Abstract Booklet

Instruct Biennial Structural Biology Conference

Muntgebouw, Utrecht, The Netherlands
19-20 May, 2022

Speakers

Posters

Poster No.	Title	Author	Page
1	Instruct Centre IL: Weizmann Institut of Science	Instruct Centre IL	33
2	Nanobodies4Instruct	Instruct Centre BE	33
3	BIOCEV / CEITEC	Instruct Centre CZ	33
4	CNB-CSIC / Instruct Image Processing Centre I2PC	Instruct Centre ES	33
5	Institut de génétique et de biologie moléculaire et cellulaire (IGBMC)	Instruct Centre FR1	33
6	Institut de Biologie Structurale (IBS)	Instruct Centre FR2	33
7	CERM/CIRMMP Instruct Centre IT	Instruct Centre IT	33
8	Instruct Centre NL is the Dutch national Instruct Centre. Instruct-NL includes three access sites offering a wide range of structural biology methods, as well as a vast national stakeholder community.	Instruct Centre NL	33
9	Instruct Centre FI is the Finnish national Instruct Centre and it was opened in 2020. Instruct-FI consists of three Centers of Excellence and offers a wide range of expert-supported structural biology and sample preparation services.	Instruct Centre FI	33
10	Astbury / Diamond / OPIC / STRUBI	Instruct Centre UK	33
11	ARIA provides a simple, single portal	ARIA Team	33

	for your facility, access and community		
12	Acinetobacter type VI secretion system (T6SS) assembles a non-canonical membrane complex	Ona KANDOLO	34
13	Cryo-EM structures of human RNA polymerase I	Agata Misiaszek	20
14	Cryo-EM and artificial intelligence visualize endogenous protein community members	Ioannis Skalidis	21
15	Mechanistic Studies on Human Prolyl Hydroxylase 2	Giorgia Fiorini	22
16	High-resolution structure of a cell-cell fusogen in nanodiscs derived from extracellular vesicles	Jo Judernatz	35
17	Structure and dynamics of a mycobacterial type VII secretion system	Catalin Bunduc	36
18	AlphaFill: Enriching the AlphaFold models with ligands and co-factors	Ida de Vries	24
19	Cryo-EM reveals the structural basis for the regulatory function of HEL1 domain in vertebrate DICERs	David Zapletal	23
20	Survival strategies in the deep - structural dynamics of a hyperthermophilic PEP-synthase	Pascal Albanese	37
21	Asymmetric cryo-EM structure of bacteriophage AP205 virion	Kaspars Tars	38
22	Characterization of the intrinsically disordered region of human NDRG1, a possible target for lung cancer therapy	Barbara Zambelli	39
23	Delineating the bacterial translation machinery with unprecedented detail using cryo-ET and sub-tomogram analysis	Liang Xue	40
24	View on molecular structures required for vision: PRPH2 and ROM1	Dounia El Mazouni	41

25	Membrane protein interactions define the tick borne encephalitis virus structure	Sarah Butcher	42
26	Baseplate structure of bacteriophage phi812 and mechanism of cell wall binding and penetration	Jan Binovsky	43
27	MATCAP, the missing tubulin detyrosinase: Structural insights	Jitske Bak	44
28	Structural analysis of a divergent endolysin from Hafnia bacteriophage Enc34	Ellna Cernooka	45
29	Structural studies on the mechanism of neuronal SALM synaptic adhesion proteins	Tommi Kajander	46
30	PyHDX: Probing Universal Protein Dynamics Using Hydrogen–Deuteriu Exchange Mass Spectrometry-Derive Residue-Level Gibbs Free Energy	Jochem H Smit	47
31	Mammalian ribosomal RNA expansion segments facilitate co-translational protein import into mitochondria	Lena Tharichen	48
32	Chaperoning of the histone octamer by the acidic domain of DNA repair factor APLF	Hugo van Ingen	25
33	Instruct and EU-LAC ResInfra	John Dolan	49
34	Plasticity of GRM2 type bacterial microcompartment shells	Gints Kalnins	50
35	Stream processing of CryoElectron Microscopy images at the acquisition site	Carlos Oscar Sanchez Sorzano	51
36	Tail proteins of Podoviridae phage SU10 reorganize into the nozzle for genome delivery	Marta Siborova	52

37	Structural and functional studies of outer surface proteins from Lyme disease causing agent *Borrelia burgdorferi*	Kalvis Brangulis	53
38	eSPC, an online data-analysis platform for molecular biophysics	Osvaldo Burastero	54
39	Albumin and ions separately prime Coxsackievirus A9 for RNA release	Zlatka Plavec	55
40	Direct observation of chemo-mechanical coupling in the Hsp70 chaperone DnaK by single-molecule force experiments	Gabriel Zoldak	56
41	Molecular mechanism of myeloma λ-type IgG light chain aggregation	Veronika Dzupponova	57
42	Structural insights into synapse formation: A compact dimer of Teneurin4	Dimphna Meijer	58
43	HIV-1 nuclear import: structural study of the interaction between hCPSF6 and HIV-1 capsid	Rui ZHU	59
44	Assessment of Structural Stability and Flexibility of Amyloid Fibril Proteins	Kartikay Sharma	60
45	Recombinant Transient Gene Expression and Preparation of HBsAg VLPs for Serology and Structural Analysis	Michael Lehky	61
46	Dual thermofluor-based assay enables extensive buffer screening for protein-DNA complexes	Ales Hnizda	62
47	Structure and proteolytic properties of Ryegrass mottle virus serine-like 3C protease in complex with and without VPg cofactor.	Eva Emilija Cesle	63
48	Dynamic assembly of the Hsp90 chaperone machinery by multi-colour single molecule FRET	Julia Schimpf	64

49	Dynamics and directionality within the multi-component Hsp90 machinery	Leonie Vollmar	65
50	Structural characterization of a mitochondrial intermembrane domain	Ramiro Illanes Vicioso	66
51	Structure and Dynamics of Huntingtin. A Segmental Labelling Approach	Xamuel Loft Lund	67
52	Alteration of the dynamic motion of the N-terminal domain of the human ryanodine receptor 2: a possible cause of several cardiac arrhythmias	Vladena Bauerova	68
53	Chemical characterization of zinc granules in the Malpighian tubules of Drosophila melanogaster	Daniele Vitone	69
54	Structural characterization of intrinsically disordered C-terminal regions of GPCRs and impact on the arrestin-dependant pathway	Assia Mouhand	70
55	Investigating gene transcription modulators inside mitochondrial gene	Michaela Krafcikova	71
56	Atomic interrogation of protein-protein interactions within intact nuclei by DNP-supported solid-state NMR	David Beriashvili	72
57	Production of stable mutants of NKp80, an activating receptor of NK cells	Kristyna Pazderova	73
58	Identification of Potentially Bioactive Argon Binding Sites in Protein Families	Islem HAMMAMI	74
59	ScipionTomo: towards cryo-electron tomography software integration, reproducibility, and validation	Marcos Gragera	75
60	CLIC1 membrane insertion and inhibition	Jose Ortega-Roldan	76

61	Protein formulation through automated screening of pH and buffer conditions using the RoboteinÂ® high throughput facility	Ernesto Ambroggio	77
62	Functional Analysis of Drosophila's Timeless Protein Domains	Enrico Bullo	78
63	19F NMR as a direct probe for proteins in human cells	Lan Pham	79
64	Seeing Double: A structural understanding of dsRNA recognition by nuclear factor 90	Atlanta Cook	80
65	CryoEM snapshots of a native lysate provide structural insights into a metabolon-embedded transacetylase reaction	Christian Tuting	81
66	Structural basis of the interaction between the adaptor protein PSTPIP1 and the LYP phosphatase: a novel mechanism of recognition of Pro-rich motifs by F-BAR domains	Jose M de Pereda	82
67	Yeast Ssd1 is a non-enzymatic member of the RNase II family with an alternative RNA recognition interface	Uma Jayachandran	83
68	The complex roles of sulphated saccharides: What we can learn from NMR and DFT	Milos Hricovi-ni	84
69	Structure of the Rap GTPase-activating protein SIPA1	Natalia Soledad Sotelo	85
70	Nanobodies for the analysis of the guanine nucleotide exchange factor C3G	Alba Moran Vaquero	86

71	New Instrumentation Available in Centre of Molecular Structure, Institute of Biotechnology CAS (Biocev) in Czech Republic	Jiri Pavlicek	87
72	Role of factor HelD in bacterial transcription	Jan Dohnalek	88
73	Activation of C3G by the adaptor protein CrkL	Antonio Rodriguez-Blazquez	89
74	Get Funding to access Instruct Structural Biology services via ISIDORe and CANserv	Pauline Audergon	90
75	High-end X-ray technologies for laboratory structural biology in INSTRUCT-CZ/BIOCEV	Jan Stransky	91
76	Cryo-CLEM reveales that increased microtubule lattice spacing correlates with selective binding of kinesin-1 in cells	Leanne de Jager	92
77	Active site complementation and oligomerization in bacterial Î±-L-fucosidases	Tomas Koval	94
78	NA on VLPs with cryo-ET by MC Roelofs	Roelofs Marc	95
79	Biophysical characterization of extracellular carbonic anhydrase IX	Alena Hofrova	96
80	Structural analysis of the bovine antibody repertoire: contributions of the heavy and light to antigenic recognition	John Clarke	97
81	RNA G-quadruplex motifs in precursor microRNAs and their	Tiago Santos	98

	interaction with small molecules and nucleolin		
82	19F Trp labelling as a selective probe into the dimeric interface	Alexandra Naplavova	99
83	14-3-3 protein isoforms: mutual dimerization propensities screening kit	Jan Simek	100
84	Bottom-up and Top-down strategies for production and characterization of human multi-protein complexes.	Arnaud Poterszman	101
85	Structural and mechanistic studies of spider silk N-terminal domains	Kristaps Jaudzems	102
86	Autotaxin is required for specific LPA cell signaling	Fernando Salgado-Polo	103
87	Bacterial nano-compartments, their function in metal homeostasis and role in the cellular response mechanisms against stress	Celia Romao	104
88	Bioinformatical analysis of Proteus bacteriophage 65APm2833 receptor binding proteins and Proteus mirabili phage receptor by genomes sequence analysis	Rafat Matusiak	105
89	Essential transcription regulator Rta of Epstein-Barr virus	Tomas Brom	106
90	Cryo-EM Facility at Instruct Center France1 for high-resolution imaging	Alexandre Durand	107
91	The Sample Preparation & Characterization facility at EMBL Hamburg	Stephan Niebling	108
92	Developing workflows to study mammalian gametes using	Ravi Teja Ravi	109

	cryogenic correlative light and electron microscopy (cryoCLEM)		
93	Fitting Side-Chain NMR Relaxation Data Using Molecular Simulations	Felix Kummerer	110
94	Structure of Engineered Protein Targeting Zika Virus Envelope Protein	Margarida Archer	111
95	Single Particle Time Resolved CryoEM study of GroEL machinery	Mugdha Kiran Dhurandhar	112
96	Application of carbon-detected NMR experiments to study the solvent paramagnetic relaxation enhancement on intrinsically disordered proteins	Lorenzo Bracaglia	113
97	Order in disorder: AUX/IAA protein and its TIR1-Aux/IAA auxin co-receptor system	Arnout P Kalverda	114
98	Keeping a secretory protein non-folded:the role of the chaperone SecB at single-molecule resolution	Guillaume Roussel	115
99	Integrative structure of a 10-megadalton eukaryotic pyruvate dehydrogenase complex from native cell extracts	Fotis Kyrilis	116
100	Cryo-EM Snapshots of Nanodisc-Embedded Native Eukaryotic Membrane Proteins	Kevin Janson	117
101	Structural insights into the diversity of photosynthetic membrane proteins revealed by cryo-electron microscopy	Dmitry Semchonok	118
102	The Mode of Action of Teixobactin	Rhythm Shukla	119

103	Structural basis of CHMP2A-CHMP3 ESCRT-III polymer assembly and membrane cleavage	Kimi Azad	120
104	Catching calcium transport in motion: Structural and dynamic investigation of LMCA1	Sara Basse Hansen	121
105	Transport Mechanism of LATs - The use of nanobodies as a tool for structural and functional studies	Nicolàs-Aragó, A	
106	Study of the human ribosomal elongation cycle in situ	Juliette Fédry	
107	iNEXT-Discovery: Infrastructure for Trans-National Access and Discovery in Structural Biology	Hans Wienk	122
108	Towards the design of macrolide inhibitors: Solution and solid-state NMR studies of the ErmB-RNA complex	Francesca Lavore	
109	Instruct EMBL Hamburg / Heidelberg / Grenoble	Instruct-EMBL	33

News from Instruct - News from Integrated Structural Biology Research

Harald Schwalbe

In the first part of this contribution, new developments in Instruct will be discussed.
In the second part, integrated structural biology approaches to study disulfide bond formation in the ribosomal exit tunnel (Schulte et al.) and to study the role of the 30S ribosome in the regulation mechanism of translational riboswitches (de Jesus et al.) will be covered.

Schulte et al. Nat Commun. 2020 Nov 4;11(1):5569.
de Jesus et al. Nat Commun. 2021 Aug 5;12(1):4723

Dynamics and Interactions of Intrinsically Disordered Proteins from Single-Molecule Spectroscopy

Ben Schuler

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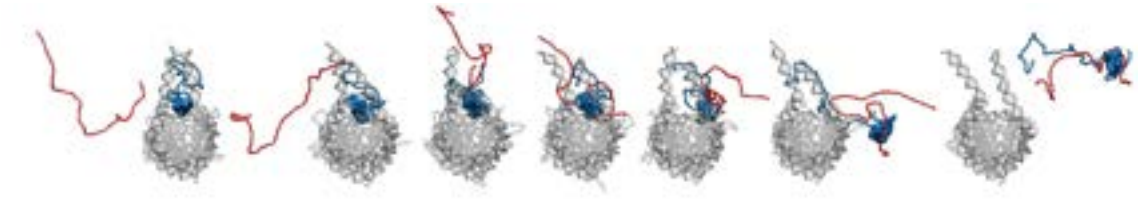
The functions of proteins have traditionally been linked to their well-defined three-dimensional, folded structures. It is now clear, however, that many proteins perform essential functions without being folded. Quantifying the highly dynamic and conformationally diverse ensembles of such unfolded or 'intrinsically disordered' proteins (IDPs) is an important aspect of understanding their functional mechanisms. Single-molecule spectroscopy is a versatile approach for investigating these systems. I will focus on highly charged IDPs and illustrate how single-molecule techniques combined with theory and simulations can be used to probe their distance distributions, dynamics, and interaction mechanisms.



Heidarsson, P.O., Mercadante, D., Sottini, A., Nettels, D., Borgia, M.B., Borgia, A., Kilic, S., Fierz, B, Best, R. & Schuler, B. (2021) Disordered proteins enable histone chaperoning on the nucleosome. Nat. Chem., https://doi.org/10.1038/s41557-021-00839-3

Sottini, A., Borgia, A., Borgia, M.B., Bugge, K., Nettels, D., Chowdhury, A., Heidarsson, P.O., Zosel, F., Best, R.B., Kragelund, B.B., & Schuler, B. (2020) Polyelectrolyte interactions enable rapid association and dissociation in high-affinity disordered protein complexes. Nat. Commun. 11, 5736.

Schuler, B., Borgia, A., Borgia, M.B., Heidarsson, P.O., Holmstrom, E.D., Nettels, D. & Sottini, A. (2020) Binding without folding – the biomolecular function of disordered polyelectrolyte complexes. Curr. Opin. Struct. Biol. 60, 66-76.

Borgia, A., Borgia, M., Bugge, K., Kissling, V.M., Heidarsson, P.O., Fernandes, C.B., Sottini, A., Soranno, A., Buholzer, K., Nettels, D., Kragelund, B.B., Best, R.B. & Schuler, B. (2018) Extreme disorder in an ultrahigh-affinity protein complex. Nature 555, 61-66.

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A Blueprint for the Structural Biology Department of the Future

Phil Selenko

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

The past decade has seen tremendous advancements in multiple areas of *in situ* Structural Biology. That is, in methods and technologies that enable direct structural investigations of biological macromolecules in their native cellular settings[1]. In these applications, spectroscopic techniques such as nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) methods, together with single- and ensemble-molecule Förster resonance energy transfer (FRET) spectroscopy play fundamental roles that provide unique and often complementary insights. In combination with cellular cryo-electron tomography (cryoET), in-cell cross-linking mass spectrometry (XL-MS), computational modeling and high-resolution optical imaging techniques, they define the toolkit that will shape the face of Structural Biology in the years to come.

Here, I present my vision for the Structural Biology Department of the future. I outline how these techniques and technologies may be best integrated to arrive at a comprehensive understanding of biological processes in their cellular settings. Because the future of Structural Biology is in the cell.

1. *Structural Biology outside the box – inside the cell.*
Plitzko JM, Schuler B, Selenko P. Current Opinion in Structural Biology *2017* 46 110-121

TITLE
Shooting with electrons to unravel the mechanism of membrane transporters - The case of the KdpFABC complex

Authors
Jakob M. Silberberg, Lisa Hielkema, Robin A. Corey, Charlott Stock, Phillip J. Stansfeld, Inga Hänelt, Cristina Paulino

My research group aims at elucidating the mechanism of action of membrane transporters on a molecular level. To this end we use an interdisciplinary approach with cryo- EM as a central technique. Several of our projects focus on membrane proteins that fall out-of-the-box, challenging conceptual boundaries present when classifying them into merely primary-active transporters, secondary-active transporters, or channels. They demonstrate how in the course of evolution, conserved protein architectures not only evolved from one another, but can merge together to adapt to different environmental and cellular requirements. A prime example for this is our research line on the bacterial emergency K^+-uptake system KdpFABC. The complex is composed of four subunits, whereby the KdpA resembles a K^+-channel and KdpB is a P-type ATPase (primary-active transporter). While it was assumed that K^+ is transported solely by the channel-like subunit[1,2], we propose a different and so far unprecedented transport mechanism, where K^+ is translocated through both subunits via two half-channels[3]. Our data show how KdpFABC func- tions as a true chimera, synergizing the best features of otherwise separately evolved transport mecha- nisms: ATP driven pumping of a P-type ATPase with the high affinity and selectivity of an ion channel. Combining cryo-EM with biochemical and MD simulation data, allowed us to elucidate in more depth how both subunits are coupled[4] and how they are regulated by an off-cycle state, strengthening the idea that KdpB is an early descendant of a common ancestor of cation pumps.

1. Bramkamp, M., Altendorf, K. & Greie, J.-C. Common patterns and unique features of P-type ATPases: a comparative view on the KdpFABC complex from Escherichia coli (Review). Mol Membr Biol 24, 375 - 386, doi:10.1080/09687680701418931 PMID - 17710642 (2007).
2. Huang, C.-S., Pedersen, B. P. & Stokes, D. L. Crystal structure of the potassium-importing KdpFABC membrane complex. Nature 90, 705 - 685, doi:10.1038/nature22970 PMID - 28636601 (2017).
3. Stock, C. et al. Cryo-EM structures of KdpFABC suggest a K+ transport mechanism via two inter-subunit half-channels. Nat Comms 9, 4971, doi:10.1038/s41467-018-07319-2 PMID - 30478378 (2018).
4. Silberberg, J. M. et al. Deciphering ion transport and ATPase coupling in the intersubunit tunnel of KdpFABC. Nat Commun 12, 5098, doi:10.1038/s41467-021-25242-x (2021).

Membrane trafficking studied by cryo-electron tomography

Giulia Zanetti

Thousands of newly synthesised proteins are transported from the ER to the Golgi apparatus in eukaryotic cells. This occurs through incorporation into COPII coated vesicles.
To understand COPII function and regulation, we reconstitute COPII budding in vitro and visualise coated membraned using cryo-electron tomography. We then apply subtomogram averaging to obtain high-resolution insights into COPII interactions. I will present recent progress on our understanding of how the COPII coat assembles.

Title: Multi-scale multi-modal electron imaging for in situ structural biology

Structures of purified proteins and protein complexes are routinely determined to atomic or near-atomic resolutions using single particle cryoEM. Structures of macromolecular assemblies that are intrinsically flexible and dynamic, and often function in higher-order assemblies that are heterogeneous, have recently been analyzed to near-atomic resolutions using cryoET and subtomogram averaging (STA). The study of native complexes in cells using cryoET STA, coupled with cryoFIB/SEM and correlative and multiscale imaging, opens a new frontier in structural cell biology. I will present our recent multi-modal, multi-scale imaging of SARS-CoV-2 infection and particulate methane monooxygenase (pMMO) in methanotrophic bacteria to demonstrate the power of combining serial cryoFIB/SEM volume imaging with cell lamellae-based cryoET STA.

Cryo-EM structures of human RNA polymerase I

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RNA polymerase I (Pol I) specifically synthesizes ribosomal RNA. rRNA constitutes 80-90% of the total RNA in mammalian cells, what is associated with high energy expenditure. Pol I upregulation is linked to cancer, making Pol I a promising drug target. Mutations in the Pol I machinery lead to developmental disorders such as Treacher Collins syndrome or Acrofacial Dysostosis. CRISPR-Cas9 technology allowed us to endogenously tag and purify native human Pol I from suspension HEK293T cells. It allowed us to obtain a cryo-EM structure of elongating human Pol I at 2.7 Å resolution. In the exit tunnel, we observe a double-stranded RNA helix that may support Pol I processivity. Our structure confirms that human Pol I consists of 13 subunits with only one subunit forming the Pol I stalk, compared to previously studied yeast which has two subunits constituting the stalk. Additionally, the structure of human Pol I in complex with the initiation factor RRN3 at 3.1 Å resolution reveals stalk flipping upon RRN3 binding. We also observe an inactivated state of human Pol I bound to an open DNA scaffold at 3.3 Å resolution. Lastly, the high-resolution structure of human Pol I allows mapping of disease-related mutations that can aid understanding of disease etiology. Comparison of the human Pol I and Pol III structures allows discerning the mode of function of certain mutations, which might specifically affect only Pol I or Pol III despite their location in a subunit shared between both polymerases.

Cryo-EM and artificial intelligence visualize endogenous protein community members

Ioannis Skalidis[1,2], Fotis L. Kyrilis[1,2], Christian Tüting[1], Farzad Hamdi[1], Grzegorz Chojnowski[3], Panagiotis L. Kastritis[1,2,4*]

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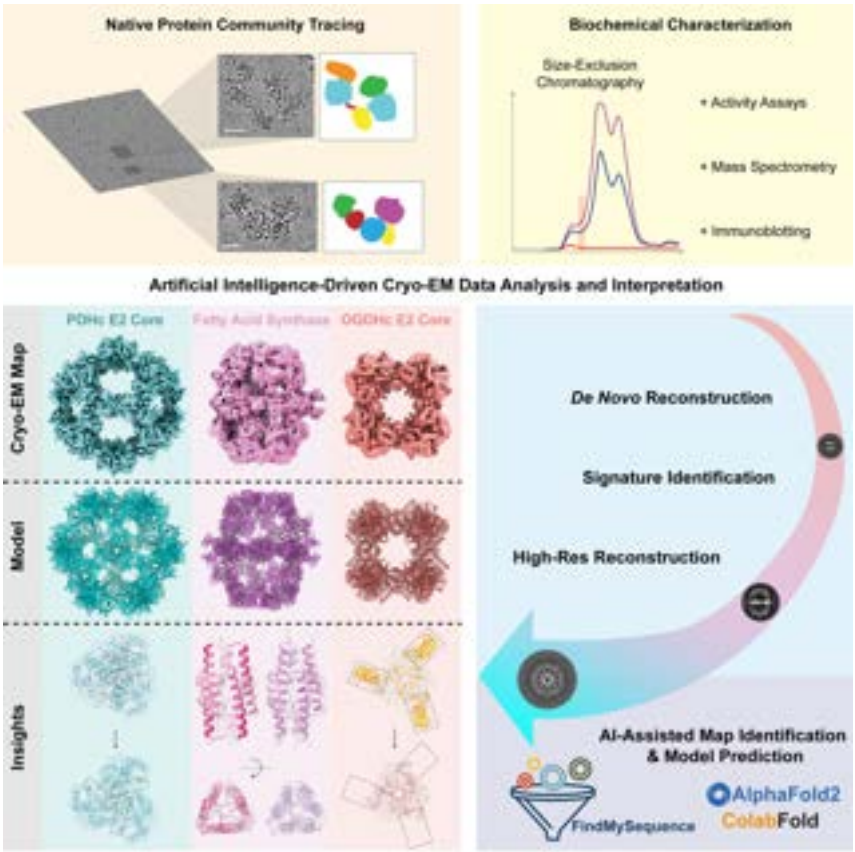
Cellular function is underlined by megadalton assemblies organizing in proximity, forming communities. Metabolons are protein communities involving metabolic pathways such as protein, fatty acid, and thioesters of coenzyme-A synthesis. Metabolons are highly heterogeneous due to their function, making their analysis particularly challenging. Here, we simultaneously characterize metabolon-embedded architectures of a 60S pre-ribosome, fatty acid synthase, and pyruvate/oxoglutarate dehydrogenase complex E2 cores de novo. Cryo-electron microscopy (cryo-EM) 3D reconstructions are resolved at 3.84–4.52 Å resolution by collecting <3,000 micrographs of a single cellular fraction. After combining cryo-EM with artificial intelligence-based atomic modeling and de novo sequence identification methods, at this resolution range, polypeptide hydrogen bonding patterns are discernible. Residing molecular components resemble their purified counterparts from other eukaryotes but also exhibit substantial conformational variation with potential functional implications. Our results propose an integrated tool, boosted by machine learning, that opens doors for structural systems biology spearheaded by cryo-EM characterization of native cell extracts.



Mechanistic Studies on Human Prolyl Hydroxylase 2

G. Fiorini[1], W. Figg[1], P. Rabe[1], M. A. McDonough[1], C. J. Schofield[1]

In humans, three HIF prolyl-hydroxylases (PHD1-3) play key roles in hypoxia sensing. The PHDs are 2-oxoglutarate (2OG)-dependent dioxygenases that catalyse *trans*-4-prolyl hydroxylation of the hypoxia-inducible factors (HIFs). Prolyl-hydroxylation enables the proteasomal degradation of HIF, causing the suppression of the hypoxic response. The aim of this work is to use time-resolved crystallography to gain insights into the mechanism of the PHDs, by characterising the reaction intermediates. High-resolution structures of $PHD2_{181-407}$:Fe(II):2OG:$HIF2\alpha_{523-542}$-CODD complex were obtained with novel anoxic crystallization conditions (**Fig. 1A**). In-house exposure of the anoxic crystals to O_2, validated the possibility of monitoring the reaction in crystallo and gave the first high-resolution structures of a PHD2:product complex (**Fig.1A**). Unexpectedly after long exposure to O_2, there was no apparent density for Fe and succinate. A room temperature crystal structure was obtained at PAL-XFEL and lays foundations for XFEL/XAS experiments that aim to investigate the changes in protein dynamics and visualise intermediates. It is unclear how O_2 diffuses through the PHDs; exposure of PHD2 crystals to a high pressure of gases (e.g. Kr, Xe) could help identify O_2 diffusion channels and potential O_2 binding sites. Preliminary data were collected after crystals exposure to high-pressure of O_2 demonstrating the feasibility of the experiment, notably, it was possible to refine a molecule of O_2 bound to the Fe. Of particular interest is PHD2 residue Thr387, reported studies suggest a role of Thr387 in the O_2 diffusion through the PHDs (Knapp et al., 2015). T387S and T387A mutants of $PHD2_{181-407}$ were produced. A structure of a $PHD2_{T387S}$-substrate complex was obtained to a resolution of 1.4 Å (**Fig.1B**). The alignment with the $PHD2_{181-407}$:substrate structures reported RMSD values of 0.084, revealing that there were no large conformational changes. Initial kinetics measurements demonstrate $PHD2_{T387A}$ can catalyse $HIF1\alpha_{556-575}$-CODD hydroxylation faster than $PHD2_{181-407}$ and $PHD2_{T387S}$ (**Fig.1D**). Further studies will clarify the role of Thr387 in PHD catalysis.

