that Dictyostelium discoideum contains two MVP isoforms: MVPα and MVPβ. It has been reported that vaults purified from MVP(-); mutant lines of either MVPα or MVPβ have lost their lobular morphology, resulting in the generation of amorphous ovoid structures (4). This suggests that both proteins are required for the structural integrity of the vault particle. If so, we consider two possible scenarios: the D39 dihedral symmetry is conserved and each halve of the particle is composed of 39 copies of MVPα and MVPβ respectively or vaults from D. discoideum present a unique architecture with both isoforms intercalating in each vault half and thus exhibiting a new rotational symmetry.

To answer these questions we have now managed to purify D. discoideum vaults from expression of both MVP in insect cells using baculovirus system and to obtained a high resolution 3D reconstruction by cryo-EM. Solving the structure of this singular vault would allow us to decipher the evolutionary relationships linking this particle from an ancient microorganism to higher eukaryotes.

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Instruct Biennial Structural Biology Conference

Alcalá de Henares, Spain | 22 – 24 May, 2019 **Poster 101**

Integrating Molecular and Cellular Structure Data for Enhanced Visualisation and Analysis

Paul Korir. EMBL-EBI

The Electron Microsopy Data Bank (EMDB; www.emdb-empiar.org) is a public archive of macromolecular and cellular structures derived through various electron microscopy imaging modalities including electron tomography. EMDB is experiencing rapid growth due to technological advances in the field, and the proliferation of high-end instrumentation. Moreover, a unanimous vote during the 2017 3DEM GRC to make the deposition of a representative tomogram mandatory is likely to lead to further growth. To date, most EMDB tools and web-services have been entry-centric, considering every entry in isolation from others. Users may therefore be unaware of the growing collection of EMDB, EMPIAR and PDB entries that are related in some way and fail to exploit the added insights that can be gained from considering these relationships. Furthermore, the data is incomplete because interpretations such as segmentations and annotations present in the corresponding publications are not currently collected.

Through a series of workshops organised by EMBL-EBI, a data model to describe segmentation and subtomogram data has been developed called the EMDB-SFF (Segmentation File Format). EMDB-SFF is designed as an open standard to enable handling and exchange of segmentation data from a variety of application-specific segmentation file formats which is extensible to subtomogram averages. In addition to its ability to hold geometrical descriptors (meshes, shapes, volumes), EMDB-SFF is designed to contain annotations enabling links to other entries as well as other resources such as GO and UniProt that provide structural and biological context and improve an entry's discoverability. We are also developing SAT, a tool for semantic annotation of segmentations, which will enable depositors to EMDB to accompany their structures with annotated segmentations. Finally, we have developed a Volume Browser which brings the information together in an intuitive graphical interface and allows users to explore the structure and biological interpretation and context of molecular and cellular 3DEM datasets.

Our poster will showcase the Volume Browser (screenshot below) which integrates annotated EMDB-SFF files with 3DEM images while linking to other related EMDB entries.

A link to a PDF version of the abstract including all authors and a relevant image may be accessed at the following link: https://drive.google.com/file/d/1ntrS5JeXhDVSSqQsU9c5uxDPYUUR-n3X/view?usp=sharing

Structural studies of the interaction between the Toxoplasma gondii protein GRA24 and MAPKs

Pauline Juyoux. EMBL

The intracellular parasite Toxoplasma gondii is the causative agent of toxoplasmosis. During host cell invasion, T. gondii secretes a number of effectors to control its host's immune response that directly alter host gene expression (Hakimi et al. 2017). In particular, T. gondii seizes control of intracellular signalling pathways, including the MAPKs (mitogen-activated protein kinases) network.
The MAPKs are a family of intracellular kinases that control fundamental mechanisms such as proliferation, differentiation, inflammation and cell death. They are activated through specific phosphorylation by MAPK kinases (MAPKKs). The docking affinity and specificity of binding partners (activating MAPKKs, inactivating phosphatases, substrates, etc) towards MAPKs is mediated by short linear motifs called Kinase Interacting Motifs (KIMs) (Garai et al. 2012).
GRA24, one of the T. gondii effectors, is mainly intrinsically disordered and contains two KIM repeats (Braun et al. 2013). GRA24 triggers the MAPKs signalling pathway by interacting with p38α as previously described in (Pellegrini et al. 2017). In this way, it triggers inflammation to repress parasite over-proliferation that would kill the host and thus facilitate long-term parasitism.
Collaborators recently showed that GRA24 also interacts with ERK1, another MAPK, during host invasion. Our work describes here the characterization of the docking interaction between the GRA24 KIM1 peptide and ERK1. Isothermal calorimetry (ITC) shows high affinity of the GRA24 KIM1 peptide for ERK1 and also no affinity for the third member of the MAPK JNK1. The structure of this docking interaction was determined at 2.4 Å. The GRA24 KIM1 peptide binds in a similar manner to the docking site of both p38α and ERK1 and induces an allosteric conformational change to a ready to be activated conformation of the MAPKs. This work gives insights into the mechanism of action of GRA24 protein on key signalling MAPKs during T. gondii host cell invasion.

