Italian Crystallographic Association

6th Meeting of the Biological MacroMolecules Section

BOOK OF ABSTRACTS

San Domenico, Firenze

February 20-21, 2025

Centro Studi Ricerca e Formazione CISL

San Domenico, Firenze (Italy)

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6th AIC-BMM Meeting – Schedule and Scientific Program

Thursday, Feb 20th 2025

13.00 Participant registration and accommodation check-in. Appetizers & light lunch

14.30 Welcome by Rita Berisio, Coordinator of AIC-BMM

Afternoon Session I – Chair: Marco Mazzorana

Molecular mechanisms in biological processes

14.40 Invited speaker **Daniele Narzi.** *Department of Physical and Chemical Sciences, University of L'Aquila, Italy.*
Title: From Structure to Function: Integrating Theoretical Approaches to Elucidate the Catalytic Mechanism of Oxygen Evolution in Photosystem".

15.10 **Noemi Destefani.** *Biocrystallography Unit, Division of Immunology, IRCCS San Raffaele Scientific Institute, Milan, Italy.*
Title: Antigen-mediated and cell-autonomous B-cell receptor signalling in chronic lymphocytic leukaemia.

15.30 **Lorenzo Antonelli**. *Institute of Molecular Biology and Pathology (IBPM-CNR) and Department of Biochemical Sciences, University La Sapienza, Rome, Italy.*
Title: Deciphering the activation mechanism of Sigma 1 Receptor and the nature of its endogenous ligands.

15.50 **Alessandro Borsellini.** *Human Technopole, Milan, Italy.*
Title: Molecular mechanisms of condensin II activity.

16.10 Coffee Break & Snack

Afternoon Session II – Chair: Veronica Morea

Macromolecular structures as a strategy against infectious pathogens

16.40 Invited speaker: **Adele Di Matteo.** *Institute of Molecular Biology and Pathology (IBPM-CNR), Rome, Italy.*
Title: Tackling Signaling Pathways in *Pseudomonas aeruginosa* to Unveil Druggable Targets.

17:10 **Cécile Exertier**. *Institute of Molecular Biology and Pathology (IBPM-CNR), Rome, Italy.*
Title: A proof-of-concept study for the design of PROTACs (PROteolysis TArgeting Chimeras) to tackle Leishmaniases.

17.30 **Mario Privitera**. *Institute of Biostructures and Bioimaging (IBB-CNR) and University of Campania Luigi Vanvitelli, Caserta, Italy*.
Title: Structural features of KP32 phage portal attacking Klebsiella pneumoniae cell wall. Integrating structural biology and AI to unravel a complex biological problem

17.50 **Valeria Napolitano**. *Institute of Biostructures and Bioimaging (IBB-CNR), Naples, Italy*.
Title*:* Structural basis of protein encapsulation in Klebsiella pneumoniae and applications against multi-drug resistant pathogens.

18.10 **Filippo Vascon**. *Department of Biology, University of Padua, Italy.*
Title: Deciphering the Bacterial SOS Response: From Structural Insights to Inhibitor Development.

18.30 Poster flash presentations. Chair: Stefano Mangani

18.30 **Bianca Braggiotti.** *Institute of Biophysics (IBF-CNR), Milan, Italy.*
Title: TRAF2 in TNF Signaling and Immune Regulation: Structural Insights and Therapeutic Potential in Cancer.

18.35 **Vittorio Brufani**. *Institute of Molecular Biology and Pathology (IBPM-CNR) and Department of Biochemical Sciences, University La Sapienza, Rome, Italy*.
Title: Investigating BiP's Function in the Regulation of S1R Activity.

18.40 **Viviana Perrotta.** *Institute of Biostructures and Bioimaging (IBB-CNR), Naples, Italy*.
Title: Understanding PE_PGRS, cell wall proteins promoting mycobacterial survival in TB.

18.45 **Chiara Marella D'Aprile.** *Institute of Crystallography (IC-CNR), Bari, Italy.*
Title: Bacteriorhodopsin/Perovskite adducts for Next-Generation of in-crystal lab-on-chip Solar Cells.

18.50 **Martina D'Auria.** *Department of Chemical Sciences, University of Naples Federico II, Italy.*
Title: A novel PET hydrolase derived from thermophilic microorganism.

18.55 **Ornella Ghilardi.** *Institute of Biostructures and Bioimaging (IBB-CNR) and Department of Medical Biotechnologies, University of Siena, Italy.*
Title: Crystal structure and biophysical characterisation of the enterococcal foldase PpiC, a cross-opsonic antigen against Gram-positive nosocomial pathogens.

19.00 **Federica Sonzini.** *Department of Biosciences, University of Milan, Italy.*
Title: The open dimeric conformation of cardiotoxic light chains: a key feature underlying soluble toxicity in cardiac AL amyloidosis.

19.05 **Doriano Lamba.** *Institute of Crystallography, National Research Council, Trieste Outstation, Trieste, Italy.*
Title: Disentangling the mechanism underlying the covalent methanesulfonyl fluoride acetylcholinesterase adduct formation and evolvement: structural and mechanistic insights into an aged-like inactive complex susceptible to reactivation by a combination of nucleophiles

19.10 **Arianna Lardieri.** *Department of Biochemical Sciences, University La Sapienza, Rome, Italy.*
Title: Cryo-EM structure of Apo-PdxR from Bacillus clausii in complex with its target DNA.

19.15 **Louiza Moudoud.** *Biocrystallography Group, IRCCS San Raffaele Scientific Institute, Milan, Italy.*
Title: Thermal and Structural Characterization of Rv0678: Insights into Multidrug Resistance in Tuberculosis.

19.20 **Patrizio Di Micco.** *Institute of Molecular Biology and Pathology (IBPM-CNR), Rome, Italy.*
Title: Face2Face: A simple tool for macromolecule interfaces analysis.

19.25 **Gianmarco Pascarella.** *Institute of Molecular Biology and Pathology (IBPM-CNR), Rome, Italy.*
Title: Consensus3D. A comprehensive pipeline for accurate detection of conserved protein regions based on structure alignment programs consensus.

19.30 **Veronica Morea.** *Institute of Molecular Biology and Pathology (IBPM), National Research Council of Italy (CNR), Rome*
Title: Structure-based design of peptides for biomedical applications

Conclusions of Day I

19.40 *Aperitif & appetizers*

20.30 *Social Dinner* @ Ristorante Pizzeria San Domenico, Piazza S. Domenico, 11, 50014 Fiesole FI, Italia (www.pizzeriasandomenico.it/)

Friday, Feb 21st 2025

Morning Session I – Chair: Massimiliano Perduca

Drug development

9.00 Invited speaker: **Grzegorz Popowicz.** *Helmholtz Zentrum München – German Research Center for Environmental Health (GmbH), Munich, Germany.*
Title: From AI crystallographer to AI drug designer? Where advanced computational tools can help us understand molecules.

9.30 **Luisa Napolitano.** *Structural Biology Laboratory, Elettra Sincrotrone Trieste and Institute of Crystallography (IC-CNR), Trieste, Italy.*
Title: High Resolution Structure of StARD3 protein in complex with VS1 inhibitor.

9.50 **Elisa Fagnani.** *Institute of Biophysics (IBF-CNR), Milan, Italy.*
Title: Development of BIR1-targeting compounds for cancer treatment: study of IAPs modulation in NF-KB pathway.

10.10 **Andrea Dalle Vedove.** *AREA Science Park and Protein Targets for Drug Discovery Lab, Elettra-Sincrotrone Trieste, Italy.*
Title: What's new in Elettra's Structural Biology Laboratory.

10.30 **James Parry.** *Unchained Labs*
Title: Meet the Aunty - Queen of Protein Stability

10.40 Coffee Break & Snacks

Morning Session II – Chair: Silvia Onesti

Large macromolecular complexes by integrated structural biology

11.00 Invited speaker: **Philipp Sebastian Herdmann**. *Human Technopole, Milan, Italy.*
Title: Towards a Biopsy at the Nanoscale.

11.30 **Luca Mazzei.** *Department of Pharmacy and Biotechnology, University of Bologna, Italy.*
Title: Structural characterization of urease activity and inhibition: an integrated X-ray crystallography and cryo-EM study.

11.50 **Christian Buratto.** *Department of Chemical Sciences, University of Padua, Italy.*
Title: Ruthenium-based polyoxometalates as a possible chaperone in cryo-EM structure determination: a case study.

12.10 **Luca Broggini.** *Department of Biosciences, University of Milan, Italy*.
Title: Structural characterization of Atrial Natriuretic Peptide amyloid fibrils from Isolated Atrial Amyloidosis patients.

12.30 **Sofia De Felice.** *Department of Biology, University of Padua, Italy.*
Title: Structural and functional characterization of hyper-stable human serum albumin variants.

13.00 Announcement of the AIC Prizes for six oral young participants

13.15 Closing remarks & light lunch

Invited Lectures
(in chronological order of presentation)

From Structure to Function: Integrating Theoretical Approaches to Elucidate the Catalytic Mechanism of Oxygen Evolution in Photosystem II

Daniele Narzi[a], Matteo Capone[b], Gianluca Parisse[a] and Leonardo Guidoni[a]

[a] Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi dell'Aquila, Italy; [b] Center S3, CNR Institute of Nanoscience, Modena, Italy; daniele.narzi@univaq.it

Water oxidation in natural oxygenic photosynthesis is catalyzed by Photosystem II (PSII), a protein-cofactor complex. The catalytic mechanism of PSII progresses through five sequential steps (S_0 - S_4) in the so-called Kok-Joliot cycle. In this cycle, after the accumulation of four oxidizing equivalents on the Mn_4Ca cluster located at the core of the reaction center, two water molecules are oxidized to yield four electrons, four protons, and O_2. Gaining a detailed understanding of the molecular mechanisms underlying PSII's catalytic function may inspire the design and development of artificial devices capable of mimicking this process and, ultimately, storing solar energy in the form of solar fuels, such as molecular hydrogen.

Throughout the years, structural information on various states of the catalytic cycle has been obtained with increasing accuracy through extended X-ray absorption fine structure experiments and X-ray crystallography. More recently, serial femtosecond X-ray crystallography (1,2) has provided additional structural insights into the (meta)stable states of the Kok-Joliot cycle (S_0 - S_3). However, the exact catalytic mechanism, particularly the final stages of the cycle, remains elusive and widely debated.

During the past 12 years, by combining multilevel theoretical approaches, ranging from classical molecular dynamics simulations to highly accurate DFT-based QM/MM calculations, we have successfully interpreted several experimental findings, providing key insights into the potential catalytic mechanism of PSII (3,4,5,6,7,8). In this presentation, I will outline the key results obtained by our group across the last decade, demonstrating how computational techniques can play a crucial role in complementing and interpreting experimental data aimed at determining the structure of the catalytic center and elucidating its catalytic mechanism.