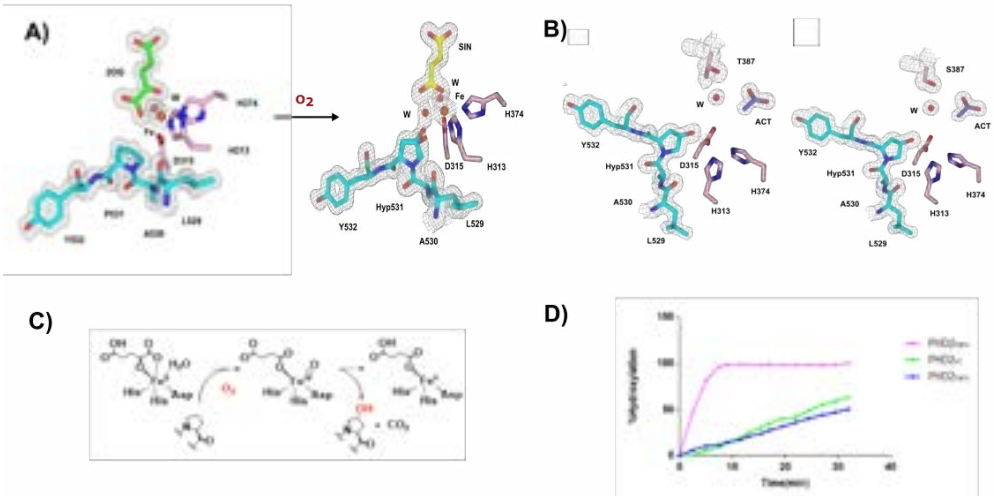


Figure 1. A) Hydroxylation in crystallo observed after oxygen exposure of anoxic **$PHD2_{181-407}$:Fe(II):2OG:$HIF2\alpha_{523-542}$-CODD** ***crystals B) Views from $PHD2_{181-407}$ and $PHD2_{181-407}$ T387S Crystal Structures C) Proposed Consensus Mechanism of Reaction for Fe and 2OG Dependent Hydroxylases D) Real-Time Hydroxylation of $HIF1\alpha_{556-575}$-CODD by $PHD2_{T387A}$, $PHD2_{181-407}$ and $PHD2_{T387S}$.***

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Cryo-EM reveals the structural basis for the regulatory function of HEL1 domain in vertebrate DICERs

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Dicer is RNase III enzyme producing small RNA in RNA silencing pathways. In mice, there are two isoforms that differ by presence or absence of the N-terminal HEL1 domain. The full-length Dicer acts in microRNA (miRNA) biogenesis while the truncated version, present in oocytes (Dicer^{O}), generates small interfering RNAs (siRNA) in RNA interference (RNAi) pathway. While models for siRNA and miRNA processing by vertebrate Dicer have emerged from the structural and biochemical studies, the active dicing state in Dicer-RNA structures has not been structurally characterized yet. In this study we used cryo-electron microscopy, AI-based prediction, and biochemical approaches to understand the structural difference between Dicer and Dicer^{O}, explaining their different *in vivo* functions. We observed that Dicer-RNA complex exists prevalently in an inactive, pre-dicing state (Fig.1b), while Dicer^{O}-RNA was observed almost exclusively in dicing-state (Fig.2c), revealing the molecular principles of substrate selection and enzymatic activity. Our data suggest HEL1 role in Dicer activity and substrate specificity by regulation of the loading of RNA substrate.

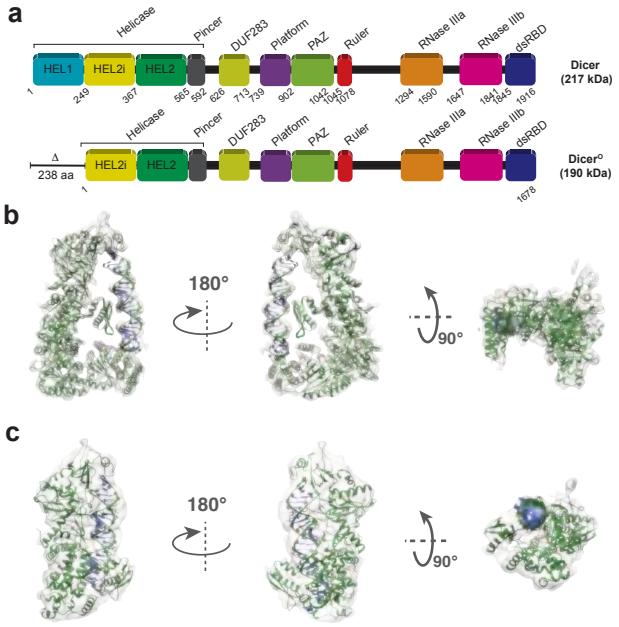

Figure 1: Cryo-EM structures of mouse Dicer and Dicer^{O}
a – Schematic depiction of domain organization of Dicer and Dicer^{O}.
b – The structure of Dicer—miR-15a complex, shown as ribbon representation fit in 4.19-Å cryo-EM density map.
c – The structure of Dicer^{O}—miR-15a complex, shown as ribbon representation fit in 6.21-Å cryo-EM density map.

AlphaFill: Enriching the AlphaFold models with ligands and co-factors

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Artificial Intelligence (AI) algorithms implemented in AlphaFold and RoseTTAFold are having transformative effect on structural biology research: 3D protein structures can now be predicted accurately based on their sequence only. Through the AlphaFold protein structure database (AFDB) over 800,000 predicted structures have become accessible. However, these proteins lack the co-factors, small molecules, and ions that are essential for structural integrity or molecular function. For example, the human myoglobin model does not contain the heme group essential for protein folding and oxygen binding (Figure 1a).

Here we present *AlphaFill*: an algorithm that "transplants" co-factors, small molecules and ions from homologous experimental structures into the AlphaFold models. Applying this algorithm to the entire AFDB has created 342,978 enriched models with 3,679,373 fitted compounds. For example, human myoglobin is now completed with heme, O_2 and CO (Figure 1b and c). All AlphaFill models are available through alphafill.eu, a new resource to help scientists in interpreting the AlphaFold models while creating new hypotheses and designing experiments.

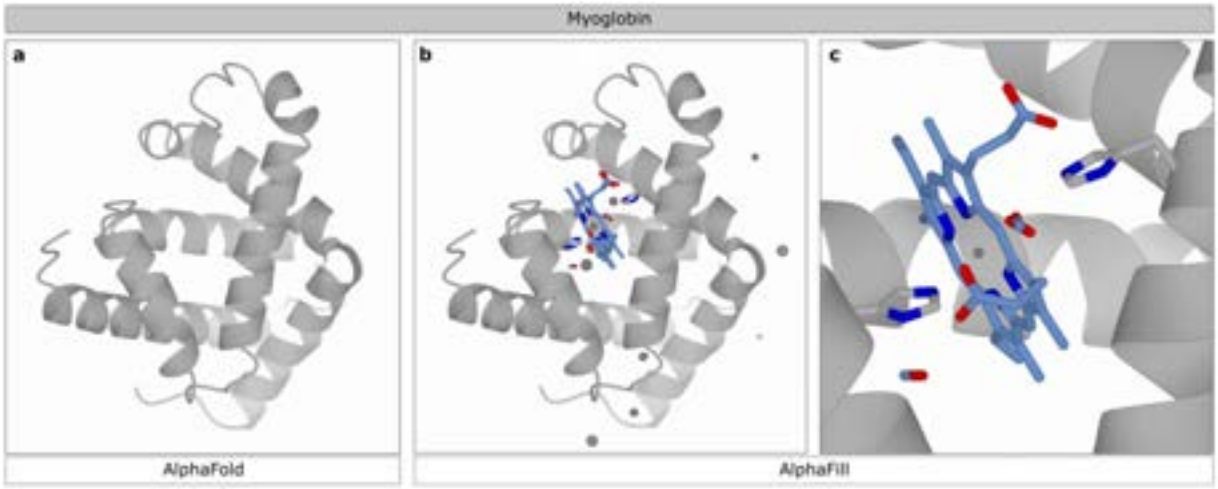

Figure 1: Human myoglobin. ***a*** *The AlphaFold model has the accurate protein structure but lacks the essential heme molecule.* ***b*** *The AlphaFill model with all transplanted compounds from homologs with >70% sequence identity.* ***c*** *The heme group as in b; only the transplanted heme group and the CO and O_2 ligands are shown.*

Chaperoning of the histone octamer by the acidic domain of DNA repair factor APLF

Ivan Corbeski[1,7], Xiaohu Guo[2], Bruna V. Eckhardt[3], Domenico Fasci[4], Melissa A. Graewert[5], Wouter Wiegant[6], Kees Vreeken[6], Hans Wienk[1,2], Haico van Attikum[6], Dmitri I. Svergun[5], Albert J.R. Heck[4], Rolf Boelens[1], Titia K. Sixma[2*], Francesca Mattiroli[3*], Hugo van Ingen[1*]

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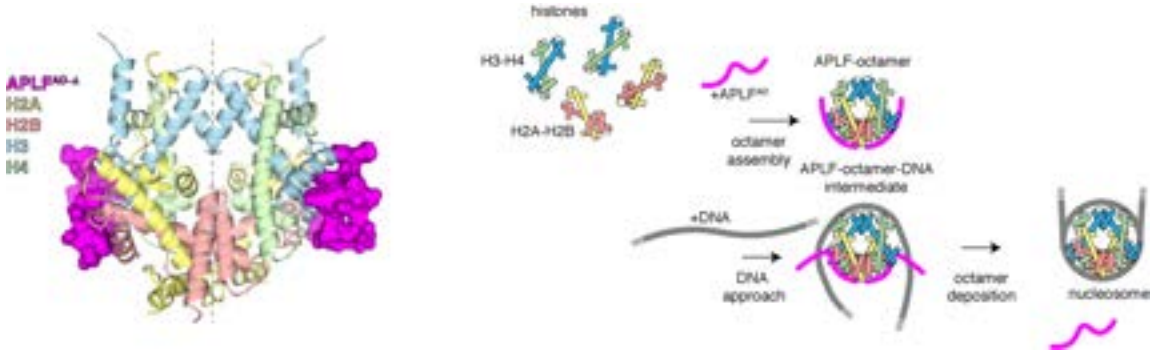
Nucleosome assembly requires the coordinated deposition of histone complexes H3-H4 and H2A-H2B to form a histone octamer on DNA. In the current paradigm, specific histone chaperones guide the deposition of first H3-H4 and then H2A-H2A. Here, we present a histone chaperone, the C-terminal acidic domain of DNA repair factor APLF (APLF^{AD}), that can assemble the histone octamer in a single step, and deposit it on DNA to form nucleosomes.

Through an integrative structural biology approach involving NMR, crystallography, XL-MS, SAXS/SANS and wide a array of biochemical experiments we dissected the histone binding of APLF^{AD} and its mechanism of chaperone activity and nucleosome assembly.[1] APLF^{AD} can bind all four core histones simultaneously with sub-micromolar affinity, and this interaction depends on four aromatic residues crucial for chaperone activity. The crystal structure of the APLF^{AD}–histone octamer complex shows that APLF^{AD} tethers the histones in their nucleosomal conformation. Mutations of key aromatic anchor residues in APLF^{AD} affect chaperone activity in vitro and in cells.

Based on additional NMR and XL-MS, we find that the APLF^{AD} envelops the octamer and screens most of the DNA binding surface except for the central H3-H3 interface region. DNA binding essays show that the APLF^{AD}–histone octamer complex can indeed form a ternary complex with DNA as intermediate in nucleosome formation.

As APLF is a scaffold protein for the non-homologous end joining (NHEJ) machinery that repairs DNA double-strand breaks, our data suggest that APLF^{AD} provides the NHEJ-machinery with the capacity to store the histone octamer during and to deposit it after DNA repair. Together, we propose that chaperoning of the histone octamer is a mechanism for histone chaperone function at sites where chromatin is temporarily disrupted.



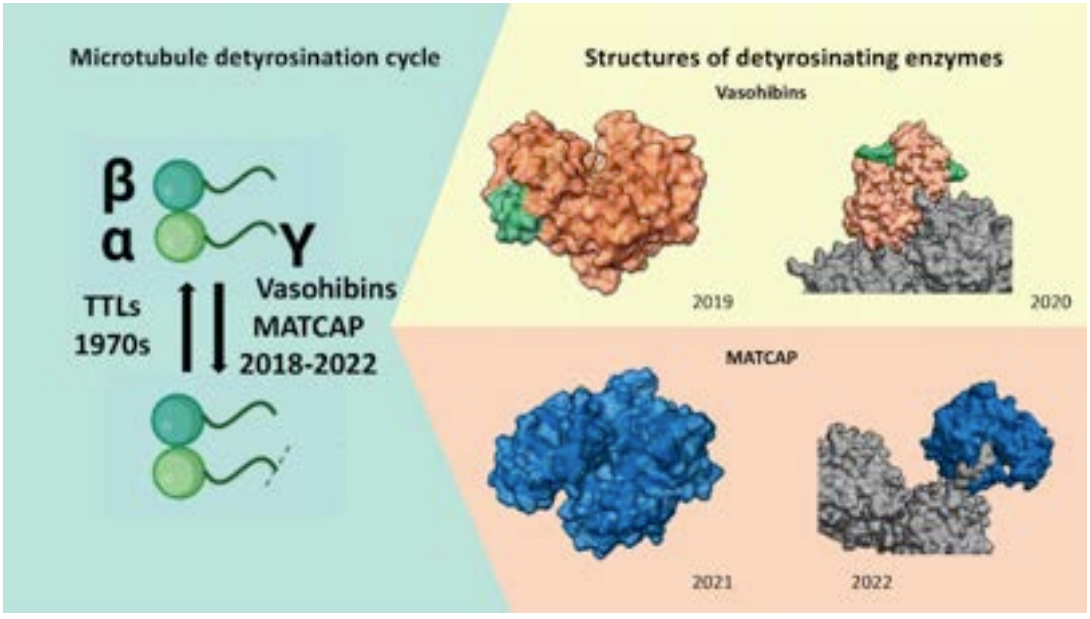
[1]. **Chaperoning of the histone octamer by the acidic domain of DNA repair factor APLF**. I. Corbeski, X. Guo, B.V. Eckhardt, D. Fasci, M.A. Graewert, W. Wiegant, K. Vreeken, H. Wienk, H. van Attikum, D.I. Svergun, A.J.R. Heck, R. Boelens, T.K. Sixma, F. Mattiroli, H. van Ingen. bioRxiv 2021.12.24.474097; doi: https://doi.org/10.1101/2021.12.24.474097

Deciphering the mechanism of microtubules detyrosination: from genetics to structures and back again

Anastassis Perrakis

The detyrosination-tyrosination cycle of microtubules involves the removal and re-ligation of the C-terminal tyrosine of α-tubulin and is implicated in cognitive, cardiac, and mitotic defects. A few years ago, others and we have described the Vasohibin-SVBP complex as the long-sought detyrosinating enzyme, that eluded identification for well over four decades after the function was shown in cells. While this was a significant step in the field, it was clear from the start that these enzymes underlies much, but not all, detyrosination in most cell types. Recently, we used haploid genetic screens in a Vasohibins-depleted cellular background, to identify an unannotated protein, MATCAP, as the remaining detyrosinating enzyme. Paradoxically, while abrogation of tyrosine re-ligation is lethal in mice, co-deletion of MATCAP and SVBP was not. Although viable, defective detyrosination caused microcephaly associated with proliferative defects during neurogenesis, and abnormal behavior.

Crystal structures of the Vasohibin-SVBP complex have revealed how SVBP acts as a folding chaperon for this assembly, and the mechanism of the VASH1 and VASH2 cysteine protease, explaining the specificity towards the α-tubulin tail. X-ray crystallography on MATCAP established MATCAP's enzymatic mechanism as an atypical metalloprotease. Computational modelling of the binding of the α-tubulin tail followed by biochemical validation experiments also revealed determinants of its substrate specificity. Cell-based imaging and a cryo-EM structure of MATCAP bound to fully detyrosinated microtubules from detyrosination-deficient cells, revealed the model of microtubule recognition, which is distinctively different compared to the Vasohibin-SVBP complex: MATCAP recognizes tubulin dimers across the same protofilament, while Vasohibins sit in between tubulin protofilaments. Thus, MATCAP is a missing component of the detyrosination-tyrosination cycle, revealing the importance of this modification in brain formation.



In-cell analysis of protein-protein interactions by crosslinking mass spectrometry

Prof Juri Rappsilber

Abstract: Crosslinking mass spectrometry is now a well-established experimental technique for investigating protein interactions, structure and function. It is an indispensable tool in support of single particle cryo-EM studies to illuminate areas with missing structural information due to flexibility. Crosslinking MS also remains unparalleled in its ability to provide structural information in complex systems. The power of the approach becomes especially apparent in conjunction with Electron Tomography for the investigation of coupled cellular processes in their native environment, such as transcription-translation in a minimal bacterium. Crosslinking MS is also relevant in the light of most recent revolutionary advancements in structural biology: Artificial intelligence program-based predictions of protein structure (e.g. by AlphaFold) are having wide-ranging impact on our ability to model protein structures but also to study protein interactions. Joining this with experimental data such as provided by crosslinking MS opens exciting avenues. However, there are also challenges associated with crosslinking MS rapidly advancing into routine use in structural biology, and large-scale studies starting to populate databases with protein-protein interaction data. The ease of obtaining crosslink data still needs to be matched by a widespread appreciation of the key factors governing the data, and data interpretation, use and sharing.

RNA Dances: How structural changes define function

Many functions of RNA depend on rearrangements in secondary structure that are triggered by external factors, such as protein or small molecule binding. These transitions can feature on one hand localized structural changes in base-pairs or can be presented by a change in chemical identity of e.g. a nucleo-base tautomer (Nature 2015). We use and develop $R_{1\rho}$-relaxation-dispersion NMR methods for characterizing transient structures of RNA that exist in low abundance (populations <10%) and that are sampled on timescales spanning three orders of magnitude (µs to s).
The characterization of different transient structures is going to be presented spanning systems from HIV virus regulation (Nature 2012) to microRNA regulation in the RISC complex (Nature 2020). We have trapped these short lived states and characterized their structure and impact on function.

References: www.petzoldlab.com

The macromolecular landscape of nidovirus replication organelles

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The formation of replication organelles is a hallmark of infection with positive-strand RNA viruses of eukaryotes. These virus-induced membrane structures support viral RNA and are thought to provide optimal microenvironments for viral replication and a shield from innate immune sensors. A fascinating example are the replication organelles induced by coronaviruses and other members of the *Nidovirale*s order. Nidoviruses generate typical double-membrane vesicles (DMVs), inside which viral RNA synthesis is presumed to take place. Strikingly, in conventional electron microscopy, these DMVs appear as closed compartments with no openings to the cytosol so, a long standing question in the field was how newly made viral RNA could be exported to the cytosol for packaging into new virions and translation.

We have used cellular cryotomography to study nidovirus-infected cells and to study the viral replication organelles with macromolecular resolution. In coronavirus-induced DMVs, this approach enabled the discovery of a crown-shaped molecular pore spanning the two membranes of the DMVs. We demonstrated that six copies of the largest viral transmembrane non-structural protein constitute the core of this pore complex. We have expanded our investigations to arteriviruses, a distantly related nidovirus family, and found similar DMV pores, often in close association with viral nucleocapsids. Moreover, DMV pore complexes were also formed when expressing only subsets of the arterivirus transmembrane nonstructural proteins. This indicates that the formation of a basic pore structure is independent of the presence of viral RNA, the replication machinery or the structural proteins. Collectively, our results suggest that DMV-spanning pore complexes is a conserved feature in nidovirus replication organelles, which highlights their important role in the viral replication cycle. These DMV pore complexes are likely RNA export channels that couple viral replication and encapsidation. They represent an intriguing novel class of viral complexes that offer new targets for future antiviral strategies.

Title: Peptide binding as monomer complementation - new approaches for blind global high-resolution peptide docking

Authors: Tsaban, Varga, Khramushin, Avraham, Ben-Aharon & **Schueler-Furman Ora ***

Affiliation: Department of Microbiology and Molecular Genetics, Institute for Biomedical Research Israel-Canada, Faculty of Medicine, Ein Kerem Campus, The Hebrew University of Jerusalem, Jerusalem, Israel

* Presenting author

Abstract

Peptide-mediated interactions play crucial roles in cellular regulation. Challenged by the flexibility of the peptide on the one hand and the often transient and weak character of the interaction on the other, they pose special challenges, both for modeling and experimental efforts. Recent advances in Deep Learning, such as by Deepmind Alphafold2, are revolutionizing computational structural biology, bringing to reach high accuracy models of full proteomes.

Evidence has accumulated that the binding of peptides to their receptors could be seen as monomer complementation, i.e., a final step of monomer folding. Based on this concept, we have developed in the recent years two novel, top-performing peptide docking protocols as assessed on a comprehensive benchmark and validated set. The first, PatchMAN(1), applies a fast search to match patches on the receptor surface for structural motifs in solved structures. These can then be used to extract complementing fragments that seve as starting point for peptide refinement. The second approach uses a slight modification of Alphafold2 to model the peptide either as separate unit, or connected by a poly-glycine linker to the c-terminus of the receptor(2). Importantly, we succeed in modeling these interactions at high accuracy, even though no information on the multiple sequence alignment of the peptide partner is available.

In my presentation, I will shortly introduce these approaches and their performance, and then discuss the underlying reasons for success, and failure. This can teach us about the basic principles of this interesting and important type of interactions between proteins.

(1) Alisa Khramushin, Ziv Ben-Aharon, Tomer Tsaban, Julia K Varga, Orly Avraham, Ora Schueler-Furman (2022). **Matching protein surface structural patches for high-resolution blind peptide docking**. *Accepted for publication in PNAS.* Original version available on Biorxiv doi: 10.1101/2021.09.02.458699

(2) Tomer Tsaban, Julia Varga, Orly Avraham Ziv Ben-Aharon, Alisa Khramushin, Ora Schueler-Furman (2022) **Harnessing protein folding neural networks for peptide–protein docking**. *Nat Commun* 2022, **13**:2021.08.01.454656.

Capturing a transient DNA zipper structure during V(D)J recombination

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Our limitless reservoir of antibodies and antigen receptors is created by stochastic recombination of gene segments (V, D and J) encoding these immunoglobulins in the process known as V(D)J recombination. To initiate V(D)J recombination, the RAG1/2 recombinase cleaves DNA at a pair of recombination signal sequences (RSSs), the 12- and 23-RSS (12/23-RSS). DNA double strand cleavage is achieved in two consecutive steps, hydrolysis and strand transfer, in a single active site. Using X-ray crystallography and cryoEM, we have determined how two RSS DNAs are paired, nicked and hairpinned at atomic resolutions. Both the protein and DNA undergo large conformational changes, and the active site of RAG1 re-arranges for DNA nicking and hairpin formation. A rare DNA zipper involving interdigitating base stacking between antiparallel DNA strands exist transiently. Combining DNA base alteration, enzyme active site engagement and reduced temperature, we captured the transient DNA conformation by cryoEM.

Cryo-Electron Tomography or the Power of Seeing the Whole Picture

Traditionally, structural biologists have approached cellular complexity in a reductionist manner by characterizing isolated and purified molecular components. This 'divide and conquer' approach has been highly successful. However, awareness has grown in recent years that only rarely can biological functions be attributed to individual macromolecules. Most cellular functions arise from their acting in concert. Hence there is a need for methods developments enabling studies performed *in situ*, i.e. in unperturbed cellular environments. *Sensu stricto* the term 'structural biology *in situ'* should apply only to a scenario in which the cellular environment is preserved in its entirety. Cryo electron tomography has unique potential to study the supramolecular architecture or 'molecular sociology' of cells. It combines the power of three-dimensional imaging with the best structural preservation that is physically possible.

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Acinetobacter type VI secretion system (T6SS) assembles a non-canonical membrane complex

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The type VI secretion system (T6SS) is a macromolecular nanomachine used by Gram-negative bacteria, notably important human pathogens. The apparent conservation of T6SS composition and architecture conceal the lack of direct investigation of T6SS species-specific adaptation. *Acinetobacter baumannii* possesses a T6SS that has been well studied for its regulation and specific activity, but little is known concerning its assembly and architecture. We have characterized for the first time the A. baumannii's specific T6SS membrane complex (AbMC). Notably, we have revealed the unique characteristic and behavior of the AbMC, its membrane localization and assembly dynamics. The AbMC is highly dynamic and undergoes a cycle of assembly and disassembly coordinated with the dynamics of the sheath. We also revealed the original composition of the AbMC, demonstrating that its biogenesis employs three Acinetobacter-specific envelope-associated proteins. We have demonstrated an intricate interaction network that connects five proteins of the AbMC, highlighting the central role of a newly discovered protein in connecting simultaneously TssL, TssM and TagX. We were able to directly visualize the motion of the C-terminal domain of TssM, a feature that could be shared by other T6SS. Our in-depth molecular study shed light on a small helical peptide, an intermolecular glycine zipper, conserved in the C-terminus of TssM and that is crucial to the functioning of the *A. baumannii* T6SS. Our work has profound impacts on our understanding of the T6SS functioning, especially on the assembly of the outer channel and paves the way to target specifically the *A. baumannii* T6SS Achilles' heel for therapeutic intervention.

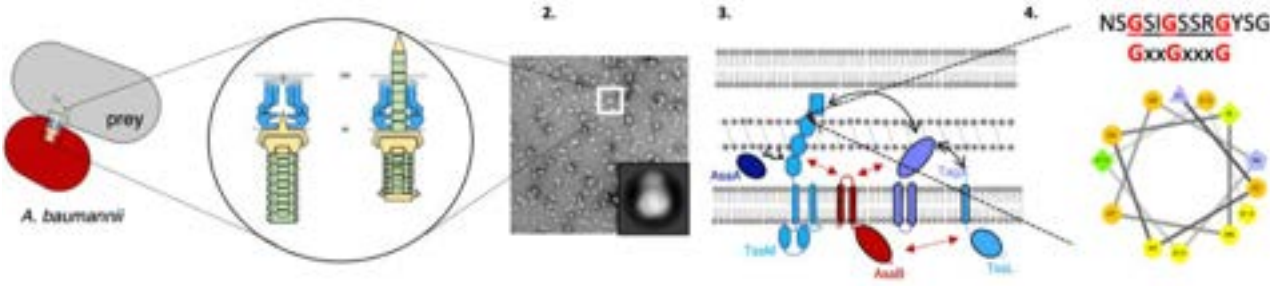

Figure 1: Structural organization of the unique *Acinetobacter baumannii* T6SS

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High-resolution structure of a cell-cell fusogen in nanodiscs derived from extracellular vesicles

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Abstract

Cell-cell fusogens are proteins that merge biological membranes. These membrane proteins are a challenging target for structure determination as they often exist in a metastable pre-fusion conformation and undergo a complex rearrangement during the membrane fusion process. Overexpression of fusogens in mammalian cells leads to secretion of membrane protein-enriched extracellular vesicles (MPEEVs). These vesicles contain a native membrane composition and present membrane proteins in their correct topology. We developed an experimental workflow to purify and reconstitute these proteins in nanodiscs obtained from MPEEVs. Using this method, we determined the post-fusion conformation structure of the cell-cell fusogen AFF1 at 3.5 Å with cryo-EM single-particle analysis. The structure revealed that AFF1 is a trimer structurally homologous to class II viral fusogens. Intriguingly, we find substantial differences between the fusion loops of AFF1 and the homologous cell-cell fusogen EFF1, supporting previously described differences in fusion efficiency between the two proteins. Electron densities between the ectodomain and the membrane suggest the presence of amphipathic helices of the membrane proximal region which were also predicted by AlphaFold. Taken together, our data provides new insights into the fusion mechanism of AFF1.

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Structure and dynamics of a mycobacterial type VII secretion system

Catalin M. Bunduc[1,2,3,4], Dirk Fahrenkamp[1,2,3], Jiri Wald[1,2,3], Roy Ummels[5], Wilbert Bitter[4,5], Edith N. G. Houben[4] & Thomas C. Marlovits[1,2,3]

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[2] Institute of Structural and Systems Biology, University Medical Centre Hamburg–Eppendorf, Hamburg, Germany.
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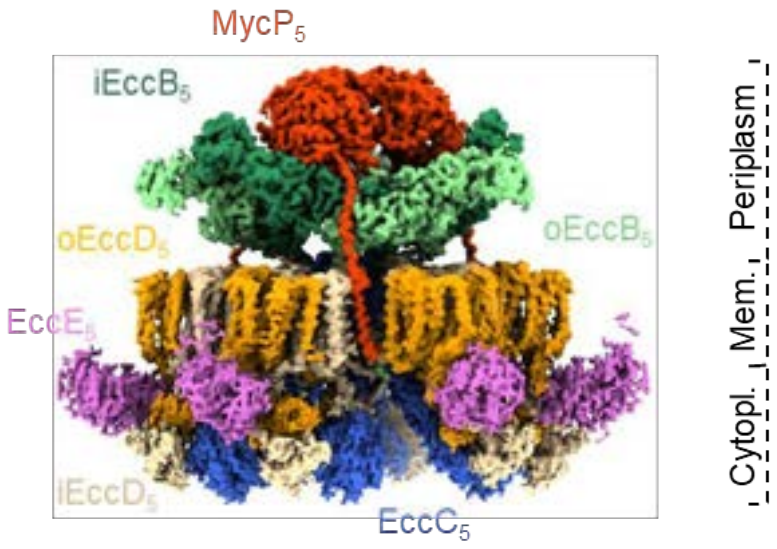
[5] Department of Medical Microbiology and Infection Control, Amsterdam Infection and Immunity Institute, Amsterdam UMC, Amsterdam, The Netherlands.