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Modified serum glycome in novel ALG12-CDG patient

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ALG12-CDG (CDG Ig) is inherited rare metabolic disease caused by defect in the function of alpha-mannosyltransferase 8, which is coded by ALG12 (22q13.33) gene. Up to this date, only 8 patients were reported worldwide. Standard procedure consisting of the isoelectric focusing of transferrin in the newborn Slovak patient revealed significant hypo-glycosylation pattern. This is consistent with CDG I subtypes of the disease that are caused by defects in glycolipid build-up prior to the glycan transfer to protein. In order to specify the diagnosis, released N-glycans from the serum proteins were permethylated and profiled by MALDI-MS. Disregulated signals that were clearly present in the mass spectra were further analysed and identified by fragmentation MALDI MS/MS analysis. The data revealed increased signals of GlcNAc2Man5-7, along with decreased signals of GlcNAc2Man8-9, that indicated the ALG12 defect. Genetic analysis of corresponding ALG12-coding region from patient´s sample led to yet unidentified homozygous substitutional variant in the exone 10, while this variant was heterozygous in the samples of both parents and asymptomatic twin. Such comprehensive glycomic and genomic approach resulted in a discovery of a new mutation in ALG12 gene responsible for the clinical manifestation of this disorder.

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Instruct Biennial Structural Biology Conference

Alcalá de Henares, Spain | 22 – 24 May, 2019 **Poster 104**

Exploring the Protein-Membrane Interactions on the Intracellular side of PRLR
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The Prolactin receptor (PRLR) is an archetypal member of the Class I Cytokine receptors, a family of single-pass transmembrane-receptors (SPTMRs) that play essential roles in growth, lactation, hematopoiesis, and immune response. Despite the wealth of information available about the function and regulation of PRLR, a complete structural picture of how the signal is transduced from the extracellular to the intracellular side and the role that membrane composition and protein/membrane interactions play in this process is still missing. In this sense, the seemingly contradictory observation that regions of the intracellular domain (ICD) of this receptor, essential for binding to JAK kinases, may also be involved in lipid-specific membrane binding, opens many questions about the dynamics and regulation of the signal transduction process at the intracellular side.

To shed light into this process, we used coarse-grained (CG) and all-atom (AA) molecular dynamics (MD) simulations to study the structure and lipid-dependent dynamics of regions of PRLR that comprise the transmembrane helix (TMD) and the lipid interaction domains (LID) of their ICD. Also, a complex of PRLR-LID1 with the FERM-SH2 domain of JAK2 has been built and simulated near membranes containing different types of lipids to study its structure, dynamics and preferential interactions and orientation with respect to the membrane.
Our results, agree with experimental observations that specific regions of the ICD of these receptors interact with the membrane forming preferred contacts with negatively charged lipids. On the other hand, the PRLR-LID1+JAK2-FERM-SH2 complex shows a preferred membrane binding orientation when negatively charged lipids are present. In this orientation, the residues that form BOX1 from PRLR interact with their binding site on JAK2 and with the membrane at the same time. These results point towards the co-existence of protein-protein and protein-membrane interactions and highlight the relevance of negatively-charged lipids such as PIP2 for receptor function and regulation. These results also provide the starting point to build more detailed models of the structural organization and dynamics of the intracellular side of these receptors.

