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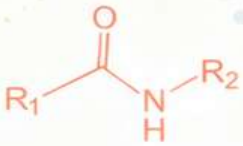
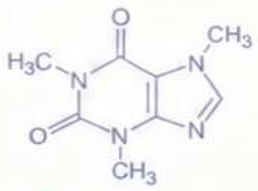
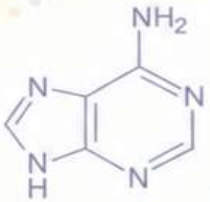


ChemBioStruct26

Book of Abstracts

Rome, Italy
October 26–27, 2026





Welcome to
ChemBioStruct26

ChemBioStruct26 is the First Joint Integrated Chemical Structural Biology Meeting, jointly organised by the BioMacroMolecular Section of the Italian Crystallography Association (BMM-AIC) and the Division of Chemistry of Biological Systems of the Italian Chemical Society (DCSB-SCI).

ChemBioStruct26 was conceived to highlight the complementary expertise of AIC and SCI and to create a forum for scientific dialogue, exchange of ideas, and professional development.

The meeting brings together researchers working at the interface of structural biology and biological chemistry, fostering scientific exchange, collaboration, and professional development.

A **Special Issue** of *Biophysical Chemistry* (Elsevier), edited by the BMM Committee, is dedicated to ChemBioStruct26. Participants are warmly invited to submit their contributions to the Special Issue entitled:

"ChemBioStruct26: From Structure to Function: Integrating Structural Biology and Chemical Biology for Therapeutic Innovation."

We wish you two days of inspiring science, stimulating discussions, fruitful collaborations, and enjoyable scientific exchange.

The ChemBioStruct26 Organising Committee

ChemBioStruct26

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ChemBioStruct26

Scientific Program

MONDAY, 26 OCTOBER 2026

12:30-13:15 Participant registration

13:15-13-30 **Welcome address — Rita Berisio (Chair and Coordinator of the Biomacromolecular section of the Italian Crystallographic Association)**

Molecular Tools for Probing Protein and Oligonucleotide Structure and Dynamics in Biology – Chair Marina Mapelli

13:30–14:00 **Invited Lecture · Massimo Degano** — Bridging crystallography and chemistry towards a dual-action ClpP-targeted multiple myeloma therapy

14:00–14:15 **Marta Palerma** — Crystal Structures of Human Histone Deacetylase 8 Reveal Stereoselective Binding of Novel Hydroxamic Acid Inhibitors

14:15–14:30 **M. Giovanna E. Papadopoulos** — Comparative Molecular Dynamics Study of MPS I-Associated Mutations in α-L-Iduronidase

14:30–14:45 **Humberto Fernandes** — From Molecular Motion to Clinical Insight: Structural Biology of PDE6-Associated Optoretinography and Retinal cGMP Restoration

14:45–15:00 **Giovanni Barra** —Structural and binding studies of the mycobacterial heat shock protein reveal a silent state and offer insights into dendritic cell activation

15:00-15:30 **Coffee Break**

AFTERNOON SESSION II

Chemical Interrogation of Interactions Shaping Molecules: From Metals and Carbohydrates to Metabolites and Protein Partners – Chair Francesco Peri

15:30–16:00 **Invited Lecture · Rachel Hevey** — Using structural information to improve the drug-like properties and target selectivity of carbohydrate-based therapeutics

16:00–16:15 **Vittorio Brufani** — Oligomerization Dynamics of Sigma-1 Receptor: Trimer, Hexamer and Nonamer Revealed by Cryo-EM and Mass Photometry

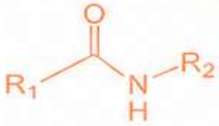
16:15–16:30 **Maria della Valle** — Atomic-level characterization of protein/nano-plastics interactions in living cells

MONDAY, 26 OCTOBER 2026 · CONTINUED

16:30–16:45 **Ilaria Di Donato** — Dissecting zinc recognition by AdcA from *Enterococcus faecium*: an integrated structural and biophysical approach

16:45–17:00 **Giuseppe Ghibellini** — From an Unexpected Crystallization Outcome to PDB Data Mining: Uncovering the ligand-protein interaction of the NADH Derived Metabolite NADHX

FLASH PRESENTATIONS · 17:00–18:16 – Chair Giancarlo Tria

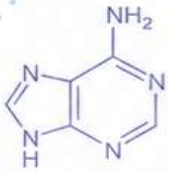


17:00–17:04 **Margherita Antonelli** — Genetic manipulation of *Chlamydia trachomatis* to investigate immune escaping strategies

17:04–17:08 **Elena Bianchi** — Ellagitannins as Potent Inhibitors of SGK1: New Insights for Therapeutic Applications

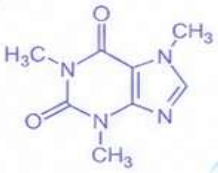
17:08–17:12 **Claudia Binda** — Monoamine Oxidases across evolution: from drug targets to biocatalysts

17:12–17:16 **Elisa Bruni** — Development of a virus-free cell assay for the evaluation of viral protease inhibitors



17:16–17:20 **Eleonora Comparini** — Targeting diadenosine tetraphosphate hydrolase in *Pseudomonas aeruginosa*: A structural path to antivirulence drug discovery

17:20–17:24 **Ionela Raluca Comnea-Stancu** — Selective Electrochemical Recognition of L- and D-Citrulline at a Poly-erythrosine/TiO2@S-Doped Graphene Interface