Mycobacterium tuberculosis causes one of the most important infectious diseases in humans, leading to 1.4 million deaths every year. Specialized protein transport systems—known as type VII secretion systems (T7SSs)—are central to the virulence of this pathogen and are also crucial for nutrient and metabolite transport across the mycobacterial cell envelope. Here we present the structure of an intact T7SS inner-membrane complex of *M. tuberculosis*. We show how the 2.32-MDa ESX-5 assembly, which contains 165 transmembrane helices, is restructured and stabilized as a trimer of dimers by the $MycP_5$ protease. A trimer of $MycP_5$ caps a central periplasmic dome-like chamber with the proteolytic sites facing towards the cavity. This chamber suggests a central secretion and processing conduit. Complexes without $MycP_5$ show disruption of the periplasmic assembly and increased flexibility, highlighting the importance of $MycP_5$ for complex integrity. Beneath the periplasmic chamber, dimers of the $EccC_5$ ATPase assemble into three bundles of four transmembrane helices each, sealing the potential central secretion channel. Individual cytoplasmic $EccC_5$ domains adopt two distinctive conformations, probably reflecting different secretion states. Our work suggests a previously undescribed mechanism of protein transport and provides a structural scaffold to aid the development of drugs against this major pathogen.



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Survival strategies in the deep - structural dynamics of a hyperthermophilic PEP-synthase

Pascal Albanese[1,2], Wenfei Song[3], Siri van Keulen[4], Jeroen Koendjbiharie[5], Sem Tamara[1,2], Serve Kengen[5], Alexandre M.J.J. Bonvin[4], Friedrich Förster[3], Richard A. Scheltema[1,2]

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[2] Netherlands Proteomics Centre, Utrecht University, Utrecht University, 3584CH Utrecht, The Netherlands
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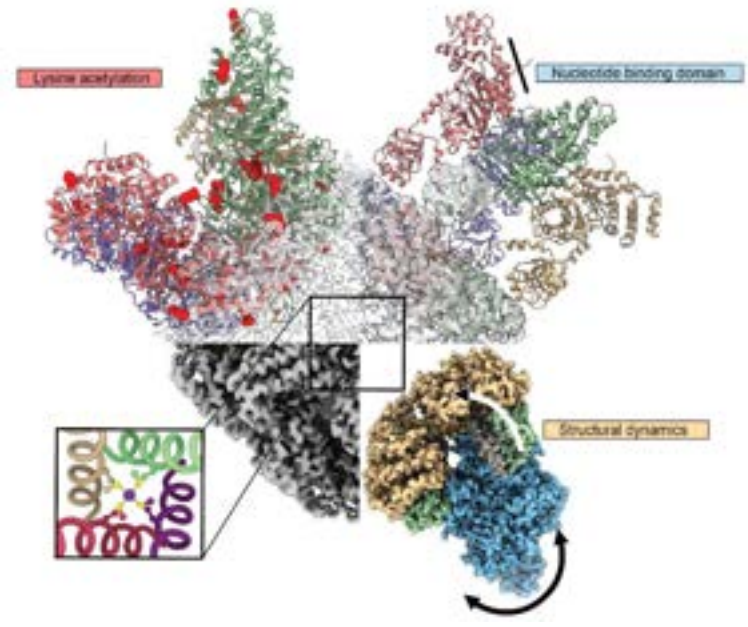
Despite life conquering nearly every habitat through adaptive evolution, the production of biomass relies on the combination of only a few metabolites: Pyruvate, Oxaloacetate and Phosphoenolpyruvate (PEP). Key metabolic enzymes of hyperthermophilic Archaea have adapted to conserve their catalytic cores to work at 90-100 °C, conditions similar to those where earliest life forms may have originated. Here we characterize an unusually abundant key enzyme of the central carbon metabolism form *P. furiosus*, through an integrative approach combining structural mass spectrometry (MS), cryo-electron microscopy (EM) and molecular dynamics (MD) simulations. From our investigation, we unveil the so far elusive structure of a 2.15 MDa phosphoenolpyruvate synthase (PPSA). Its 24-meric folding has an ancient origin and harbors extremely flexible distal N-terminal domains. The structure of the stable core, solved by Cryo-EM at ~3.4 Å, is assembled as a structure of six tetramers comprising the C-terminal domain of the protein, the PEP binding domain. The structure is stabilized by the planar coordination of an iron by four methionine residues and inter-chain salt bridges. We additionally uncovered a widespread non-enzymatic lysine acetylation of PPSA, as well as the major complexes of P. furiosus, suggesting an overall stabilizing effect on its structural dynamics. Integration of Crosslinking MS and co-evolution analyses provided the spatial distance restraints to model the felxible nucleotide binding domain, the other catalytic core of the protein, ultimately defining 2 possible arrangements within the full 24-meric assembly. These were further subjected to half millisecond long coarse-grained MD simulations, conducted in at 90°C in the presence or absence of lysine acetylation to assess its role on the overall stability. Results indicate that the inter-domain interfaces within each tetramer are maintained only in the presence of this post-translational modifications (PTMs). Even though no co-factor were included in the simulation, some catalytic cores starts forming functional contacts between them, resembling known structures of homologous enzymes in mesophilic organisms. This work ultimately provides insights on the role of extensive non-enzymatic acetylation in hyperthermophiles and paves the way for characterizing the effect of PTMs on large and flexible macromolecular complex integrating structural MS, cryo-EM and MD simulations.



Integrative structural modelling of *P. furiosus* PEP-synthase - Combining MS, EM and MD to unveil the stabilization and dynamics of a 2.15 MDa enzyme

Asymmetric cryo-EM structure of bacteriophage AP205 virion

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The bacteriophages with single-stranded RNA (ssRNA) genomes are among the simplest and smallest of the known viruses. Structurally, ssRNA phage particles consist of a genomic RNA molecule enclosed by 178 copies of the coat protein (CP) and a single copy of the maturation protein (MP). The MP is responsible for adsorbing the virion to the cellular receptor – a pilus on the surface of the host bacterium – and subsequent delivery of the RNA genome into the cell. We have determined the cryo-EM structure of Acinetobacter phage AP205 which has revealed significant differences compared to the previously determined structures of Escherichia ssRNA phages MS2 and Qβ. Notably, the AP205 virion contains not one, but two copies of the MP assembled in an asymmetric dimer. Furthermore, the genomic RNA inside the AP205 particle is arranged in a very different manner: while in MS2 and Qβ the majority of genome-capsid interactions are formed by RNA hairpin loops, in AP205 RNA-CP interactions are mediated by double-stranded RNA segments laying tangentially with respect to the inner surface of the virion. The AP205 structure demonstrates the considerable structural flexibility for receptor recognition and genome packaging among the evolutionary highly diverse ssRNA phages.

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Characterization of the intrinsically disordered region of human NDRG1, a possible target for lung cancer therapy

Ylenia Beniamino[a], Vittoria Cenni[b], Elisabetta Mileo,[c] Stefano Ciurli[a*], Barbara Zambelli[a*]

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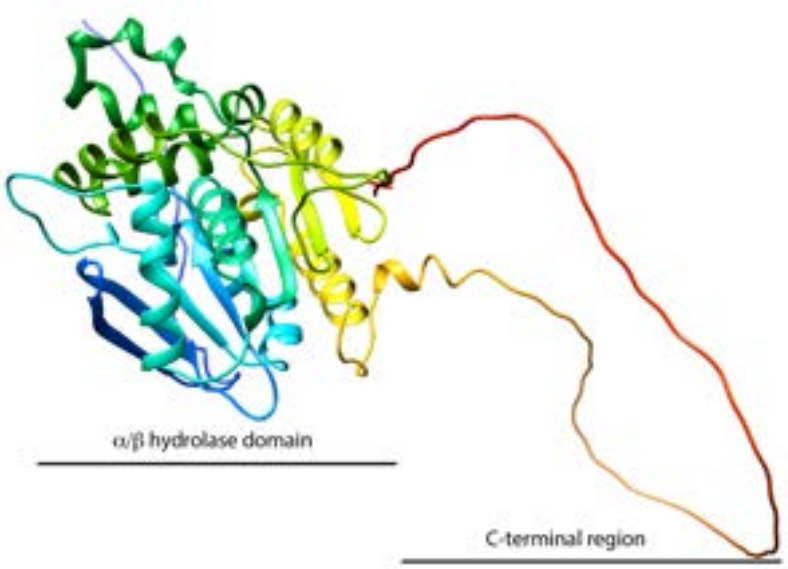
Nickel compounds, found in cigarette smoke and in fine dusts, are classified as class I lung and nasal carcinogens by IARC and exert its tumorogenic potential by activating the cell hypoxia response. This results in the up-regulation of the human N-myc downstream regulated gene 1 (*h*NDRG1), which is linked to poor prognosis, higher tumor aggressiveness and resistance to chemotherapy in lung cancer.

*h*NDRG1 share a non-enzymatic α/β hydrolase globular domain with other three members of the *h*NDRG family. In addition, it contains a unique C-terminal sequence of 83 residues, rich in charged residues and featuring a three-times repeated decapeptide. This region binds transition metal ions such as Ni(II), is regulated by post-translational phosphorylation and interacts with lipids, suggesting a key role of this region in the physiological function of *h*NDRG1.

In the present work, the structural, biochemical, and biophysical characterization of the C-terminal sequence of *h*NDRG1 (here named *h*NDRG1*C) is reported. Analysis of the sequence assigned it to the family of the intrinsically disordered regions (IDRs). The polypeptide was expressed and purified from *Escherichia coli* and experiments of isothermal titration calorimetry, light scattering, circular dichroism and SDSL-EPR were carried out to establish its metal-binding activity, as well as secondary and quaternary structure. A thorough analysis of the spectroscopic fingerprint of ^{1}H and ^{13}C detected NMR spectra provided detailed information on the effect of pH and Ni(II) binding on the structure. The biophysical data were integrated with the analysis of the Ni(II)-induced expression, subcellular localization and oligomeric states of *h*NDRG1 in a cell line of lung adenocarcinoma. The results are discussed considering the possible role of *h*NDRG1*C in the Ni(II)-driven lung cancer progression.



Delineating the translation machinery inside the genome-reduced bacterial cell

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To structurally and quantitatively delineate molecular processes inside the cell with atomic detail is the ultimate goal of structural biology. With large cryo-electron tomography (cryo-ET) data of the genome-reduced *Mycoplasma pneumoniae* cells and the advanced sub-tomogram analysis workflow, I determined an in-cell ribosome structure at 3.0 Å resolution, which is the highest for complex structures resolved inside the cell. The derived atomic model of mycoplasma ribosomes revealed novel structural features of ribosomal protein extensions. In particular, atomic detail on antibiotic binding and ion coordination in the ribosome were clearly visualized. Extensive sub-tomogram classification of ribosomes in native cells determined more than 13 translation intermediate structures (4-10 Å resolutions), which not only visualizes structural dynamics of translation elongation but also provides unique insights into their energy landscapes inside cells. Moreover, the spatial and functional organization of translating ribosomes revealed by mapping back the classified structures into the cellular tomogram, unraveled a novel elongation coordination mechanism mediated within polysomes. In addition to ribosomes, a variety of supramolecular assemblies formed by ribosomes and other molecular machines were captured, which include transcription-translation coupling complexes, ribosome-membrane translocon complexes, and other supercomplexes that may only exist within the cellular context. Furthermore, I resolved more than 17 ribosome intermediate structures in three antibiotic-treated datasets, and demonstrated how the drug molecules fundamentally reshape structural and functional landscapes of translation inside bacterial cells. Our work pushed the limits of cryo-ET and sub-tomogram analysis, and herald a new era in the emerging field of integrative in-cell structural biology.

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Instruct Biennial Structural Biology Conference
24
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

View on molecular structures required for vision: PRPH2 and ROM1

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Normal vision relies on continuous renewal of hundreds of tightly-packed membranes discs of outer segments (OS) in rod and cone cells. A broad range of genetic mutations disrupt OS morphology, weaken rod and cone function, and ultimately leads to blindness. Large number of those mutations is located in peripherin-2 (PRPH2) gene and is connected with a multitude of inherited human retinal diseases. The retina-specific tetraspanins PRPH2 and retinal outer segment protein 1 (ROM1) are key players in the distinctive architecture of photoreceptors OS, both required for the initialization, maturation and stability of its morphology. PRPH2 and ROM1 carry an odd number of cysteines in their extracellular loop that allows hetero-oligomerization via intermolecular disulfide bonds, a prerequisite for OS morphogenesis. However, the molecular basis and mechanisms underlying OS essential component of disk morphogenesis and maintenance remains unresolved.

Here we present single-particle cryo–electron microscopy (cryo-EM) structures of PRPH2-ROM1 hetero-dimer and higher-order oligomers. The cryo-EM map at 3.7Å resolution of the smallest molecular-weight species revealed a molar-like molecular shape hetero-dimer of ROM1 and PRPH2 (~76kDa). Our results highlight that high-risk pathogenic PRPH2 mutations map to the protein-dimer interface. Negative stain electron microscopy micrographs of purified ROM1-PRPH2 revealed various order of particles sizes of hetero-oligomers, forming large highly curved continuous chains. We solved the cryo-EM structures of ROM1-PRPH2 asymmetric tetramers, hexamers, octamers, hold together exclusively via disulfides-bridges. Our work reveals curved ROM1-PRPH2 oligomers and twisted ribbon-like structures of higher-order oligomers. Together the data allow us to model a mechanism for OS morphogenesis where PRPH2-ROM1 hetero-dimers assemble into twisted and layered complexes, shaping and maintaining the unique photoreceptor-cell membranes.

Membrane protein interactions control the tick borne encephalitis virus structure

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Tick-borne encephalitis virus (TBEV) is a pathogenic, enveloped, positive-stranded RNA virus in the family *Flaviviridae*. Structural studies of flavivirus virions have primarily focused on mosquito-borne species with only one cryo-electron microscopy structure of a tick-borne species published. Here, we present a 3.3 Å cryo-electron microscopy structure of the TBEV virion of the Kuutsalo-14 isolate, confirming the overall organisation of the virus. We observe conformational switching of the peripheral and transmembrane helices of M protein, which can explain the quasi-equivalent packing of the viral proteins, and their importance in stabilizing the raft assembly in the virion. The residues responsible for the M protein interactions are highly conserved in sequenced TBEV isolates but not in the structurally studied Hypr strain, nor in mosquito-borne flavivirus species. These interactions may compensate for the lower number of hydrogen bonds between E proteins in TBEV compared to the mosquito-borne flaviviruses. The structure clearly reveals two lipids bound in the E protein, which are likely to be important in virion stabilisation, as mutation of just one key tryptophan within the pocket can destroy the infectivity. The lipid pockets are comparable to those recently described in Zika, Spondweni, Dengue, and Usutu viruses. Our results thus advance the understanding of tick-borne flavivirus architecture and virion-stabilising interactions.

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Instruct Biennial Structural Biology Conference
26
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

Baseplate structure of bacteriophage phi812 and mechanism of cell wall binding and penetration

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Antibiotic-resistant strains of *Staphylococcus aureus* cause human infections that are difficult to treat and lead to death [1]. Host-range mutants of bacteriophage (phage) phi812 infect 90 % of *S. aureus* isolates and, therefore, are promising phage therapy agents [2]. As the phage approaches its host cell, phage receptor-binding proteins attach to the cell wall. This interaction triggers a cascade of structural changes in the baseplate, resulting in phage tail contraction and genome ejection into the host cytoplasm [3]. Mechanistic description of the baseplate re-organization, however, remains unknown.

Using cryo-electron microscopy (cryo-EM), we reconstructed the phage baseplate in extended and contracted states. The quality of reconstructed maps enabled us to assign individual proteins to their densities. Selected proteins involved in the host cell wall binding and penetration were produced in recombinant form and their structures were solved using X-ray crystallography and cryo-EM single-particle reconstruction.

We present the first detailed structural characterization of a contractile phage infecting Gram-positive bacterium. Comparison of the two distinct baseplate states allows the description of the initial stage of phage infection on the molecular level. Finally, our results provide a framework for engineering phage particles to combat *S. aureus* infections in humans.

1. D. M. Lin et al., *World J. Gastrointest. Pharmacol. Ther.*, **8**, (2017).

2. R. Pantůček et al., *Virology*, **246**, (1998).

3. J. Nováček et al., *Proc. Natl. Acad. Sci.*, **113**, (2016).

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MATCAP, the missing tubulin detyrosinase: Structural insights

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The microtubule detyrosination cycle - the enzymatic removal and re-addition of the C-terminal tyrosine encoded in most α-tubulin isoforms - is important for e.g. neuronal differentiation, cardiomyocyte contraction, and mitosis. The tyrosinase (TTL) of the cycle has been long known, and is studied elaborately; the detyrosinases however, have only been identified recently. Vasohibins were identified and analyzed as tubulin tyrosine carboxypeptidases in our department. However, our analysis suggested the existence of another, yet to be discovered, detyrosinating enzyme, which we now identified: MATCAP. X-ray crystallography establishes MATCAP as a metal-dependent carboxypeptidase and molecular modeling show that MATCAP recognizes the negative charged α-tubulin tail and C-terminal tyrosine distinctly different from Vasohibins. The cryo-EM structure reveals the MATCAP-microtubule binding interface, and hunts upon the microtubule-preference of MATCAP. Together with biochemical assays to portray substrate preference and specificity as well as in cellulo localization we characterized MATCAP as a new enzyme of the tubulin code.

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28
Instruct Biennial Structural Biology Conference
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

Structural analysis of a divergent endolysin from *Hafnia* bacteriophage Enc34

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Endolysins are bacteriophage-encoded peptidoglycan-degrading enzymes which at the end of the viral replication cycle digest the bacterial cell wall, leading to cell lysis. As a consequence, they could be used as new proteinaceous drugs to treat antibiotic-resistant bacterial infections and contamination. *Hafnia* phage Enc34 encodes a structurally unusual endolysin with an N-terminal enzymatically active domain which has no recognizable sequence similarity to other known endolysin types, and a C-terminal transmembrane domain with, as of now, unclear biological role. The crystal structure of the enzymatically active domain shows a distinctive topology that distantly resembles the α-lobe of the lysozyme fold, but lacks any β-structured elements. The analysis of conserved catalytically important residues, however, indicate a shared evolutionary history between the Enc34 endolysin and GH73 and GH23 family glycoside hydrolases and propose a molecular signature for substrate cleavage for a large group of peptidoglycan-degrading enzymes.

Structural studies on the mechanism of neuronal SALM synaptic adhesion proteins

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Synaptic adhesion molecules play an important role in the formation, maintenance and refinement of neuronal connectivity. Recently, several leucine rich repeat (LRR) domain containing neuronal adhesion molecules have been characterized including netrin G-ligands, SLITRKs and the synaptic adhesion-like molecules (SALMs). Dysregulation of these adhesion molecules have been genetically and functionally linked to various neurological disorders. We investigated the molecular structure and mechanism of ligand interactions for the postsynaptic SALM3 adhesion protein with its presynaptic ligand, receptor protein tyrosine phosphatase σ (PTPσ). We solved the crystal structure of the dimerized LRR domain of SALM3, revealing the conserved structural features and mechanism of dimerization. Furthermore, we determined the complex structure of SALM3 with PTPσ using small angle X-ray scattering, revealing a 2:2 complex similar to that observed for SALM5, and determined the crystal structure of the complex at 6.5 Å resolution. Solution studies unraveled additional flexibility for the complex structure, but validated the uniform mode of action for SALM3 and SALM5 to promote synapse formation. Together the SAXS and crystal structures confirm the molecular mode of action. The relevance of the key interface residues was further confirmed by mutational analysis with cellular binding assays and artificial synapse formation assays. Collectively, our results suggest that SALM3 dimerization is a pre-requisite for the SALM3-PTPσ complex to exert synaptogenic activity.

PyHDX: Probing Universal Protein Dynamics Using Hydrogen–Deuterium Exchange Mass Spectrometry-Derived Residue-Level Gibbs Free Energy

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Hydrogen–deuterium exchange mass spectrometry (HDX-MS) is a powerful technique to monitor protein intrinsic dynamics. The technique provides high-resolution information on how protein dynamics are altered in response to biological signals, such as ligand binding, oligomerization, or allosteric networks. However, identification, interpretation, and visualization of such events from HDX-MS data sets is challenging as these data sets consist of many individual data points collected across peptides, time points, and experimental conditions. Here, we present PyHDX[1], an open-source Python package, that allows the extraction of the universal quantity Gibbs free energy at residue level over multiple protein conditions and homologues. PyHDX includes an interactive webserver, enabling users to upload, process and interact with their data, thereby lowering accessibility barriers and increasing overall HDX-MS experimental throughput. We present existing PyHDX functionality and applications and explore how obtained ΔG values relate to orthogonal descriptions of protein dynamics[2]–[4]. Finally, we pitch an initial draft for an HDX-MS protein Gibbs free energy database.

[1] J. H. Smit, S. Krishnamurthy, B. Y. Srinivasu, R. Parakra, S. Karamanou, and A. Economou, 'Probing Universal Protein Dynamics Using Hydrogen–Deuterium Exchange Mass Spectrometry-Derived Residue-Level Gibbs Free Energy', *Anal. Chem.*, vol. 93, no. 38, pp. 12840–12847, Sep. 2021, doi: 10.1021/acs.analchem.1c02155.

[2] S. Krishnamurthy *et al.*, 'A nexus of intrinsic dynamics underlies translocase priming', *Structure*, vol. 29, no. 8, pp. 846-858.e7, Aug. 2021, doi: 10.1016/j.str.2021.03.015.

[3] B. Yuan *et al.*, 'Structural Dynamics of the Functional Nonameric Type III Translocase Export Gate', *J. Mol. Biol.*, vol. 433, no. 21, p. 167188, Oct. 2021, doi: 10.1016/j.jmb.2021.167188.

[4] S. Krishnamurthy *et al.*, 'Preproteins couple the intrinsic dynamics of SecA to its ATPase cycle to translocate via a catch and release mechanism', Aug. 2021. doi: 10.1101/2021.08.31.458331.

Mammalian ribosomal RNA expansion segments facilitate co-translational protein import into mitochondria

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Mitochondria, the vital organelles for chemical energy production in eukaryotic cells, harbour more than 1000 different proteins that are necessary for their function. Although mitochondria possess their own translation machinery, more than 99 % of their proteome is synthesized by cytosolic ribosomes and imported into the organelles. This process can occur post-translationally after release from the ribosome or it can occur co-translationally where translating cytosolic ribosomes localize to the outer mitochondrial membrane (OMM) for simultaneous protein import. But how do ribosomes interact with the mitochondrial surface and which structural features are involved in the context of co-translational import? To answer these questions, we performed *in situ* structural analysis by cryo-electron tomography and subtomogram averaging. Intriguingly, we found mammalian OMM-associated cytosolic ribosomes in a very different orientation compared to yeast mitochondria or the endoplasmic reticulum membrane. We identified two ribosomal RNA expansion segments that facilitate membrane attachment and co-translational targeting and import into mammalian, but not yeast mitochondria. Our findings exemplify the concept of functional extension to core machineries in higher eukaryotes.

Instruct and EU-LAC ResInfra

John Dolan, Claudia Alen Amaro

The main goal of the EU-LAC ResInfra project, 'Towards a new EU-CELAC partnership in Research Infrastructures', is to enhance the bi-regional collaboration regarding Research Infrastructures. Within this project Instruct-ERIC is responsible for the Pilot on Infrastructure cooperation and coordination on Structural Biological for Health. The project is a Horizon 2020 Coordination and Support Action that will run until 28 February 2023.

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Plasticity of GRM2 large type bacterial microcompartment shells

Bacterial microcompartments (BMCs) are prokaryotic organelles consisting of a protein shell and an encapsulated enzymatic core. These organelles are involved in such biochemical processes as choline, glycerol, ethanolamine, fucose and rhamnose degradation and carbon fixation. Encapsulation of the enzymatic pathway inside the protein shell has the benefit of increasing the local substrate concentrations and containing toxic and/or volatile metabolites. Since nonnative enzymes can also be encapsulated in BMCs, an improved understanding of BMC shell assembly and encapsulation processes could be useful for synthetic biology applications.

The *Klebsiella pneumoniae* GRM2 type BMC is a choline degrading metabolosome. Its shell consists of two types of BMC structural proteins: the hexameric BMC-H and pentameric BMC-P proteins. In our previous research we have created a system for production of recombinant empty BMC shells and determined the structure of the predominant small type pT=4 particle. However, the sample of these empty particles turned out quite heterogenic, with larger particles present. In this study we analyzed a material containing these larger BMC forms and managed to calculate four novel 3D cryo-EM maps of larger BMC shell particles with the resolution in range of 9 to 22 Å and identify nine novel 2D classes corresponding to discrete BMC shell forms. These structures reveal icosahedral, elongated, oblate, multi-layered and polyhedral traits of BMCs, indicating considerable variation in size and form as well as plasticity during shell formation processes.

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instruct-eric.org
35
Instruct Biennial Structural Biology Conference
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

Stream processing of CryoElectron Microscopy images at the acquisition site

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Abstract

Cryo-Electron Microscopy (CryoEM) has established as one of the key techniques in Structural Biology. The throughput of electron microscopes for cryo-samples of biological macromolecules is steadily increasing in image size and rate. This large increase in acquisition speed must be accompanied by a tight control on the quality of the acquisition so that the amount unusable data is minimized. This is accomplished by an online analysis of the images being acquired. The two most typical monitored parameters are the average frame drift over time and the microscope defocus as proxies of the stability of the acquisition and correction of the optical setup. In this abstract, we show that the quality of the sample itself can also be monitored by automatically finding particles in the micrographs, classifying them in two-dimensions, and assigning in real-time the newly acquired particles to the found classes. This quality may change over time depending on the ice thickness and the quality of the region of the grid being analyzed. If the quality is low, then the acquisition may be shifted to some other region or the grid changed.

In this poster, we introduce the use of Scipion for this purpose. Scipion is a workflow engine especially designed for image processing in CryoEM. We have carefully analyzed the bottlenecks affecting our software performance and optimized it so that the image analysis can be performed in real-time. The executed workflows are highly configurable and every facility can design its own image analysis pipeline including the most popular image analysis packages (motioncorr2, relion, cistem, cryoSPARC, Xmipp, gctf, gautomatch, sphire, etc.). Additionally, Scipion and Xmipp bring quality control protocols that warns the microscope operator if the acquisition goes out of specifications. Finally, HTML reports of the acquisition status can be generated and, if desired, made accessible from outside the facility, so that the user can have automatic feedback on his/her acquisition in real-time.

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instruct-eric.org
36
Instruct Biennial Structural Biology Conference
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

Tail proteins of *Podoviridae* phage SU10 reorganize into the nozzle for genome delivery

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Abstract

Escherichia coli phage SU10 belongs to the genus *Kuravirus* from the family *Podoviridae* of phages with short tails. *Kuraviruses* have the potential to be used in phage therapy against antibiotic-resistant bacterial infections, however, their structure and genome delivery mechanism have not been characterized yet. Here we used cryo-electron microscopy of purified SU10 particles and cryo-electron tomography of infected *E. coli* cells to describe the structural changes of the phage tail that are required for its genome ejection and delivery. The binding of the long tail fibers to the receptors in the outer bacterial membrane is followed by the straightening of nozzle proteins and rotation of short tail fibers by 135°. In the new arrangement, the nozzle proteins and short tail fibers alternate to form a nozzle that prolongs the tail by 28 nm. To open the tail channel, the tail needle detaches from the nozzle proteins. The inner core proteins of five types, one of which has the predicted peptidoglycan-degradation activity, are ejected from the SU10 head before or together with the genome. The nozzle with the putative extension formed by the inner core proteins enables the delivery of the SU10 genome into the bacterial cytoplasm. Tails of podoviridae phages are defined as short and non-contractile, however, here we show that upon infection kuravirus tails extend into a nozzle for genome delivery.