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The R2SP co-chaperone: expression, purification, biophysical analysis and preliminary crystallization

Sara Silva. ITQB-NOVA

R2TP, as part of the R2TP/Prefoldin-like/HSP90 complex, is involved in regulation of DNA damage response of ATM, ATR and DNA-PKcs (PIKKs), in the biogenesis of small nucleolar ribonucleoprotein (snoRNP) and pre-ribosomal RNA processing, and plays roles in apoptosis. This complex also takes part in the assembly of other ribonucleoprotein complexes, such as Telomerase and spliceosomal U4 snRNA. While R2TP is spread throughout the organism, its homolog R2SP is located mostly in the brain and testes. R2TP and R2SP are involved in many pathways of disease, namely cancer-related. However, the mode of action of these important co-chaperones is still poorly understood.
The structure of R2TP has been extensively studied, mainly by cryo-EM, but no structure exists yet for R2SP. In this work, we tackle the expression and biophysical characterization of R2SP complex. We further show preliminary results in structure determination by X-ray crystallography.
Our results show that a complex can be obtained in two ways: either by co-expression of the four components, or by incubation of truncated RuvBL1/RuvBL2 complex with truncated versions of Spagh1_PIH1D2 complex. Dynamic Light Scattering analyses show similar profiles between the two complexes. However, Differential Scanning Fluorimetry data points to possible differences in structure, possibly due to the different modes of assembly. Finally, the crystal optimisation process is described. We collected diffraction data at 4,9 Å. With this resolution, we obtained a solution by Molecular Replacement, using an R2TP complex as model. This preliminary result shows the probable stacking of the molecules in the crystalline mesh. Interestingly, the molecules are tilted, suggesting an asymmetric placement of the Spagh1_PIH1D2 dimer on the RuvBL1/RuvBL2 hexamer.

A synthetic biology toolbox for antiviral antibacterial C-nucleosides
Sisi Gao. University of St Andrews.

There is an urgent need for new antibiotics against the so called ESKAPE pathogens and demands for potent antivirals against emerging filoviruses. The C-nucleotide class of natural products are known to possess both these activities. These promising molecules are likely to remain largely unused which given their known biological potency is a serious missed opportunity.
C-nucleoties, such as formycin, were first discovered as natural product antibiotics but due to off target activities, are little used and the complex synthetic chemistry surrounding their de novo synthesis has made them unattractive. However, recent work at Gilead has developed C-nucleotide drug to treat previously untreatable Ebola infections. This has driven a resurgence of medicinal chemical interest in C-nucleoside analogs.

All nucleosides that have been licensed and commercialised for clinical use have a heterocyclic moiety linked to sugar through a C-N bond, hence termed ”N-nuleotides”. This C-N linkage can be broken, however, when these compounds are either exposed to mild acid or react with cellular phosphate in a phosphorylase-catalysed transformation. These problems are eliminated by replacing the C-N linkage by a C-C bond to give a class of compounds known as ”C-nucleotides”.

Forymycin A and its analogues have been shown to be a potent inhibitors of bacterial purine nucleoside phosphorylase, whereas they are inactive against mammalian enzymes. Therefore, formycine A is of interest for its potential in selective drug design. Further- more, it has been reported that formycin A exhibits antiproliferative properties with respect to various cancer cells and antiviral activity against influenza virus A14 and human immunodeficiency virus type 1.

Although extensive research has been devoted to study the biological activities of formycins, investigation of their biosynthesis has been rare and slow. Early in vivo and in vitro studies have shown that the pyrazolopyrimidine ring of formycin A is derived from L-glutamate and L-lysine. However, the biosynthetic gene cluster and enzymes responsible
for such transformations between these precursors and the final product have not yet been identified.

The genes encoding for formycin have been identified by our collaborator and others, but the actual pathway remains elusive. There have been no systematic studies of their structures and chemical mechanisms, indeed the true substrates for many of these enzymes remain to be identified. I have already begun to study the entire formycin pathway and have determined stuctures of two key enzymes, a PLP dependent enzyme and a flavin dependent enzyme, with crystals of the third C-C bond forming enzyme.