17:24–17:28 **Martina D'Auria** — Structural characterization of a thermophilic PETase isolated from a geothermal environment

17:28–17:32 **Andrea Dalle Vedove** — A Structural Biology Facility at Elettra 2.0

17:32–17:36 **Emily Freeman** — Capturing protein dynamics through room temperature and time-resolved serial crystallography on ID29 at the ESRF

17:36–17:40 **Luca Gessa** — Structural Insights into RBP3 and Its Potential Interaction with GLUT1

FLASH PRESENTATIONS · CONTINUED

17:40–17:44 **Ramona Georgescu-State** — Green-engineered nanostructured electrochemical interface for stochastic recognition of IL-6 and IL-8 in complex biological matrices

17:44–17:48 **Ornella Ghilardi** — Structural and functional insights into peptidoglycan hydrolases involved in Gram-positive cell division

17:48–17:52 **Wawrzyniec Haberek** — NAT2 mediated acetylation of vorinostat

17:52–17:56 **Zbigniew Kaczynski** — Structural diversity of O-polysaccharides from plant-pathogenic bacteria

17:56–18:00 **Shangkun Li** — Bio-Inspired Stable {Co4O4} Molecular Catalyst for the Oxygen Evolution Reaction

18:00–18:04 **Viviana Perrotta** — AI-guided identification of novel phosphoethanolamine transferase inhibitors as potential colistin adjuvants against antimicrobial resistance

18:04–18:08 **Filippo Piazza** — Harnessing Cashew NutShell Liquid for Target-Based Sustainable Antiviral Drug Discovery via Ugi 4-C reaction

18:08–18:12 **Mario Privitera** — Deciphering the KP32 Phage Architecture: Implications for Antimicrobial Strategies against *Klebsiella pneumoniae*

18:12–18:16 **Alessia Ruggiero** — The dual role of Rv3463 in *Mycobacterium tuberculosis*: bridging redox metabolism to host immune response

TUESDAY, 27 OCTOBER 2026

MORNING SESSION I

Precision Chemistry: Antibodies, Chemical Probes and Beyond as Next-Generation Therapeutics – Chair Cecilia Pozzi

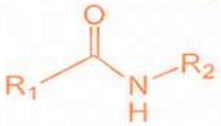
09:00–09:30 **Invited Lecture · Johannes Huebner** — Monoclonal Antibodies and Beyond: Novel Anti-Infective Strategies Targeting Bacterial Surface Structures of multidrug-resistant pathogens

09:30–09:45 **Prabhanshu Tripathi** — Dual Homotypic Antibody Interfaces Enable Broad Recognition of the Malaria Circumsporozoite Protein

09:45–10:00 **Karthik Ramanadane** — Integrative structural analysis of the LRP2-LRPAP1 complex reveals multiple regulatory sites

10:00–10:15 **Md Saiful Islam** — Structural Basis of ATM Activation by MRN and DNA multiple regulatory sites

10:15–10:45 **Coffee Break**

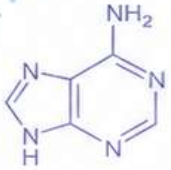


MORNING SESSION II

Integrating Chemical Biology and Structural Analysis to Accelerate Drug and Vaccine Discovery – Chair Rita Berisio

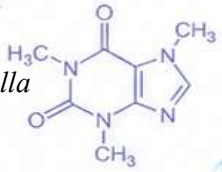
10:45–11:15 **Invited Lecture · Maria Grazia Pizza** — Shaping Immunity: Structural Vaccinology for Next-Generation Vaccine Antigens

11:15–11:45 **Invited Lecture · Juan Hermoso** — From Antibiotic Sensing to Cell Envelope Protection: Structural Biology of Bacterial Resistance Mechanisms



11:45–12:00 **Michelle Fornasier** — Structure-Guided Development of Selective Pyrazine-Based CK1δ Inhibitors

12:00–12:15 **Federica Gabriele** — Site-Selective Targeting of Cryptosporidium parvum Thioredoxin Reductase by Gold Compounds: Structural and Mechanistic Insights



12:15–12:30 **Laura Nagy** — Looking for the next target: The story of a 70-year-old *Shigella sonnei* mutant

12:30–12:45 **Renata Ceragno** — A Modular Synthetic Strategy for Late-Stage Functionalisation of *Staphylococcus aureus* Capsular Polysaccharide Type 1 Oligosaccharides

12:45–13:00 **Rita Berisio** — Conclusions and Announcement of AIC, SCI and Elsevier Young Researcher Awards

ChemBioStruct26

Invited Speakers

A Mitochondrial Protease in Motion: Structural Mechanisms of Inhibition and Functional Consequences in Disease

Marco Patrone[a], Flavio Di Pisa[a], Tommaso Perini[b], Laura Tosatto[c], Paolo Swuec[c], Francesca Coscia[c], Simone Cenci[b], Massimo Degano[a]

[a]Institute of Immunology and Infectious Diseases, Università Vita-Saluta San Raffaele, Milano, Italy; [b]Division of Genetics and Cell Biology, IRCCS Ospedale San Raffaele, Milan, Italy; [c]Structural Biology, Human Technopole, Milan, Italy