Structural and functional studies of outer surface proteins from Lyme disease causing agent *Borrelia burgdorferi*

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Lyme disease is a common tick-borne infection caused by a spirochete *Borrelia burgdorferi* which is transferred from infected *Ixodes* ticks to the vertebrate host during the ticks blood meal. *B. burgdorferi* is exceptionally rich in outer surface lipoproteins, which are attached to cell membrane covalently by an N-terminal lipid modification. However, for most of the *B. burgdorferi* outer surface proteins the exact function/ligand/receptor is unknown, and they do not possess any homology with proteins in any other organism whose genome has been sequenced, and that also prevents to make any assumption about the potential function. Our aim is to study structural/functional details of the outer surface proteins to reveal the molecular details of pathogenesis of Lyme disease which remains poorly understood. Recently our attention has been paid to *Borrelia* proteins that belong to paralogous gene family 12 (PFam12) since the structural details revealed the potential function of the proteins that may be related to biofilm formation.

This work was supported by the LZP grant Nr. lzp-2021/1-0068.

eSPC, an online data-analysis platform for molecular biophysics

Osvaldo Burastero, Angelica Struve, Stephan Niebling, Maria Garcia Alai

All biological processes rely on the formation of protein-ligand, protein-peptide and protein-protein complexes. Studying the affinity, kinetics and thermodynamics of binding between these pairs is critical for understanding basic cellular mechanisms. Many different technologies have been designed for this purpose, each based on measuring different signals (fluorescence, heat, thermophoresis, scattering and interference, among others). Evaluation of the data from binding experiments and their fitting is an essential step towards the quantification of binding affinities. Here, user-friendly online tools to analyze biophysical data from steady-state fluorescence spectroscopy, microscale thermophoresis and differential scanning fluorimetry experiments are presented. The modules of the data-analysis platform (https://spc.emblhamburg.de/) contain classical thermodynamic models and clear user guidelines for the determination of equilibrium dissociation constants (Kd) and thermal unfolding parameters such as melting temperatures (Tm).

Title: Albumin and ions separately prime Coxsackievirus A9 for RNA release

Authors: Aušra Domanska[1,4], Visa Ruokolainen[2,4], Zlatka Plavec[1,4], Benita Löflund[1], Rabah Soliymani[3], Varpu Marjomäki[2], Sarah Butcher[1,5],

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Abstract:

Coxsackievirus A9 (CVA9) is classified into a family Picornaviridae comprising small, about 30 nm in diameter, icosahedrally symmetric viruses with ss(+)RNA genomes. CVA9 genome is translated into a polyprotein, which is further cleaved to 7 non-structural and 3 structural proteins (VP0, VP1 and VP3). VP0 further matures to VP2 and VP4. Previous research has shown that certain conditions such as receptor binding, heat, and acidic pH can trigger expansion of the capsid in some picornaviruses. Expanded picornavirus capsids are characterized by 4% increase in their diameter and prominent pores at 2-fold axes compared to native capsids. In expanded virions, RNA moves towards the edges of the capsid and VP1 N-termini are externalized next to the pores at 2-fold axes. In addition, in cryo-EM reconstructions, the density for VP4 is not resolved in expanded particles. Here we show that fatty-acid free BSA or endosomal ionic conditions can separately prime CVA9 for uncoating and RNA release. Using real-time spectroscopy to measure viral RNA accessibility to the fluorescent dye SYBR Green II (indicating particle expansion), we determined optimal conditions to achieve capsid expansion in most of the particle population. Analysis of particle expansion by faf-BSA or endosomal ion treatment using cryo-EM and single-particle reconstruction revealed significant particle heterogeneity in faf-BSA or ion-treated as well as non-treated CVA9 data sets. To achieve complete separation of different particle states we will utilize ultracentrifugation in sucrose gradients followed by drop-by-drop fractionation. Fractions will be tested for infectivity and mass spectrometry will be used for detection of VP4 in expanded particles. Latest results will be presented at the conference.

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Instruct Biennial Structural Biology Conference
40
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

Direct observation of chemo-mechanical coupling in the Hsp70 chaperone DnaK by single-molecule force experiments

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Protein allostery implies regulation between distant sites within a protein. In the molecular chaperone Hsp70, a two-domain enzyme, the ATP/ADP status of an N-terminal nucleotide-binding domain regulates the substrate affinity of a C-terminal substrate-binding domain. Recently available three-dimensional structures of Hsp70 in ATP/ADP states have provided deep insights into molecular pathways of allosteric signals[1,2]. However, direct mechanical probing of long-range allosteric coupling between the ATP hydrolysis step and domain states is missing.

Using laser optical tweezers, we examine the mechanical properties of a truncated two-domain DnaK(1-552ye) in apo/ADP/ATP and peptide-bound states. We find that in the apo and ADP states, DnaK domains are mechanically stable and rigid. However, in the ATP-state, SBD*ye is mechanically destabilized as the result of interdomain docking followed by the unfolding of the α-helical lid. By observing the folding state of the SBD, we could observe the continuous ATP/ADP cycling of the enzyme in real time with a single molecule. The SBD lid closure is strictly coupled to the chemical steps of the ATP hydrolysis cycle even in the presence of peptide substrate.

References:

[1] Qi R., et al., *Nature structural & molecular biology*, 2013, **20 (7),** 900-907.
[2] Bertelsen E. B. et al., *Proc Natl Acad Sci U S A*, 2009, **106 (21)**, 8471-8476.

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Molecular mechanism of myeloma λ-type IgG light chain aggregation

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Stability is the essential property for the proper biological function of proteins. Low protein stability leads to partial or complete unfolding, which can dramatically speed up the formation of aggregates. As non-physiological forms of proteins, aggregates are often toxic and pathological to the body and cause severe diseases in many cases. Examples of protein diseases include amyloidosis and multiple myeloma of the light chain of immunoglobulin G. The malignancy is manifested by uncontrolled proliferation and accumulation of the damaged plasma cells in the bone marrow. Myeloma plasma cells produce light chains of IgG in huge concentrations, subsequently secreted into the bloodstream. In the bloodstream, the light chain can start to aggregate and is subsequently trapped in vital organs. Over a long-time, they form microscopic fibrils of amyloid deposits in these organs[1-3].

In our laboratory, we developed a method for rapid and effective purification of a soluble form of IgG light chain, which yields 23 mg of pure protein per 1 liter of bacterial culture. A pure and correctly folded light chain was characterized by standard biophysical techniques, and we developed mathematical models to understand the kinetics of the aggregation. Firstly, we focused on solving conformational and colloidal stability contributions to the aggregation process. In our study, we measured the conformational stability of the light chain by biophysical methods, such as circular dichroism, tryptophan fluorescence and differential scanning calorimetry. In the next part, we determined the amount of soluble form of LC after exposing the protein to elevated temperatures, and by this way, we characterized the colloidal stability of the protein. Obtained data were used to calculate activation energy for the unfolding of LC (conformational stability) and the formation of the aggregates of the unfolded LC (colloidal stability). Based on our results, we prepared a kinetic model at different LC concentrations at 37 °C. Our mathematical model simulates the formation of insoluble aggregates[4]. Next, we looked at how the reduction of disulfide bonds contributes to the formation of aggregates. Aggregation kinetics in the presence of different types of reducing agents (TCEP, DTT and GSH) were analyzed by extended Finke-Watzky model, which additionally includes pre-step of monomer-dimer equilibrium. The analysis reveals the critical steps that lead to the formation of aggregates; aggregated forms display vastly different morphology and fractal properties (Fig. 1).

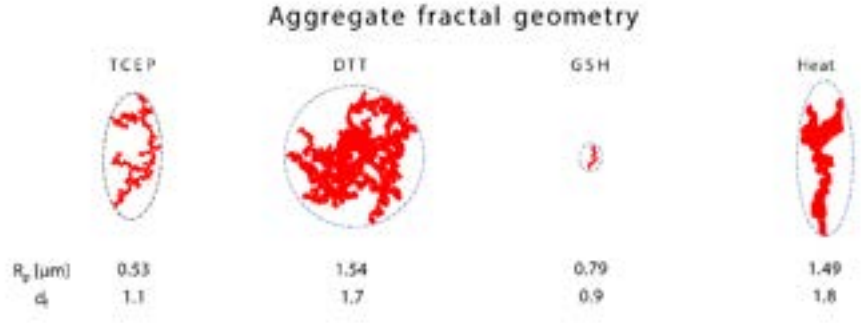

Fig. 1. A comparison of the morphologies of light chain aggregates formed by different chemical reducing agents: TCEP, DTT, GSH or by temperature. 3D morphologies were obtained by STEDYCON microscope.

References:

[1] Andrich K., et al., *Journal of Biological Chemistry*, 2016, **292(6)**, 2328–2344
[2] Chiti F., Dobson C. M., *Annual Review of Biochemistry*, 2017, **86**, 27–68.
[3] Blancas-Mejia L. M., et al., *Chemical Communications*, 2018, **54**, 10664-10674.
[4] Džupponová V., Huntošová V., Žoldák G., *Protein Science*, 2020, **29 (12)**, 2408 – 2421.

Structural insights into the Teneurin-4 dimer reveal a compact conformation

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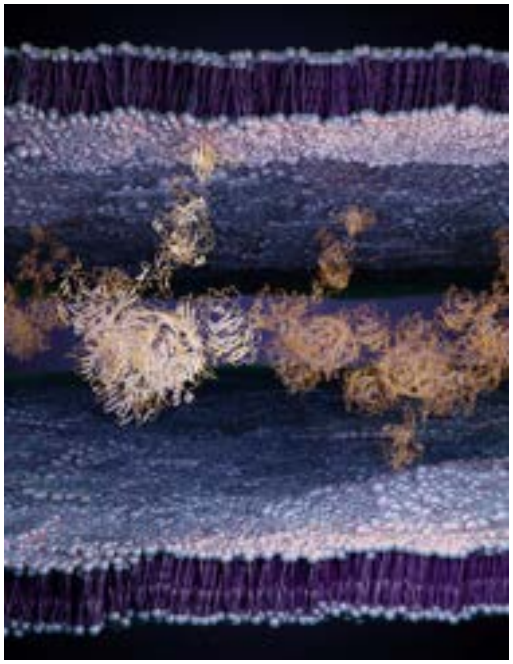
Abstract
Synaptic partner matching is a crucial step during neural circuitry formation. The neuronal transmembrane protein family of Teneurins is required for partner finding in the visual and hippocampal systems in vertebrates. It remains unclear how individual Teneurin molecules form macromolecular *cis*- and *trans*-synaptic protein complexes. Here, we present a 2.7 Å cryo-EM structure of the dimeric ectodomain of human Teneurin4. The structure reveals a compact conformation of the dimer that is stabilized by C-rich, YD-shell and ABD-mediated interactions. A 1.5 Å crystal structure of the C-rich domain shows three conserved calcium binding sites and we demonstrate using thermal unfolding and SAXS-based rigid-body modelling that the compactness and stability of Teneurin4 dimers is calcium-dependent. Cellular assays reveal that the compact *cis* dimer is compatible with homomeric *trans* interactions. Together, these findings support a role for Teneurins as a scaffold for macromolecular complex assembly and the establishment of *cis*- and *trans*-synaptic interactions to construct functional neuronal circuits.



HIV-1 nuclear import: structural study of the interaction between hCPSF6 and HIV-1 capsid

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Lentiviruses such as HIV-1 can infect non-dividing cells, which are different from other retroviruses, implying that lentiviruses employ specific mechanisms to traverse the nuclear pore complex. HIV-1 capsid (CA) protein and host cell factor Cleavage And Polyadenylation Specific Factor 6 (CPSF6) are demonstrated to play a key role in nuclear transport of HIV-1. We are working on the interaction of the two proteins with integrative structural biology tools. Attempts failed to form and purify the complex of CA hexamer-CPSF6, which is consistent with a previous report. As many host factors such as TRIM5α, TRIMCyp, and MxB, recognize assembled capsid interfaces that are not presented in unassembled capsid subunits, we tested CA in supramolecular assembled forms including the CA rods and the viral capsid core. CA rod-CPSF6 complex and capsid core-CPSF6 complex can be formed under our conditions. We are employing single particle analysis and subtomogram averaging cryoEM to resolve the structures.

Assessment of Structural Stability and Flexibility of Amyloid Fibril Proteins

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Amyloid fibrils are fibrillar polypeptide aggregates consisting of cross-β sheet structure. They are pathological hallmarks of human diseases from Alzheimer's to amyotrophic lateral sclerosis and systemic amyloidosis. To investigate the structural stability of amyloid fibrils we recently started to use cryo-electron microscopy. Studies carried out across different fibril systems show that fibrils encompass regions of very high structural stability as well as regions of structural heterogeneity, if not random coil-like structural disorder. Comparison of fibrils formed in vitro and in vivo from the same precursor protein revealed structural differences along with different lengths of the structurally disordered segments; this is, ex vivo fibrils tend to have longer regions of high structural stability. Pathological amyloids are also more resistant to proteolytic digestion than the structurally different in vitro fibrils, suggesting that the lower levels of structurally disordered segments rendered specific fibril morphologies more resistant to proteolysis and enabled them to become pathogenic in vivo.

Abstract – Recombinant Transient Gene Expression and Preparation of HBsAg VLPs for Serology and Structural Analysis

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Hepatitis B virus (HBV) is a major threat to humankind since it is causing one of the most frequent chronic viral infections. Development of chronic infection may lead to liver cirrhosis and hepatocellular carcinoma. Thus, many research groups around the world try to find new vaccines, diagnostic methods or a cure to hepatitis B. One major player in these strides is the hepatitis B surface antigen (HBsAg) which exists in three versions small (S), middle (M) and large (L). It is an important serological marker for the progression of infection. This protein can form non-infectious virus-like particles (VLP). It is possible to produce and assemble them using recombinant expression systems. Most recombinant expressed HBsAg is S-HBsAg, since it is the minimum prerequisite for particle formation. The overall 3D-structure and symmetry of the HBsAg particle is solved at ~12-Å resolution [1]. However, the 3D-atomic-structure of the correctly folded, antigenic determinant is not resolved at this resolution and therefore left to be elucidated. In multiplex serological assays, most commercially available recombinant HBsAg proteins and VLPs did not allow sufficient discrimination between positive and negative sera. Serum isolated HBsAg particles are only limited reliable as a reference, since the antigenicity varies from batch to batch. Thus, reproducible production of recombinant HBsAg VLPs (with solved epitope structure for quality control) would be a powerful reference in serological assays. The correct folding of the epitopes is required for proper presentation of the antigenic determinant. Only after confirmation of fully antigenic HBsAg it is possible to solve the correct 3D-atomic-structure of the antigenic loop. Here, we established a fast and easy-to-perform production and folding strategy for HBsAg VLPs. S-HBsAg was extracted from HEK293 suspension cell line following transient gene expression. The VLPs were not formed during expression. After purification, the S-HBsAg was assembled into VLPs with subsequent epitope maturation. Upon comparison of the produced recombinant S-HBsAg VLPs to commercial serum-isolated and yeast-produced HBsAg VLPs in serological assays, we could show that the tag-size matters in regards to quality of the VLPs and their reactivity with standard anti-HBsAg serum samples.

[1] Gilbert RJ, Beales L, Blond D, et al. Hepatitis B small surface antigen particles are octahedral. *Proc Natl Acad Sci U S A*. 2005;102(41):14783-14788. doi:10.1073/pnas.0505062102

Dual thermofluor-based assay enables extensive buffer screening for protein-DNA complexes

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Selection of optimal buffer is an important prerequisite for structural studies of macromolecular complexes. For this purpose, differential scanning fluorimetry (DSF, also known as thermofluor) using Sypro Orange dye has been recognized as a valuable technique with a high capacity.

In our work, we established a dual thermofluor-based assay to determine an optimal buffer conditions for protein-DNA complexes. We combine a traditional procedure used in thermofluor assay (orangeDSF), which monitors thermally induced unfolding of proteins using the Sypro Orange, with an approach using a DNA intercalator (dsGreen) to record stability of double strand DNA in temperature gradient (greenDSF). Using two independent runs by the orangeDSF and the greenDSF, we were able to record conformational stability of both components in protein-DNA complexes.

Applicability of this approach was tested on the DNA-binding domain of FOXO4 bound to the oligonucleotide containing its recognition motif. For both protein and DNA, we observed significant stabilization of respective components in the complex compared to their apo-forms. Specifically, melting temperatures were increased from 46 °C to 60 °C and from 59 °C to 64 °C in case of the protein and DNA, respectively. Similar results were obtained using circular dichroism spectroscopy, which supported validity of dual thermofluor assay. In the next step, we used this assay for searching optimal conditions using the Rubic buffer screen. Buffer conditions were stratified according to structural stability and homogeneity of both protein and DNA as judged by melting temperature and a number of transition points of recorded denaturation curves. We identified two potentially suitable buffers, specifically HEPES and potassium phosphate. These were further assessed by DNA binding assay using fluorescence anisotropy. Finally, we selected HEPES as an optimal buffer in which the K_D value was determined to be 100 nM.

In conclusion, we established a dual thermofluor assay for an extensive buffer screen to probe each component in protein-DNA complexes. Currently, we are testing general applicability of this approach with a set of diverse transcription factors bound to target DNA.

This work was funded from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101003406 (HIPPOSTRUCT) and by EPIC-XS - grant agreement ID: 823839*. We acknowledge CMS-Biocev ("Biophysical techniques, Crystallization, Diffraction, Structural mass spectrometry") of CIISB, Instruct-CZ Centre, supported by MEYS CR (LM2018127).*

Structure and proteolytic properties of Ryegrass mottle virus serine-like 3C protease in complex with and without VPg cofactor.

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Sobemoviruses encode serine-like 3C proteases (Pro) which participate in polyprotein processing and maturation of other virus encoded proteins. Its activity *in cis* and *trans* is modulated by naturally unfolded virus-genome liked protein (VPg). Previous NMR studies with other viruses has demonstrated Pro-VPg complex interaction and VPg tertial structure, but still the information regarding structural changes of Pro-VPg complex during interaction is missing. We have solved a full Pro-VPg 3D structure in three different conformations that demonstrates the flexibility of the overall fold and illustrates conformational changes due to VPg interaction with Pro. This is the first report of full plant protease crystal structure with its VPg cofactor. Also, we had confirmed previously not mapped cleavage site for sobemovirus protease in transmembranal domain - E/A, and demonstrated that Pro activity *in trans* do not require fused VPg at its C-terminus.

Dynamic assembly of the Hsp90 chaperone machinery by multi-colour single molecule FRET

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Proteins are important building blocks that play a major role in all domains of life. However, they seldomly work alone but rather form complexes and assemble into molecular machineries in order to fulfil their respective functions.

Hsp90 forms various chaperone machineries with different co-chaperones for a plethora of clients. Especially its involvement in neurodegenerative diseases like Alzheimer's, as well as various forms of cancer require an in-depth understanding of these machineries. The first step towards this goal is gaining knowledge of these machineries' assemblies and the dynamics of the interactions within. Therefore, it needs to be clarified whether the proteins involved have random encounters and form complexes by chance or if there is an underlying, sequential mechanism that includes one protein recruiting another and then the next and so on.

We are studying the interaction between Hsp90, a co-chaperone and a substrate in real time with multi-colour single molecule FRET. To overcome low mutual affinities, linked protein fusions with orthogonal labelling sites are used. Three-colour single molecule traces show interactions between all three proteins in vitro. First results point towards a highly dynamic recruiting process by Hsp90. Together, this now enables us to directly observe and quantify assembly pathways.

Dynamics and directionality within the multi-component Hsp90 machinery

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The molecular chaperone Hsp90, together with its co-chaperones, processes many clients (e.g. kinases) and is abundant in eukaryotic cells. Hereby, Hsp90 exhibits dynamics on a wide range of timescales, ranging from nanoseconds {1} to many minutes {2}. These dynamics are visible in absence of any energy source (ATP). This detailed balance even holds in presence of ATP, i.e. no directionality in Hsp90's conformational dynamics could be observed {3}.

We and others are convinced that the presence of co-chaperones and/or clients will at some point break detailed balance to reveal a clear, directed conformational cycle in Hsp90. Therefore, we use single molecule TIRF-FRET in combination with maximum likelihood analysis to investigate directionality in Hsp90-cochaperone and Hsp90-client constructs.

First we have investigated the effect of the co-chaperone Aha1 in different stoichiometries on Hsp90. A clear shift in the populations of conformations were observed and the changes in the kinetics quantified. Surprisingly, again there was no clear directionality in the coordinates we investigated. Similar results could be obtained for a Hsp90-co-chaperone-client complex. These findings challenge our understanding of the Hsp90 machinery and point to other conformational coordinates to show directionality.

{1} B. Sohmen, C. Beck, T. Seydel, I. Hoffmann, B. Hermann, M. Nüesch, M. Grimaldo, F. Schreiber, S. Wolf, F. Roosen-Runge & T. Hugel
Nanosecond structural dynamics of the chaperone Hsp90
arXiv (2021). https://arxiv.org/abs/2110.10483

{2} W. Ye, M. Götz, S. Celiksoy, L. Tüting, C. Ratzke, J. Prasad, J. Ricken, S.V. Wegner, R. Ahijado-Guzmán, T. Hugel & C. Sönnichsen
Conformational Dynamics of a Single Protein Monitored for 24 h at Video Rate
Nano Lett., 18 (10), 6633 (2018).

{3} S. Schmid, Sonja, M. Götz, T. Hugel
Single-Molecule Analysis beyond Dwell Times: Demonstration and Assessment in and out of Equilibrium
Biophysical Journal, Volume 111, Issue 7, 1375 - 1384 (2016)

Structural characterization of a mitochondrial intermembrane domain

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Our object of study is a human protein anchored at the mitochondrial inner membrane. This protein consists of three domains whose function is still unknown. The N-terminal domain (N) spans through the intermembrane space, whereas the C-terminal domain (C) faces the mitochondrial matrix, both regions being connected by a transmembrane helix embedded in the inner membrane. Several constructs of both the N and C domains were generated to obtain stable protein fragments suitable for expression and crystallization. However, only a few N-terminal constructs were soluble, but most of them aggregated during purification. Construct N4 showed the highest expression levels in *Escherichia coli* and could be reasonably purified. Nevertheless, once pure, N4 had a high tendency to precipitate, which led us to explore new approaches to improve its stability. We will present the re-design approaches of the constructs to improve solubility and solve aggregation and precipitation.

Structure and Dynamics of Huntingtin. A Segmental Labelling Approach

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Huntington's Disease (HD) is a genetically inheritable neurodegenerative disorder caused by a mutation in the gene encoding the protein Huntingtin (Htt)[1]. The mutation causes an increase in CAG trinucleotides in the first exon, which increases the number of glutamines in the poly-glutamine (Poly-Q) tract of the intrinsically disordered N-terminal region of the protein[2]. HD symptoms only manifest in individuals with a poly-Q tract of more than 35 consecutive glutamines. The length of the Poly-Q tract beyond the threshold is correlated with the age of onset and the severity of the pathology. The exon-1 of Htt is a low complexity region that contains the N-terminal 17 residues, the poly-Q tract and a proline rich region. My project aims at elucidating the structural differences between non-pathogenic and pathogenic Htt exon-1 constructs using Small-Angle Neutron Scattering (SANS) measurements in amino-acid specific deuterated samples. Profiting of the distinct scattering properties of deuterium and hydrogen, we aim at extracting valuable structural information of the Poly-Q region. Constructs with specific deuteration patterns (Gln/Pro) are produced using the Cell-Free protein expression system.

SANS data, collected at the D22 Beamline at ILL, are combined with Small-Angle X-ray Scattering (SAXS) data measured at the Swing beamline at Soleil Synchrotron and atomistic models[3]. Synergistic analyses of the data are performed using the ensemble optimization method (EOM)[4]. Eleven SANS samples have been measured for pathogenic (HttQ36) and non-pathogenic (HttQ16) constructs of the protein and ensemble analyses are in progress.

Keyswords: Hungtington's Disease, Cell-free, SANS

1. Saudou F, Humbert S. The Biology of Huntingtin. Neuron. 2016 Mar 2;89(5):910–26.
2. Zuccato C, Valenza M, Cattaneo E. Molecular Mechanisms and Potential Therapeutical Targets in Huntington's Disease. Physiol Rev. 2010 Jul 1;90(3):905–81.
3. Estaña A, Sibille N, Delaforge E, Vaisset M, Cortés J, Bernadó P. Realistic Ensemble Models of Intrinsically Disordered Proteins Using a Structure-Encoding Coil Database. Structure. 2019 Feb 5;27(2):381-391.e2.
4. Bernadó P, Mylonas E, Petoukhov MV, Blackledge M, Svergun DI. Structural Characterization of Flexible Proteins Using Small-Angle X-ray Scattering. J Am Chem Soc. 2007 May 1;129(17):5656–64.

Alteration of the dynamic motion of the N-terminal domain of the human ryanodine receptor 2: a possible cause of several cardiac arrhythmias

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Cardiac arrhythmias are one of the most serious illnesses, decreasing the quality of the life or, in some cases, causing death. Among the most malignant and difficult to treat of these is catecholaminergic polymorphic ventricular tachycardia (CPVT), which has an estimated frequency of 1:10,000. The human cardiac ryanodine receptor (hRyR2), the ion channel responsible for the release of Ca^{2+} ions from the sarcoplasmic reticulum into the cytosol, plays an important role in cardiac muscle contraction. Mutations of this channel are associated with inherited cardiac arrhythmias, including CPVT1 and arrhythmogenic right ventricular dysplasia, as well as syncopes of unknown origin, sudden cardiac death and sudden infant death syndrome. These mutations appear to cluster in distinct parts of the hRyR2 channel: the N-terminal, central and C-terminal. Here, we used molecular dynamics simulation to examine the effects of three disease-associated mutations in the N-terminal region, R414L, I419F and R420W, have on the dynamics of a model containing residues 1–655 of hRyR2. We find that the R414L and I419F mutations diminish the overall amplitude of motion without greatly changing the direction of motion of the individual domains, whereas R420W both enhances the amplitude and changes the direction of motion. Based on these results, we hypothesize that R414L and I419F hinder channel closing, whereas R420W may enhance channel opening. Overall, it appears that the wild-type protein possesses a moderate level of flexibility which allows the gate to close and not easily open without an opening signal. These mutations, however, disrupt this balance by making the gate either too rigid or too loose, causing closing to become difficult or less effective.

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53
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Chemical characterization of zinc granules in the Malpighian tubules of *Drosophila melanogaster*

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The storage of zinc (Zn) in *Drosophila melanogaster* depends on the consumption of tryptophan [1]. Specifically, the tryptophan metabolite kynurenine (kyn) is released from insect fat bodies and induces the formation of Zn storage granules in the Malpighian tubules, where 3-hydroxykynurenine and xanthurenic acid act as endogenous Zn chelators. Confocal microscopy and Zn K-edge X-ray absorption spectroscopy (XAS) were performed on the Malpighian tubules to assess Zn coordination *in situ*. Moreover, the chemical characterization of Zn complexes with *kyn* metabolites was carried out in solution by electrospray ionization mass spectrometry (ESI-MS), electronic absorption and nuclear magnetic resonance (NMR) spectroscopy. Finally, *ab initio* density functional theory (DFT) calculations yielded a structure of Zn complexes with metal-ligand distances consistent with those determined experimentally by XAS analysis. The discovery that the tryptophan metabolites 3-hydroxykynurenine and xanthurenic acid are the major Zn-binding ligands in insect cells establishes the kynurenine pathway as a regulator of systemic Zn homeostasis. These novel direct molecular links will allow the elucidation of many biological processes modulated by Zn and the kynurenine pathway, such as immunity, blood pressure, aging and neurodegeneration [2-7].