Discovering Novel Ligands for Mosquito Odorant Binding Proteins (OBPs) using a combined computational methodology

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Abstract:
Mosquitoes and other hematophagous arthropods, the primary vectors of multiple parasites and viruses, are responsible for the transmission of serious diseases to humans such as malaria, dengue fever, Zika, West Nile fever, leishmaniasis etc. Nowadays the interest is focused on the development of novel repellents with advanced properties to the existing ones in terms of protection duration, minimum effective dose, efficacy against a wide variety of insects' bites, including resistant or insensitive species, and safety. Towards this direction, the present study attempts the discovery of novel hit compounds which may evolve as insect repellents using a combined methodology of different computational techniques. Particularly, a pharmacophore-based virtual screening of synthetic and natural compound libraries was performed, followed b y molecular docking to identify compounds with binding affinity to Odorant binding protein 1 (OBP1). In total, 6 compounds were picked and tested for their potential insect repellency activity in vivo using behavioral assays against females of the mosquito species Aedes albopictus. Results revealed that two of the examined compounds presented considerable insect repellent activity compared to naked hand (untreated hand) and could serve as new scaffolds for further structure optimization in order to improve their repellent activity.

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Human Telomeric G-quadruplex structures containing 8-oxo-7,8-dihydroguanine (oxoG) – one of the most common oxidation product of guanine

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Oxidative stress causes many types of DNA lesions, to which cells respond by activating relevant DNA repair pathways or apoptosis, if repair is unsuccessful. Among the four DNA nucleobases, guanine has the lowest redox potential and is therefore most prone to oxidation. Furthermore, guanines in tracts are more susceptible to oxidation than isolated guanines. Consequently, G-rich DNA regions could serve as trapping sites for oxidative damage caused by one-electron via radical cation migration through the DNA. Through the process of ageing, ends of chromosomes (telomeres) are shortening under conditions of high oxidative stress. Also, due to their high G content, human telomeric repeats d(T2AG3)4 in solutions containing K+ ions adopts hybrid fold of G-quadruplex, which feature three G-quartets planes with mixed parallel/antiparallel G-tract directionalities and G-tracts are connected by two edgewise and one double-chain-reversal loops.

Guanines at all 12 positions in oligonucleotide d[T2G3(T2AG3)3A] were individually substituted with 8-oxo-7,8-dihydroguanine (oxoG), which is one of the most common oxidation products of guanine. G-quadruplex forming ability of modified oligonucleotides was evaluated through examination of 1D 1H NMR spectra. A loss of G-quadruplex structure was observed for most oligonucleotides containing oxidative lesions, presumably due to reduced hydrogen-bonding capability caused by incorporation of oxoG. However, some positions in the hTel sequence can tolerate substitutions with oxoG. Two of those positions are G10 and G21, which adopt anti and syn glycosidic conformations in the parent hTel G-quadruplex, respectively. The two oligonucleotides containing individual modifications were selected for the further analysis and determination of high-resolution structures by solution-state NMR. However, due to oxoG's preference for the syn conformation, distinct responses were observed when replacing guanines with different glycosidic conformations. Accommodation of oxoG at sites in syn or anti in nonsubstituted hTel G-quadruplex requires a minor structural rearrangement or a major conformation shift, respectively. The system responds by retaining or switching to a fold where oxoG is in syn conformation. Importantly, both G-quadruplex structures with oxoG substitution at G10 or G21 positions are still stable at physiological temperatures and should be considered detrimental in higher-order telomere structures.

The first case of active site complementation and novel oligomeric state in family GH29 revealed by crystal structure of α-L-fucosidase isoenzyme 1 from Paenibacillus thiaminolyticus

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α-L-Fucosidase isoenzyme 1 from bacterium Paenibacillus thiaminolyticus catalyses hydrolysis of L-fucose from nonreducing termini of oligosaccharides and glycoconjugates. It also catalyses transglycosylation reactions [1]. This enzyme is a member of the glycosyl hydrolase family GH29 (CAZy classification). The structure of this enzyme revealed the first case of active site complementation and a novel quaternary state within the family GH29. The enzyme is active as a hexamer and its active site is complemented by tryptophan 392 from a neighbour molecule. The complementing tryptophan residue is affecting the catalytic properties but has no effect on the stability of the protein. The influenced catalytic properties include pH optimum, reaction rate in reaction using natural substrate 2'-fucosyllactose, and affinity towards artificial substrate α-L-fucopyranoside. The effect of the active site complementation on substrate specificity and transglycosylation abilities of α-L-fucosidase isoenzyme 1 was also analysed. A variety of naturally occurring substrates and different types of acceptor molecules were used for comparison of activity of the wild type and a mutant with the complementing tryptophan residue mutated to alanine. The assay showed that the mutation did not affect substrate specificity or the type of molecule recognised as acceptor for transglycosylation. However, it did change the yield of the reactions. [2]