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Mitochondrial protein homeostasis is increasingly recognized as a vulnerability in cancer [1,2], yet the structural and mechanistic basis of targeting mitochondrial proteases across neoplasias remains poorly understood. Here, we show that the mitochondrial caseinolytic protease ClpP is upregulated in hematological malignancies, including multiple myeloma, and represents a tractable therapeutic target. We report the crystal structure of truly unliganded human ClpP at 2.4 Å resolution, revealing a symmetric ground state with an unoccupied active site and a disengaged catalytic triad. Using a boronic acid dipeptide inhibitor, VT7-56, we determined a 1.9 Å cryo-electron microscopy structure of inhibited ClpP, capturing a ligand-induced conformational transition characterized by pronounced rotation between the two heptameric rings and complete ordering of the active site. Although VT7-56 also exhibits activity toward the 20S proteasome, genetic ablation of ClpP attenuates its cytotoxic effects, indicating a significant contribution of ClpP inhibition to the cellular response. Consistently, a selective ClpP inhibitor recapitulates key phenotypes in multiple myeloma cells, including apoptosis, reduced proliferation, and accumulation of mitochondrial ClpP substrates previously implicated in metabolic rewiring and activation of immune-associated transcriptional programs. Together, our findings define the structural basis of human ClpP inhibition, provide an experimental framework for its targeting, and establish disruption of mitochondrial proteostasis as a mechanistic driver of multiple myeloma cell death.

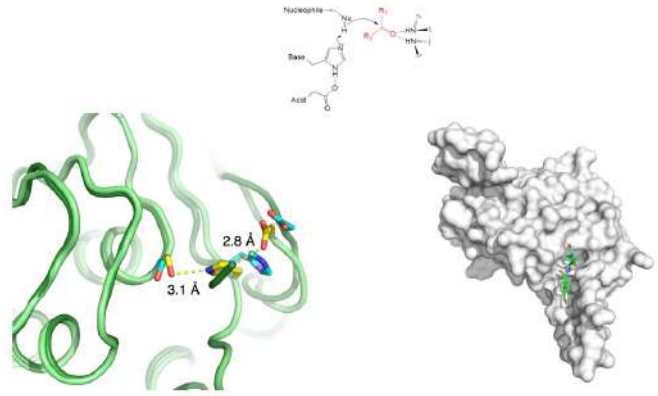

Figure 1. Restructuring of the human ClpP structure and active site upon binding of inhibitor

[1] Cole A. *et al.*, *Cancer Cell*. (2015), 27, 864.
[2] Perini T. *et al*. *Blood.* (2025), 146, 1286.

Using structural information to improve the drug-like properties and target selectivity of carbohydrate-based therapeutics

Rachel Hevey[a]

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Carbohydrates are an important family of natural products which coat all cell surfaces and play essential roles in diverse biological processes, such as immune regulation, infection, and cancer metastasis. Many diseases are characterized by changes in the composition of cell-surface carbohydrates, suggesting their potential utility as a therapeutic target.

Although carbohydrate structures have extensive structural diversity, they suffer from weak binding affinities and inherently poor pharmacokinetic properties. To enhance their usefulness as small molecule pharmaceuticals, 'glycomimetic' compounds can be designed which incorporate more drug-like molecular features yet mimic the structure and function of native carbohydrates [1]. Central to this approach is the use of structural information to guide molecular design, enabling optimization of ligand conformation, polarity, bioisosteric substitutions, and interactions with extended protein binding sites to improve both drug-like properties and target selectivity.

These principles will be illustrated using examples from an ongoing project aimed at developing antagonists of collectin-11 (CL-11), an innate immune protein that has been linked to ischemia-reperfusion injury (IRI) and organ rejection in the context of kidney transplantation [2-4]. Structural models of the CL-11–ligand complex were developed through X-ray crystallography and molecular dynamics simulations to define the carbohydrate binding mode and identify opportunities to exploit adjacent binding site features.

These insights guided the design and synthesis of focused glycomimetic libraries incorporating rational modifications predicted to enhance affinity and selectivity. Compounds were evaluated using thermal denaturation and competitive binding assays, leading to early lead molecules with approximately 100-fold improvements in binding affinity. Beyond advancing the development of CL-11 antagonists for transplantation-associated IRI, this work highlights how structure-guided glycomimetic design can overcome key limitations of carbohydrate-based therapeutics and provides a general framework for improving their drug-like properties and target specificity.

[1] R. Hevey *Pharmaceuticals (*2019), *32*, 55.
[2] C.A. Farrar, *et al.*, *J. Clin. Invest. (*2016), *126*, 1911.
[3] W. Wu, C *et al.*, *J. Am. Soc. Nephrol. (*2018), *29*, 168.
[4] M.C. Howard, *et al.*, *FASEB J. (*2020), *34*, 822.

Novel anti-infective strategies targeting bacterial surface structures of multidrug-resistant pathogens

Johannes Hübner

Dr. von Hauner Children's Hospital · LMU Klinikum München

Vancomycin-resistant *Enterococcus faecium* ranks among the highest Gram-positive priority pathogens on the 2024 WHO bacterial priority pathogens list. Mean vancomycin resistance across the EU/EEA is 19.8% and the incidence of bloodstream infection rose by 25.9% between 2019 and 2023. More than 10,000 deaths were attributable to the organism worldwide in 2021. *Pseudomonas aeruginosa* presents a comparable problem among Gram-negative organisms: intrinsic and acquired resistance mechanisms combine, the species accounts for a substantial proportion of AMR-attributable deaths, and no monoclonal antibody or vaccine has been licensed despite more than a decade of clinical development. In both species the available therapeutic options are narrowing as the burden increases.