References

[1] Garay, E., Schuth, N., Barbanente, A., Tejeda-Guzmán, C., Vitone, D., Osorio, B., Clark, A.H., Nachtegaal, M., Haumann, M., Dau, H., Vela, A., Arnesano, F., Quintanar, L., Missirlis, F. Tryptophan regulates *Drosophila* zinc stores. *Proceedings of the National Academy of Sciences*, in press (2022).
[2] Haase, H., & Rink, L. *Immunity & Ageing*, **6**(1) (2009)
[3] Cronin, S. J. F. et al. *Nature*, **563**(7732) (2018)
[4] Campesato, L. F. et al. *Nature Communications*, **11**(1) (2020)
[5] Worton, S. A. et al. *Circulation Research*, **128**(11) 1679–1693 (2021)
[6] Fazio, F. et al. *Frontiers in Pharmacology*, **8**. 214 (2017)
[7] Betrie, A. H. et al. *Nature Communications*, **12**(1)3296 (2021)

Structural characterization of intrinsically disordered C-terminal regions of GPCRs and impact on the arrestin-dependant pathway

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Abstract

G protein-coupled receptors (GPCRs) are important signaling machineries and can activate G-protein independent pathways. These signaling pathways result from the coupling of GPCRs with their physiological partner arrestins [1]. The interaction with arrestins is modulated by the phosphorylation of the intrinsically disordered C-terminal region of GPCRs (C-ter) regulated by specific class of kinases [1–3]. The so-called phospho-barcode model [4] states that the pattern of phosphorylation controls the conformation of arrestin and therefore downstream cellular events. The impact of phosphorylation on the conformation of GPCR C-ter and its interaction with arrestin is not yet well understood due to its disordered nature. We have studied three different class A receptors representative of the two different classes of arrestin binder. The characterization by Nuclear Magnetic Resonance (NMR) of these three truncated GPCR C-terminal domains in their wild-type and phosphomimetic forms allowed us to compare their content in secondary structure elements in order to better understand the structural impact of phosphorylation. We have shown that all C-termini undergo a structural transition, which is located in the arrestin binding region. Thus, the phospho-barcodes would induce a specific structural conformation recognized by different stabilized arrestin conformations, which will trigger diverse signaling pathways.

References:

1. Guillien, M.; le Maire, A.; Mouhand, A.; Bernadó, P.; Bourguet, W.; Banères, J.L.; Sibille, N. *IDPs and their complexes in GPCR and nuclear receptor signaling*; 1st ed.; Elsevier Inc., 2020; Vol. 174; ISBN 9780128226155.
2. Sente, A.; Peer, R.; Srivastava, A.; Baidya, M.; Lesk, A.M.; Balaji, S.; Shukla, A.K.; Babu, M.M.; Flock, T. Molecular mechanism of modulating arrestin conformation by GPCR phosphorylation. *Nat. Struct. Mol. Biol.* **2018**, *25*, 538–545, doi:10.1038/s41594-018-0071-3.
3. Gurevich, V. V; Gurevich, E. V GPCR Signaling Regulation: The Role of GRKs and Arrestins. *Front Pharmacol* **2019**, *10*, 125, doi:10.3389/fphar.2019.00125.
4. Nobles, K.N.; Xiao, K.; Ahn, S.; Shukla, A.K.; Lam, C.M.; Rajagopal, S.; Strachan, R.T.; Huang, T.Y.; Bressler, E.A.; Hara, M.R.; et al. Distinct phosphorylation sites on the β 2-adrenergic receptor establish a barcode that encodes differential functions of β-arrestin. *Sci. Signal.* **2011**, *4*, doi:10.1126/scisignal.2001707.

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55
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Investigating gene transcription modulators inside mitochondrial genes

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A mitochondrion is a semi-autonomous double-membrane organelle found in most eukaryotic organisms, including humans. The mitochondrion is responsible for various functions ranging from cellular differentiation, signaling, cell death, maintaining control of the cell cycle and growth, to generating most of the cell's supply of adenosine triphosphate (ATP). Numerous connections have been discovered between mitochondria and crippling human diseases including cardiac dysfunction, heart failure, or autism.

The mitochondrial genome encodes proteins involved in the oxidative phosphorylation system. The transcription of the mtDNA strands results in the single polycistronic transcript, which is subsequently processed by RNases excising the tRNA sequences. One copy of encoded mRNA, rRNA, and tRNA for each transcription cycle is being created.[1] How do mitochondria manage to translate their protein-coding genes whereas only one set of tRNAs is produced in each transcription cycle and how mitochondria accumulate enough tRNAs for translation remains unknown or is poorly understood.[2]

A possible explanation would be that mitochondrial genomes are locked in the "futile" transcription cycles. They generate tRNAs through a not-yet-understood mechanism for the selective transcription of mitochondrial tRNA genes. Regulation of such a selective transcription could be controlled by the environmentally promoted stabilization of non-canonical DNA/RNA structures, G-quadruplexes (Fig. 1).[3] DNP (Dynamic Nuclear Polarization[4]) enhanced solid-state NMR spectroscopy is a unique tool capable of non-invasively probing live mitochondria extracted from cells. We report on recent progress to employ DNP to prove the hypothesis of G-quadruplex structures existing in mitochondrial genomic DNA/RNA, playing a crucial role in the RNA transcription process and even influence the process by possible formation of a G-quadruplex-ligand complex.

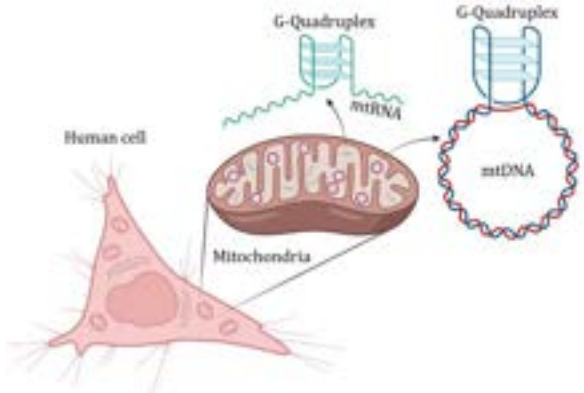

Figure 1| Scheme of potential G-Quadruplex structures inside of mitochondria's DNA and RNA. The Figure was created with BioRender.

References: [1]A. R. D'Souza and M. Minczuk, *Essays Biochem.*, 2018, 62, 309–320; [2]E. M. Novoa, M. Pavon-Eternod, T. Pan and L. Ribas de Pouplana, *Cell*, 2012, 149, 202–213; [3]M. Falabella, R. J. Fernandez, F. B. Johnson and B. A. Kaufman, *Curr. Med. Chem.*, 2019, 26, 2918–2932. [4]S. Narasimhan, S. Scherpe, A. Lucini Paioni, J. van der Zwan, G. E. Folkers, H. Ovaa, M. Baldus, *Angew. Chem. Int. Ed.*, 2019, 58, 12969.
Acknowledgement: This project was supported by EMBO postdoctoral fellowship (ALTF 410-2021) and an internal UU fund for EM access. Furthermore, it was supported in part by the Dutch Research Council (NWO, projects 700.26.121 and 700.10.443 to M.B.).

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56
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Atomic interrogation of protein-protein interactions within intact nuclei by DNP-supported solid-state NMR

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Investigating protein-protein interactions at atomic levels of resolution whilst maintaining native conditions constitutes the cutting-edge of structural biology.[1–3] To this end, we detail a methodology coupling recent advances in ultra-high field dynamic nuclear polarization-supported solid-state NMR (DNP ssNMR) with physical enrichment of sub-cellular compartments to study protein-protein interactions *in situ* – in our specific case intact nuclei. For a proof of concept, we utilized our organelle-NMR approach to visualize the nuclear landscape of the omnipresent post-translational modifier ubiquitin.[4] We observe that physical enrichment coupled with ultra-high magnetic fields yields significant gains both in terms of sensitivity and resolution, consequently allowing for cellular detection of vital ubiquitination events like iso-peptide bonds. Altogether our method lays the foundation for investigation of nuclear proteins in a broader cellular context and can be expanded on further to investigate other sub-cellular compartments.

[1] Nature **530**, 45–50 (2016), [2] Nat. Protoc. 1–26 (2021), [3]Angew. Chem. Int. Ed. **58**, 12969–12973 (2019), [4] Angew. Chem. Int. Ed. **49**, 10149–10153 (2010).

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57
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Production of stable mutants of NKp80, an activating receptor of NK cells

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Abstract

Natural killer (NK) cells are effector lymphocytes capable of destroying aberrant and potentially harmful cells such as malignant or virus-infected cells spontaneously[1]. Their cytotoxic activity can be stimulated by activating receptors on their surface, such as receptor NKp80 (Natural Killer protein 80 kDa). The human NK cell receptor NKp80 stimulates cytotoxicity upon engaging its genetically linked ligand AICL (Activation Induced C-type Lectin)[2]. AICL is naturally expressed by all myeloid cells. Nevertheless, in cancerous or damaged cells, AICL is often overexpressed, leading to the lysis of such cells by NK cells expressing NKp80. Ultimately, the NKp80:AICL complex is a potential target for the immunotherapeutic treatment of myeloid leukemia.

However, the structure of both proteins remains unknown. So far, we have been focused on successfully producing the extracellular domain of NKp80 in sufficient quality and quantity. Here, we introduced a series of mutations in the stalk region to study their effect on the production, stability, and homodimer formation. Altogether, we produced seven variants of NKp80 replacing cysteines with serines using stably transfected HEK293S GnTI- cells. The proteins were analyzed using size-exclusion chromatography, differential scanning fluorimetry, analytical ultracentrifugation, and mass spectrometry.

In our hands, for six out of the seven mutants, the production yield has at least doubled, and for certain variants even quintupled compared to the wild-type NKp80 extracellular domain. Therefore, we chose the two most successful variants to be produced large-scale for crystallization trials to obtain the unknown structure of NKp80. In our further research, we plan to determine also other biophysical properties of the NKp80 mutant variants. We aim to solve the structure of NKp80, prepare its ligand AICL, and study this receptor:ligand interaction with a prospect to investigate its therapeutical potential fully.

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References

1. Vogler, I. & Steinle, A. Vis-à-Vis in the NKC: Genetically linked Natural killer cell receptor/ligand pairs in the Natural killer gene complex (NKC). *J. Innate Immun.* **3**, 227–235 (2011).
2. Dennehy, K. M., Klimosch, S. N. & Steinle, A. Cutting Edge: NKp80 Uses an Atypical Hemi-ITAM To Trigger NK Cytotoxicity. *J. Immunol.* **186**, 657–661 (2021).

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58
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Identification of Potentially Bioactive Argon Binding Sites in Protein Families

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Abstract

Argon belongs to the group of chemically inert noble gases, which display a remarkable spectrum of clinically useful biological properties. In an attempt to better understand noble gases, notably argon's mechanism of action, we mined a massive noble gas modelling database which lists all possible noble gas binding sites in the proteins from the Protein Data Bank. We developed a method of analysis to identify among all predicted noble gas binding sites, the potentially relevant ones, based on their conservation, binding energy, hydrophobicity, shape and localization within structurally aligned protein families which are likely to be modulated by Ar.[1]

This method allowed us to identify relevant noble gas, in particular Ar binding sites that have potential pharmacological interest in several protein families. The potential Ar targets are currently undergoing crystallographic studies under Ar pressure to confirm our *in silico* predictions. *In vitro* validation experiments are being performed with Ar in order to improve the understanding of its interaction mechanism with the identified putative physiological targets.

(1) Hammami, I.; Farjot, G.; Naveau, M.; Rousseaud, A.; Prangé, T.; Katz, I.; Colloc'h, N. Method for the Identification of Potentially Bioactive Argon Binding Sites in Protein Families. *J. Chem. Inf. Model.* **2022**. https://doi.org/10.1021/acs.jcim.2c00071.

ScipionTomo: towards cryo-electron tomography software integration, reproducibility, and validation.

Marcos Gragera Cabezudo, Pablo Conesa Mingo, Carlos Oscar Sanchez Sorzano, Jose Carazo, Roberto Melero

Image processing in cryogenic electron tomography (cryoET) is currently in a similar situation as Single Particle Analysis (SPA) in cryogenic electron microscopy (cryoEM) was a few years ago. Their workflows are far from being well defined and the user experience is still not very smooth. One of the main reasons is the heterogeneity among the different software packages developed by different groups and that each of them is focused on different steps of the data processing pipeline. Even more, file formats and their associated metadata are far from being standardised.
The Scipion framework, which was originally developed for SPA, has a generic python workflow engine that gives it the versatility to be extended to other fields, as we demonstrated for model building. In this article, we provide an extension of Scipion based on a set of tomography plugins (referred to as ScipionTomo from now on), with the same purpose: to allow users to be focused on the data processing and analysis instead of having to deal with multiple software installation issues and the inconvenience of switching from one to another, converting metadata files, managing possible incompatibilities, scripting etcetera. Additionally, having all the software available in an integrated platform allows comparing the results of different algorithms trying to solve the same problem. In this way, the commonalities and differences between estimated parameters throw light about which results can be more trusted than others. ScipionTomo is developed by a collaborative multidisciplinary team composed of Scipion team engineers, structural biologists and in some cases the developers whose software packages have been integrated. It is open to anyone in the field who wants to contribute to this project.
The result is an extension that combines the acquired knowledge of Scipion development, the close collaboration with third-party developers, and the on-demand design of functionalities requested by beta testers applying this solution to actual biological problems.

Elucidation of the mechanism of CLIC1 membrane insertion and its inhibition

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The CLIC protein family displays the unique feature of altering its structure from a soluble form to a membrane-bound chloride channel. CLIC1, a member of this family, is found in the cytoplasm or in internal and the plasma membranes, with membrane relocalisation linked to endothelial disfunction, tumour proliferation and metastasis. The mechanism of membrane insertion, as well as the molecular switch promoting CLIC1 activation, remain unclear. We have used an integrated approach combining immunofluorescence microscopy, biochemical assays, solution NMR and SAXS to identify the mechanism of CLIC1 membrane insertion. This mechanism involves a complex equilibrium between a major closed state and a minor open state in different oligomeric species which, upon binding to Zn^{2+}, leads to the insertion in the membrane into an inactive state of the chloride channel form, which is only activated at low pH (1). Using NMR RDCs and SAXS we have obtained structural models and dynamics information of the different states of CLIC1 in solution, including the low populated open state, providing the first molecular detail of the membrane insertion process. We also show that Zn^{2+} binding increases both the oligomerisation rates as well as the overall CLIC1 oligomerisation into the tetrameric form required for chloride channel activity (2). Using the structural models and the dynamics obtained for the opening equilibrium of the CLIC1 structure, we have adopted an in-silico screening approach to identify a set of inhibitors targeting specific areas of CLIC1. Our top hits are able to bind CLIC1 and halt its insertion in the membrane in HUVEC cells.

In summary, we have elucidated the mechanism of activation and membrane insertion of CLIC1 with molecular detail and have used this information to develop novel inhibitors of CLIC1 membrane insertion, paving the way for the development of new treatments of glioblastoma and other types of endothelial disfunction.

1. Medina-Carmona E., Varela l., Hendry A.C., Thompson G.S., White L.J., Boles J.E., Hiscock J.R & Ortega-Roldan J.L. (2020) A quantitative assay to study the lipid selectivity of membrane-associated systems using solution NMR. Chemical Communications - 10.1039/d0cc03612a
2. Lorena Varela, Alex C. Hendry, Joseph Cassar, Ruben Martin-Escolano, DiegoCantoni, John C Edwards, Vahitha Abdul-Salam, Jose L. Ortega-Roldan. (2022) Zn^{2+} triggered two-step mechanism of CLIC1 membrane insertion and activation into chloride channels. bioRxiv - 10.25.465729; doi: https://doi.org/10.1101/2021.10.25.465729

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61
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Protein formulation through automated screening of pH and buffer conditions using the Robotein® high throughput facility

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Among various factors, the direct environment (e.g. pH, buffer components, salts, additives, etc.) is known to have a crucial effect on both the stability and activity of proteins. In particular, proper buffer and pH conditions can improve their stability and function significantly during purification, storage and handling, which is highly relevant for both academic and industrial applications. It can also promote data reproducibility, support the interpretation of experimental results and, finally, contribute to our general understanding of the biophysical properties of proteins.

In this study (Kellner *et al.*, 2021), we have developed a high throughput screen of 158 different buffers/pH conditions in which we evaluated: i) the protein stability, using differential scanning fluorimetry and ii) the protein function, using either enzymatic assays or binding activity measurements, both in an automated manner. The modular setup of the screen allows for easy implementation of other characterization methods and parameters, as well as additional test conditions. The buffer/pH screen was validated with five different proteins used as models, i.e. two active-site serine β-lactamases, two metallo-β-lactamases (one of which is only active as a tetramer) and a single-domain dromedary antibody fragment (V_HH or nanobody). The formulation screen allowed automated and fast determination of optimum buffer and pH profiles for the tested proteins.

Besides the determination of the optimum buffer and pH, the collection of pH profiles of many different proteins may also allow to delineate general concepts to understand and predict the relationship between pH and proteins. In addition, our screen could be useful for protein crystallization, as proteins with higher apparent thermal stabilities (i.e., T_m values) were found to be more prone to crystallization (Boivin *et al.*, 2013).

Boivin, S., Kozak, S., Meijers, R. (2013) Optimization of protein purification and characterization using Thermofluor screens, *Protein Expr. Purif.* **91**:192–206.

Kellner, R., Malempré, R., Vandenameele, J., Brans, A., Hennen, A._F., Rochus, N., Di Paolo, A., Vandevenne, M., Matagne, A. (2021) Protein formulation through automated screening of pH and buffer conditions, using the Robotein® high throughput facility, *Eur. Biophys. J.* **50**, 473-490.

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FUNCTIONAL ANALYSIS OF TIM PROTEIN DOMAINS

Circadian rhythms are behavioural and molecular oscillations displayed by a wide range of organisms, from cyanobacteria to humans. These rhythms persist under constant conditions, with a free-running period (*Tau*, τ) of roughly 24 hours, and are temperature compensated, that is the whole system "keeps ticking" in a temperature-independent manner. External cues, such as daily light-dark cycles or temperature, can entrain this biological clock by resetting its phase. This synchronization allows the organism to be temporally coordinated with the different external conditions in order to anticipate and promptly respond to the environmental changes.

Our research is focused on *Drosophila melanogaster* circadian rhythms, with a particular interest in a pivotal protein, Timeless (TIM), which expression in fly's ~150 clock neurons is essential for the circadian clock. TIM oscillates in abundance and subcellular localization during the day, interacting together with many different proteins, including Period (PER), Clock (Clk), and Cycle (CYC). This creates a negative feedback loop, which is the core of the *Drosophila* circadian clock. Furthermore, the interaction of TIM with Cryptochrome (CRY) is key for the light-mediated resetting of the clock. Despite the importance of TIM and long term-interest from top laboratories, the structure of this 1421-amino acid protein remains elusive. To circumvent difficulties of structural biology with TIM, we applied functional genetic approaches and created several unique TIM mutations including a large in-frame deletion, all of which specifically alter the free-running period and point to a specific region in TIM that seems to be crucial for the temperature compensation of the circadian clock. In addition, we have developed an effective experimental knock-in strategy by which we can modify large TIM regions without compromising essential gene components including temperature-dependent splicing. Despite the absence of experimentally-identified TIM structure, some homologous proteins were successfully analysed and might serve as a guide to our experimental design. Using prediction software utilizing sequence homology (JPred4, Phyre2, DeepView–Swiss-PdbViewer), we were able to obtain a model with >90% identity of a region roughly corresponding to the PAB domain of distantly-related human TIM (hTIM). Importantly, this region contains mutations that were independently identified in our group as temperature-compensation affecting substitutions. The model suggests a local arrangement of beta sheets and alpha helices with an outside-protruding loop. Our objective is to test this predicted structure experimentally by designing a pair of mutations (See Fig.1) that when applied individually should affect temperature compensation, but their combination should produce a compensatory effect that will have a mild or even no impact on the circadian clock. While we have a strong background in *Drosophila* genetics and well-prepared functional assay, our limitation is *in silico* analyses, structure predictions, and (structural) data interpretation. Participating in the 2022 ERIC conference on structural biology will help us in predicting and defining optimal experimental strategies that will be the foundation for intended hypothesis-driven experimental tests.

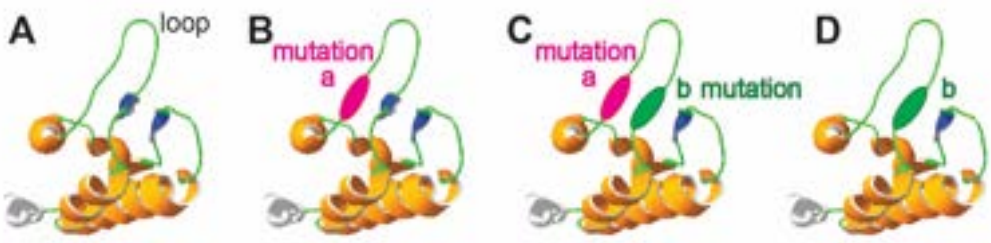

Fig. 1 3-Dimensional structure of PAB domain from hTIM protein and planned mutations
*Picture **A** shows the wild-type protein folding of PAB domain region from hTIM. Orange: α-helices; Blue: β-sheets.*
***(B)**We plan to introduce a single mutation (mutation a) in a conserved region of this domain, to induce temperature compensation defects. **(C)** An opposing mutation (mutation b) will be introduced in a region interacting with the first mutated amino acid, in order to restore a wild-type phenotype.*
***(D)** The mutation b, by itself, will elicit temperature compensation defects. (Created with Swiss-PdbViewer, Guex, N. and Peitsch, M.C. (1997))*

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63
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^{19}F NMR as a direct probe for proteins in human cells

Lan Pham, Letizia Barbieri, Enrico Luchinat and Lucia Banci

Abstract

^{19}F NMR is a sensitive method to study biomolecules. The technique has been widely applied in medicinal chemistry and used to characterise over 70 proteins. As being the second most sensitive stable NMR-active nucleus with 100% natural abundance, ^{19}F carries favourable magnetic resonance properties and its signals can be readily analysed in one-dimensional spectrum. One of the advantages of ^{19}F over ^{1}H NMR in macromolecule studies is the hyperresponsiveness to changes in the surrounding chemical environment. For example, the chemical shifts arising from fluorinated residues of a target protein would display an appreciably wider signal dispersion while they are likely indistinguishable in ^{1}H spectrum. Furthermore, the absence of fluorine in biological systems greatly benefits the protein-observed experiments, especially in-cell NMR studies. Compared to other types of isotopic labelling, ^{19}F in-cell NMR offers spectra with little background noise, hence facilitating the identification of the signals of interest, the observation of lineshape and chemical shift changes.

Taking advantage of ^{19}F NMR, we examined the feasibility of this technique in the in-cell study using human cells. Owing to the tolerance of biosynthetic machinery, we succeeded to incorporate ^{19}F-labelled amino acid analogues into different proteins, varying from 14 to 30 kDa. We also examined the efficiency of fluorination according to the incubation time of ^{19}F-amino acid containing medium by NMR and mass spectrometry. After defining a suitable time of labelled medium incubation, the current workflow is being applied to studying protein-ligand interactions and the structural stability of mutants in living cells.

Since this method relies on endogenous labelling, achieving a complete fluorination at all interested residues in the protein sequence poses a challenge for the desired optimisation. Nonetheless, our present protocol could gain a high percentage of ^{19}F-incorporation, the stochastic labelling gives rise to signals resulting from the residues of interest in the protein sequence. As a result, we were able to detect the ^{19}F-signals from all expected positions on the target protein, which is comparable to the published ^{19}F chemical shift assignments obtained in vitro.

At present, we could demonstrate the feasibility of endogenous ^{19}F-labelling in mammalian cells for in-cell signal investigation. The choice of analogues will be dependent on the protein nature as well as the study of interest. We envision that this method will have broad applicability to in-cell NMR protein studies.

Seeing Double: A structural understanding of dsRNA recognition by nuclear factor 90

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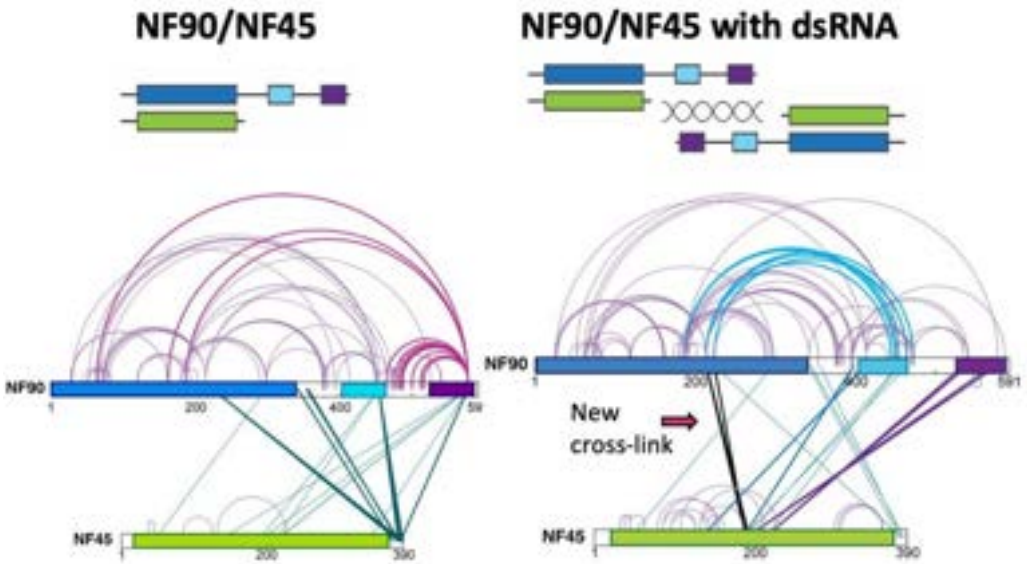
Nuclear factors NF90 and NF45 are essential vertebrate proteins that are implicated as regulators in many different RNA processing pathways including alternative splicing, circRNA biogenesis and adenosine-to-inosine (A-to-I) editing. The NF90/NF45 complex is known to bind dsRNA but how this multidomain protein complex recognises cellular dsRNAs to alter the outcome of post-transcriptional processes is not well understood. Previously, our co-crystal structure showed that NF90 and NF45 dimerise through their pseudo-nucleotidyltransferase domains. We also showed that the two dsRNA binding domains of NF90 interact with A-form dsRNA with some base preferences. We have now used an integrative structural biology approach, combining our crystal structures with cross-linking mass spectrometry, modelling and electron microscopy. Our integrative structural approach suggests that NF90/NF45 complexes may oligomerise on dsRNA and this is supported by negative stain electron microscopy. This suggests a mechanism for how this protein complex might alter accessibility to RNA editing sites.



Cryo-EM snapshots of a native lysate provide structural insights into a metabolon-embedded transacetylase reaction

Christian Tüting, Panagiotis Kastritis

Found across all kingdoms of life, 2-keto acid dehydrogenase complexes possess prominent metabolic roles and form major regulatory sites. Although their component structures are known, their higher-order organization is highly heterogeneous, not only across species or tissues but also even within a single cell. Here, we report a cryo-EM structure of the fully active Chaetomium thermophilum pyruvate dehydrogenase complex (PDHc) core scaffold at 3.85 Å resolution (FSC = 0.143) from native cell extracts. By combining cryo-EM with macromolecular docking and molecular dynamics simulations, we resolve all PDHc core scaffold interfaces and dissect the residing transacetylase reaction. Electrostatics attract the lipoyl domain to the transacetylase active site and stabilize the coenzyme A, while apolar interactions position the lipoate in its binding cleft. Our results have direct implications on the structural determinants of the transacetylase reaction and the role of flexible regions in the context of the overall 10 MDa PDHc metabolon architecture.