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Insight in to the function and structure of HelD, the interaction partner of RNA polymerase from Bacillus subtilis

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Transcription in Gram-positive bacterium Bacillus subtilis is facilitated by RNA polymerase - the essential multisubunit protein complex the core of which consists of β, β', ω and two α subunits. While the RNAp core is capable of transcription elongation, for its initiation RNAp must form holoenzyme by integration with one of the σ factors. RNAp is also capable of interaction with several other proteins. These complexes form in different stages of transcription and have regulatory functions. One of the important interaction partners of RNAp is HelD [1, 2], which stimulates transcription by enhancing transcriptional cycling and elongation. HelD is an ATP-dependent helicase-like protein capable of binding the core of RNAp, but also free DNA. Although HelD is not an essential protein, its deficiency decreases the ability of cells to adapt [2]. The recent findings also suggest other possible roles of HelD [3]. The structure of HelD at atomic resolution is not known and function cannot be easily deduced from the knowledge about homologues (e.g. helicases from E. coli) as their sequence similarity with HelD is low. Recently we have performed studies of HelD structure and function using SEC–SAXS (beamline P12, EMBL Hamburg at the PETRA III, DESY, Hamburg, Germany), mutational studies and bioinformatic analysis. Our results revealed ligand dependent conformational changes of HelD possibly related to its function and further broaden our understanding of the structural and functional relationship of HelD to the UvrD and RapA helicases.

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Instruct Biennial Structural Biology Conference

Alcalá de Henares, Spain | 22 – 24 May, 2019 **Poster 111**

Crystal Structures of Porcine Pancreatic Elastase and Human Neutrophil Elastase in Complex with Novel 3-Oxo-β-Sultams Inhibitors

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Crystal Structures of Porcine Pancreatic Elastase and Human Neutrophil Elastase in Complex with Novel 3-Oxo-β-Sultams Inhibitors

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Human Neutrophil Elastase (HNE) is a serine protease responsible for cleavage of peptide bonds conferring elasticity to the connecting tissues. For this reason, this enzyme is mainly found in the lungs, arteries and ligaments. When unregulated, HNE leads to the onset of some diseases, such as rheumatoid arthritis and arteriosclerosis. Porcine Pancreatic Elastase (PPE) is commonly used as a model for HNE, sharing 40% of amino acid sequence homology.
The 3-Oxo-β-Sultams are chemical compounds capable of inactivating serine hydrolases irreversibly. They are characterized by a 4-member ring warhead with 2 electrophilic centers (carboxyl and sulfonyl groups), allowing for two possible reaction mechanism paths: acylation or sulfonylation of the catalytically active serine of the enzyme [1-2].
In order to clarify the dispute concerning the action mechanism of 3-Oxo-β-Sultams, X-ray crystallographic structures of elastases bound to various 3-Oxo-β-Sultam inhibitors were characterized. Diffraction data were collected at European Synchrotron Radiation Facility (ESRF, Grenoble, France) and Diamond Light Source (DLS, Oxford, United Kingdom). Three crystal structures of PPE:inhibitor complexes were determined to resolutions better than 1.4 Å, allowing the characterization of the interactions between protein and inhibitor at atomic detail. Moreover, one crystal structure of HNE:Inhibitor complex was obtained to 2.6 Å resolution.
Structural analysis of all complexes revealed the protein nucleophilic attack to 3-Oxo-β-Sultams to occur via sulfonylation, contradicting previously published data and highlighting the potential use of these inhibitors [1].

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Poster 112

Pdr17 - yeast phosphatidylinositol transfer protein.