References: 1. J. Kern, *et al. Nature* (2018) 563:421-425 2. A. Bhowmick, *et al. Nature* (2023) 617:629-636 3. D. Bovi, D. Narzi and L. Guidoni *Angew. Chem. Int. Ed.* (2013) 52: 11744 4. D. Narzi, D. Bovi and L. Guidoni *Proc. Natl. Acad. Sci. USA.* (2014) 111: 8723 5. M. Capone, *et al*. J. Phys. Chem. Lett. (2016) 7: 592, 6. D. Narzi, *et al*. Chem. – Eur. J. (2017) 23: 6969 7. M. Capone, *et al. Biochemistry* (2021) 60: 2341 8. P. Greife, *et al. Nature* (2023) 617: 623

Tackling signaling pathways in Pseudomonas aeruginosa to unveil druggable targets

G. Pistoia[a], F. Giordano[a], L. Caruso[b], M. Mellini[b], M. Cervoni[b], G. Rampioni[b], F. Imperi[b], C. Travaglini[a], A. Coluccia[c], J.B. Vicente[d], E. Forte[a], A. Giuffrè[e], F. Troilo[e], G. Giardina[a], A. Di Matteo[e]

[a]Department of Biochemical Sciences, Sapienza University of Rome; [b]Department of Science, University Roma Tre, Rome; [c]Department of Drug Chemistry and Technologies, Sapienza University of Rome; [d]Institute of Chemical and Biological Technology António Xavier (ITQB NOVA), Portugal; [e]CNR Institute of Molecular Biology and Pathology, Rome, Italy.
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Multidrug-resistant (MDR) bacterial pathogens are rapidly spreading, while only a few new antibacterial drugs are in the pipeline.
Pseudomonas aeruginosa (PA) is a major opportunistic human pathogen responsible for over 300,000 deaths annually and one of the leading causes of chronic pulmonary infections in cystic fibrosis (CF) patients (1). Its high metabolic plasticity, ability to thrive in diverse environments, extensive arsenal of virulence factors, and resistance to multiple antibiotics highlight the need for innovative therapeutic strategies, including the design of adjuvants that can restore the efficacy of existing antibiotics.
In this context, using a multidisciplinary approach integrating microbiological, biochemical, and structural studies, we investigate proteins involved in the metabolism of two key signaling molecules: H_2S and the second messenger diadenosine tetraphosphate (Ap4A). H_2S is a pleiotropic signaling molecule whose metabolism is tightly regulated to prevent toxic effects. Its intracellular physiological levels result from a balance between biosynthesis and degradation pathways. Ap4A, in the other hand, is linked to stress resistance and virulence traits in PA. Recent studies have shown that ApaH, the enzyme responsible for Ap4A degradation, contributes to PA virulence, and its loss significantly impairs the bacterium's ability to cause infections (2).
In my presentation, I will focus on the structural and functional characterization of the Persulfide Dioxygenase (PDO), an enzyme involved in H_2S catabolism, and the Ap4A-hydrolyzing enzyme (ApaH), being both key players in signaling pathways of PA.

References: 1. Reynolds D, Kollef M. The Epidemiology and Pathogenesis and Treatment of Pseudomonas aerugi- nosa Infections: An Update. Drugs. 2021, 81(18):2117–2131; 2. Cervoni M, Sposato D, Ferri G, Bähre H, Leoni L, Rampioni G, Visca P, Recchiuti A, Imperi F. The diadenosine tetraphosphate hydrolase ApaH contributes to Pseudomonas aeruginosa pathogenicity. PLoS Pathog. 2024, 19;20(8):e1012486.

From AI crystallographer to AI drug designer? Where advanced computational tools can help us understand molecules.

Grzegorz M. Popowicz

Institute of Structural Biology, Helmholtz Zentrum München – German Research Center for Environmental Health (GmbH), Munich, Germany.

The application of machine learning (ML) in structural biology has already been transformative to the field.
Not only because of improved accuracy but with much better accessibility of structural models to life scientists. Meanwhile, ML models gain accuracy in predicting not only single domain structure but also multimeric complexes of diverse molecules. Moreover, a reverse folding models are able to generate realistic structure with pre-programmed properties without biological template.
Yet, the progress of structure-based drug discovery seems to lag behind the overall wave of ML developments. This can be attributed to much noise in the training data and poor coverage of nearly infinite chemical combinatorial space by knowns experimental structure.
We present an ML model designed to circumvent these limitations. The approach, called Target Preference Mapping, treats each interface between biomolecule and a drug as a large set of small microenvironments. They are then used to predict biomolecule preference for specific ligand chemistry. Chemical connectivity and binding energy are not used for the training to avoid overtraining of the model on the known complexes. We show that this approach allows us to predict drug optimization and perform virtual screening at unprecedented speed and accuracy.
Similar approach was recently tested for Protein-protein interactions, we how some preliminary observations on these interactions as well.

SOLIST: Towards a Biopsy at the Nanoscale

Philipp Erdmann[a], Jasmine Nguyen[a], Gaia Perone[a]

[a] Human Technopole, Palazzo Italia, Via Rita Levi Montalcini, 1, 20157 Milan

Deciphering molecular processes within complex biological systems requires imaging techniques that combine nanoscale resolution with mesoscale context. To address this need, we developed Serialized On-Grid Lift-In Sectioning for Tomography (SOLIST)[1], an innovative cryo-lift-out method that overcomes key limitations in cryo-electron tomography (cryo-ET) of high-pressure frozen samples, such as low throughput, ice contamination, and sample instability, which have historically restricted cryo-ET to simpler systems such as single cells.

SOLIST introduces a tenfold increase in the number of lamellas generated per lift-out compared to traditional methods, significantly streamlining the workflow and reducing the time required for sample preparation. With SOLIST, we achieve subnanometer resolutions within just one day of sample preparation and data acquisition. This efficiency is critical for optimizing the use of precious biological specimens, including patient-derived tissue biopsies and iPSC-derived organoids. Our method also improves the consistency and quality of cryo-ET datasets by reducing ice contamination and enhancing sample stability. These advances enable the detailed visualization of cellular structures in their native environment. With SOLIST, we reveal, for example, the architecture of phase-separated chromatin droplets in NUT carcinoma, map the structural organization of neuronal precursor cells in human brain organoids, and resolve ribosome-rich regions in native mouse liver tissue. Additionally, we observe sarcomeric thin and thick filaments in native mouse heart muscle from just a handful of tomograms, demonstrating SOLIST's broad applicability.

By bridging the gap between nanoscale resolution and mesoscale context, our method extends the reach of cryo-ET to medically relevant systems, for example, providing insights into human brain organoids, an important model system for neurodegenerative disorders. SOLIST, therefore, facilitates studies of human disease mechanisms and structural biology at an unprecedented complexity and scale.

With its serialized approach, our method maximizes data yield from every sample while minimizing waste and preserving the native molecular and structural integrity of complex biological systems. SOLIST, therefore, pushes the boundaries of cryo-electron tomography, offering a robust platform for high-resolution, in situ imaging and bringing us closer to achieving a "Biopsy at the Nanoscale."

References: 1. Nguyen, H. T. D. *et al.* Serialized on-grid lift-in sectioning for tomography (SOLIST) enables a biopsy at the nanoscale. *Nat. Methods* **21**, 1693–1701 (2024).

Oral Presentations
(in chronological order of presentation)

Antigen-mediated and cell-autonomous B-cell receptor signalling in chronic lymphocytic leukaemia

Noemi Destefani[a], *Louiza Moudoud*[a], *Marco Patrone*[a], *Nicholas Chiorazzi*[b], *Massimo Degano*[a,c]

[a]*Biocrystallography Unit, Division of Immunology, IRCCS San Raffaele Scientific Institute, via Olgettina 58, 20132 Milan, Italy;* [b]*The Feinstein Institute for Medical Research, Manhasset, New York 11030, USA;* [c]*Università Vita-Salute San Raffaele, via Olgettina 58, 20132 Milan, Italy; destefani.noemi@hsr.it*

Chronic lymphocytic leukaemia (CLL) is the most common type of leukaemia in the western world, characterized by the expansion of neoplastic $CD5^+$ B lymphocyte clones with a constitutively active B-cell receptor (BCR) mediated signalling, and heterogeneous clinical manifestations (1). The classification of stereotyped BCRs based on sequence homology in the heavy chain complementarity-determining region 3 (HCDR3) allowed the identification of several CLL subsets associated with a more homogeneous clinical outcome. In most CLL cases, the BCR signal is cell-autonomous, ensuing from homotypic interactions between BCRs that are distinctive in each subset (Figure 1). On the other hand, the roles of other self- and non-self-antigens in the disease have not yet been fully elucidated. We wish to further investigate BCR signalling in CLL by defining the intermolecular interactions of BCRs belonging to different subsets.

The immunoglobulin light chain gene 3-21 (IGLV3-21) bearing the G110R mutation is known to favour BCR self-recognition when paired with the homologous heavy chains IGHV3-21 or IGHV3-48 (2). We plan to characterize the structure and self-association of IGLV3-21 G110R paired to different heavy chains to better elucidate their relevance in BCR self-recognition.

In subset 4 BCRs, the HCDR3 mediates the self-recognition of a composite epitope comprising both the variable and constant BCR domains (3). Recently, a viral antigen was shown to bind to subset 4 BCRs homodimers, possibly contributing to clonal selection and disease onset. Therefore, we plan to characterize this ternary complex using cryoEM to provide the first description of a non-self-antigen interacting with a CLL-associated BCR.

The BCR antigen binding fragments (Fab) were expressed in Expi293F cells and purified through affinity and size exclusion chromatography. We obtained crystals of a IGLV3-21 BCR Fab and are currently determining its structure. Surprisingly, and in this case unwelcomely, the subset 4 BCR Fab crystallized spontaneously after affinity purification, requiring an extensive search of conditions to maintain the protein soluble.

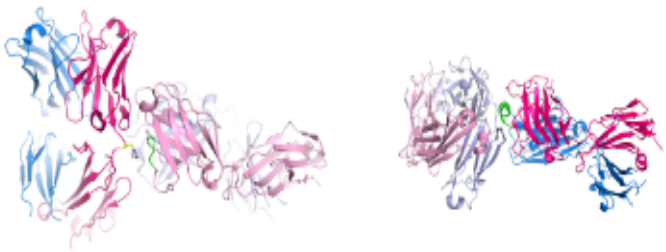

Figure 1. CLL BCR structures. Left, homotypic interactions between IGLV3-21-expressing BCRs, mediated by the light chain CDR2 (green) and the R100 side chain (yellow). Right, subset 4 receptors interacting via the HCDR3 loop (green).

References: 1. Nicholas Chiorazzi, et al. (2021). *Cold Spring Harb Perspect Med 11*:a035220; 2. Maity PC, et al. (2020) *Proc Natl Acad Sci USA 117*:4320-4327. 3. Minici, et al. (2017) *Nat Commun 8*, 15746.