The bacterial cell surface is accessible to antibodies and partly conserved between strains and species. The enterococcal surface glycome can be divided into three systems: capsular polysaccharides, glycerophosphate lipoteichoic acid (LTA), and the rhamnan-based enterococcal polysaccharide antigen (Epa) with its wall teichoic-acid decorations. Antibodies to LTA are opsonic and cross-protective, including across genera against *Staphylococcus aureus*, but do not kill CPS-C/D strains. This observation led to the identification of the capsular diheteroglycan (DHG) as a target. These carbohydrate antigens are complemented by a panel of protein antigens that are targets of opsonic/protective antibodies (AdcA/PsaA, PpiC/PrsA, EbpA, SagA, TraM).

A pathogen-agnostic pipeline for human monoclonal antibody discovery combines functional donor screening (opsonophagocytic killing, C1q deposition, antigen-to-B-cell flow cytometry) with single-cell BCR sequencing, using monobiotinylated synthetic glycans as bait for antigen-specific B cells. Glycoconjugates in which the carrier protein is itself protective (DHG–SagA, DHG–PpiC), multivalent protein formulations ranging from trivalent to pentavalent, and antibodies against the conjugation-machinery protein TraM, which mediate opsonophagocytic killing and interfere with plasmid transfer, extend the approach from elimination of the pathogen to interference with the horizontal spread of resistance.

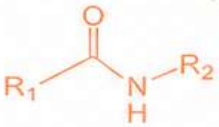
Synthetic glycochemistry, structural biology and immunology thus contribute to a common set of approaches: human and humanized monoclonal antibodies, structure-guided glycoconjugates, and chemical probes directed at conserved surface structures of multidrug-resistant pathogens.

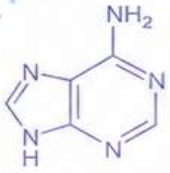
Shaping Immunity: Structural Vaccinology for Next-Generation Vaccine Antigens

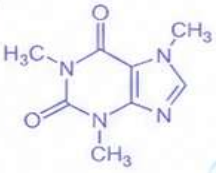
Mariagrazia Pizza[a]

[a]*Imperial College London, UK*

Discovering a new antigen does not necessarily make it an effective vaccine target. Protective immunity depends on which epitopes antibodies recognise, whether these are accessible on the surface of the pathogen, and how antibody binding can block infection. Structural vaccinology brings these questions into antigen design, enabling protective epitopes to be preserved and presented effectively to the immune system, enhancing antigen stability, and helping to overcome mechanisms of immune evasion. These examples show how structural biology can support the development of next-generation vaccines, particularly against antimicrobial-resistant bacterial pathogens.







From Antibiotic Sensing to Cell Envelope Protection: Structural Biology of Bacterial Resistance Mechanisms

Juan A. Hermoso

Department of Crystallography and Structural Biology, Instituto de Química-Física "Blas Cabrera", CSIC, Madrid. (Spain).

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Antimicrobial resistance is driven by the remarkable ability of bacteria to sense, adapt to, and ultimately withstand antibiotic challenge. At the same time, the bacterial cell envelope is a dynamic and highly organized structure whose integrity is essential for survival. Understanding the molecular mechanisms that connect antibiotic sensing, resistance and cell-envelope homeostasis is therefore critical for identifying new opportunities for therapeutic intervention. In this lecture, I will discuss how structural biology can uncover these mechanisms at the molecular level and provide a framework for their pharmacological exploitation.

Our structural studies on methicillin-resistant *Staphylococcus aureus* (MRSA) of the resistance determinant PBP2a have revealed the molecular basis of β-lactam resistance and its allosteric regulation [1-4]. I will then present our recent work on BlaR1, the membrane-associated receptor that senses β-lactam antibiotics and initiates the resistance response. We identified a small-molecule inhibitor that blocks BlaR1 signaling and restores susceptibility of MRSA to β-lactam antibiotics [5]. X-ray crystallography of BlaR1 in complex with the inhibitor revealed its molecular mode of recognition and provided a structural framework to explain inhibition and guide the development of new resistance-modifying compounds.

Finally, I will discuss how structural biology can also illuminate mechanisms that protect the bacterial cell envelope in Gram-negative pathogens. In *Pseudomonas aeruginosa*, we recently identified PA2854 as the enzyme responsible for covalently anchoring the outer-membrane lipoprotein OprI to peptidoglycan [6]. Structural and biochemical analyses reveal the molecular basis of substrate recognition and catalysis, providing new insights into how bacteria reinforce the architecture of their cell envelope.

Together, these studies illustrate how structural biology can move from the visualization of molecular interactions to the mechanistic understanding of bacterial resistance and envelope homeostasis, revealing new vulnerabilities that may be exploited for the development of next-generation antibacterial strategies.

[1] Otero *et al. PNAS* (2013) 110, 16808-16813.
[2] Fishovitz *et al. JACS* (2014) 136, 9814-9817.
[3] Bouley *et al. JACS* (2015) 137, 1738−1741.
[4] Mahasenan *et al. JACS* (2017) 139, 6795-6798.
[5] Nguyen *et al. Nature Chem Biol* (2025) 21, 882-489.
[5] El-Araby *et al. JACS* (2026) 148, 25740-25756.