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Many lipid transfer proteins operate at membrane contact sites where two membranes come close together to facilitate exchange of material and information. Protein domain responsible for transfer PI, PC, sterol is called CRAL/TRIO. In *Saccharomyces cerevisiae*, Pdr17p (also known as Sfh4p) is a member of CRAL/TRIO containing proteins and acts at the membrane contact sites between the endoplasmic reticulum (ER) and the Golgi/endosomes and is required for transfer of phosphatidylserine (PS) from the ER to the membrane where decarboxylation of PS to PE by phosphatidylserine decarboxylase 2 (Psd2p) takes place. We generated Pdr17 mutant proteins to better understand the mechanism by which Pdr17p facilitates inter-membrane transfer of PS at membrane contact sites. One of the Pdr17 mutant proteins is not capable of binding phosphatidylinositol (PI) by using permeabilized human cells and complementation assays *in vivo*. These mutations changed only the lipid binding cavity of Pdr17p and not the surface properties of the protein. In contrast to all other previously tested yeast PITPs, Sec14p, Pdr16p, and Sfh5p, the ability of Pdr17p to bind PI is not required for Pdr17p major cellular function in the inter-membrane transfer of PS.

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Towards structure determination of plant membrane-anchored calpain DEFECTIVE KERNEL 1

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DEFECTIVE KERNEL 1 (DEK1) is 240 kDa membrane protein with essential functions in plant growth and development. DEK1 has been implicated in positional sensing, cell division plane orientation and cell fate maintenance throughout the plant lineage ranging from mosses to crop plants including wheat and maize. Despite its essential functions and extensive genetic studies, the molecular mechanism of DEK1 activation and substrate recognition is still poorly understood. DEK1 consists of a predicted 23-spaning transmembrane domain with long central Loop and segments showing homology to MFS transporters, a cytosolic Linker segment containing LaminiG-like domain and a C-terminal Calpain protease that represents an effector module of DEK1. In collaborative efforts supported by participating labs and INSTRUCT networks, we have initiated HTP expression and purification screens for production of individual DEK1 domains, as well as the full-length DEK1 protein, for structural studies. Currently we use heterologous expression systems including E. coli, yeasts, insect Sf9 cells and mammalian HEK cells. In addition, we use a native source of DEK1, the moss Physcomitrella patens, modulated as a plant cell culture. Here, we present successes and challenges of the individual approaches and discuss further directions based on experiments and results achieved so far. This work is supported by the Slovak Research and Development Agency grant APVV-17-0570.

Unique crystal structure of human derived antimicrobial peptide reveals an outstanding hexameric formation.

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Antimicrobial peptides (AMPs) are vital key components of the innate immune system of host. For over more than 60 years, over than 2000 different AMPs, from all kingdoms of life. AMPs identified and are classified into four main groups according to their secondary structure. LL37 is an AMP, alpha-helical and cationic amphipathic which found in human as well. This peptide plays a critical role in host defense mechanisms through a direct antimicrobial activity and through regulation of the immune system activity. Nevertheless, to its acute role in health conditions, LL37 is expressed and secreted in many tissues and organs such as lungs, head, skin, intestine etc. Moreover, it is known to be naturally cleaved in the host's body to more than 50 segment peptides which hold an individual biological function. Unfortunately, less than 1% of the known AMPs have a determined atomic crystal structure and so none of the known cleaved peptides of LL37. High tendency to aggregate and poor solubility in water revenue low crystallization rates which poorly diffract. In this work, we present a breakthrough structural work being done on a segment peptide of LL37. LL3717-29 is a 13 residues long segment peptide with antimicrobial activity against both gram positive and negative bacteria. It is poorly crystallizing and the diffraction data collected several times at the micro-focus beamline P14 operated by EMBL Hamburg at the PETRA III storage ring (DESY, Hamburg, Germany), couldn't yield a fine crystal structure. Therefore, we crystallized a gorilla homolog of LL3717-29 which sequence is altered in the first amino acid, from phenylalanine to serine. This homolog's crystal structure was determined using the mentioned beam in a tremendous resolution of 1.15Å. The crystal structure of the human peptide was then also determined, in a resolution of 1.5Å using the gorilla structure as a reference model for molecular replacement. Amazingly, the two atomic structures present crystal packing of P6211 where peptides assemble into four-helical bundles sharing a strong hydrophobic core. Those helixes bundles are then positioned in spiral orientation and form a novel type of fibril with a core of a nanochannel in the center of it. More astonishing to find was that the substitution in the first amino acid alters the physical properties of the core, such as hydrophobicity and diameter, suggesting to affect the biologic function as well. Correlatively, antimicrobial studies of the human and gorilla forms of LL3717-29 and also of single point mutations array reveal a tight relationship between the atomic structure and the functions of AMPs.