Deciphering the activation mechanism of Sigma 1 Receptor and the nature of its endogenous ligands.

Lorenzo A., Annarita F., Gianmarco P., Giancarlo T., Cécile E., Vittorio B., Veronica M. and Andrea I.

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The Sigma-1 receptor protein (S1R) is a transmembrane protein resident in the endoplasmic reticulum, involved in various molecular mechanisms, including those related to neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis's disease (1). The neuroprotective role of S1R has led to increasing pharmacological interest. Significant advances have been made in understanding the biological role of the protein, and structural biology has made a strong contribution by solving crystallographic structures in complex with some molecules identified as agonists and antagonists of hS1R (2).
However, despite the progress made in recent years, some of the most important hS1R features, required to fully understand its function namely the receptor's endogenous ligand(s), the molecular mechanism of ligand access to the binding site, and the oligomerization mechanism influenced by agonists and antagonists have not yet been unequivocally determined. Using computational techniques such as molecular dynamics and virtual screening, and experimental techniques such as cryogenic electron microscopy (Cryo-EM) and fluorescence assays, we aimed at addressing these key characteristics of hS1R. To shed light on the nature of the endogenous hS1R ligand(s), we used a combination of computational virtual screening (VS), electron density maps fitting, and fluorescence titration assays to measure ligand binding to hS1R in vitro. We found that the ligands with the highest affinity for the receptor were molecules with a steroid motif, and among them, 16,17-didehydroprogesterone was shown by fluorescence titration to bind hS1R with significantly higher affinity than the prototypical hS1R ligand pridopidine in the same assay (3). Through molecular dynamics simulations, we investigated the mechanism of ligand entry into the binding site. It was hypothesized that ligands access the protein's binding site through a cavity that opens on the surface in contact with the membrane. This was supported by molecular dynamics studies that revealed conformational changes in the hS1R structure, particularly in the membrane-interacting helices, in agreement with previous structural studies on S1R from Xenopus laevis. In parallel, Cryogenic Electron Microscopy (Cryo-EM) was used to study the oligomeric structure of S1R in solution. Cryo-EM particle analysis revealed two main protein states: a trimer and a hexamer. The trimer conserves the same conformation of that observed in X-ray crystallography, whereas in the hexamer the trimers assume different conformations. In particular, the N-terminal α-helices assume a different conformation with respect to the trimer, used to form the oligomeric interface. These results are particularly important since they contribute to disclose the nature of the endogenous ligand, its entry pathway, and the mechanism of oligomerization, which are at the basis of the S1R activation mechanism and are important for the developing of new therapeutic compounds aimed at modulating the receptor in neurodegenerative diseases.

References: 1. Battista,T.; et al. *Int. J. Mol. Sci.* **2021**,22, 1293. 2. Schmidt, H.R.; et al. *Nature*. **2016**, 532, 527–530. 3. Antonelli, L.; et al. *Int. J. Mol*. Sci. **2023**, 24, 6367.

Molecular mechanisms of condensin II activity

Alessandro Borsellini, Valentina Cecatiello, Joanna J. Andrecka and Alessandro Vannini

Human Technopole; alessandro.borsellini@fht.org

Condensin II is a multi-subunit protein complex that, along with condensin I, cohesin, Top2A, and Kif4A, plays a crucial role in the proper compaction and organization of the genome during cell division in humans (1). As a member of the SMC family of proteins, condensin II utilizes energy from ATP in a process known as loop extrusion to generate loops of DNA, which then compact the disordered genome into chromosomes (2). Because condensin II is continuously present in the nucleus throughout the cell cycle, its activity must be precisely regulated to ensure that chromosome compaction occurs only during mitosis. It is known how the activity of condensin II is repressed by a protein named microcephalin (MCPH1), which prevents chromosome condensation during interphase (3).

Through proteomic approaches we have identified M18BP1, a protein previously associated with centromere identity, as the elusive factor required for condensin II localization to chromatin, and its activation at the onset of mitosis.

Using Cryo-EM, we determined multiple structures of condensin II at different stages of the ATP hydrolysis cycle, both in the presence and absence of DNA and M18BP1. Additionally, we employed optical trap technology to examine the activity of condensin II on a single DNA molecule, providing insight into how condensin II cofactors regulate the condensation process during mitosis. Together, our results offer new insights into the molecular mechanisms governing condensin II activation.

References: 1. Paulson JR, Hudson DF, Cisneros-Soberanis F, Earnshaw WC. Mitotic chromosomes. Semin Cell Dev Biol. 2021;117:7-29. doi:10.1016/j.semcdb.2021.03.014; 2. Mahipal Ganji et al. Real-time imaging of DNA loop extrusion by condensin. Science 360,102-105(2018).DOI:10.1126/science.aar783;1 3. Yamashita D, Shintomi K, Ono T, et al. MCPH1 regulates chromosome condensation and shaping as a composite modulator of condensin II. J Cell Biol. 2011;194(6):841-854. doi:10.1083/jcb.201106141

A proof-of-concept study for the design of PROTACs (PROteolysis TArgeting Chimeras) to tackle Leishmaniases

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Leishmaniases are neglected tropical diseases that are endemic in developing and under-developed countries and that affect up to one million people each year according to the World Health Organization. Leishmaniases are caused by protozoan parasites transmitted during the bloodmeal of infected female sandflies which natural habitat expands owing to environmental changes. Notably, visceral leishmaniasis, induced by *Leishmania infantum* infection, is characterized by a fever and the enlargement of the spleen and liver, which most of the time leads to death if untreated. So far, treatment against Leishmania infection showed poor efficacy, high toxicity and increasing resistance (1).

Leishmania parasites are capable to survive despite the oxidative environment inside host macrophages owing to their peculiar redox system, which relies on trypanothione, a variant of glutathione. Trypanothione is maintained reduced by the transfer of two electrons from NADPH through the redox action of the FAD-dependent trypanothione reductase (TR), a homolog of the glutathione reductase (2). TR is considered a very promising target since i) it is essential for parasite survival, ii) it diverges enough from the host glutathione reductase to offer a good selectivity and iii) it is rather easily druggable. Unfortunately, only compounds decreasing TR redox activity of ~90% may efficiently affect parasite survival. Despite the considerable efforts gathered over the last decades to design compounds inhibiting TR, none of the proposed ones has yet been accepted for clinical trials due to sub-optimal efficacy or high toxicity and therefore alternatives need to be explored (3).

Recently, targeted protein degradation has emerged as a promising strategy to traditional drug design. This methodology, which has proven its efficiency against cancer and neurodegenerative diseases, is based on the utilization of bifunctional molecules, namely PROTACs (PROteolysis Targeting Chimeras), to induce the ubiquitination of a target protein yielding its proteasome-dependent degradation (4).

We rationally designed PROTACs to lead to the ubiquitination and therefore to the degradation of *Leishmania infantum* TR. We assessed the biding capacity of promising PROTACS and characterized their interaction with TR by X-ray crystallography. Finally, we tested them against *Leishmania infantum* parasites and demonstrated the effective degradation of TR using western blot analysis and proteomics. These first results represent the proof-of-concept that the rational design of PROTACs is a valid strategy to tackle parasitic diseases.

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Structural features of KP32 phage portal attacking *Klebsiella pneumoniae* cell wall. Integrating structural biology and AI to unravel a complex biological problem

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Phage-derived depolymerases are an attractive option to adjuvate the effect of antibiotics as they can render antibiotic-resistant bacteria sensitive to the action of antibiotics. Only recently, a limited number of K. pneumoniae-targeting depolymerases have been structurally characterised (1,2). We previously demonstrated that KP32 phage, belonging to group A KP32 Podoviruses, encodes for two proteins with different CPS-degrading activity, KP32gp37 and KP32gp38, which degrade K3 and K21 capsular serotypes, respectively (1). By combining x-ray crystallography with mass spectrometry, we studied the mode of action of these depolymerases as antibiotics adjuvants. Furthermore, thanks to artificial intelligence, we provide a better understanding of the architecture of depolymerase containing RBPs in the Klebsiella phage KP32. On analogy with T7 phage active against E. coli, KP32 has a portal attached to its icosahedral capsid shell, formed by a dodecameric KP32gp11 and a hexameric KP32gp12. This portal forms a channel for viral DNA packaging and ejection. Being a tailed bacteriophage, KP32 exploits the KP32gp37-KP32gp38 depolymerase system to recognize host cells. This branching model allows the KP32 phage to carry 12 depolymerase molecules on a single phage, of which 6 with K21 and 6 with K3 serotype specificity. In this complex arrangement, communication between the portal, DNA, and the enclosing capsid shell may be a normal part of phage biology (3). In conclusion, our results highlight several novel features of the KP32 depolymerase system that, acting as a complex branched molecular machinery, allows the KP32 phage to efficiently recognize and hydrolyse bacterial CPS.

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Structural basis of protein encapsulation in Klebsiella pneumoniae and applications against multi-drug resistant pathogens

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Vaccines are an effective tool in combating antimicrobial resistant pathogens. Maximizing immunogenicity while ensuring safety and tolerability is the primary goal in vaccine design. This can be achieved by designing vaccines that mimic the essential characteristics of pathogens (such as size, shape, and surface molecule organization) without causing disease. It is well known that the size and geometry of vaccine antigens are pivotal in eliciting an effective immune response, with nanoparticle-sized antigens being particularly optimal (1). In this work we identified Kp-lin, an encapsulin from *K. pneumoniae* as a promising candidate for a vaccine delivery platform. Indeed, crystallographic and biophysical studies show that Kp-lin is a protein cage of 24 nm diameter with a highly repetitive architecture. Additionally, we demonstrated that Kp-lin can encapsulate a dye-decolorizing (DyP)-type peroxidase, encoded upstream of Kp-lin and likely protecting cells against oxidative stress. This peroxidase exists in multiple oligomeric species in solution and therefore has propensity to assemble into large oligomeric species within the Kp-lin cage. Our results provide structural insights into encapsulation processes in *K. pneumoniae* and a strong tool for the rational design of vaccine antigens against multiple nosocomial pathogens (2).

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Deciphering the Bacterial SOS Response: From Structural Insights to Inhibitor Development

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The bacterial SOS response to genotoxic stress is governed by the interplay between the DNA damage sensor RecA and the transcriptional repressor LexA. Oligomerizing on single-stranded DNA (a hallmark of genotoxic stress), RecA promotes LexA autoproteolysis, leading to the derepression of SOS genes, many of which drive disease-related phenomena such as hypermutagenesis, antibiotic resistance, and virulence. Therefore, the SOS response has been proposed as a target for innovative anti-evolutive and anti-virulence strategies. To advance the development of SOS response inhibitors, a deeper structural and functional understanding of its architecture is essential. In particular, the molecular details of the RecA-LexA interaction have remained a long-standing enigma.