Structural basis of the interaction between the adaptor protein PSTPIP1 and the LYP phosphatase: a novel mechanism of recognition of Pro-rich motifs by F-BAR domains

José A Manso[1], Tamara Marcos[2], Virginia Ruiz-Martín[2], Javier Casas[2], Pablo Alcón[1], Mariano Sánchez Crespo[2], Yolanda Bayón[2], José M de Pereda[1], Andrés Alonso[2]

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PSTPIP1 (proline–serine–threonine phosphatase interacting protein 1) is an adaptor protein expressed in most immune cell lineages. It consists of an N-terminal F-BAR domain and a C-terminal SH3 domain. PSTPIP1 binds to the PEST subfamily of protein tyrosine phosphatases (PTPs): PTPN12 (PTP-PEST), PTPN18, and the lymphoid phosphatase (LYP). These phosphatases bind to the F-BAR of PSTPIP1 through a short and conserved Pro-rich C-terminal homology domain (CTH). Mutations in PSTPIP1 cause a spectrum of rare autoinflammatory diseases. Likewise, LYP is involved in autoimmune diseases with a significant inflammatory component, like arthritis or systemic lupus erythematosus. To better understand the PSTPIP1/LYP interaction and the mechanisms by which mutations cause autoinflammatory disorders, we solved the structure of the F-BAR domain of PSTPIP1 alone and bound to the CTH of LYP. This is the first high-resolution structure of an F-BAR domain bound to its cognate ligand. The F-BAR forms homo-dimers with a curved shape; the concave side has electropositive character and binds to membranes. LYP-CTH binds to the convex side of the F-BAR dimer, at the rim of the dimerization interface. The LYP-binding site is characterized by three pockets: LYP-CTH prolines P795 and P798 dock in two shallow pockets while a third and deeper cavity in the F-BAR accommodates a PxxW motif of LYP. The interaction is further stabilized by ionic interactions. The two-fold axis that relates the two protomers of the F-BAR dimer lies in the first Pro-binding pocket of the LYP-binding site. Consequently, only one molecule of LYP can bind to PSTPIP1 at the time, as confirmed by pull-down assays, fluorescence anisotropy, and ITC. Pathogenic mutations R228C, D246N, E250Q, E250K, and E257K directly target the LYP-binding site and compromise binding. This provides a link between the disruption of the PSTPIP1/LYP interaction and a group of immune diseases associated to PSTPIP1. Finally, a PSTPIP1 homologue protein, PSTPIP2, which only has a highly similar F-BAR domain to PSTPIP1, exhibits a similar mode of interaction with PEST phosphatases. Furthermore, the determinants of the interaction are also present in the F-BAR domain of Cdc15 and its ligand Cdc12. Collectively, the PSTPIP1/LYP interaction emerges as a general mode for the recognition of Pro-rich motifs by a group of F-BAR domains.

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67
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Yeast Ssd1 is a non-enzymatic member of the RNase II family with an alternative RNA recognition interface

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Abstract:

Yeast Ssd1 is a conserved fungal RNA binding protein belonging to the RNase family II. It is a protein that is involved in virulence, cell wall remodelling and stress responses in fungi. Previous reports have suggested that Ssd1 lacks catalytic activity and yet Ssd1 has been shown to be involved in translational repression of cell wall remodelling proteins. We solved the crystal structure of *S. cerevisiae* Ssd1 at 1.9Å resolution and show that the catalytic residues are absent. The channel in which RNA binds is blocked by regulatory sequences. The structure reveals patches of charged residues on the outer surface of Ssd1 that are conserved, suggesting RNA binding is likely directed to this region on Ssd1. UV-based *in vivo* crosslinking and cDNA analysis (CRAC) identified a highly conserved bipartite motif in the 5'UTRs of Ssd1-associated transcripts. Using bioinformatics and biochemical experiments we show that the length and sequence of these tandem motifs are important for Ssd1 recognition. Furthermore, using structure-based mutation studies we show that the RNA binding is located on the outer surface of the cold shock domain (CDS). *In vivo* experiments confirm that the disruption to RNA binding causes loss of Ssd1 function in yeast. Finally, we conclude that Ssd1 has likely evolved from a previous ancestral DIS3 family of enzymes. It does so by fixing regulatory elements in place to close off the classical RNA channel, with gain of a new RNA binding site on the CSDs.

Structure of the Rap GTPase-activating protein SIPA1

Natalia S. Sotelo[1], Alba Morán-Vaquero[1,2], Antonio Rodríguez-Blázquez[1,2], Carmen Guerrero[1,2,3], José M de Pereda[1]

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Signal-induced proliferation-associated protein 1 (SIPA1) is a GTPase-activating protein (GAP) that stimulates the intrinsically slow GTP-hydrolase activity of small GTPases promoting the transition from the active GTP-bound state to the inactive GDP-bound form. SIPA1 inactivates the GTPases Rap1 and Rap2 that regulate diverse functions including cell adhesion and organization of the cytoskeleton. SIPA1 is highly expressed in lymphoid tissue such as thymus, spleen, and peripheral blood lymphocytes. The deregulated Rap1 activation in SIPA1 deficient mice causes enhanced expansion of bone marrow hematopoietic progenitors and given rise myeloproliferative disorders of long latency (Chronic Myeloid Leukaemia; Chronic Lymphoid Leukaemia). There is evidence that SIPA1 expression in the bone marrow (BM) niche is critical for maintaining BM niche homeostasis, and the normal lymphocyte development. SIPA1 has been found upregulated in melanoma, breast cancer, colorectal cancer, and prostate cancer, which is involved in the regulation of tumour cell adhesion and invasion.

While the roles of SIPA1 in lymphoid tissues has been characterized extensively, little is known about the structure of SIPA1 or its regulation. SIPA1 is a 112 kDa multidomain protein. SIPA1 contains a Rap-GAP catalytic domain, a PDZ domain, and a C-terminal coiled-coil domain. The Rap-GAP domain is inserted in a region referred as dimerization domain (DD) by analogy with the equivalent DD of the related protein Rap1GAP. Here we present the X-ray crystal structures of 70 kDa fragment of human SIPA1 in two crystal forms that belong to space groups P1 and $C222_1$, respectively. Diffraction of all crystals was strongly anisotropic; the P1 data extends to 3.2, 2.8 and 2.4 Å resolution in the main directions. The structure was very similar in the two crystal forms. SIPA1 adopts a compact structure with extensive inter-domain interactions. The so-called DD does not participate in homotypic interactions as in Rap1GAP, suggesting that it does not mediate dimerization of SIPA1. In summary, the structure of SIPA1 reveals the inter-domain architecture and paves the way for the structure-based analysis of SIPA1 function and regulation.

Structure of the Rap GTPase-activating protein SIPA1

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Nanobodies for the analysis of the guanine nucleotide exchange factor C3G

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Abstract:

C3G (RapGEF1) is a guanine nucleotide exchange factor (GEF) that activates the small GTPase Rap1. Similarly to other GEFs, C3G promotes the transition of Rap1 from an inactive GDP-bound state to the active GTP-bound form. C3G is a 120 kDa multi-domain protein with three distinct regions: an N-terminal domain (NTD), a central SH3b domain, and a C-terminal catalytic region consisting of a REM (Ras exchange motif) domain and a Cdc25 homology domain (Cdc25HD). The Cdc25HD is responsible for the GEF activity. We have showed recently that a segment of the SH3b domain binds to the Cdc25HD domain and blocks its GEF activity; hence, we have named this segment the autoinhibitory region or AIR [1]. Two missense mutations identified in non-Hodgkin's lymphoma (Y554H and M555K), which target residues in the AIR, disrupt the AIR/Cdc25HD autoinhibitory interaction and cause constitutive activation of C3G. To investigate the structure and function of C3G we have developed C3G-specific nanobodies at the Nanobodies4Instruct Center. Here, we present the characterization of anti-C3G nanobodies. We have identified 12 nanobodies representing as many nanobody families that bind to the Cdc25HD catalytic domain of C3G. Analysis of the interaction between nanobodies and the Cdc25HD using fluorescence anisotropy revealed that binding occurs with low-micromolar affinity. Three of the anti-C3G-Cdc25HD nanobodies block the GEF activity with a half-maximal inhibitory concentration (IC50) between 0.5 and 7 µM. These nanobodies also inhibit the GEF activity of the constitutively active point mutant C3G-Y554H. In summary, these anti-C3G nanobodies are valuable tools for the analysis of the structure and function of C3G.

Reference:

1. Carabias A, Gómez-Hernández M, de Cima S, Rodríguez-Blázquez A, Morán-Vaquero A, González-Sáenz P, Guerrero C, de Pereda JM **(2020)** "Mechanisms of autoregulation of C3G, activator of the GTPase Rap1, and its catalytic deregulation in lymphomas." ***Science Signaling*** 13(647):eabb7075.

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71

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New Instrumentation Available in Centre of Molecular Structure, Institute of Biotechnology CAS (Biocev) in Czech Republic

J. Pavlíček, J. Stránský, T. Charnavets, O. Dmitruk, P. Pompach, P. Vaňková, T. Nepokojová, Ľ. Škultétyová, M. Schneiderová and J. Dohnálek

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The core facilities for structural biology in BIOCEV are organized under the Centre of Molecular Structure, run by the Institute of Biotechnology, Czech Academy of Sciences and belong to the Czech Infrastructure for Integrative Structural Biology. The Centre of Molecular Structure (CMS) encompasses several laboratories providing a complex approach to studies of three-dimensional structure, function and biophysical properties of biological molecules.

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72
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Role of factor HelD in bacterial transcription

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Regulation of bacterial transcription performed by RNA polymerase (RNAP) is facilitated by various transcription factors (e.g. σ factors), which interact with RNAP. HelD is a transcription-associated protein present in many Gram-positive bacteria (e.g. *Bacillus subtilis* or *Mycobacterium smegmatis*) [1]. HelD forms complexes with RNAP, the function of which is not yet fully understood. Using data from X-ray crystallography (one HelD domain), cryo-EM, small-angle X-ray scattering, homologous modelling, and functional transcription assays [2, 3] we are trying to explain the relationship between structural observations and functional manifestation of HelD behaviour.

HelD interacts with RNAP in different stages of transcription. It binds deep in the primary and secondary channels of RNAP and via the primary channel it reaches the polymerase active site. Interactions of HelD with RNAP are incompatible with the binding of DNA or product RNA in the RNAP active site and surrounding channels. HelD was shown to effectively clear RNAP of nucleic acids by dismantling RNAP-DNA complexes [3]. HelD itself is a multi-domain protein capable of structural changes both in solution [2] and in complex with RNAP [3]. The structural flexibility and dynamics of HelD must be linked to its function. It is a GTPase/ATPase but its enzymatic activity and putative power strokes have not yet been connected to the cycle of binding and unbinding to *M. smegmatis* RNAP. Interestingly, the roles and structural behaviour of *B. subtilis* and *M. smegmatis* HelD differ significantly, as can be demonstrated by structural comparison and differences in effects on transcription [1,3-5].

1. Wiedermannová, J., Sudzinová, P., Kovaľ, T., Rabatinová, A., Šanderova, H., Ramaniuk, O., Rittich, Š. & Dohnálek, J. (2014). *Nucleic Acids Res.* **42**, 5151-5163.
2. Kovaľ, T., Sudzinová, P., Perháčová, T., Trundová, M., Skálová, T., Fejfarová, K., Šanderová, H., Krásný, L., Dušková, J. & Dohnálek, J. (2019). *FEBS Lett.* **593**, 996-1005.
3. Kouba, T., Koval', T., Sudzinová, P., Pospíšil, J., Brezovská, B., Hnilicová, J., Šanderová, H., Janoušková, M., Šiková, M., Halada, P., Sýkora, M., Barvík, I., Nováček, J., Trundová, M., Dušková, J., Skálová, T., Chon, U., Murakami, K.S., Dohnálek, J. & Krásný, L. (2020). *Nat Commun.***11**, 6419.
4. Pei, H.H., Hilal, T., Chen, Z.A., Huang, Y.H., Gao, Y., Said, N., Loll, B., Rappsilber, J., Belogurov, G.A., Artsimovitch, I., Wahl, M.C. (2020). *Nat Commun.* **11,** 6418.
5. Newing, T.P., Oakley, A.J., Miller, M., Dawson, C.J., Brown, S.H.J., Bouwer, J.C., Tolun, G., Lewis, P.J. (2020). *Nat Commun.* **11,** 6420.

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Activation of C3G by the adaptor protein CrkL

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Abstract:
C3G is a guanine nucleotide exchange factor (GEF) that activates the small GTPase Rap1. C3G facilities the transition of Rap1 from an inactive (GDP-bound) to an active (GTP-bound) state, we refer to this as the GEF catalytic activity. C3G (120 kDa) is a modular protein with three distinct segments: an N-terminal region, a flexible central SH3b domain that contains five proline-rich motifs (PRMs, P0 to P4), and a C-terminal region that consists of a REM (Ras Exchange Motif) and a GEF-catalytic Cdc25 homology domain (Cdc25HD). In absence of stimulus, C3G adopts an autoinhibitory conformation stabilized by an intramolecular interaction between the final segment of the SH3b domain (known as Auto-Inhibitory Region or AIR) and the Cdc25HD. One missense mutation identified in non-Hodgkin´s lymphoma and located in the AIR (Y554H) disrupts the AIR/Cdc25HD interaction and cause a constitutive active C3G [1]. Physiological activation of C3G involves two stimuli: phosphorylation in tyrosine residues by Src-family kinase and binding to members of the Crk adaptor protein family (CrkI, CrkII and CrkL). Here we present a detailed characterization of the binding of CrkL to C3G and its role in the activation of C3G. CrkL contains two SH3 domains (SH3N and SH3C) but only the SH3N binds to PRMs. The CrkL-SH3N binds the isolated PRMs P1, P2, P3, and P4 with similar affinity (*Kd* 1.3-3 µM) as determined by fluorescence anisotropy. However, analysis of the binding of CrkL to autoinhibited full-length C3G by isothermal titration calorimetry (ITC) revealed differences in the recognition of the PRMs by CrkL. PRMs P1 and P2 are mostly accessible to CrkL, while the P3 and P4, which are next and within the AIR respectively, are only partially accessible to CrkL. Analysis of the binding of CrkL to these PRMs in phosphorylated C3G and in the constitutively active C3G-Y554H mutant revealed that the cryptic P3 site becomes exposed in activated C3G. We have also analysed the activation of C3G by CrkL. Activation occurs by binding of CrkL to the P3 and P4 sites; phosphorylation by Src primes C3G for a more efficient activation by CrkL. In summary, our results reveal a mechanism for the required convergence of two stimuli at signalling sites to achieve the activation of C3G. Our work paves the way to tackle the structural basis of C3G activation by CrkL.

Reference:
1. Carabias A, Gómez-Hernández M, de Cima S, Rodríguez-Blázquez A, Morán-Vaquero A, González-Sáenz P, Guerrero C, de Pereda JM **(2020)** "Mechanisms of autoregulation of C3G, activator of the GTPase Rap1, and its catalytic deregulation in lymphomas." ***Science Signaling*** 13(647):eabb7075.

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Authors: Pauline Audergon, Claudia Alen Amaro, Natalie Haley*

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High-end X-ray technologies for laboratory structural biology in INSTRUCT-CZ/BIOCEV

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The Centre of Molecular Structure (CMS) provides services and access to state-of-art instruments, which cover a wide range of techniques required by not only structural biologists. CMS operates as part of the Czech Infrastructure for Integrative Structural Biology (CIISB), and European infrastructures Instruct-ERIC and MOSBRI. CMS is organized in 5 core facilities: CF Protein Production, CF Biophysics, CF Crystallization of Proteins and Nucleic Acids, CF Diffraction Techniques, and CF Structural Mass Spectrometry.

CF Diffraction Techniques employs two laboratory X-ray instruments equipped with high flux MetalJet X-ray sources: a single crystal diffractometer D8 Venture (Bruker) and a small angle X-ray scattering instrument SAXSpoint 2.0 (Anton Paar). The configurations of both instruments represent the top tier of possibilities of laboratory instrumentation. Apart from standard applications, the instruments are also extended for advanced experiments: the diffractometer is equipped with the stage for in-situ crystal diffraction and crystal dehydration, SAXS is equipped with in-situ UV-Vis spectroscopy and a liquid chromatography system for SEC-SAXS. The setups enable easy access and fast turn-around of samples under different conditions, but also collection of high quality end-state data without further need for synchrotron data collection in many cases. CF Diffraction Techniques provides services in synergy with the other CFs on-site, therefore scientific questions can be quickly answered as they emerge from the experiments.

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Increased microtubule lattice spacing correlates with selective binding of kinesin-1 in cells

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Abstract

Within the cell cargo is transported via motor proteins walking along microtubules. The affinity of motor proteins for microtubules is controlled by various layers of regulation like tubulin isoforms, post-translational modifications and microtubule associated proteins. Recently, the conformation of the microtubule lattice has also emerged as a potential regulatory factor, but to what extent it acts as an additional layer of regulation has remained unclear. In this study, we used cryo-correlative light and electron microscopy to study microtubule lattices inside cells. We find that, while most microtubules have a compacted lattice (~41 Å), a significant proportion of the microtubule cores have expanded lattice spacings and that these lattice spacings could be modulated by the microtubule stabilizing drug Taxol. Furthermore, kinesin-1 predominantly binds microtubules with a more expanded lattice spacing (~41.6 Å). The different lattice spacings present in the cell can thus act as an additional factor that modulates the binding of motor proteins to specific microtubule subsets.

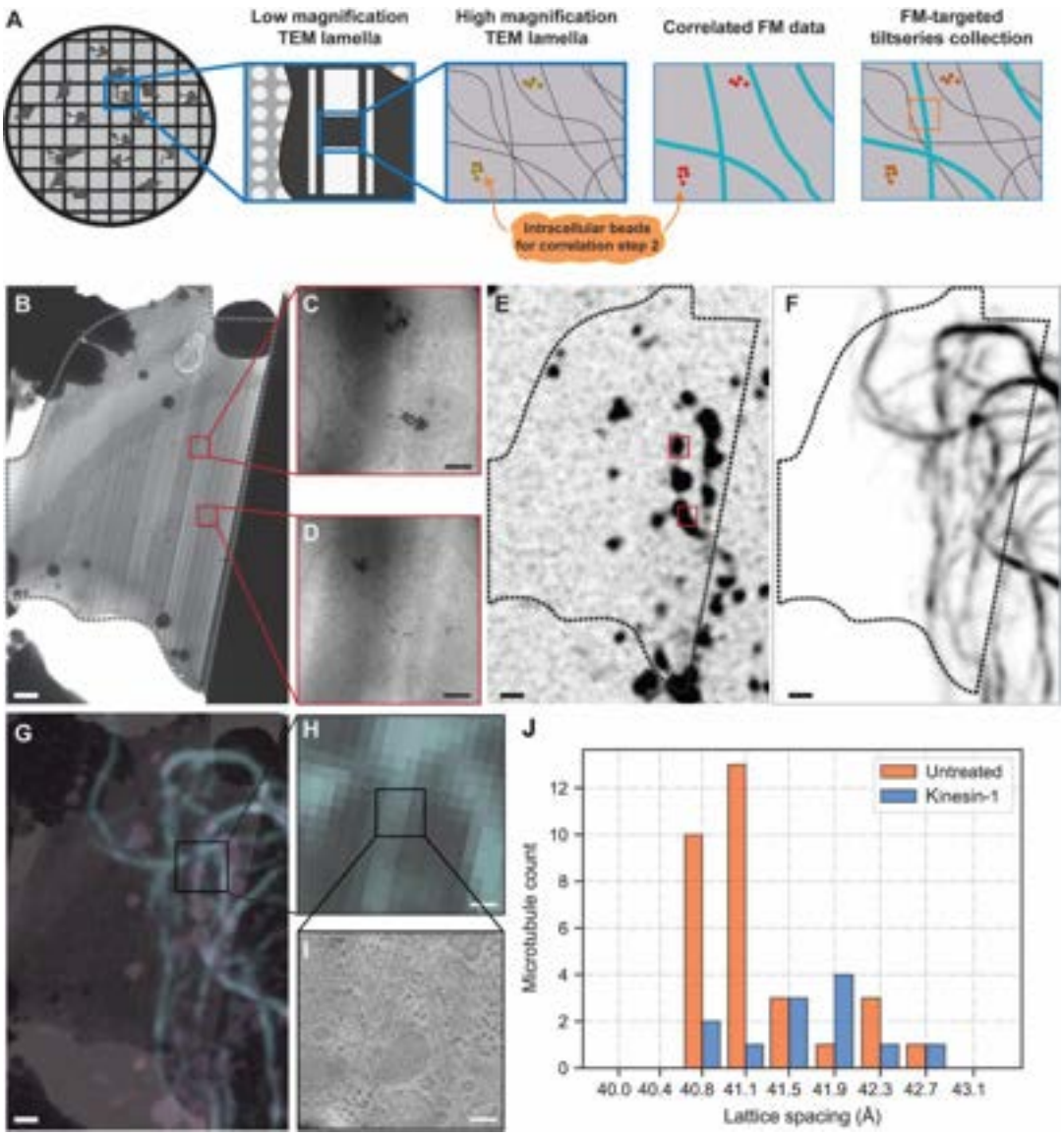

Figure 1. Kinesin-1 bound microtubules have a slightly expanded lattice compared to the GDP-compacted lattice. (**A**) Cartoon describing the FM-TEM correlation performed using intracellular beads. (**B**) TEM overview image of a lamella, dotted line indicates outline of the lamella, red squares the location of the fBSA-Au^5 beads. (**C and D**) Zoom in of the fBSA-Au^5 beads found both in the TEM lamella in B and in the correlated fBSA-Au^5 FM data in E. (**E**) Correlated fBSA-Au^5 FM data, red squares indicate fBSA-Au^5 location. (**F**) Correlated rigor-bound microtubules. (**G**) Overlay of TEM lamella and correlated FM data of both fBSA-Au^5 (pink) and rigor-bound microtubules (blue). (**H and I**) Two step zoom of two microtubules overlapping with the rigor-bound FM data. (**J**) Histogram showing the untreated lattice spacings (N=31, 12 tomograms) and the lattice spacings of the kinesin-1 bound subset of microtubules (N=12, 6 tomograms). Scale bars: 1 µm (**B, E, F and G**), 100 nm (**C,D and I)**, 400 nm (**H**). Untreated distribution is significantly different from kinesin-1 distribution (p-value = 0.045, unpaired t-test based permutation test).

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Active site complementation and oligomerization in bacterial α-L-fucosidases

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Keywords: α-L-fucosidase, GH151, glycosyl hydrolase, crystal structure, active site complementation

α-L-Fucosidases (EC 3.2.1.51) catalyse hydrolysis of the α-L-fucosyl moiety from the non-reducing terminus of oligosaccharides and glycoconjugates. They belong to glycosyl hydrolase families GH29, GH95, GH139, GH141, and GH151. In our studies we focused on the structure-function analyses of two isoforms of α-L-fucosidases present in bacterium *Paenibacillus thiaminolyticus* [1,2,3]. α-L-Fucosidase isoenzyme 1 belongs to the GH29 family, isoenzyme 2 to GH151. Both enzymes use the same retaining double-displacement mechanism of enzymatic cleavage but have different substrate specificities and have distant sequences. We solved structures of both enzymes using X-ray crystallography, determined their oligomerisation state using small angle X-ray scattering supported by several biophysical methods and further analysed connection between observed structural features and enzymatic activities using mutational studies, biochemical methods and *in silico* analyses. Both structures show a new and unusual organization of the enzyme in oligomers. Isoenzyme 1 (GH29) is a hexamer, isoenzyme 2 (GH151) functions as tetramer. Interestingly, active sites of both structures show catalytically important complementation. These are the first observed cases of active site complementation in α-L-fucosidases [2, 3]. Moreover, structure of isoenzyme 2 presents the first structural insight into the GH151 family [3]. The structural, functional, and biophysical data for both enzymes answer some questions about α-L-fucosidase substrate specificity, while other questions regarding the complemented active sites within α-L-fucosidases remain open.

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1. Benešová E, Lipovová P, Krejzová J, Kovaľová T, Buchtová P, Spiwok V & Králová B (2015) α-L-Fucosidase isoenzyme iso2 from *Paenibacillus thiaminolyticus*. *BMC Biotechnol* 15, 36.

2. Kovaľová T, Kovaľ T, Benešová E, Vodičková P, Spiwok V, Lipovová P, Dohnálek J (2019) Active site complementation and hexameric arrangement in the GH family 29; a structure–function study of α-L-fucosidase isoenzyme 1 from *Paenibacillus thiaminolyticus*. *Glycobiology,* 29(1), 59–73.

3. Koval'ová T, Kovaľ T, Stránský J, Kolenko P, Dušková J, Švecová L, Vodičková P, Spiwok V, Benešová E, Lipovová P, Dohnálek J. The first structure-function study of GH151 α-l-fucosidase uncovers new oligomerization pattern, active site complementation, and selective substrate specificity. FEBS J. 2022 Feb 3. doi: 10.1111/febs.16387.

Studying Neuraminidase on VLPs with cryo-electron tomography

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Influenza A viruses (IAVs) cause significant morbidity and mortality in yearly global outbreaks. On the surface it contains two major glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Whereas HA mediates attachment and viral entry, NA is involved in catalysing the removal of terminal sialic acids and thus functions as a receptor-destroying enzyme. NA's structure consists of a cytoplasmic tail, transmembrane domain, stalk and head domain, and forms a tetramer on the virion's surface. The stalk has been shown to contribute to NA stability and to play an important role in NA activity. Deletion of NA stalk, accompanied with decreased NA activity, is a well-known adaptation of avian IAVs to chickens. All human viruses have a long stalk. Previous efforts have produced crystal structures of the head domain, but structures of the stalk have not been reported. Here, we use cryo-EM to study NA expressed on virus-like-particles (VLPs). With tomography, we show NA forms a highly compacted pseudo-hexagonal lattice on the VLPs. Focusing on a single NA spike, we are able to confirm the proteins expressed on the vesicles are indeed tetramers and are in line with literature in terms of size and height. Furthermore, we can resolve a part of the stalk, providing more insight into the connection to the head. By increasing our understanding of the NA stalk at the structural level we aim to provide novel insights into NA function.

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79
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

Biophysical characterization of extracellular carbonic anhydrase IX

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Human carbonic anhydrase IX (CA IX) is a dimeric transmembrane zinc metalloenzyme catalyzing the extracellular conversion of CO_2 and H_2O into HCO_3^- and H^+. CA IX expression in advanced tumors maintains optimal intracellular pH of cancer cells while acidifying the extracellular environment and promoting cancer cell survival, migration, and metastasis.[1] Extracellular CA IX consists of well-characterized catalytic domain and intrinsically disordered proteoglycan-like (PG) domain – a structural feature unique among other human CAs. Although the PG domain is functionally essential its structural and biophysical properties within CA IX are insufficiently investigated.[2, 3] We have determined biophysical properties of the extracellular part of CA IX which is crucial for subsequent inhibitor design. As structures of human CAs isoforms are highly conserved, it is important to design specific inhibitors that target solely to CAs upregulated in cancer cells (e.g., CA IX, CA XII) while not interacting with other CAs expressed in healthy cells.[4] Thus, the determination of CA IX biophysical properties will lead to specific inhibition of overexpressed CA IX and contribute to cancer cell elimination.

[1] S. Pastorekova and R. J. Gillies, "The role of carbonic anhydrase IX in cancer development: links to hypoxia, acidosis, and beyond," *Cancer and Metastasis Reviews*, vol. 38, no. 1–2. Springer New York LLC, pp. 65–77, Jun. 15, 2019, doi: 10.1007/s10555-019-09799-0.

[2] V. Alterio *et al.*, "Crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 106, no. 38, pp. 16233–16238, 2009, doi: 10.1073/pnas.0908301106.

[3] K. Koruza *et al.*, "Biophysical characterization of cancer-related carbonic anhydrase ix," *Int. J. Mol. Sci.*, vol. 21, no. 15, pp. 1–20, Aug. 2020, doi: 10.3390/ijms21155277.

[4] S. Singh, C. L. Lomelino, M. Y. Mboge, S. C. Frost, and R. McKenna, "Cancer Drug Development of Carbonic Anhydrase Inhibitors beyond the Active Site," *Molecules*, vol. 23, no. 5, 2018, doi: 10.3390/molecules23051045.

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Structural analysis of the bovine antibody repertoire: contributions of the heavy and light to antigenic recognition

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A fraction of bovine antibodies comprise heavy chains characterised by 'ultralong' CDR3H loops of up to 70 residues, which extend above the antigen-binding surface of the heavy and light chain variable domains. These so-called ultralong heavy chains are paired with a highly conserved light chain, raising questions about the role of the light chain in bovine antigenic recognition.

Specificity of antibody secreting cells isolated from animals serially vaccinated with Foot and Mouth Disease Virus-like Particles (VLPs) of multiple serotypes were screened using a fluorescent-based assay, and subsequently sequenced. 9 ultralong antibodies were expressed as Fabs. 7 Fab structures were solved by X-Ray diffraction, and 2 were predicted using AlphaFold. Additionally, 1 ultralong CDR3H identified from a non-immunized animal was produced, along with 2 chain-exchanged variants. Interaction between Fabs and VLPs were measured using Bio-layer Interferometry. Fab-VLP interactions were then studied using cryogenic electron microscopy.

143 unique antibodies were identified from 349 B cells, including 22 ultralong CDR3H. A pair of antibodies sharing an identical ultralong heavy chain gave differing specificity screening profiles and have differentially encoded light chains. Different light chains induce varying degrees of twist to the CDR3H stalk, which affects relative positions of residues within the knob domain and may explain the observed specificity differences.