ChemBioStruct26

Oral Presentations

Crystal Structures of Human Histone Deacetylase 8 Reveal Stereoselective Binding of Novel Hydroxamic Acid Inhibitors

Marta Palerma[a,b], Luke N.M. Harding[c], Samuel Y. Aboagye[d], Valentina Z. Petukhova[c], Federica Gabriele[a], Matteo Ardini[a], Jala A. Bogard[d], Natalia Makaro[c], David L. Williams[d], Pavel A. Petukhov[c], Francesco Angelucci[a]

[a]Dept. of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy; [b]Dept. of Science, Technology and Society, University School for Advanced Studies of Pavia, Pavia, Italy; [c]Dept. of Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, United States of America; [d]Dept. of Microbial Pathogens and Immunity, Rush University Medical Center, Chicago, United States of America.

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Histone deacetylases (HDACs) are enzymes that catalyze the removal of acetyl groups from acetyl-lysine residues in histones and non-histone proteins and, as part of the epigenetic machinery, play a key role in the regulation of many biological processes. Among the different HDAC isoforms, HDAC8, a Zn^{2+}-dependent class I HDAC, has emerged as a promising drug target for the treatment of several diseases, including cancer and parasitic diseases.

In cancer, HDAC8 is implicated through its overexpression or deregulation in several tumor types, making the development of inhibitors with selectivity over other isoforms a major therapeutic objective to overcome the adverse effects and limited selectivity of FDA-approved human HDAC inhibitors [1].

In parallel, HDAC8 plays a crucial role in parasite survival in Schistosoma mansoni, the etiological agent of schistosomiasis, a Neglected Tropical Disease for which a single drug is currently available. In schistosomes, HDAC8 is highly expressed throughout all stages of the life cycle, and its inhibition leads to parasite death. In this context, the availability of potent and selective S. mansoni HDAC8 (SmHDAC8) inhibitors is of particular importance [2].

Novel tetrahydroisoquinoline (TIQ)-based HDAC8 inhibitors have shown an improvement in potency and selectivity for human HDAC8 (hHDAC8) compared to other HDAC8 inhibitors studied [3], also showing activity against SmHDAC8 with micromolar IC_{50} values.

We solved several X-ray crystal structures of hHDAC8 in complex with TIQ-based inhibitors. The structures reveal the typical hHDAC8 fold, with an α/β domain and multiple loop regions. The inhibitors bind at the catalytic site, occupying the hydrophobic tunnel, with their hydroxamic acid group chelating the catalytic Zn^{2+} ion. The structures provide insights into the stereoselectivity of these compounds. Co-crystallization with racemic mixtures reveals preferential binding of the R-stereoisomer over the S-stereoisomer, consistent with functional data showing higher activity of the R-enantiomer. Crystal structures of hHDAC8 in complex with the pure enantiomers further support these findings, revealing a different substituent orientation for the S-enantiomer.

These structural insights provide a basis for the rational design of HDAC8 inhibitors with improved isoform selectivity, species selectivity, and stereoselectivity for anticancer and antischistosomal drug discovery.

[1] Banerjee S, *et al. Eur J Med Chem*. (2019),164:214-240.
[2] Chakrabarti A, *et al*. *Trends Pharmacol Sci* (2015);36(7):481-92.
[3] Taha TY, *et al*. *ACS Med Chem Lett.* (2017); 8(8):824-829.

ChemBioStruct26

Comparative Molecular Dynamics Study of MPS I-Associated Mutations in α-L-Iduronidase

M. Giovanna E. Papadopoulos[a], Giuseppe Bonapace[b], Lorenzo Taglietti[c], Barbara La Ferla[d], Michele Pitaro[e], Daniela Concolino[b], Rosanna Rizzi[a], Domenico Alberga[a], Giuseppe F. Mangiatordi[a]

[a]CNR - Institute of Crystallography; [b]Department of Health Sciences, University Magna Graecia; [c]Department of Biotechnology and Biosciences, Milano-Bicocca University; [d]Department of Earth and Environmental Sciences; [e]Consorzio Interuniversitario "Istituto Nazionale Biostrutture e Biosistemi" (INBB)

Mucopolysaccharidosis type I (**MPS I**) is a rare autosomal recessive lysosomal storage disorder caused by mutations in the **IDUA** gene, which encodes α-L-iduronidase [1]. This is a **glycosylated** enzyme that degrades glycosaminoglycans (GAGs) [2]. Enzymatic deficiency leads to GAG accumulation in lysosomes, disrupting cellular function and causing multisystemic damage with clinical severity ranging from mild (Scheie) to severe (Hurler) [3]. Among pathogenic IDUA variants, **L490P** and **P533R** are commonly linked to attenuated forms. These mutations retain partial α-L-iduronidase activity, reducing systemic severity but still causing subtle neurological impairments, such as cognitive delays and attention deficits, due to GAG accumulation in the central nervous system (CNS) [3].

We employed unbiased molecular dynamics (**MD**) simulations to compare the wild-type (WT) protein with the **L490P** and **P533R** mutants, aiming to uncover potential microswitches that lead to protein misfolding. The simulations were performed at neutral and acidic pH to mimic the microenvironment of the endoplasmic reticulum (ER) and of the lysosomes, where the enzyme exerts its function. We performed three replicates of 1.6 μs (400 ns equilibration and 1.2 μs production) for the WT and the two mutants at **pH 7.5** and **4.5** (6 systems, 18 simulations). The root mean square deviation (**RMSD**) and fluctuation (**RMSF**) analyses performed to evaluate the protein's secondary-structure stability did not reveal major differences between the WT and the two mutants. We also employed **RMSF** analyses of the **N372-linked glycan** for all systems at both pH values. Interestingly, the glycan of **L490P** and **P533R** showed lower fluctuation only at acidic pH compared to the WT. We, therefore, proceeded with **cluster analyses** of the WT and mutants at acidic pH to characterize the different conformational states experienced by the glycan in the mutants compared to the WT (**Figure 1; representative frames**). Finally, the **hydrogen bond** analysis of the **N372-linked glycan** with the enzyme in the simulation of the **P533R** mutant revealed interactions with specific amino acidic sites that are not present in the other systems, responsible for the lower fluctuation of the glycan. This may be an indication of the enzyme's inability to degrade GAGs.