To address this gap, we applied an integrative structural biology approach to characterize the SOS response in *Pseudomonas aeruginosa*. Using X-ray crystallography, we solved the structure of the LexA autoproteolytic domain, while cryo-electron microscopy (cryo-EM) provided insights into the RecA/ssDNA complex and the elusive RecA-LexA interaction. Our cryo-EM structure revealed a unique binding mechanism, that stabilizes LexA in the conformation required for self-cleavage. Fluorescence polarization-based binding and self-cleavage assays and analysis of RecA mutants further supported this model, shedding light on the molecular basis of RecA-induced LexA autoproteolysis (1).

In parallel, we developed potent anti-LexA nanobodies using llama immunization and phage display selection. LexA autoproteolysis assays demonstrated that these nanobodies are the most effective LexA inhibitors identified to date, while SOS gene expression profiling confirmed their ability to suppress the SOS response in antibiotic-stressed bacterial cells. X-ray structures of LexA-nanobody complexes revealed that these nanobodies, instead of targeting the LexA catalytic site, stabilize LexA's cleavable loop in an inactive conformation (2). This structural information fostered nanobodies optimization by computer-aided protein design, leading to variants with improved affinity and inhibitory activity on LexA.

Beyond answering long-standing questions on the SOS response, this work lays the foundation for innovative anti-evolution and anti-virulence strategies in antimicrobial research.

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High Resolution Structure of StARD3 protein in complex with VS1 inhibitor

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StAR-related lipid transfer domain-3 (STARD3) is a sterol-binding protein, anchored at the membrane of late endosomes. Initially identified as a lutein-binding proteins due to its high-degree of homology to the carotenoid-binding proteins found in silkworm (1), StARD3 has been proposed to be specifically involved in cholesterol transport, transferring such sterol from endoplasmic reticulum and vice versa (2). Interestingly, StARD3 is overexpressed in different human cancers like colorectal, gastric and prostate cancer and in particular in HER2-overexpressing breast cancer. For this reason, StARD3 is a very attractive candidate as a target for cancer therapy. In 2019, the group of Flavio Rizzolio has identified the first StARD3 inhibitor using a Virtual Screening (namely VS1 compound) that has been demonstrated to have a relevant biological activity in cancer cell lines (3). Here we have crystallized the Lutein Binding Domain (LBD) of StARD3 protein in a presence of twofold molar excess of VS1. The structure of the complex has been resolved using the previously determined apo-structure as a model (4; PDB ID: 5i9j). A superimposition of the ligand-bound and apo copies of $StARD3_{LBD}$ domain reveals a conformation change in the Ω1 loop upon the VS1 compound which occupies the cavity base. Our results demonstrate that $StARD3_{(LBD)}$ interacts with the VS1 compound in a specific manner opening the way to the creation of a novel class of anti-cancer therapeutics.

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Development of BIR1-targeting compounds for cancer treatment: study of IAPs modulation in NF-KB pathway

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Inhibitor of Apoptosis Proteins (IAPs) are a conserved family of proteins that act as negative regulators of apoptosis and are frequently overexpressed in cancer cells. Their inhibitory function is primarily mediated by Baculovirus IAP Repeat (BIR) domains, classified into type I (BIR1) and type II (BIR2 and BIR3) (1). Type II BIR domains directly bind and inhibit caspases, preventing proteolytic activation, while the BIR1 domain facilitates the assembly of signaling complexes that regulate the NF-κB pathway. Specifically, XIAP-BIR1 (X-chromosome linked IAP) in its dimeric form interacts with TAB1, a kinase activator, modulating pro-survival signaling. Similarly, cIAP2-BIR1 recognizes TNF Receptor Associated Factors (TRAFs), facilitating its recruitment to the TNF-α receptor signaling complex and functioning as an E3 ubiquitin ligase in the extrinsic apoptotic pathway (2,3).

A well-established strategy to inhibit IAPs involves the development of Smac-mimetic (SM) small molecules, which mimic the endogenous IAP antagonist Smac/DIABLO by targeting type II BIR domains (4). However, resistance mechanisms in certain cancer cell lines, linked to residual cIAP1/2 E3 ligase activity, have limited their efficacy (5). This has led to the identification of the BIR1 domain as an alternative therapeutic target for modulating IAP-mediated protein interactions.

Following a virtual screening of compound libraries against the BIR1 domain of cIAP2 and XIAP, the molecule FC2 was identified, demonstrating modulatory activity on the NF-κB pathway in MDA-MB-231 adenocarcinoma cells. Furthermore, FC2 exhibited a synergistic cytotoxic effect when combined with SMs and TNF-α (6). Building on the FC2 scaffold, a new library of compounds was designed and optimized. The cytotoxic effects of these derivatives were evaluated across different cancer cell lines, while their binding affinities for isolated BIR1 domains of cIAP2 and XIAP were characterized through fluorescence-based assays and virtual docking studies (7). The most promising compounds demonstrated improved efficacy in cancer cells and binding affinities in the low micromolar range, providing a foundation for further structural optimization.

To deepen our understanding of how FC2 derivatives modulate the NF-κB pathway through IAP interactions, we aim to: (i) optimize the production of full-length IAP proteins (cIAP1, cIAP2, XIAP) and their partner proteins TRAF2 and TAB1; (ii) investigate the effects of selected compounds on isolated proteins and their corresponding complexes in vitro; (iii) structurally characterize full-length IAPs in the presence and absence of ligands; and (iv) evaluate the cytotoxicity of lead derivatives in diverse cancer cell models. Moreover, the insights gained will support the rational design of hybrid compounds combining SMs with BIR1-targeting molecules, paving the way for novel anticancer strategies.

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What's new in Elettra's Structural Biology Laboratory

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Elettra Sincrotrone Trieste is a multidisciplinary research center for materials and life sciences, which is based on a third generation source and includes 28 beamlines and 6 support laboratories.
One of such labs is the Structural Biology Laboratory, which comprises research groups working on the structure-based discovery of new drugs and on the structural analysis of protein-nucleic acid interactions.
For the synchrotron itself, 2025 will be a hallmark year, as it will be the year of Elettra 2.0: the light source will be switched off to perform substantial works, with the purpose of boosting its capabilities and implementing new beamlines.
This substantial upgrade of the synchrotron, however, is not the only news: in the last year, taking advantage of NextGeneration EU funding (PNRR - PRP@CERIC), the Structural Biology Laboratory of Elettra has been expanded and upgraded. The acquisition of new instruments and personnel now allow an increased output and enable the research groups to perform new and better experiments. The upgrades of the Laboratory not only improve the possibilities of the internal research projects, but have also been implemented to offer more facility-like services, that span from protein production and purification, to protein crystallization and the solution of the tridimensional structure, in the view of facilitating the access to the structural biology field and, along with the macromolecular crystallography beamline, provide a more complete structural biology workflow at Elettra. Examples will be shown on some collaborations and results obtained in this past year.

Structural characterization of urease activity and inhibition: an integrated X-ray crystallography and cryo-EM study

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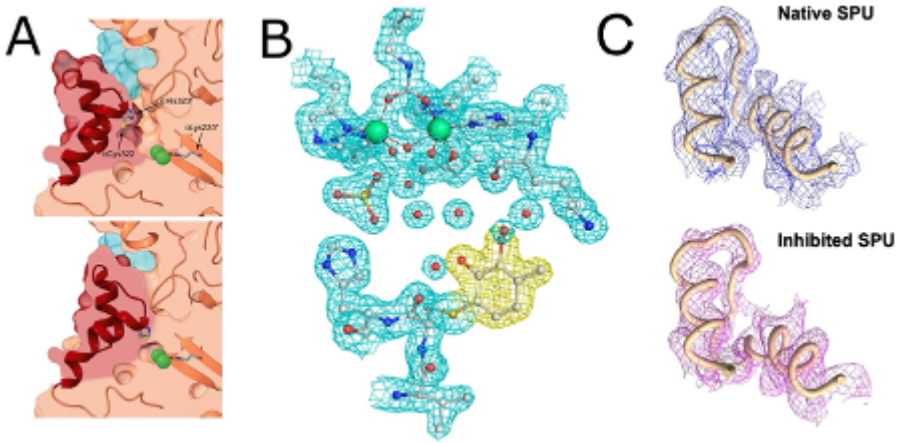
Urease, a Ni(II)-enzyme that plays a prominent role in the global nitrogen cycle by catalyzing the hydrolysis of urea to give ammonia and carbon dioxide, is responsible for negative consequences on human health and agro-environment (1). In the past ten years, a strategy for urease inhibition has emerged that targets an enzyme helix-turn-helix motif (called mobile flap), the latter covering the active site pocket and having been reported to be directly involved in the catalytic mechanism by switching from an *open* to a *closed* state (Fig. A) (2). In this integrated work, the X-ray crystal structures of urease from *Sporosarcina pasteurii* (SPU, 250 kDa) in complex with a series of urease inhibitors targeting the mobile flap are provided (Fig. B), thus illustrating the potential of this alternative inhibition strategy, at the molecular level (3). Moreover, two cryo-EM structures of SPU, recently determined both in the native state and inhibited by a reaction intermediate analogue, are presented, which provide new insights into the role of the apical portion of the helix-turn-helix motif in urease catalysis (Fig. C) (4).



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Ruthenium-based polyoxometalates as a possible chaperone in cryo-EM structure determination: a case study

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Polyoxometalates (POMs) are emerging as versatile tools in both biomedical applications and structural biology. These molecular clusters, typically composed of early transition metals (e.g., W, Mo, V) and oxygen, exhibit remarkable structural diversity and functionality, enabling their use in catalysis, drug development, and macromolecular structure determination (1). In structural biology, POM are tools able to aid protein crystallography by stabilizing proteins and resolving the "phase problem" as heavy atom derivatives (1). POMs are also emerging as metallodrugs, showing anticancer, antiviral, and antimicrobial properties (2). Functionalization, such as organic-inorganic hybridization, enhances their bioactivity and reduces toxicity, broadening their potential in therapeutic applications. (2) In this context, we extended the use of ruthenium-based POM (Ru_4POM) to facilitate cryo-EM data collection of ACAD9 (acyl-CoA dehydrogenase family member 9), a challenging target with a molecular mass of 120 kDa and several disordered regions (3). Its preferential orientation further complicates cryo-EM studies. To address these challenges, our aim was to utilize Ru_4POM to stabilize the disordered regions of ACAD9 and facilitate particle alignment during 2D classification in cryo-EM data processing. Complementary biochemical assays have been conducted to elucidate the impact of Ru_4POM on ACAD9 enzymatic activity and ligand-binding affinity. These findings highlight the potential of POMs in advancing both structural biology and drug discovery.