Antigenic interaction involving ultralong CDR3H exclusively relies upon the CDR3H, though the light chain and other CDRH loops affect its precise positioning, offering insight to bovine immunological mechanisms. Ongoing investigations hope to further clarify the structural and functional differences between near identical antibodies.

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81
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RNA G-quadruplex motifs in precursor microRNAs and their interaction with small molecules and nucleolin

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While DNA G-quadruplexes (G4s) have attracted considerable interest over the last two decades, G4s in non-coding RNAs have recently emerged as significant agents in biology. The formation of G4s has been described in the regulation of RNA metabolism, including the miRNA pathway, where such structures play essential roles in many steps of miRNA biogenesis and function. Indeed, miRNA biogenesis can be regulated by influencing the equilibrium G4-stem-loop of pre-miRNAs. Developing new small molecules (ligands) that can specifically and selectively target those G4s and modulate their structure and function has recently attracted the attention of researchers. However, the structure and function of G4s can also be regulated by endogenous proteins and vice-versa. Nucleolin is an important RNA-binding protein with a high affinity and preference for both DNA and RNA G4 architectures. The influence of nucleolin on microRNA biogenesis has also been studied, and since some pre-miRNAs can adopt a G4 structure, it is expected that the protein binds tightly to these structures. Moreover, upon binding to G4s, the structure and functions of nucleolin can be influenced, and vice versa. As a result of the biological significance of nucleolin/G4 partnership, its modulation for therapeutic and/or diagnostic purposes has tremendous potential. Herein, we have studied the formation of G4 structures in the G4 motifs found in pre-miRNAs, as well as their interaction with ligands and nucleolin. To this purpose, we employed several experimental techniques, including circular dichroism, fluorescence spectroscopy, nuclear magnetic resonance, and confocal microscopy. Altogether, the results of our study demonstrated the binding of the G4 structures to the ligands, NCL and ligand/NCL complex both *in vitro* and in cells, which supports the hypothesis according to which the biogenesis of miRNAs could be modulated by targeting its G4 structure with small molecules.

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82
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

^{19}F Trp labelling as a selective probe into the dimeric interface

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The self-association of proteins is a fundamental mechanism in cell. The oligomerization offers a way of protein regulation, often broadening their functionality [1]. One of the rarest amino acids tryptophan (Trp) has a unique role in protein self-association [2]. It has been previously described that Trp is commonly present in so called dimerization hot spots partaking in dimer formation [3,4]. A method that could selectively focus on Trp could therefore offer a unique probe into protein dimerization.

Nuclear magnetic resonance is a powerful tool in structural biology. Traditional double ^{13}C, ^{15}N labelling can be complemented by the ^{19}F labelling which is a simple and straight-forward method [5]. The biggest advantage of ^{19}F labelling is high signal intensity and a selective labelling of only chosen amino acid (usually Trp, Tyr or Phe), leading to less convoluted spectra without background noise. For these reasons, the ^{19}F labelling may present a technique especially useful for determination of parameters connected to protein dimerization.

In this work, we explore application of ^{19}F Trp labelling on example of 14-3-3 proteins. The eukaryotic 14-3-3 proteins are important regulators involved in number of processes. Their dimeric form is crucial for proper function and the mechanism of dimer-ligand interaction has been thoroughly described [6]. Upon study of post-translational modifications, it has been discovered that phosphorylation of 14-3-3 at Ser58 located at dimeric interface leads to monomerization [7]. Intriguingly, residue neighbouring Ser58 is Trp59. We are thus comparing ^{19}F Trp labelled dimeric 14-3-3 wild type and monomeric 14-3-3 phosphorylated at Ser58 and showing the possibilities that such labelling offers for study of dimerization.

References:

1 N. J. Marianayagam, M. Sunde, J. M. Matthews, Trends in Biochemical Sciences. (2004) **29**, 618–625.
2 S. Barik, International Journal of Molecular Sciences. (2020) **21**, 8776.
3 I. S. Moreira, J. M. Martins, R. M. Ramos, P. A. Fernandes, M. J. Ramos Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics. (2013) **1834**, 404–414.
4 A. A. Bogan, K. S. Thorn, Journal of Molecular Biology. (1998) **280**, 1–9.
5 N. G. Sharaf, A. M. Gronenborn in (2015) Methods in Enzymology, pp. 67–95.
6 M. B. Yaffe How do 14-3-3 proteins work?, FEBS Letters. (2002) **513**, 53–57.
7 J. M. Woodcock, J. Murphy, F. C. Stomski, M. C. Berndt, A. F. Lopez, Journal of Biological Chemistry. (2003) **278**, 36323–36327.

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14-3-3 protein isoforms: mutual dimerization propensities screening kit

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14-3-3 is a group of eukaryotic regulatory proteins, well known for its irreplaceable role in regulation of cell cycle and metabolism. 14-3-3 family involves 7 isoforms of this protein: ε, γ, ζ, β, θ, η and σ.[1] These isoforms differ in their sequences, expression levels in various tissues and interactomes, but their structures and general biophysical properties are similar. Function of 14-3-3 protein is determined by its dimeric character. [2] It was observed that 14-3-3 proteins are capable of both homo- and heterodimerization. Due to different sequences of dimeric interfaces, monomeric subunits of different isoforms have different mutual affinities.[3], [4]
Target of this work is to compare dimerization propensities of all possible pairs of 14-3-3 monomers. In our research group, the dimerization of the ζ isoform was deeply studied, using FRET fluorescence assay in 1.5 ml cuvette, and evaluated applying complex mathematical model. This experiment provides quantitative description of dimerization, but can be performed only for one dimerization pair and is relatively time consuming. [5] Therefore, in this study, fluorimetric screening on 96 well plate was developed to describe mutual affinities of all 14-3-3 isoforms. Main output is rough information about which pairs could not form dimers, and which are candidates for further studies.

[1] T. Ichimura et al., „Molecular cloning of cDNA coding for brain-specific 14-3-3 protein, a protein kinase-dependent activator of tyrosine and tryptophan hydroxylases", Proc Natl Acad Sci U S A, č. 19, s. 7084–7088, říj. 1988, doi: 10.1073/pnas.85.19.7084.
[2] N. N. Sluchanko a N. B. Gusev, „Oligomeric structure of 14-3-3 protein: what do we know about monomers?", FEBS Lett, roč. 586, č. 24, s. 4249–4256, pro. 2012, doi: 10.1016/j.febslet.2012.10.048.
[3] X. Yang et al., „Structural basis for protein-protein interactions in the 14-3-3 protein family", Proc Natl Acad Sci U S A, roč. 103, č. 46, s. 17237–17242, lis. 2006, doi: 10.1073/pnas.0605779103.
[4] Z. Jandova, Z. Trosanova, V. Weisova, C. Oostenbrink, a J. Hritz, „Free energy calculations on the stability of the 14-3-3ζ protein", Biochim Biophys Acta Proteins Proteom, roč. 1866, č. 3, s. 442–450, bře. 2018, doi: 10.1016/j.bbapap.2017.11.012.
[5] Z. Trošanová et al., „Quantitation of Human 14-3-3 ζ Dimerization and the Effect of Phosphorylation on Dimer-monomer Equilibria", Journal of Molecular Biology, roč. 434, č. 7, s. 167479, dub. 2022, doi: 10.1016/j.jmb.2022.167479.

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84

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Title: Bottom-up and Top-down strategies for production and characterization of human multi-protein complexes.

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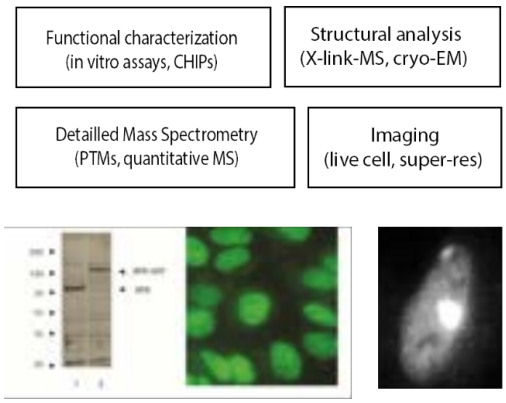
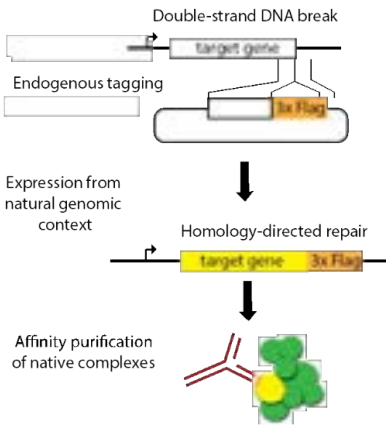
Abstract:

Macromolecular complexes govern the majority of biological processes and are of great biomedical relevance as factors that perturb interaction networks underlie a number of diseases, and inhibition of protein-protein interactions is a common strategy in drug discovery. Recombinnant approaches in insect and mammalian cells for the production and reconstitution of multiprotein complexes but are not generally applicatble, in particular for large macromolecular assemblies. Genome editing technologies enable precise modifications in protein coding genes in mammalian cells, offering the possibility to introduce affinity tags or fluorescent reporters for proteomic or imaging applications in the bona fide cellular context. Here we describe a streamlined procedure which uses the CRISPR/Cas9 system for efficient generation of homozygous endogenously tagged human cell lines. Establishing cellular models that preserve native genomic regulation of the target protein is instrumental to investigate protein localization and dynamics using fluorescence imaging but also to affinity purify associated protein complexes using anti-GFP antibodies or nanobodies.





References:

Geny S, Pichard S, Poterszman A, Concordet JP Gene tagging with the CRISPR-Cas9 system to facilitate macromolecular complex purification. Methods Mol Biol. Structural Proteomics. Methods Mol Biol. 2021;2305:153-174. doi: 10.1007/978-1-0716-1406-8_8. PMID: 33950389

Geny S, Pichard S, Brion A, Renaud JB, Jacquemin S, Concordet JP and Poterszman A, Tagging proteins with fluorescent reporters using the CRISPR/Cas9 system and double stranded DNA donors Multiprotein complexes: Methods and Protocols. Methods Mol Biol. 2021;2247:39-57. doi: 10.1007/978-1-0716-1126-5_3. PMID: 33301111

Sandoz, J., Nagy, Z., Catez, P., Caliskan, G., Geny, S., Renaud, J.B., Concordet, J.P., Poterszman, A., Tora, L., Egly, J.M., et al. (2019). Functional interplay between TFIIH and KAT2A regulates higher-order chromatin structure and class II gene expression. Nat Commun 10, 1288.

Structural and mechanistic studies of spider silk N-terminal domains

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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 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 major ampullate spidroin (MaSp) NT was early on found to be pH sensitive. In the ampulla of the spider silk gland, where spidroins are stored, MaSp NT forms a monomeric five-helix bundle and promotes solubility of the spidroins. As spidroins are pulled through the spider silk duct, the environmental pH is reduced, which causes protonation of a cluster of glutamic acids on the NT's surface. This disrupts several charge interactions leading to dimerization of the domain. The NT dimerization results in firmly interconnected spidroins, which ensures propagation of pulling forces during the silk fiber formation. Here, we compare the pH dependent dimerization mechanism between NTs from different silk types by studying the solution structures and behaviour of the wild type proteins as well as several site-directed mutants bearing glutamate to glutamine substitutions. This shows that the pH dependent dimerization mechanism is conserved among the different spider silk types despite significant sequence differences.

Autotaxin is required for specific LPA cell signaling

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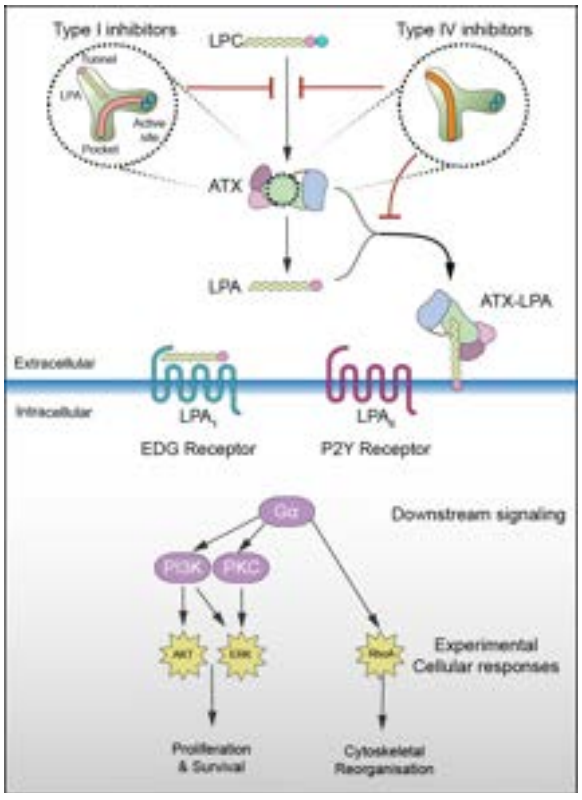
Autotaxin (ATX; ENPP2) produces the lipid mediator lysophosphatidic acid (LPA) that signals through disparate EDG (LPA_{1-3}) and P2Y (LPA_{4-6}) G protein-coupled receptors. ATX/LPA promote several (patho)physiological processes, including in pulmonary fibrosis, thus serving as attractive drug targets. However, it remains unclear if clinical outcome depends on how different ATX inhibitors modulate the ATX/LPA signaling axis. Here, we show that inhibitors binding to the ATX "tunnel" specifically abrogate key aspects of ATX/LPA signaling. We find that the tunnel is essential for signaling efficacy and dictates cellular responses independent of ATX catalytic activity, with a preference for activation of P2Y LPA receptors. These responses are abrogated by tunnel-binding inhibitors, but not by inhibitors that exclusively target the active site, as shown in primary lung fibroblasts and a murine model of radiation-induced pulmonary fibrosis. Our results uncover a receptor-selective signaling mechanism for ATX, implying clinical benefit for tunnel-targeting ATX inhibitors.

Keywords: ENPP2, lysophosphatidic acid, lipid chaperone, pulmonary fibrosis, G protein-coupled receptor, drug development, small-molecule inhibitor, structure–function



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87
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Bacterial nano-compartments, their function in metal homeostasis and role in the cellular response mechanisms against stress

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Bacterial nano-compartments are known to exist in bacteria. We have studied the electron dense granules (EDG), from the radiation resistant bacteria *Deinococcus radiodurans* and the involvement of both DNA-binding proteins Dps1 (*dr2263*) and Dps2 (*drb0092*) [1-3] on these cellular substructures. *Dr*Dps are ferritin-like proteins, with the ability to store Mn and Fe as well as to bind/protect DNA under *in vitro* conditions [3]. Formation of EDG in *Dr*Dps knockout mutants is abolished, suggesting that these proteins play an important role on the formation and regulation of these bacterial nano-compartments. Using X-ray fluorescence nano-imaging data (ID16A-NI, ESRF), we have investigated the metal content in these nano-compartments, and our results showed that these are element-rich regions, namely with phosphorous, calcium and manganese [4]. Therefore, these nano-compartments act as element-rich regions under control conditions, which are triggered to release the different elements when cells are subject to stress [4]. In order to increase our molecular and structural insights on these regions, we applied for an ACCESS Instruct-Eric proposal to perform STEM analysis coupled with Electron Dispersive X-ray Spectroscopy (EDS) on the *Dr*Cells. We will present our recent results, where we will show the details of these nano-compartments regarding its heterogeneous metal composition across different cells.

References

1. Romao et al (2006) *JBIC*, 11, 891.
2. Cuypers et al., (2007) *JMB*, 371, 787.
3. Santos et al (2015) *FEBS J*, 282, 4307.
4. Santos et al (2019) *Scient Rep,* 9, 17217.

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88
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Title: Bioinformatical analysis of *Proteus* bacteriophage **65APm2833** receptor binding proteins and *Proteus mirabilis* phage receptor by genomes sequence analysis

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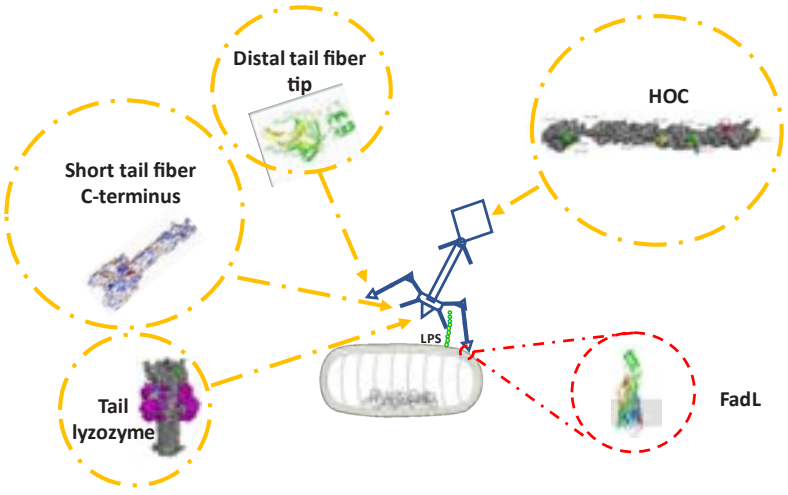
Presenter: **Rafał Matusiak,** Proteon Pharmaceuticals S.A., Łódź Poland, rmatusiak@proteonpharma.com

Proteus mirabilis is a Gram-negative, facultatively anaerobic, rod-shaped bacterium. It shows swarming motility and urease activity. *P. mirabilis* causes 90% of all *Proteus* infections in the urinary tracts of humans. Almost all *P. mirabilis* strains are sensitive to, fluoroquinolones, aminoglycosides, β-lactams, and trimethoprim/sulfamethoxazole, but are resistant to tetracycline and nitrofurantoin. The AMR (antimicrobial resistance) refers to the ability of microorganisms to withstand antimicrobial treatments which is the effect of overuse or misuse of antibiotics. The effect of this action is the emergence and spread of microorganisms that are resistant to them, rendering treatment ineffective and posing a serious risk to public health. The solution for increasing AMR is among other bacteriophage therapy. The bacteriophage therapy uses natural bacterial antagonists (bacteriophages), which are viruses killing bacteria, collected and mixed in a special cocktail that can kill AMR pathogenic strains.

In this study, we present results of the bioinformatic genome and structural analysis of P. mirabilis phage 65APm2833 and its host and phage resistant mutant of the host. The bioinformatic analysis predicted that this phage has T4 like morphology and belongs to unclassified Tevenvirinae. We detected and characterized regions encoding virion structural proteins involved in interaction with the environment and phage receptor recognition and predicted their structures by homology modelling and de novo modelling using AlphaFold2 software. Bioinformatic analysis showed that this bacteriophage needs two receptors for attachment to the cell's surface (outer membrane protein and lipopolysaccharide core). As a result of bioinformatic analysis, the regions encoding e.g.: short tail fiber involved in recognition of lipopolysaccharide core and tail fiber distal tip involved in interaction with some outer membrane proteins were described. The analysis of single nucleotide polymorphism (SNP) allowed detecting crucial mutations in phage resistant mutant of *P. mirabilis*. The first noticed mutation was the in-frame insertion of region encoding four amino acids, in the region encoded phosphoethanolamine transferase MCR-3.4. Structure prediction and bioinformatical functional analysis showed that this mutation is in the cell membrane attachment subunit and probably increases binding affinity to the inner cell membrane. The second detected mutation is the insertion in the region encoded FadL (Long-chain fatty acid transport protein). This protein is involved in the translocation of long-chain fatty acids across the outer membrane. This mutation inserts additional adenine which makes frameshift and generates a stop codon. Bioinformatic analysis showed that mutated protein loses 38% of the sequence, which encode part of β-barrel. These changes decrease predicted binding affinity to the outer cell membrane and destroy protein structure.

The results of the conducted bioinformatics analyses allowed for the characterization of virion and identification of bacteriophage receptors (LPS core and FadL) for *P. mirabilis* phage 65APm2833, which is crucial for the development of more effective bacteriophage cocktails against antibiotics resistant *Proteus mirabilis* strains.

Graphical abstract:



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89
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Essential transcription regulator Rta of Epstein-Barr virus - functional and structural implications for new antiviral strategy

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The Epstein-Barr virus (EBV) is one of the most common human viruses that infects more than 90% of the world population during their lifetime. EBV causes 200,000 cancer cases per year. EBV is associated with various premalignant lymphoproliferative diseases, including Hodgkin's lymphoma, gastric cancer, and nasopharyngeal carcinoma and is infectious mononucleosis [1]. Besides cancer, infectious mononucleosis and multiple sclerosis are linked to EBV [2, 3].

Replication and transcription activator (Rta) is essential for the EBV life cycle because Rta reactivates lytic phase of EBV from latency. Rta binds to Rta Response Element (RRE) localized on viral DNA and transactivates a series of lytic genes, including the viral lytic gene PAN [4]. Rta has not been structurally characterized yet. Furthermore, no direct homologies were identified compared to other known DNA binding or dimerization motifs [5].

Rta targeting with small molecules presents a new potential approach in the fight against EBV-associated diseases. Thus, a detailed understanding of the Rta structure and oligomeric state is critical for future rational anti-EBV drug design.

We will present the first structural characterization of Rta, Rta interactions with DNA and host proteins along with Rta localization in human cells.

1. Rezk S.A., Zhao X., and Weiss L.M., Epstein-Barr virus (EBV)–associated lymphoid proliferations, *Human Pathology*, **79**, (2018), 18-41.
2. Williams H., Crawford D.H., Epstein-Barr virus: the impact of scientific advances on clinical practice, *Blood*, **107** (2006), 862–869.
3. Bjornevik, K., Cortese, M., Healy, B.C., Kuhle, J., Mina, M.J., Leng, Y., Elledge, S.J., Niebuhr, D.W., Scher, A.I., Munger, K.L., Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis, *Science*, **375**, (2022), 296-301.
4. Feederle R., and Delecluse H., The Epstein-Barr virus lytic program is controlled by the co-operative functions of two transactivators, *The EMBO Journal*, **19**, (2000), 3080-3089.
5. Necasova I., Stojaspal M., Motycakova E., Brom T., Janovic T., Hofr C., Transcriptional regulators of human oncoviruses: structural and functional implications for anticancer therapy, *NAR Cancer*, **4**, (2022)

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Cryo-EM Facility at Instruct Center France1 for high-resolution imaging

Alexandre Durand, IGBMC/CBI, Illkirch Graffenstaden

The Cryo-EM facility of the Instruct Center France 1, located in the IGBMC/CBI (Institute of Genetic, Molecular and Cellular Biology/ Integrated Biology Center, Illkirch-Graffenstaden France) is part of the Integrated Structural Biology and provides a fantastic technological and scientific environment for integrative structural biology approaches.

The cryo-EM facility provides access to state-of-the-art electron microscopes for structure determination of molecular and cellular machineries by electron microscopy approaches such as single particle analysis and cryo-electron tomography. The CBI houses a Cs-corrected Titan Krios equipped with a BioQuantum imaging filter and a Gatan K3 direct electron detector, a 200kV Glacios TEM equipped with a K2 Summit camera and a Zeiss Auriga for FIB-SEM imaging and lamella milling.

The facility has developed and set-up unique approaches in sample preparation methods for cryo-electron microscopy. The facility is the first one, in Europe, to house a Chameleon freezing device. This next generation vitrification robot uses a fast-freezing approach to overcome the usual limits of the classical “plunge-freezing” method, namely preferential orientations and denaturation at the air-water interface. In addition, we have developed robust protocols for affinity grids production. Streptavidin crystals are immobilizing on a TEM grid and used to immobilize labeled samples, with high affinity and specificity. This tool offers unique possibilities in order to address challenging, fragile, or of low-abundance samples, by tackling the problems of preferential orientations and denaturation at air-water interface, or allowing concentration of diluted samples as well as and on-grid purification.

The Glacios microscope is equipped with an additional camera, the Ceta-D, and has been optimized for electron diffraction experiments. Complementary to x-ray crystallography also available in house, this new technic allows to record diffraction data on small crystals which could not be handled with the classical approach. In addition, sample thickness is usually the limiting factor and small crystals still have a thickness in the hundreds-of-nanometer range. To overcome this limit, the FIB-SEM can be used to mill bigger crystals in order to produce fine crystal lamellas, in cryo-condition, which can be transferred to the electron microscope.

Eventually, the facility has setup the computational resource required for addressing the challenge of the large data volume produces by recent cryo-electron microscopes, and for on-the-fly processing. This step allows to immediately monitor the quality of the data collected by the microscope and to tune the acquisition parameters in order to obtain the best possible data. It also allows to stop data acquisition if sample is not promising in order to optimize the microscope time. On-the-fly processing allows to obtain sub-3A resolution map within 24h when sample permits it.

The Sample Preparation & Characterization facility at EMBL Hamburg

Angelica Struve García, Stephan Niebling, David Ruiz Carillo and María García-Alai

The EMBL Sample Preparation & Characterization (SPC) facility is located within the DESY Campus in Hamburg, Germany. Our main task is supporting academic and industry researchers carrying out structural biology studies. In addition to that, our facility has a strong record in developing and implementing new technologies and approaches in the biophysics field.

Our high-throughput crystallization (HTX) laboratory offers a large array of commercial crystallization screens as well as customized screens for optimization of initial hits. Located next to the EMBL beamlines, users have access to automated crystal harvesting and data processing for streamlined crystallization experiments and synchrotron data collection. In addition, we offer assistance to perform SAXS batch measurements with near-real-time outputs of macromolecular structural parameters and low resolution solution-state structures.

The biophysical platform of the SPC includes cutting-edge technologies to measure biomolecular interactions and to precisely determine the stability, shape and size of different biomolecules and biomolecular assemblies. We offer a wide range of services, from initial protein quality control to more sophisticated biophysical approaches. Our online data analysis platform, eSPC, is available at spc.embl-hamburg.de and allows users to analyze and visualize biophysical data from anywhere in the world.

The SPC core facility is a partner of the MOlecular-Scale Biophysics Research Infrastructure (MOSBRI) and a member of other complementary research networks like iNEXT-Discovery, INSTRUCT-Eric and HALOS. These networks offer researchers from different fields to get fully-funded access to a wide range of methods to support their scientific projects.

Developing workflows to study mammalian gametes using cryogenic correlative light and electron microscopy (cryoCLEM)

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Mammalian gametes are highly specialized cells which represent extremes of cellular organization. Mature sperm have lost most of their cytoplasm and many of their organelles becoming one of the smallest cell, on the other hand the egg accumulates cytoplasm in preparation for embryonic development, thus becoming one of the largest cell types. Knowledge of gamete ultrastructure is vital for understanding how these special cells function. We are developing multiple workflows that allow imaging mammalian gametes at close to native conditions and at molecular resolution. Sperm cells are plunge frozen and if localisation is needed they are first imaged under a cryo-fluorescence microscope. Thin regions (<250nm), such as the distal part of the flagella, are imaged directly, while thicker regions, such as the midpiece or head, are either imaged with a volta phase plate (VPP), if the whole cell volume is needed, or are first thinned with cryo-focused ion beam (cryo-FIB) milling. Neural-network based segmentations are used to quantify organelle morphometrics and subtomogram averaging for determining molecular structures. By adopting a holistic approach, imaging sperm from 'head to tail', we revealed conserved as well as species-specific structural modifications that affect sperm motility. Oocytes, on the other hand, need to be high-pressure frozen due to size (~150 µm). Our attempts for FIB milling oocytes showed that it is limited to <50 µm. As such we are now implementing the waffle method for milling.

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93
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Fitting Side-Chain NMR Relaxation Data Using Molecular Simulations

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Proteins display a wealth of dynamical motions that can be probed using both experiments and simulations. We present an approach to integrate side-chain NMR relaxation measurements with molecular dynamics simulations to study the structure and dynamics of these motions (Figure 1A). The approach, which we term ABSURDer (average block selection using relaxation data with entropy restraints), can be used to find a set of trajectories that are in agreement with relaxation measurements. We apply the method to deuterium relaxation measurements in T4 lysozyme and show how it can be used to integrate the accuracy of the NMR measurements with the molecular models of protein dynamics afforded by the simulations. We also show how fitting of dynamic quantities leads to improved agreement with static properties.
In addition, we highlight areas in need of further improvements and explore these by optimising force field parameters using NMR relaxation data (Figure 1B). Applying those optimised parameters improves the estimation of NMR relaxation rates from molecular dynamics simulations. Finally, we demonstrate that the optimised force field becomes even more effective in combination with our reweighting approach.