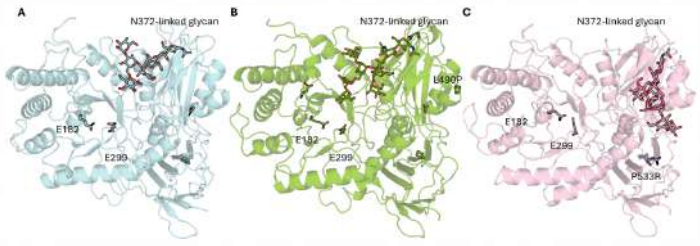

Figure 1. Representative frames of the most populated cluster of each system trajectory at pH 4.5 showing the different conformational space occupied by the N372-linked glycan, namely **A**) WT (in cyan), **B**) L490P (in green), and **C**) P533R (in pink). The catalytic residues and mutated residues are labelled and shown in sticks.

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This project is funded by the European Union – Next Generation EU, under the National Recovery and Resilience Plan (PNRR), Mission 4, Component 1, Project CUP 2022S2M7E5.

From Molecular Motion to Clinical Insight: Structural Biology of PDE6-Associated Optoretinography and Retinal cGMP Restoration

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Vision begins when light activates phototransduction in the highly ordered photoreceptor outer segment. Phosphodiesterase 6 (PDE6) converts transducin activation into cyclic GMP (cGMP) hydrolysis and channel closure. Light also induces nanometre-scale changes in photoreceptor shape, recorded in vivo by optoretinography (ORG). Their molecular origin remains unresolved, limiting diagnostic interpretation. Our main work addresses PDE6, and we showed that sildenafil attenuates photopic flicker-driven length modulation [1], supporting a PDE6-dependent contribution without proving that PDE6 is the physical force generator. We are now testing this hypothesis using cryo-electron tomography and complementary imaging to quantify disc organization and outer-segment changes under dark, light-stimulated and inhibitor-treated conditions. We are complementing these structural observations with HPLC measurements of cGMP hydrolysis by native PDE6, together with transducin-dependent reconstitution. We aim to connect Gαt stoichiometry, PDE6γ rearrangement, and membrane-associated ensembles with disc spacing and ORG kinetics. This is more than a structural curiosity. In retinitis pigmentosa, ORG can reveal functional loss before conventional structural markers change [2]. Molecular attribution could make ORG a mechanism-informed biomarker for early dysfunction, stratification, and treatment monitoring, but such attribution requires causal perturbation and orthogonal validation. Recovery of photoreceptor sensitivity requires replenishment of the cGMP pool. Both insufficient and excessive retinal guanylyl cyclase (RetGC) activity can disrupt cGMP homeostasis and cause inherited retinal diseases. Therefore, we have initiated a structural and biochemical project on RetGCs, which are the enzyme that restores cGMP during photoreceptor recovery. We expressed full-length human RetGC1; purified its catalytic region, and regulatory proteins such as GCAP1, and RD3; and established HPLC assays of regulated cGMP synthesis. This platform provides a basis for examining how disease-associated RetGC1 variants alter GCAP-dependent regulation, including through impaired GCAP affinity and how these molecular defects relate to clinical phenotype [3]. Pathological cyclase output may also be restrained by regulator-derived inhibition [4], or compensatory hydrolysis elsewhere in the circuit [5]. Together, structural studies of intact complexes and quantitative measurements of cGMP hydrolysis and synthesis may reveal whether cGMP signaling in disease requires restoration, restraint, or rebalancing, while the PDE6-ORG studies aim to relate molecular mechanisms to diagnostic signatures.

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Structural and binding studies of the mycobacterial heat shock protein reveal a silent state and offer insights into dendritic cell activation

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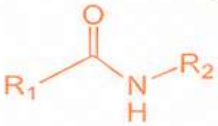
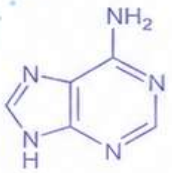
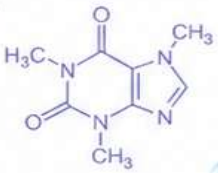


HtpGMtb of *Mycobacterium tuberculosis* is an ATP-dependent heat shock protein that assists the correct folding of nascent and stress-accumulated misfolded proteins, in concert with other chaperones. Beside playing a role in stress response, it is able to elicit an immune response against *M. tuberculosis* infection by activating Dendritic Cells in a Toll Like Receptor 4-mediated manner [1]. However, the molecular basis underlying both its catalytic activity and Toll Like Receptor 4 activation remains poorly understood due to the lack of structural and biophysical data. Despite the limited structural information available, the first crystal structure of HtpGMtb in complex with a non-hydrolysable ATP analogue was successfully determined using X-ray crystallography [4]. The structure reveals that the HtpGMtb dimer adopts a conformationally silent structure, that precludes the dimerisation of the chaperone catalytic domains needed for ATP hydrolysis. Also, binding studies show that HtpGMtb directly interacts with Toll Like Receptor 4 with a nanomolar affinity indicating that a single HtpGMtb dimer can engage two receptor molecules simultaneously. This finding suggests that activation of Toll Like Receptor 4 by HtpGMtb is due to its ability to induce the dimerisation of the host receptor, an essential step for initiating the entire signalling cascade [2-3].