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Structural characterization of Atrial Natriuretic Peptide amyloid fibrils from Isolated Atrial Amyloidosis patients

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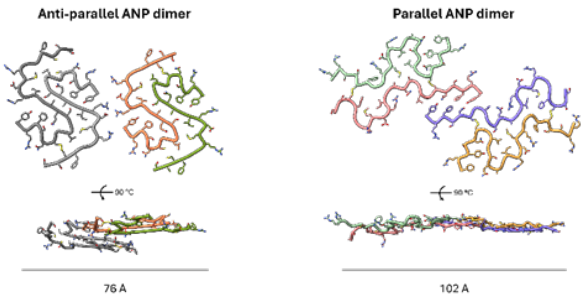
Isolated atrial amyloidosis (AANP) is an age-related disorder that primarily affects the atria of the heart, where atrial natriuretic peptide (ANP) forms the main component of amyloid fibrils (1). ANP, a 28-amino-acid peptide hormone secreted by atrial cardiomyocytes, plays a key role in regulating blood volume and pressure. In circulation, ANP exists in three molecular forms: mature ANP (28 amino acids) with an intramolecular disulfide bond, dimeric ANP (56 amino acids), and pro-ANP (126 amino acids), the latter containing the sequence of mature ANP (2). Under pathological conditions, increased production and secretion of both monomeric and dimeric ANP contribute to amyloid fibril formation, disrupting atrial conduction and increasing the risk of atrial fibrillation (3). Despite its clinical significance, the structural characterization of pathological ANP fibrils has remained elusive, preventing a clear understanding of the aggregation mechanism and its impact on atrial fibrillation. To address this, we collected atrial appendage samples from three patients during open-heart surgery. Immunohistochemical analysis confirmed the presence of ANP amyloid deposits in these tissues. Cryo-electron microscopy (cryo-EM) of extracted fibrils revealed two distinct polymorphs (A and B) of ANP fibrils in all three patients, resolved at 2.9 Å and 3.3 Å, respectively. De novo model building identified covalent dimeric ANP in both polymorphs. Polymorph A displayed an anti-parallel orientation of the two chains, while polymorph B exhibited a parallel orientation. These findings highlight the pivotal role of the ANP dimer in protein aggregation. Upon reduction of the intramolecular disulphide bond, mature ANP can dimerize—either in a parallel or anti-parallel orientation—forming a covalent dimer that self-assembles forming the amyloid core. These novel insights into the structural basis of ANP aggregation offer a deeper understanding of the role of ANP amyloid fibrils in AANP and atrial fibrillation.



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Structural and functional characterization of hyper-stable human serum albumin variants

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Human serum albumin (hSA) is the most abundant protein in blood plasma, serving as the natural transporter of several endogenous ligands and being well reported to influence drugs bioavailability and pharmacokinetics [1]. Indeed, the interaction with the neonatal Fc receptor (FcRn), rescues albumin from lysosomal degradation, leading to its persistence in the serum for up to three weeks [1]. The prolonged half-life sparked an increasing interest in utilizing albumin-conjugated compounds in the drug discovery field. Such unique feature offers significant benefits, including reduced drug administration frequency for patients, enhancing their well-being and contributing to a more patient-friendly healthcare system.

In this poster we present the *structural and functional characterization* of a panel of *hSA variants,* from now on named hSA1, hSA2, hSA3 (hSA*), engineered *in silico* by the group of Prof. Sarel Fleishman at the Weizmann Institute of Science (Rehovot, Israel), to be hyper soluble, stable and expressible in *Escherichia coli* with a high yield (up to 100 mg/L), [2]. Specifically, hSA* were designed using the PROSS algorithm based on sequence and structure similarity to other albumins and incorporating mutations spread across its structure, 18, 25 and 73 respectively [2].

On the structural side, hSA *wt* resists crystallization in its apo-form and requires supplementation with myristic acid (Myr) and concentration up to 100 mg/mL to precipitate in an order manner. Due to their high solubility, with a melting temperature higher than 90°C, the crystallization of hSA variants was even a harder task and proved to be successful just for hSA1 [2]. To overcome this problem, we decided to exploit Cryo-Electron Microscopy (Cryo-EM), emerged as a valuable complementary technique for proteins like hSA, difficult to crystallize due to their flexibility and extreme stability [3]. Here I present for the first time, the Cryo-EM structure of one of hSA3, in complex with a megabody (MbAlb1) selected to be cross-reactive for both mouse and human albumin [4], yielding a consensus map at a 3.9 Å overall resolution (structure under deposition). 0,3 mg/mL solution of hSA6/MbAlb1gel filtrated complex was applied to a glow discharged Quantifoil R 1.2/1.3 Cu300 holey carbon grid. The excess sample was blotted and plunge-freezed into liquid ethane using a Leica plunger (5.0 s blot time, at 25°C under 95% humidity) at the Kavli Institute of Nanoscience (TU Delft). The grids were imaged on the 300 kV Titan Krios microscope (Thermo Fisher Scientific) of the Netherlands Centre for Electron Nanoscopy (NeCEN) facility with a K3 direct electron camera in the super resolution mode at 0.418 Å per pixel. A total of 3732 movies was collected with 60 frames each, a dose of 1 e/Å−2 per frame and a defocus range from -2.4 to -1.6 um. Overall, hSA3 atomic structure is highly conserved with the hSA *wt* one, maintaining the global 3D arrangement and the position of the respective domain. To explore all the hSA* possible applications, we functionally characterize hSA2 and hSA3 performing a panel of affinity measurements by isothermal titration calorimetry (ITC) with the two-gold standard albumin-binding drugs (i.e., warfarin and ibuprofen). Besides, a preliminary size exclusion chromatography (SEC) showed that hSA1 retains the capability of binding its receptor, hFcRn, an appealing feature in the context of its possible drug delivery application. The hFcRn binding will be tested also for hSA2 and hSA3 and surface plasmon resonance (SPR) measurements will be carried out to measure the binding affinity of all hSA variants

for the hFcRn. To test whether the hSA* are not toxic to human cells and can be used in cell culture medium, we are setting up a viability test in collaboration with a group in the Department of Biomedical Science (University of Padova), adding hSA* variants to the growth medium of primary cells.

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Flash Presentations
(in chronological order of presentation)

TRAF2 in TNF Signaling and Immune Regulation: Structural Insights and Therapeutic Potential in Cancer

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The tumor necrosis factor receptor-associated protein TRAF2 is a key regulator of signaling pathways involved in cell death, inflammation, and cancer. As a critical adaptor in the TNF system, TRAF2 bridges TNF receptors to downstream effectors, including cellular inhibitors of apoptosis (cIAPs), glutathione transferase P1-1, zinc finger protein A20, and the kinase RIP1, ensuring precise control over apoptosis and NF-κB signaling. Structurally, molecular models indicate that TRAF2 forms a homotrimer, comprising a globular TRAF-C terminal domain and an extended coiled-coil TRAF-N terminal region. Key interaction sites on TRAF-C and TRAF-N mediate its binding to trimeric TNF receptors and allow hetero-trimerization with TRAF1, which enhances cIAP recruitment. The dissociation of TRAF2-C into monomeric or dimeric forms promotes membrane interactions, suggesting that oligomerization plays a regulatory role in TNF signaling. However, the molecular mechanisms governing TRAF2's activity, structural dynamics, and roles in membrane association and vesicle trafficking remain poorly understood. To address these gaps, we are investigating different TRAF2 constructs, including the TRAF2-C (residues 267-501) and full-length TRAF2 (1-501), to expand structural insights and characterize its conformational stability. We successfully produced TRAF2-C and are optimizing full-length TRAF2 production. Preliminary Small-Angle X-ray Scattering (SAXS) experiments confirmed that TRAF2-C forms a stable homotrimer, and crystallization trials are underway to obtain high-resolution structural data. These studies contribute to a clearer understanding of TRAF2's role in inflammation and immune responses, particularly in TNF-induced signaling.

Beyond its role in TNF signaling, TRAF2 has emerged as a promising therapeutic target in cancer. Indeed, the interaction between the BIR1 domain of cIAP2 and TRAF2 is essential for recruiting cIAPs to TNF receptors, thereby activating NF-κB and promoting cancer cell survival. Given the validated role of cIAPs in cancer therapy and their contribution to chemoresistance, disrupting these interactions represents a potential therapeutic strategy. To modulate NF-κB signaling, we identified the small molecule FC2 (1) through virtual screening of the Chembridge library against the BIR1 domain of cIAP2/TRAF2 interaction surface. FC2 exhibits cytotoxic effects in MDA-MB-231 adenocarcinoma cells, with enhanced activity when combined with TNF. To improve its efficacy, we designed a library of dozens of FC2 (2) derivatives, which are currently undergoing structural and functional characterization. By targeting TRAF2-cIAP interactions, these findings provide a foundation for developing novel therapeutics aimed at modulating NF-κB-mediated inflammation and cancer cell survival.

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Investigating BiP's Function in the Regulation of S1R Activity.

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The endoplasmic reticulum (ER) is a versatile organelle essential for protein synthesis and calcium (Ca^{2+}) homeostasis. Proteins are translocated into the ER lumen during translation, where molecular chaperones facilitate their proper folding into functional conformations. Under stress, the ER activates the unfolded protein response (UPR), increasing chaperone expression to manage protein folding demands. Binding immunoglobulin protein (BiP), a member of the Hsp70 chaperones, plays a central role in this process by regulating UPR through ATP-driven cycles of protein binding (1) (Gething MJ.). BiP transiently interacts with three ER membrane proteins—activating factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and PKR-like ER kinase (PERK)—that act as UPR sensors. During stress, BiP dissociates from these sensors, enabling their activation, which triggers pathways to enhance protein folding, ER volume, and degradation of misfolded proteins via ER-associated degradation (ERAD) (2) (Lewy TG, Grabowski JM, Bloom ME.).

BiP also contributes to ER function in conjunction with the sigma-1 receptor (S1R), an enigmatic ER transmembrane protein implicated in conditions such as depression, drug addiction, neurodegenerative diseases and neuropathic pain. BiP forms a complex with S1R at mitochondria-associated membranes (MAM) of the ER. Under Ca^{2+}depletion and/or S1R agonist binding, BiP dissociates from S1R, allowing its chaperone activity and its redistribution to the entire ER, where it may contribute to UPR, autophagy activation and calcium flux toward the mitochondria (3) (Hayashi T, Su TP.). Additionally, S1R/BiP interactions appear to be modulated by S1R ligand binding.

Given the role of BiP and S1R in maintaining cell survival and mitochondrial function, as well as their association with neurological and neurodegenerative diseases, significant research efforts have focused on uncovering their mechanisms of action. While the cellular functions of this complex are relatively understood, the structural characterization of the interaction between these two proteins remains incomplete.

To produce BiP we expressed a recombinant form of the protein Smt3-BiP, fusing a SUMO protein at the N-terminal. The SUMO moiety also presents a His-tag. The protein was cloned in BL21 E. coli cells and expressed under IPTG induction. The purification protocol requires a passage on HisTrap, followed by cleavage of SUMO moiety and further passage on HiTrap Q column. A final passage on SEC assures a pure sample.