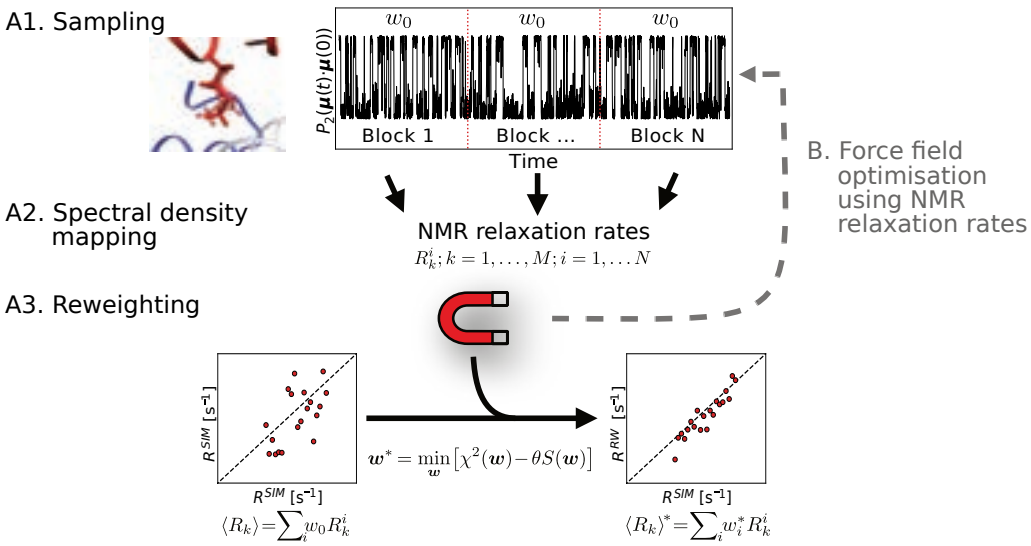

Figure 1. (A) Schematic representation of the ABSURDer workflow. Step 1 involves sampling protein dynamics using one or more longer MD simulations and calculating bond vector orientations for the resulting trajectories. These are then divided into blocks, and in step 2, we calculate correlation functions, spectral densities, and NMR relaxation rates for each block. In step 3, we optimize the agreement between the average calculated rates and experimentally measured values by changing the weights of the different blocks. (B) For the force field optimisation we use the same NMR relaxation data to optimise potential energy barriers of methyl group rotation.

Structure of Engineered Protein Targeting Zika Virus Envelope Protein

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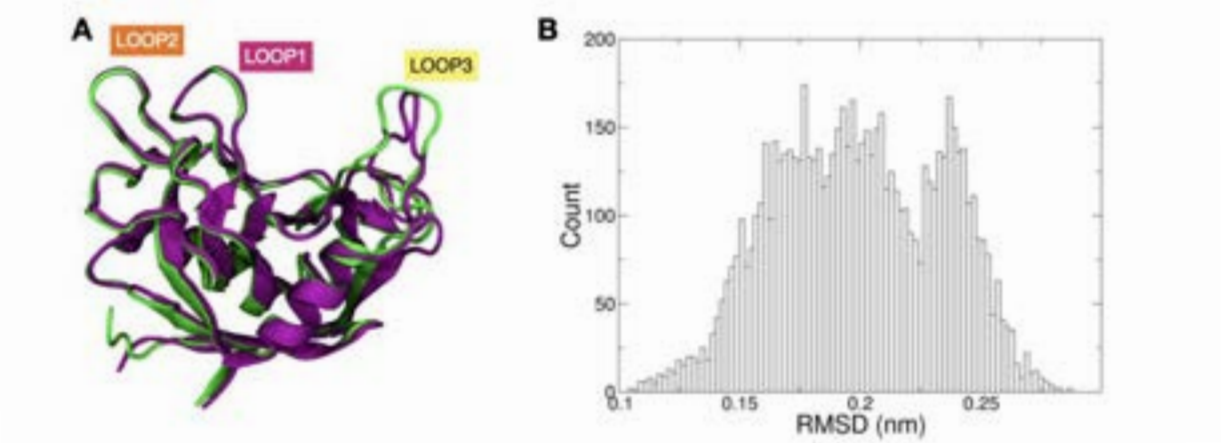
Synthetic proteins were computationally engineered to specifically bind to the Zika virus (ZIKV) fusion loop (FL) located on the (E) envelope protein. FL is a highly conserved region among flaviviruses that mediates cell infection and target of neutralising antibodies. These engineered proteins contain the immunogenic region of human neutralising antibodies (NAbs) grafted into selected scaffold proteins, such as cyclophilin, galectin-8 or interleukin. The calculated binding affinity of the engineered proteins to the FL was used as selection criterium. The best candidate (ZVPA3) was experimentally synthesized. It was shown to bind to its target with high-affinity, and it was able to efficiently neutralise ZIKV *in vitro*. The protein structure was determined by X-ray crystallography to validate the computational design, as well as to offer additional insights into the structural basis of flaviriral neutralisation targeting the FL envelope protein.
Studies are on-going to produce ZIKV E, NS1 proteins and virus-like particles (VLPs) in mammalian cell lines and form complexes with the synthetic proteins mimicking NAbs. The goal is to elucidate their 3D structures by X-ray Crystallography or single particle analysis cryo-electron microscopy (cryo-EM). understanding of the structural and molecular basis that dictate high affinity interactions between antigens and their respective antibodies and establishing a platform to engineer improved vaccine antigens.



Superposition of crystal structure of ZVPA3 with the predicted computational one (A), RMSD distribution of a trajectory of 100 ns of molecular dynamics using the experimental structure (B)

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95
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Single Particle Time Resolved CryoEM study of GroEL machinery

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Abstract:

GroEL is the most studies chaperonin system by far. However, even after decades of research, exact mechanism by which GroEL machinery works is still being debated. To carry out the function of protein folding, GroEL, in presence of ATP undergo several conformational changes. Many of these states are short lived and thus hard to capture by conventional structural biology techniques. In this work, we have applied method of time resolved Cryo-EM to elucidate the structural changes and the kinetics of GroEL-ES machinery. GroEL-ES samples were plunged at multiple time-points. Resulting structures resolve the structure of ATP-bound GroEL and kinetics of formation of GroEL-GroES complex. With this study we would like to bring the focus back to chaperon mediated protein folding using GroEL as an example.

Application of carbon-detected NMR experiments to study the solvent paramagnetic relaxation enhancement on intrinsically disordered proteins.

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Intrinsically disordered proteins (IDPs) lack a stable tertiary structure and can sample many conformations with different local structure and dynamic features. Nuclear magnetic resonance (NMR) spectroscopy has emerged as a leading technique for the characterization of structural and dynamic features of IDPs at atomic resolution.[1] Adding a small paramagnetic compound to the protein solution causes a paramagnetic relaxation enhancement (solvent PRE) that can be experimentally determined to obtain information about the properties of the protein.[2] Although this experimental approach usually relies on the measurement of PRE of amide protons, this strategy is not well suited for disordered proteins, due to the exchange with solvent. We used ^{13}C-detected exclusively heteronuclear NMR experiments,[3] modified by inclusion of a saturation recovery pulse sequence element, to measure the solvent PRE on the disordered protein α-synuclein and on one of the linkers of the CREB-binding protein (CBP-ID4). This alternative approach exploits carbon detection to avoid chemical exchange with the solvent and improve the resolution of NMR spectra.

1. Felli, I. C. & Pierattelli, R. (Eds) *Intrinsically Disordered Proteins Studied by NMR Spectroscopy*. Springer (2015).
2. Spreitzer, E., Usluer, S. & Madl, T. Probing Surfaces in Dynamic Protein Interactions. *Journal of Molecular Biology* vol. 432 2949–2972 (2020).
3. Felli, I. C. & Pierattelli, R. ^{13}C Direct Detected NMR for Challenging Systems. *Chemical Reviews* (2022).

Order in disorder: AUX/IAA protein and its TIR1-Aux/IAA auxin co-receptor system

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Auxin is a central signalling molecule in plant biology with roles in both the patterning of developmental events and the regulation of cellular growth. This is achieved via the TIR1/AFB-auxin-Aux/IAA co-receptor complex. Within this ternary complex, auxin acts as a molecular glue to promote binding of Aux/IAA transcriptional repressor proteins to $SCF^{TIR1/AFB}$ ubiquitin-ligase complexes, thereby catalysing their ubiquitin-mediated proteolysis. A conspicuous feature of the crystal structure of the complex is a rare *cis*W-P bond within the binding site. This binding site is centred on a 13 amino acid motif called the degron. We have used a combination of NMR and Molecular Dynamics simulations to gain insight into the solution structure of the amino-terminal half of the Aux/IAA-protein AXR3/IAA17 and its binding in complex with TIR1 and auxin. We show that while the protein presents as intrinsically disordered by NMR, still the critical degron W-P bond occurs with an unusually high (1:1) ratio of *cis* to *trans* isomers. While the WPP sequence is one of the most strongly *cis* proline promoting elements. In peptides containing this motif the population of the *cis* conformer is at most 36%. This demonstrates that there must be at least a transient structural element promoting the population of the *cis* conformer beyond this. Analysis of RDC's confirm a deviation of random coil stucture both in the degron motif and near the N-terminus, where a transient helix is formed that provides a key interface for the recruitment of the co-repressor TOPLESS. Molecular dynamics simulations give a view of the protein populating fluctuating secondary structure elements that associate into transiently structured motifs and imply a richness to the structural ensemble containing higher order structural elements that vary in nature. We then show that assembly of the co-receptor complex involves both auxin-dependent and -independent interaction events. Further, using the synthetic auxin molecule cvxIAA we show that a subset of auxin-dependent binding events occur away from the base of the canonical auxin binding pocket in TIR1. Our results suggests that the complex regulation of auxin dependent events is mirrored in the complex behaviour of the intrinsically disordered Aux/IAA-proteins that are central to the signalling cascade.

Acknowledgements: We acknowledge the Astbury BioStructure Laboratory (ABSL) for access to the 950 MHz spectrometer which was funded by the University of Leeds. The ABSL NMR, Cryo-EM and MassSpec facilities are a member of INSTRUCT-ERIC and accepts applications for measurement time from European based scientists. Contact the presenter for more information.

Keeping a secretory protein non-folded: the role of the chaperone SecB at single-molecule resolution

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About a third of the bacterial proteome is exported outside the cytoplasm. All these secretory proteins are synthetized in the cytoplasm and the vast majority (95%) is exported outside by the evolutionary conserved Sec translocase. However, this membrane-embedded channel only allows the secretion of non-folded clients, meaning that the synthetized client need to remain unfolded in the cytoplasm until its final delivery the secretion channel.

In that context, we are focusing on the role cytoplasmic chaperones to maintain a model secretory client in a dynamic non-folded state. We combined single-molecule Forster Resonance Energy Transfer (smFRET) and local hydrogen-deuterium exchange (HDX). The unique combination of both methods is powerful to capture some folding intermediates and observe the effect of different chaperones by smFRET since only the client is labeled and therefore its refolding observed. The addition of local HDX allowed us to describe the intrinsic dynamics of some folding intermediate.

Using this *in vitro* approach, we demonstrated that chaperones involved in the secretory pathway have some limited action on the refolding kinetics of the client, with SecB being the ideal chaperone. Indeed, SecB acts as a holdase, keeping the protein in an expanded and very dynamic state, competent for secretion and preventing its folding; but also, and for the first time observed, as a denaturase by reversing the preliminary folding of the protein and bringing intermediates to the expanded state. We also showed that the interaction with the mature domain (MBP) alone is different than with the form carrying the signal peptide (preMBP) to target to the secretion channel, meaning that the chaperone can sense its client, probably through folding kinetics.

Title: "Integrative structure of a 10-megadalton eukaryotic pyruvate dehydrogenase complex from native cell extracts"

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Oxidative decarboxylation of pyruvate is a key metabolic reaction during pyruvate catabolism and a vital step in aerobic metabolism performed by a giant enzymatic complex, the pyruvate dehydrogenase complex (PDHc). PDHc belongs to the α-keto acid/2-oxo-acid dehydrogenase complex (OADH complexes) family together with the 2-oxoglutarate dehydrogenase complex (OGDHc, also known as α-ketoglutarate dehydrogenase complex) and branched-chain keto-acid dehydrogenase complexes (BCKDHc). PDHc components have been characterized in isolation, but its quaternary structure remains elusive due to sheer size, heterogeneity and plasticity. By utilizing mass spectrometry, activity assays, crosslinking, electron microscopy and computational modeling, we identified fully assembled Chaetomium thermophilum α-keto acid dehydrogenase complexes in native cell extracts and characterized their domain arrangements. We reported the cryo-EM structure of the PDHc core and observed unique features of the previously unknown native state. We reconstructed asymmetrically the 10-MDa PDHc and resolved spatial proximity of its components, in agreement with stoichiometric data (60 E2p:12 E3BP:~20 E1p:≤12 E3), and proposed a minimum reaction path among component enzymes. PDHc shows the presence of a dynamic pyruvate oxidation compartment, organized by core and peripheral protein species. Our data provide a framework for further understanding PDHc and α-keto acid dehydrogenase complex structure and function.

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100
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Cryo-EM Snapshots of Nanodisc-Embedded Native Eukaryotic Membrane Proteins

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New membrane complex purification technologies in combination with cryo-electron microscopy (cryo-EM) recently allowed the exploration of near-native membrane protein complex architectures. Polymer nanodiscs in particular provide the basis to study overexpressed membrane proteins at high resolution while retaining protein—protein and protein—lipid interactions. However, how the majority of endogenous membrane proteins are organized remains elusive, mainly due to the inherent complexities that a hydrophobic environment poses to biochemical preparations.
In this work, we combined biochemical enrichment protocols for native membrane complexes together with amphiphilic polymers to increase the quality of recovered endogenous membrane complexes. The derived protein-encapsulated nanodiscs were identified by mass spectrometry and imaged with cryo-EM. This set of technologies is applied to *Chaetomium thermophilum*, a thermophilic fungus, that confers additional advantages for protein structure determination due to the increased thermal stability of its biomolecular assemblies.
Our results show a highly efficient recovery of protein-encapsulating nanodiscs, amenable to structural and biophysical characterization with a multitude of methods. Initial mass spectrometry results reveal ~1300 proteins while multiple 2D class averages from cryo-EM data show prominent nanodisc-embedded structural signatures. This combined methodological approach to isolate multiple endogenous membrane protein complexes provides unprecedented opportunities for a deeper understanding of the membrane proteome of a eukaryote.

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101
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Structural insights into the diversity of photosynthetic membrane proteins revealed by cryo-electron microscopy.

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Abstract

Photosynthesis is a global process during which light energy is harvested and converted into chemical energy and is achieved, at the molecular level, by assemblies of photosynthetic membrane proteins. Their basic architecture comprises antenna subunits and reaction centres, the former providing light absorption and transportation functions while the latter performs the light-induced charge separation followed by energy (electron) transfer. In this work, we present cryo-EM structures from different photosynthetic organisms across the tree of life that highlights the extreme architectural diversity of endogenous photosynthetic complexes. The resolution achieved allows us to disentangle stoichiometries, subunit proximities, interfaces and localization of reaction centres and antennas.
Our work reveals a structural basis of a distinct adaptation strategy to deal with diverse environmental conditions reflected by the assembly and reorganization of key photosynthetic membrane proteins. This study discloses the logic behind the adaptability and efficient energy transfer mechanisms across ecosystems engineered by nature.

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Instruct Biennial Structural Biology Conference

102

Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

The Mode of Action of Teixobactin

Rhythm Shukla[1,3], Francesca Lavore[1], Sourav Maity, Maik Derks[1,3], Alexandre MJJ Bonvin[1], Moreno Lelli[6], Harold D. MacGillavry[5], Wouter H. Roos[4], Eefjan Breukink[3], Kim Lewis[2], Markus Weingarth[1]

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Teixobactin, produced by unculturable bacteria and considered the first novel antibiotic in 30 years, has aroused tremendous interest worldwide (L.Ling *et al*, *Nature*, 2015). While it was known that teixobactin targets an essential lipid (called Lipid II) in the bacterial plasma membrane, the mechanism of teixobactin remained obscure because of technical problems to study drugs in biological membranes.

Here, by combining solid-state NMR (ssNMR) with modern microscopy methods, we resolve the mechanism of teixobactin from the micrometer to the atomistic scale (Figure 1). Thereby, we show that teixobactin self-assembling into fibrils that destroy the bacterial membrane, Is a novel mechanism and a paradigm shift in understanding how antibiotics kill bacteria[1,2]. Advanced microscopy techniques (super-resolution microscopy and high-speed atomic force microscopy) disclosed that teixobactin forms large fibrillar structures on the bacterial surface. Strikingly, these fibrillar assemblies severely damage the membrane, killing the bacteria. Using comprehensive ssNMR studies, we solved the high-resolution structure of teixobactin-Lipid II complex in membranes. This structure shows that teixobactin specifically targets an immutable part of Lipid II, explaining why bacteria has major difficulty to develop resistance against the drug.

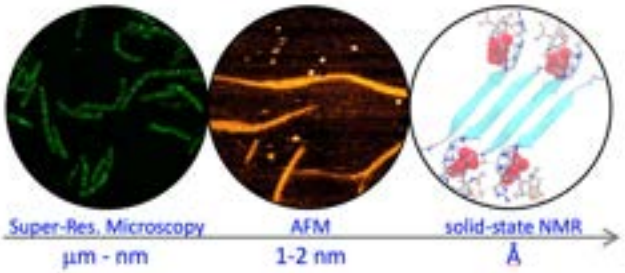

Fig 1. The killing mechanism from the micrometre to the atomistic scale.

Altogether, we have shown that teixobactin uses a novel dual killing mechanism by trapping Lipid II in an irreversible mesh of lethal fibrils that damage the membranes. This explains the excellent bactericidal activity of teixobactin and is a paradigm shift how antibiotics kill bacteria. This knowledge serves as a steppingstone towards the development of better antibiotics to tackle the urgent problem of antimicrobial resistance.

1) Shukla, R., Medeiros-Silva, J., Parmar, A., Vermeulen, B.J.A., Das, S., Paioni, L.A., Jekhmane, S., Lorent, J., Bonvin, A.M.J.J., Baldus, M., Lelli, M., Veldhuizen, E., Breukink, E., Singh, I., Weingarth, M. (2020) Nature Communications, 11, 2848, *Mode of action of teixobactins in cellular membranes*

2) Shukla, R., Lavore, F., Derks, M., Becker, L.M., Vermeulen, B., Wang, X., Kumar, R., Medeiros-Silva, J., Bonvin, AMJJ, Lorent, J., Lelli, M., Peoples, AJ., Spoering, AL., Ling, LL., Hughes, DE., Breukink, E., Lewis, K., Weingarth, M., *Teixobactin kills bacteria by a two-pronged attack on the cell envelope*, in revision

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103
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

Structural basis of CHMP2A-CHMP3 ESCRT-III polymer assembly and membrane cleavage

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The endosomal sorting complex required for transport (ESCRT) is a highly conserved multi-protein complex machinery that drives a divers set of physiological and pathological membrane remodeling processes. Membrane remodeling is essentially catalyzed by ESCRT-III filaments adopting different geometries that stabilize either negatively or positively curved membranes. The filaments are remodeled and ultimately recycled by the AAA-type ATPase VPS4, which sets the stage for membrane fission. However, the structural basis of ESCRT-III polymers stabilizing and constricting negatively curved membranes is unknown. Here we reconstituted the ESCRT-III CHMP2A and CHMP3 polymer within narrow membrane tubes. Cryo-electron microscopy structures of the membrane-coated CHMP2A-CHMP3 filaments of two different diameters at 3.3 and 3.6 Å resolution, respectively, show helical filaments assembled by CHMP2A-CHMP3 heterodimers in the open ESCRT-III conformation. Polymerization via helical hairpin stacking generates a partially positive charged membrane interaction surface, positions short N-terminal motifs for membrane interaction and the C-terminal VPS4 target sequence towards the tube interior. Inter-filament interactions are electrostatic, which can facilitate filament sliding upon VPS4-mediated polymer remodeling. Fluorescence microscopy as well as high speed atomic force microscopy imaging corroborate CHMP2A-CHMP3 membrane tube constriction and cleavage by VPS4. Our data thus reveal how CHMP2A-CHMP3 helical polymers and VPS4 can constrict narrow membrane necks and act as a minimal ESCRT-III membrane fission machinery resembling the ancestral ESCRT-III-VPS4 function.

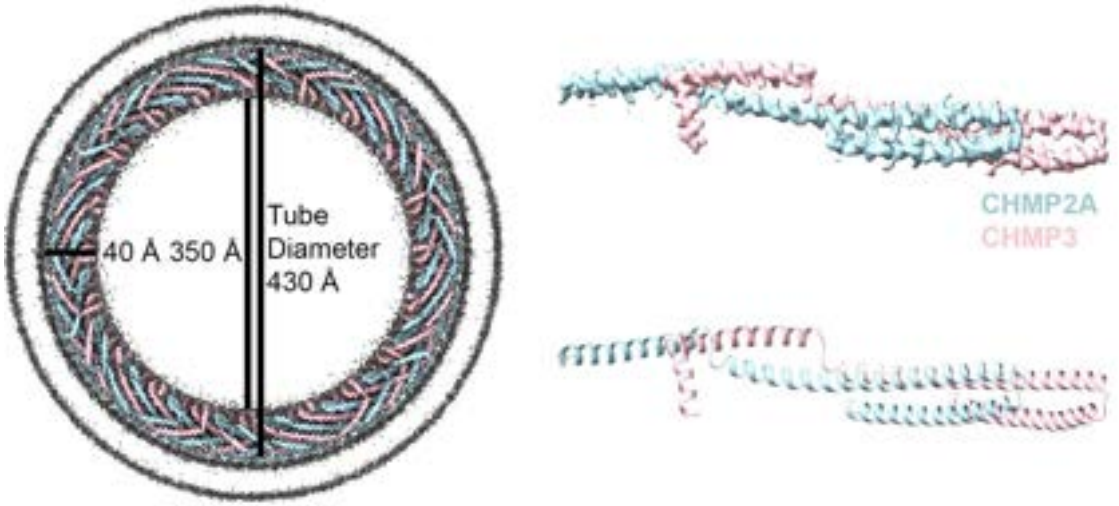

Figure: Cryo-EM structure of CHMP2A-CHMP3 membrane-coated helical polymer (left) and its repeating unit (right).

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Instruct Biennial Structural Biology Conference
104
Muntgebouw, Utrecht, The Netherlands I 19-20 May, 2022

Catching calcium transport in motion: Structural and dynamic investigation of LMCA1

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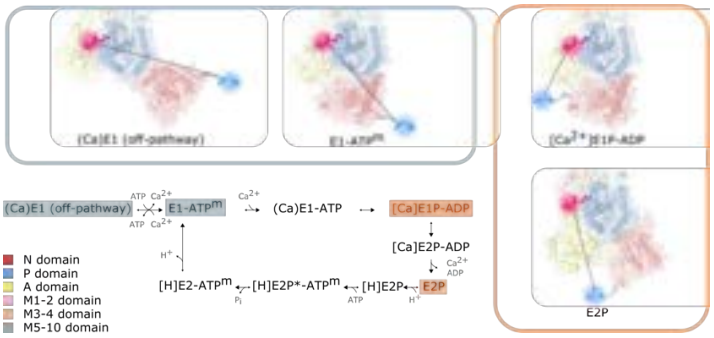
Ca^{2+} ATPases use ATP hydrolysis to transport Ca^{2+} across the cell membrane against a twenty thousand-fold concentration gradient. It is crucial that all steps in the pumping cycle are not reversible, thus the irreversible step enables Ca^{2+} transport against this concentration gradient.



Single-molecule Förster Resonance Transfer (smFRET) measurements have revealed that the intermediate [Ca]E2P-ADP state is important for the irreversibility of Ca^{2+}-ATPase 1 from *Listeria Monocytogenes* (LMCA1)[1]. ADP release from the nucleotide binding (N) domain is the potential point of no return of Ca^{2+} transport. smFRET measurements can determine the dynamics of the N domain during E1P to E2P transition and disclose the importance of ADP release. To investigate this further, cryogenic electron microscopy (cryo EM) of LMCA1 in the [Ca]E2P-ADP state can uncover which structural interactions are essential to pump against this steep concentration gradient.

Ca^{2+} transport is not continuously active; hence the pump needs to be turned off to conserve energy when not needed. However, the inhibitory mechanism of Ca^{2+} ATPases without a tethered inhibitory domain is poorly understood. Nucleotide binding to the N domain could be involved in switching the pump between active and inactive states. From crystal structures it seems that nucleotide binding facilitates a compact conformation between the N domain and the actuator (A) domain[2] in contrast to a detached N domain when nucleotide is absent[3]. smFRET measurements can determine the dynamics of the N domain and illuminate the regulatory mechanism of LMCA1.

Altogether, the smFRET measurements will elucidate how nucleotide binding can regulate both the irreversibility and activity of LMCA1, and in combination with structural information from cryo EM, we can uncover an important area of Ca^{2+} transport.

1 Dyla, M., Terry, D. S., Kjaergaard, M., Sorensen, T. L., Lauwring Andersen, J., Andersen, J. P., Rohde Knudsen, C., Altman, R. B., Nissen, P. & Blanchard, S. C. Dynamics of P-type ATPase transport revealed by single-molecule FRET. *Nature* **551**, 346-351, doi:10.1038/nature24296 (2017).
2 Sorensen, T. L., Moller, J. V. & Nissen, P. Phosphoryl transfer and calcium ion occlusion in the calcium pump. *Science* **304**, 1672-1675, doi:10.1126/science.1099366 (2004).
3 Toyoshima, C., Nakasako, M., Nomura, H. & Ogawa, H. Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 A resolution. *Nature* **405**, 647-655, doi:10.1038/35015017 (2000).

iNEXT-Discovery: Infrastructure for Trans-National Access and Discovery in Structural Biology

Hans Wienk[1] & the iNEXT-Discovery Consortium

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iNEXT-Discovery, the “infrastructure for transnational access and discovery in structural biology”, is the EC-funded Horizon2020 project for increasing the overall impact of structural biology research for society. The consortium combines thirty well-known academic European structural biology partners, willing to share and develop with others their structural biology expertise, and to open up access to advanced research equipment.

Trans-national access for external scientists and research groups is available to the state-of-the-art X-ray synchrotrons, ultra-high field NMR instruments, modern cryo-electron microscopes, and molecular biophysics instrumentation included in the project, either through physical visits or remotely. Our access is essentially free of costs after short user proposals have been submitted and approved upon peer-review. Special attention is given to attract all kinds of research sectors, including but not limited to biomedicine, biotechnology, agrofood sciences and biomaterial development. Therefore, one of the challenging objectives of iNEXT-Discovery is to provide access services to non-experts, while we also cater for experts in structural biology. As a result, our expert staff is used to be involved in many different topics and at different stages of research, ranging from proposal preparation to data acquisition, sometimes even assisting data analysis and integration of different methods. The iNEXT-Discovery access catalogue has been composed to allow for all different methods and expertise levels and uses an efficient access portal for continuous proposal submission. To specifically address certain research communities or topics, several calls for access are being issued, often connecting our access opportunities to services from other research infrastructures. Overall, iNEXT-Discovery has the capacity to support over 150 external user projects annually, until the end of the project in early 2024!

Besides the provision of access services, all our consortium partners heavily engage in networking, not just between them, but - perhaps even more important - with local, regional and international communities from academia and industry, with researchers from other research fields, and with the general public. With this, iNEXT-Discovery creates awareness about all benefits of structural biology with an ever-increasing part of society, at the same time attempting to include them actively in our project. Scientific meetings, and theoretical and practical workshops are other major focal points, as well as on-site research visits to the research facilities. Such training activities can be of particular interest to early career researchers, to expand their research methods portfolio by learning routine or emerging structural biology technologies.

In a different direction, we discuss and develop with each other and with other stakeholder groups the ‘structural biology research of the future’, creating outlook for instance towards long-term

sustainability of specific methods and efficient data management and curation. Related to that, our partners collaborate in joint research for method development. Together they (i) create new software for streamlining fragment-based drug discovery, (ii) increase throughput for data acquisition and analysis in the fields of cryo-EM and serial crystallography, (iii) improve light-induced, real-time, and in-cell solution and solid state NMR, and (iv) prepare and automate workflows for emerging and challenging technologies such as correlating soft X-ray tomography with super-resolution fluorescence microscopy, integrating solution X-ray scattering and NMR, and solid-state NMR dynamics and integration with cryo-EM.

The long-term objective of the vast collection of iNEXT-Discovery access, networking and joint research activities is to optimize and widen the structural biology access offered by our facilities to external researchers, and to promote and advance the field of structural biology worldwide.