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Oligomerization Dynamics of Sigma-1 Receptor: Trimer, Hexamer and Nonamer Revealed by Cryo-EM and Mass Photometry

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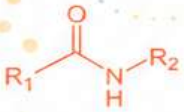


The Sigma-1 receptor (S1R) is an evolutionarily conserved ER-resident membrane protein acting as a pluripotent chaperone and signalling modulator. It maintains cellular homeostasis by orchestrating ER-mitochondria communication at MAMs, sensing lipid microenvironments, calcium flux, and protein quality control as a stress-response hub[1].

Structurally, S1R is a ~25 kDa single-pass transmembrane protein forming homo-oligomers. Crystallography reveals a trimeric architecture with a transmembrane helix and β-barrel-like ligand binding domain (PDB: 5HK1, 6DK1)[2]. Ligand binding triggers conformational changes that regulate oligomerization and localization, linking chaperone activity to signalling.

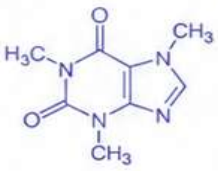
S1R interacts with the ER chaperone BiP; under stress or ligand binding, S1R dissociates from BiP to stabilize client proteins at MAMs, enhancing cellular resilience.

We used mass photometry to investigate S1R oligomerization states in detergent solution, identifying distinct peaks with masses consistent with S1R monomer, trimer, hexamer, and nonamer, along with undefined higher molecular weight species.

Single-particle cryo-EM analysis of S1R in detergent enabled reconstruction of three 3D volumes: a trimer matching prior crystallographic structures, plus hexamer and nonamer forms composed of related trimers with two-fold and three-fold symmetry, respectively. We resolved the trimer and hexamer structures to 2.7 Å and 3.3 Å, respectively. Notably, no density appears in the hexamer active site, while density for an undefined ligand appears in the trimer volume.

Mass photometry on BiP characterized its oligomerization and ATP-hydrolysis cycle, revealing monomerization upon ATP binding with an apparent Kd of 0. 6μM.

We are now employing mass photometry to biophysically probe BiP-S1R interactions across the BiP ATP-hydrolysis cycle and varying Ca^{++} concentrations.



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Atomic-level characterization of protein/nano-plastics interactions in living cells

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Exposure to nano-plastics (NPs) is currently an emerging issue with potential biological implications that are still poorly understood at the molecular level. It has been demonstrated that nanoscale surfaces can interact with proteins, altering their folding, dynamics and stability, but a high-resolution quantitative description of the conformational perturbations induced in the cellular environment is lacking [1].

In this context, human ubiquitin will be used as a molecular model due to its central role in cellular proteostasis, its well-defined structural properties, and its responsiveness to small conformational changes [2].

Here, a description of the significant molecular events regulating protein/NPs interactions will be presented. Advanced solution-state Nuclear Magnetic Resonance (NMR) methodologies will be combined with state-of-the-art in-cell NMR approaches, enabling the direct observation of protein behavior both in controlled environments and within living cells. This integrated strategy is expected to provide unprecedented atomic-resolution insights into the biological impact of nano-plastics and establish a mechanistic framework for understanding their potential molecular toxicity.

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ChemBioStruct26

Dissecting zinc recognition by AdcA from *Enterococcus faecium*: an integrated structural and biophysical approach

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The increasing prevalence of antimicrobial resistance has prompted the search for alternative therapeutic strategies targeting bacterial processes that are essential for survival and virulence [1]. Among these, zinc homeostasis has emerged as a promising target. During infection, the host limits zinc availability through nutritional immunity, requiring pathogens to rely on high-affinity uptake systems to acquire this essential micronutrient [2]. In *Enterococcus faecium*, a multidrug- resistant member of the ESKAPE group, zinc uptake is mediated by the AdcABC transporter, in which AdcA functions as the extracellular solute-binding protein responsible for zinc recognition and delivery to the membrane transport machinery [3]. AdcA displays a bimodular architecture comprising an N-terminal ZnuA domain and a C-terminal ZinT domain connected by a flexible linker (Figure 1) [3]. This modular organization offers the opportunity to investigate the structural and functional properties of the full-length protein together with those of its individual domains, providing insight into the structural determinants governing zinc recognition and protein stability. In this work, the full-length AdcA protein together with its isolated domains were recombinantly expressed and purified for an integrated structural and biophysical characterization. Circular Dichroism (CD) spectroscopy and Differential Scanning Calorimetry (DSC) were employed to assess protein secondary structure and thermal stability in both the apo and zinc-bound states. These studies were complemented by Isothermal Titration Calorimetry (ITC), Microscale Thermophoresis (MST) and fluorescence spectroscopy to investigate zinc recognition and its associated thermodynamic properties. The knowledge gained from this work may also support comparative investigations of homologous zinc uptake systems in other clinically relevant pathogens, including *Klebsiella pneumoniae.* Overall, this work aims to provide a comprehensive structural and biophysical characterization of AdcA and its functional modules, contributing to the development of a mechanistic model of the AdcABC zinc transport system and supporting future antimicrobial strategies targeting bacterial zinc homeostasis [4].