To study the interaction between Bip and S1R, 10 peptides were designed on the basis of the S1R X-ray structure, corresponding to the S1R surface, which may interact with BiP. Using SPR we have been studying the binding of these peptides to Smt3-BiP, evaluating the affinity and binding parameters. We intend then to further characterize structurally the binding of BiP-S1R, as this may contribute to understanding the mechanisms upon its activation and sensitivity toward Ca^{2+} and S1R ligands binding.

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Understanding PE_PGRS, cell wall proteins promoting mycobacterial survival in TB

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Tuberculosis, caused by Mycobacterium tuberculosis (Mtb), has been known to mankind since ancient times, and yet it is the leading cause of death by a single bacterium, infecting over 10 million and killing more than 1 million annually. A remarkable feature of TB is the dormancy, where Mtb establishes a dynamic equilibrium with the host immune system that lasts for a lifetime, although the immunological mechanisms governing the host-pathogen interaction remain poorly understood as are the virulence factors responsible for these distinct features [1].

The complete sequencing of the Mtb genome revealed the existence of a unique family of genes, the PE_PGRS family, containing about 65 members, 51 of which are thought to express functional proteins. PE_PGRS proteins play critical roles in bacterial pathogenesis and immune evasion. Their high abundance in pathogenic mycobacterial strains and the diversification of their functional roles are central elements in the evolution and virulence of Mtb [2].

The PE_PGRS family shares a peculiar modular structure constituted by a conserved N-terminal PE domain, a polymorphic glycine-rich domain (PGRS), and a C-terminal domain that varies in length and uniqueness among different PE_PGRS proteins. Yet, the lack of structural data of these proteins has so far hampered a satisfying understanding of their role in Mtb pathogenesis. Recently, we proposed the "sailing" model to describe how PE_PGRS proteins navigate the mycobacterial membrane exposing structural motifs for host interactions and/or deliver functional protein modules at their C-terminus [3]. This model, supported by AlphaFold 2.0 predictions, offers new insights into their dynamic behavior.

In collaboration with the Catholic University of Rome, we are investigating a panel of PE_PGRS proteins to tackle general features of these important virulence factors [4]. To this end, we integrate structural and biochemical approaches to characterize these modular domains and explore the structure-function relationship of this enigmatic family. Based on bioinformatic analysis, we have recombinantly produced both the PE domains and the C-terminal domains of selected PE_PGRS proteins. Our ongoing research is aiming to deepen the understanding of the PE_PGRS protein family and their interactions with host molecules, offering key insights into *Mycobacterium tuberculosis* pathogenesis.

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Bacteriorhodopsin/Perovskite adducts for Next-Generation of in-crystal lab-on-chip Solar Cells

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The development of advanced solar cells is essential to meet global energy demands and face climate change. Solar cells are a sustainable solution as they harness energy without relying on fossil fuels, significantly reduce greenhouse gas emissions, and enable off-grid energy supplies, features that make such devices essential in the transition to a green economy. The integration of bacteriorhodopsin (bR) into perovskite solar cells (PSCs) offers a great opportunity to improve the efficiency of energy transport due to the ability of such membrane protein to act as a highly efficient photosensitiser.(1) Here, we propose the development of a bio-inspired in-crystal lab-on-chip device in which the well-known technology of PSCs is integrated with bR acting a photoactive protein to increase PSC efficiency.(2) Crystals of bR/perovskite will be grown in lipidic cubic phase (LCP) (3) a matrix that is expected to extend the surface for the capture of sunlight by aligning protein units(4) and reduce the well-known water dependent degradation of perovskite. bR/perovskite crystals will be prepared by using perovskites selected among the ones having good band alignment with bR and proven water tolerance. Once obtained, bR/perovskite crystals will be investigated to unravel perovskite binding site on the surface of the protein (by X-ray crystallographic technique) and any inclusion of perovskite in the defects of the bR crystals (by electron microscopy). The device will be evolved with microfluidic techniques, by which a layer of LCP-embedded bR/perovskite crystals will be stacked on a TiO_2 layer deposited on an ITO substrate. Such device will enable crystal growth control as well as to control soaking procedure to introduce perovskite precursor in the bR crystal. Photoelectric properties of the device will be assessed. The lab on-chip in-crystal device is expected to have a higher efficiency than the perovskite-based device photosensitized by bR in amorphous state and to reduce the risk of water and oxygen dependent degradation of perovskite with respect to traditional PSCs. Together, these efforts aim to advance the field of solar cell technology and to contribute to the global transition to sustainable energy sources.

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A novel PET hydrolase derived from thermophilic microorganism

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Plastics have become ubiquitous in our daily life, due to their significant properties. However, the nature of the polymers constituting plastic materials makes them persistent in the environment. The resulting growth in the quantity of plastics waste has emerged as a pressing issue (1). In recent years, biodegradation approaches have demonstrated valuable potential in degrading plastics through the action of enzymes, bringing low environmental impact (2). In 2016, a bacterial strain called *Ideonella sakaiensis* 201-F6 was identified for its abilities to produce two unique enzymes, the polyethylene terephthalate hydrolase (PETase) and the mono (2-hydroxyethyl) terephthalic acid hydrolase (MHETase). These enzymes enable the bacteria to utilize polyethylene terephthalate (PET) as their sole carbon source (3). In this scenario, extensive research has been carried out for the identification of other bacterial strains able to produce enzymes exploitable for plastic degradation (1).
Starting from these bases, the identification of new bacterial hydrolases deriving from thermophilic microorganisms can give new insight into plastic biodegradation in harsh conditions, such as high temperatures. Through meta-genomic approaches, a novel thermophilic PET hydrolase has been recently identified from geothermal samples. This enzyme, denominated PP_EG PETase, was produced in recombinant form and purified to perform physico-chemical analyses and assess its thermal stability and structural features. Furthermore, crystallization trials are ongoing to provide deeper insights into its structural and functional properties.

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Crystal structure and biophysical characterisation of the enterococcal foldase PpiC, a cross-opsonic antigen against Gram-positive nosocomial pathogens

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Enterococcus faecium infections have high rates of antibiotic resistance, with vancomycin resistance acknowledged as one of the most important in medical practice. In 2017, the WHO declared vancomycin resistant *E. faecium* a threat to humankind for which rapid actions are needed, through the identification and inactivation of key molecules for the bacterial survival. PpiC is a peptidyl-prolyl isomerase (PPIase) from *E. faecium*, with the key role of contributing to the folding of proteins translocated across the bacterial membrane. It is a membrane bound lipoprotein at the membrane–wall interface, therefore easily accessible to the action of inhibitors or to the immune system. Consistently, we previously identified PpiC as an important cross-opsonic vaccine antigen. We here report the crystal structure of PpiC and its biophysical characterisation (1). Consistent with PpiC folding activity, the biological assembly of PpiC is a bowl-shaped structure containing two parvulin-type peptidyl-prolyl cis/trans isomerase domains. We also dissected the role of N- and C-terminal regions of the molecule on its dimerization, an event which is predicted to play an important role in the folding of client proteins.

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The open dimeric conformation of cardiotoxic light chains: a key feature underlying soluble toxicity in cardiac AL amyloidosis

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Light chain (AL) amyloidosis is a rare systemic disease characterized by the misfolding and aggregation of immunoglobulin light-chains (LCs)amyloid fibrils that deposit in various organs severely affecting their functions and leading to poor prognosis, especially when hearth involvement is severe. Besides fibrillar deposition, the toxicity of soluble pre-amyloid LCs is another important pathogenic factor. Although the mechanisms underlying LC cardiotoxicity remain unclear, several biochemical and biophysical traits were recently found to be typical of amyloidogenic LCs and favoring their tendency to misfold (1).

Recently, we reported for the first time a partially open conformation of a patient-derived cardiotoxic LC in complex with nanobodies (Nbs). In this conformation, the canonical homo-dimeric fold of LC is disrupted, causing the two variable domains to move apart and expose highly hydrophobic and aggregation prone patches (2). Interestingly, we hypothesized that this open conformation may play a crucial role in toxicity and aggregation mechanisms associated with cardiotoxic LCs.

Here, we further investigated this aspect by studying five additional patient-derived amyloidogenic LCs from a biochemical and biophysical point of view. Firstly, biolayer interferometry experiments between LCs and Nbs revealed a strong 1:2 binding of Nbs, confirming the existence of the open-to-close equilibrium also in the set of cardiotoxic LCs.

Moreover, through limited proteolysis experiments we found that all the five LCs are characterized by high susceptibility to protease cleavage, suggesting high dynamics and flexibility. Interestingly, the same experiments in presence of Nbs showed an increase LC stability, indicating a marked reduction in their flexibility and dynamics by stabilizing the equilibrium between closed and open LC states. Lastly, functional assays using the in vivo model *C. elegans* indicated that the transition to open state is central to LC-mediated cardiotoxicity. Indeed, while all five LC showed an evident toxic activity, no major effects were observed with LC-Nb complexes, in which the LC open state is stabilized and neutralized.

Overall, we added additional evidences on the pivotal role of closed-open conformation in LC cardiotoxicity, suggesting it may be a further trait of amyloidogenic LCs. In addition, we further demonstrate the potentiality of Nbs as LC stabilizers, paving the way for the design of future therapeutics directed towards the stabilization of native LC conformation.

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Disentangling the mechanism underlying the covalent methanesulfonyl fluoride acetylcholinesterase adduct formation and evolvement: structural and mechanistic insights into an aged-like inactive complex susceptible to reactivation by a combination of nucleophiles

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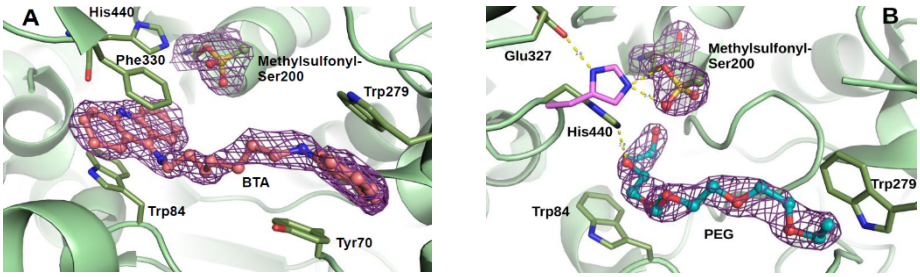
Chemical warfare nerve agents and pesticides, known as organophosphorus compounds inactivate cholinesterases (ChEs) by phosphorylating the serine hydroxyl group located at the active site of ChEs. Over the course of time, phosphorylation is followed by loss of an organophosphate-leaving group and the bond with ChEs becomes irreversible, a process known as aging. Differently, structurally related irreversible catalytic poisons bearing sulfur instead of phosphorus convert ChEs in its aged form only by covalently binding to the key catalytic serine.
Kinetic and crystallographic studies of the interaction between *Torpedo californica* (*Tc*) acetylcholinesterase and a small organosulfonate, methanesulfonyl fluoride (MSF), indeed revealed irreversibly methylsulfonylated serine 200 (Fig.1B), to be isosteric with the bound aged Sarin/Soman analogues. The potent bulky reversible inhibitor 7-bis-tacrine (BTA) adopts, in the active site of the crystal structure of the MSF-enzyme adduct (Fig.1A), a location and an orientation that closely resemble the one being found in the crystal structure of the BTA-enzyme complex (1). Remarkably, the presence of BTA accelerates the rate of methanesulfonylation by a factor of two. This unexpected result can be explained on the basis of two facts: i) the steric hindrance exerted by BTA to MSF in accessing the active site and ii) the acceleration of the MSF-enzyme adduct formation as a consequence of the lowering of the rotational and translational degrees of freedom in the proximity of the catalytic serine.
It is well known that Pralidoxime alone or in the presence of the substrate acetylcholine cannot reactivate the active site serine of the *Tc*AChE-MSF adduct. We show that the simultaneous presence of Pralidoxime and the additional neutral oxime, 2-[(hydroxyimino)methyl]-l-methylimidazol, triggers the reactivation process of *Tc*AChE within the hour timescale.
Overall, our results pave the way toward the likely use of a cocktail of distinctive oximes as a promising recipe for an effective and fast reactivation of aged cholinesterases (2).



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Cryo-EM structure of Apo-PdxR from *Bacillus clausii*

in complex with its target DNA

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PdxR is a bacterial transcription factor that regulates the production of pyridoxal-5-phosphate (PLP) by binding to specific DNA sequences and modulating the expression of the *pdxST* biosynthetic operon, responsible for PLP synthesis, and its own *pdxT* gene, which encodes the PdxR protein itself. Depending on intracellular PLP concentrations, PdxR exists either in an apo form that supports the transcription of PLP biosynthetic genes or in a holo form that represses this pathway (1,2). In this research, cryo-electron microscopy (cryo-EM) and biochemical approaches were used to investigate the structural mechanisms underlying PdxR function and its interaction with nucleic acid. We collected and analyzed a dataset of a sample of apo-PdxR in complex with a 48bp dsDNA containing the sequences which are specifically recognized by PdxR. Data analysis revealed a major population of single-apo-PdxR in complex with its DNA molecule, and a minor population of a double-apo-PdxR complex (two molecules of PdxR) bound to two DNA molecules. Within the single complex population, there seem to be multiple subpopulations representing different conformational states. Ongoing analyses focus on improving the resolution of the cryo-EM maps to facilitate 3D model reconstruction and enable comparisons of apo- and holo-PdxR complexes (2). Planned functional assays will assess whether the double-apo-PdxR-DNA complex contributes to gene regulation *in vivo* or it represents an artifact of elevated protein concentrations *in vitro*. These results could offer insights into the conformational dynamics of PdxR and its role in bacterial PLP biosynthesis.

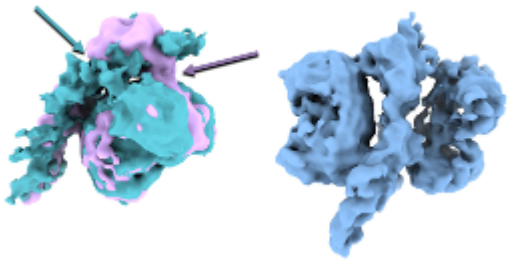

Figure. *Cryo-EM 3D maps representing single-apo-PdxR-DNA complex in two conformations (a); Cryo-EM 3D map representing double-apo-PdxR-DNA complex (b).*

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Thermal and Structural Characterization of Rv0678: Insights into Multidrug Resistance in Tuberculosis

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Every year, more than 10 million people worldwide are affected by tuberculosis (TB) caused by infection with *Mycobacterium tuberculosis* (MTB) (1). While several anti-TB drugs allowed an enhanced global disease control, the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains poses a significant challenge. Drug resistance mechanisms include mutations in specific genes, altered drug targets, and increased activity of efflux pumps (2). The cell wall plays a key role in the virulence of *M. tuberculosis*. Central to the function of the mycobacterial cell wall are the MmpL and MmpS transporters, which are involved in exporting fatty acids and other lipid components essential for MTB virulence. The Rv0678 regulator, a transcriptional repressor, controls and regulates the levels of expression of the MmpS-MmpL transport system (3).
In this work, we provide a characterization of the effect of single point mutations on the structure and stability of Rv0678. We wish to contribute to the improvement of the WHO mutation catalogue by identifying resistance mutations and thus inform advanced sequencing tests (tNGS), useful for quickly identifying patients that may not respond to the bedaquilin/pretomanid/linezolid (BPaL) treatment.
We expressed the Rv0678 protein and its mutants in bacterial cells and purified through affinity and size exclusion chromatography. First, we investigated their thermostability using both differential scanning fluorimetry (DSF) and circular dichroism (CD). DSF was performed using a Cys-specific dye (BODIPY), since hydrophobic dyes such as SYPRO Orange yielded uninterpretable melting curves.
Circular dichroism spectra of each sample have been recorded in the 180-260 nm range, and thermal unfolding was monitored at three wavelengths (Figure 1). Furthermore, we are in the process of crystallizing Rv0678 and its variants to gain a better understanding of the mechanisms leading to drug resistance.

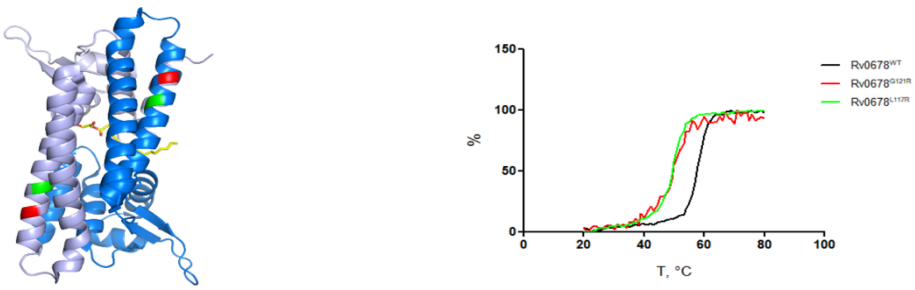

Figure 1. (A) Rv0678 WT dimer structure (4). The location of the G121R (red) and L117R (green) mutations are shown. **(B)** Corresponding melting curves derived using CD.

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Face2Face: A simple tool for macromolecule interfaces analysis

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Analyzing interactions that stabilize tertiary and quaternary structures or macromolecular complexes is crucial in structural biology. Existing tools for macromolecular interface analysis often lack comprehensive features, such as compatibility with all molecule types and customizable 2D/3D outputs in user-friendly formats.

To address these limitations, we developed FACE2FACE, a web-based tool for detailed interface analysis between macromolecules and their ligands. FACE2FACE accepts proteins, nucleic acids, or their segments and calculates interfaces with other molecules, including proteins, nucleic acids, and small ligands.

The tool generates diverse output formats for comprehensive visualization and analysis. Text files provide lists of polar and non-polar contacts at both residue and atom levels, filtered by a user-defined threshold (3.5-4Å). 2D contact maps, unlike fixed images provided by other tools, are delivered as spreadsheet-ready files, enabling users to customize and modify the data to fit specific analysis needs or create publication-ready visuals. For 3D visualization, FACE2FACE offers ready-to-use scripts for PyMol and Chimera, where interacting partners are highlighted for easy examination.

The interface is interactive and includes a "hub-and-spoke" visualization model, where hub sizes reflect the number of contacts, allowing users to quickly identify interaction hotspots. Outputs can be visualized directly on the web page or downloaded for further analysis.

FACE2FACE's versatility and comprehensive outputs make it valuable for structural bioinformatics applications, including analyzing interaction hotspots, designing experiments, and generating visuals for publications. By integrating a wide range of features, FACE2FACE provides a robust solution for studying macromolecular interfaces in detail.

Examples of the program's applications further demonstrate its utility in advancing structural biology research.

Availability: FACE2FACE is available at https:/face2face.ibpm.cnr.it/.

Consensus3D. A comprehensive pipeline for accurate detection of conserved protein regions based on structure alignment programs consensus..

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Protein structure comparison is at the core of most applications in Structural Bioinformatics. For this reason, a plethora of protein structure alignment programs (PSAPs) have been developed over the years. While most PSAPs correctly identify most of the structurally conserved regions (SCRs) between highly similar structures, the fraction of correctly aligned SCRs diminishes markedly as the structural similarity between the two proteins decreases, and so does the concordance of SCRs identified by different PSAPs. Unfortunately, the comparative accuracy of the available PSAPs in identifying SCRs for specific protein families, or in general, remains undetermined.
To provide a comprehensive approach to accurate SCR identification, we developed Consensus3D, a computational pipeline that integrates outputs from the 18 PSAPs that are currently available for download and whose output comprises a structure-based sequence alignment. Since different PSAPs are unlikely to make the same mistakes by chance, "consensus" SCRs, i.e., SCRs identified by multiple PSAPs, are expected to be more reliable than the SCR provided by any single PSAP.
The Consensus3D pipeline can be run either in pairwise or in multiple mode.
In pairwise mode, the pipeline: accepts either two PDB IDs or two coordinate files as input; launches all PSAPs; collects structure-based sequence alignments; and aligns them to one another.

The output includes:

(i) The structure-based sequence alignment, highlighting the consensus SCRs among all PSAPs (SCRs-all).
(ii) The coordinates of the two proteins, superimposed using the SCRs-all, and scripts for visualization in PyMol and Chimera.
(iii) Structural similarity parameters, such as SCRs-all length, RMSD and TM-score.
(iv) A table that reports structural similarity parameters obtained for the SCRs resulting from the consensus among different subsets of PSAPs (e.g., SCRs-17, SCRs-16, SCRs-15, etc.) and for the SCRs detected by each PSAP, as well as for the SCRs-all. From this table, it is possible to: immediately evaluate the relative accuracy of the different SCRs; generate output (i-iii) based on selected SCRs-subsets; and directly access each PSAP through a link.

The multiple mode accepts multiple PDB IDs or coordinate files as input, and combines their pairwise alignments based on SCRs-all.

The output comprises:

- the SCRs-all based multiple sequence alignment;
- conservation of residue types in the SCRs-all shared by all proteins;
- phylogenetic trees based on either RMSD values or TM-scores.

We tested the accuracy of the SCRs-all identified by the pipeline by comparing them with those identified by detailed hand-based procedures. For 140 protein structures belonging to ten different families, the overlap was 97.5%.

The Consensus3D website is free, open to all users and without login requirement at the address: https://consensus3d.ibpm.cnr.it.

Structure-based design of peptides for biomedical applications

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Peptides are highly effective research tools and attractive therapeutic agents per se, as well as convenient lead compounds for the rational development of non-peptide small molecule drugs. Peptides mapping on protein regions involved in interactions with macromolecular partners can be highly effective interaction mimics and/or inhibitors. For these reasons, whenever the experimentally determined 3D-structure of a protein complex or a reliable molecular model is available, peptides aimed at inhibiting this interaction, and/or at binding one of the two molecular partners, can be most effectively designed by taking advantage of structural information and of a few empiric rules. Even when a 3D structure or model of a protein is only available in the free state, peptides aimed at inhibiting protein interactions and/or binding the interaction partners of the protein can be identified by designing a set of peptides covering essentially the whole protein surface and testing them experimentally.

In the past years, our manual structure-based design procedure has allowed us to obtain peptides endowed with several biomedical activities: i) Peptides endowed with either pro- or anti-angiogenic properties, due to their ability to directly interact with VEGFR-1 binding partners or interfere with interactions involving VEGFR-1 (1-5); ii) Peptides able to correct the pathological phenotype caused by single point-mutations in mitochondrial tRNAs (6-10).

To increase the speed of peptide design procedure and make it available to the scientific community, we have developed MinePept a novel fast, user-friendly and comprehensive tool for peptide design. MinePept can be used in two modes: fully automated and interactive.

In the fully automated mode, the user selects the protein of known structure based on which the peptides must be designed. If the structure of the selected protein has been determined in the free state, the program returns a set of peptides mapping on the whole protein surface; if the structure of the selected protein has been determined in complex with a ligand of interest, the program returns a set of peptides mapping in the interaction regions.

In the interactive mode, the user can perform specific choices at several steps, such as: peptide length; overlap between consecutive peptides; peptide positioning with respect to secondary structure elements; threshold values of interatomic distance and/or solvent accessible surface area, which are used to identify intermolecular interaction and solvent accessibility, respectively.

The MinePept website is free, open to all users and without login requirement at the address: https://minepept.ibpm.cnr.it/.

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Other contributions

Structural Insights into the Bathochromic Shift of Astaxanthin in Homarus americanus Unravels the Molecular Basis of *marine invertebrate colouration*

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The study of naturally occurring lipid bioactive compounds and their interactions with proteins has become a critical area of research, particularly in understanding the mechanisms underlying color changes in biological systems. Carotenoproteins, common throughout marine invertebrates, are essential in generating vibrant colors through complex interactions between carotenoids and proteins. One key example is the Homarus americanus (American lobster), whose distinctive blue coloration arises from the interaction of the carotenoid pigment astaxanthin (AXT) with the α-crustacyanin (α-CR) protein complex. α-CR are constituted of multiple copies of the heterodimer β-crustacyanin (β-CR) in which the chromophore, namely the carotenoid astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione), is associated stoichiometrically with the protein subunits H1 and H2 (1). Astaxanthin exhibits a characteristic red-orange color, absorbing at 472 nm in its free form. However, when bound to the crustacyanins, it undergoes a bathochromic shift, shifting its absorption maximum from red-orange (λmax = 472 nm) to purple-blue (λmax = 591 nm) to dark-blue (λmax = 631 nm) upon interaction respectively with β-CR and α-CR, resulting in the blue hue of the lobster carapace. β-CR has been extensively studied and structural data provided insight into the molecular basis of the spectral shift to 591 nm, but detailed understanding of α-CR, the main complex responsible for the larger shift, has been lacking so far. Recently, we obtained a high-resolution cryo-electron microscopy structure of α-CR from *H. americanus* complemented by small angle X-ray scattering and X-ray diffraction, revealing groundbreaking key features behind the molecular mechanisms responsible for the significant bathochromic shift to 631 nm. The cryo-EM structure of H. americanus α-CR to a resolution of 2.75 Å reveals, besides the expected crustacyanin subunits H1 and H2, the presence of a third protein belonging to the heptatricopeptide repeat protein (HPR) family, which interconnects a variable number of β-CR heterodimers and interacts asymmetrically with the two astaxanthin molecules, leading to the observed bathochromic shift to λmax = 631 nm. The study provides not only a successful understanding of the lobster blue coloration but also an exhaustive advance of how protein-carotenoid interaction influences the spectral properties of astaxanthin laying the foundation for the rational design of astaxanthin-based scaffolding compounds. Such compounds hold promises for applications in nanotechnology with the possibility of manipulating the bathochromic shift across the entire visible spectrum.

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Exploring the role of metals in Carbonic Anhydrase 2

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Carbonic anhydrases (CAs) are ubiquitous metalloenzymes that catalyse the reversible conversion of CO_2 into bicarbonate (HCO_3^-), crucial for pH homeostasis in the bloodstream, especially in the lungs and kidneys.
Their active site uses a zinc ion (Zn^{2+}) to deprotonate water, generating a hydroxide ion (OH^-) that attacks CO_2. This process is regulated by amino acid residues and water networks that orient substrates or partake in the proton shuttle system.
CA dysregulation is linked to diseases like glaucoma, epilepsy, sleep apnea, and high-altitude sickness, making CAs key therapeutic targets. The human isoform 2 (hCA2), central to renal function, has been extensively studied, with many inhibitor-bound structures resolved. Sulfonamide-based inhibitors, such as acetazolamide, are used to treat glaucoma by reducing intraocular pressure and to manage epilepsy by preventing pH-induced seizures. These studies have advanced both understanding of CA biology and the development of targeted therapies.
A less investigated area involves the substitution of metals within this enzyme. Some studies have observed the loss of the native esterase activity when Zn^{2+} is replaced by other transition metals. Others have discovered new functionalities, such as nitrite reductase activity in Cu^{2+}-substituted CA2 and peroxidase activity in the Mn^{2+}- variant.
We investigated the structure and properties of human hCA2with various metal substitutions, confirming the existence of a secondary metal-binding site for Cu^{2+} and uncovering a similar site for Zn^{2+}. Through mutagenesis, biophysical analyses, and a comprehensive set of high-resolution structures, we gained deeper insights into the metal coordination geometries and their relationship with distinct catalytic mechanisms.

Structural insights into the Regulator of Telomer Length 1 helicase

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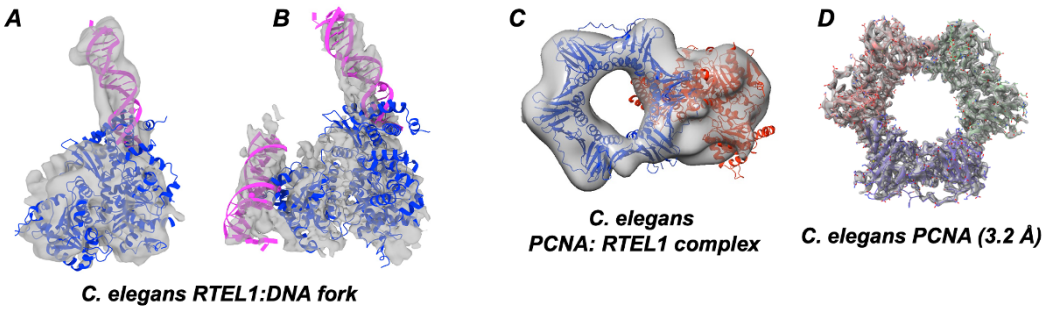
The RTEL1 (Regulator of telomere length 1) helicase belongs to the Iron-Sulphur (FeS) cluster helicase family and is involved in the stability and elongation of telomeres, DNA replication and repair, stability of fragile sites and removal of G4-associated R-loops. It has been implicated in a number of genetic diseases characterized by accelerated telomere shortening. Rtel1 gene amplification has been observed in gastrointestinal cancer and adrenocortical carcinoma and Single Nucleotide Polymorphisms have been associated with increased susceptibility to glioma, underlying a key role for RTEL1 in cancer development.
In addition to the catalytic core, the protein includes a long C-terminal region, which has a modular structure with folded domains interspersed by disordered sequences. We expressed and purified a variety of fragments encompassing the folded domains and the unstructured regions of the C terminal domain of human RTEL1. Using a combination of crystallography, SAXS, NMR and biochemistry, we obtained a comprehensive picture of the architecture and possible function of the C terminal region of human RTEL1. We determined the crystal structure of one repeat, folding as an harmonin homology domains, and obtained SAXS data on all the fragments. NMR data provide experimental information on the interaction between PCNA and the RTEL1 C-terminal region, revealing a putative low-affinity additional site of interaction. Biochemical analysis show high affinity for R-loops and D-loops, as well as telomeric RNA and DNA G-quadruplexes, consistent with the role played by the RTEL1 helicase in homologous recombination, telomere maintenance and preventing replication-transcription conflicts (1).
We have obtained a preliminary structure of the catalytic domain of *C. elegans* RTEL1, in a complex with a DNA fork, to a resolution of about 4-5 Å. Interestingly two distinct complexes can be visualised on the EM grids, with one (Fig. 1A) and two (Fig. 1B) DNA substrates respectively.
We also expressed and purified *C. elegans* PCNA and showed that it physically interacts with the *C. elegans* RTEL1 N-terminal domain; a preliminary very low resolution map pf the PCNA:RTEL1 complex is shown in Fig 1C. We have also solved the 3.2 Å structure of *C. elegans* PCNA, both by CryoEM, and crystallography, in a complex with a PIP peptide (Fig. 1D).



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Structural and Functional Insights into Human Aromatic L-amino Acid Decarboxylase: step-by-step Serial Crystallography on Serotonin Biosynthesis

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Human aromatic L-amino acid decarboxylase (AADC) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme essential for the biosynthesis of neurotransmitters dopamine and serotonin, from the decarboxylation of the corresponding L-DOPA and 5-hydroxy-L-tryptophan (L-5HTP) substrates.
The high-resolution X-ray crystallographic structures of both the apo and holo forms of human AADC were already solved, elucidating the enzyme's conformational dynamics and active site architecture (1, 2). Here, we provide clues into substrate specificity reporting the first AADC crystallographic structures obtained with the indolic substrate, L-5HTP, highlighting the indole side chain binding mode, which differs from that of the catechol moiety of L-DOPA. In addition, we present a detailed structural analysis of AADC during the conversion of L-5HTP to serotonin, using a step-by-step serial X-ray crystallography experiment, elucidating its molecular architecture and catalytic mechanism by isolating decarboxylation intermediates. High-quality crystals of AADC in complex with PLP were obtained and soaked with the substrate; then, they were frozen after different reaction times and subjected to diffraction at ESRF. We could solve the enzyme atomic structure in complex with different intermediates and identify the role of specific amino acid residues and substrate interactions involved in enzyme activity.
Furthermore, the structural analysis, combined with kinetic studies, highlights the conformational changes useful to the steps of the mechanism of decarboxylation. These findings provide a comprehensive understanding of AADC structural determinants for catechol and indole substrate binding, offering potential therapeutic targets for neurometabolic disorders associated with pathogenic variants of AADC deficiency, a recessive inherited disease mainly characterized by motor and neurodevelopmental symptoms associated to mood alterations and serotonin-imbalance disorders.

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