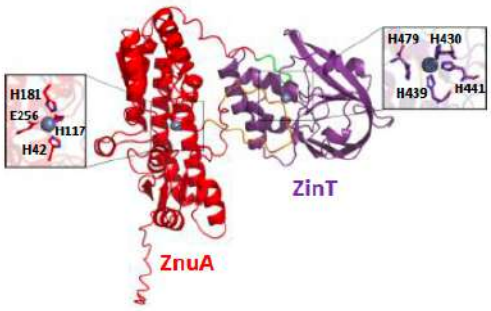

Figure 1. Predicted three-dimensional structure of AdcA

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From an Unexpected Crystallization Outcome to PDB Data Mining: Uncovering the ligand-protein interaction of the NADH Derived Metabolite NADHX

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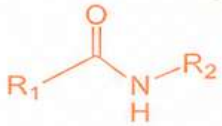
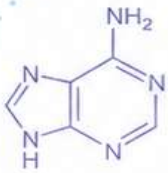
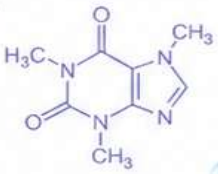


6β-Hydroxy-1,4,5,6-tetrahydronicotinamide adenine dinucleotide (NADHX) is a metabolite formed under stress conditions from the much more common reduced β-nicotinamide adenine dinucleotide (NADH). It can also exist in its phosphorylated form, 6β-hydroxy-1,4,5,6-tetrahydronicotinamide adenine dinucleotide phosphate (NADPHX), which is generated from reduced β-nicotinamide adenine dinucleotide phosphate (NADPH). NAD(P)HX is a highly toxic metabolite associated with acute encephalopathy, skin lesions, and, in severe cases, death [1]. Several proteins have been identified as potential targets of NAD(P)HX mediated inhibition [2–4]. Although NAD(P)HX is a well-characterized metabolite, its relevance from a protein– ligand interaction perspective has received comparatively little attention. Accordingly, at the time of writing, the only NAD(P)HX containing structures deposited in the Protein Data Bank correspond to the two enzymes responsible for its repair: NAD(P)HX dehydratase (NAXD) and NAD(P)HX epimerase (NAXE) [5]. The initial aim of our work was to characterize novel interactions between an alcohol dehydrogenase from the model species *Arabidopsis thaliana* (ADH1) and NADH. We soon discovered that, despite using NADH as a co-crystallization agent, the resulting protein structure contained NADHX. Investigation of the crystallization conditions, the protein–ligand interactions, and inhibition kinetics revealed that NADHX can spontaneously form in specific conditions and can strongly stabilize specific protein conformations even at very low concentrations. Using this knowledge, we first obtained the NADHX bound structure of ADH2, an orthologous alcohol dehydrogenase related to ADH1.



We then applied the insights gained from the ADH structures to systematically search the Protein Data Bank for analogous cases involving other protein classes in which NADH had been used as a co-crystallization agent. This analysis allowed us to identify additional cases resembling that one observed for ADH1. Overall, our results provide the first experimental characterization of the protein–ligand interactions of NADHX with potential target proteins.



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ChemBioStruct26

Dual Homotypic Antibody Interfaces Enable Broad Recognition of the Malaria Circumsporozoite Protein

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The malaria-protective monoclonal antibody L9 preferentially recognizes NVDP-containing minor repeats within the *Plasmodium falciparum* circumsporozoite protein (PfCSP) [1,2]. To broaden L9 recognition across naturally variable PfCSP repeat architectures, we performed site-saturation mutagenesis followed by yeast-display screening and identified affinity-enhanced variants with altered repeat specificity [3]. One variant, L9_yd19, retained potent activity against the benchmark 3D7 strain and showed improved protection against transgenic *Plasmodium* expressing a chimeric CSP containing only two widely separated minor repeats derived from a Kenyan parasite strain. Unexpectedly, L9_yd19 also acquired high-affinity recognition of NANP major repeats and protected against parasites expressing a NANP12 CSP knock-in lacking NVDP repeats [3]. Cryo-electron microscopy revealed that this broadened recognition is associated with an unusual higher-order antibody assembly. L9_yd19 engages repetitive PfCSP while simultaneously forming two distinct homotypic antibody-antibody interfaces. These interactions organize the antibodies into two layers of asymmetric trimers that associate in a head-to-head arrangement, coupling asymmetric three-antibody assemblies through an overall twofold architecture [3]. This organization provides a structural mechanism by which cooperative antibody-antibody interactions can stabilize recognition of repetitive PfCSP epitopes with different sequence compositions and spatial distributions. Together, our results show that affinity maturation of L9 expands recognition across distinct PfCSP repeat architectures while promoting a previously unrecognized supramolecular antibody assembly. These findings highlight homotypic antibody interactions as an additional determinant of recognition of repetitive antigens and provide a structural framework for engineering broadly protective antibodies against malaria.

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ChemBioStruct26

Integrative structural analysis of the LRP2-LRPAP1 complex reveals multiple regulatory sites

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The low density lipoprotein receptor-related protein 2 (LRP2), commonly known as megalin, is a large single transmembrane glycoprotein (~1.2 MDa dimer) that mediates extracellular ligand uptake *via* clathrin-mediated endocytosis. It is primarily localized at the apical membrane of epithelial cells in the kidney, brain, and thyroid, where it mediates the internalization of various ligands, releasing them in endosomes upon vesicle acidification before recycling back to the membrane. Unlike other low density lipoprotein receptor (LDLR) family members, LRP2 interacts with an exceptionally diverse range of ligands, including secreted proteins, hormones, and small molecules. However, it remains unclear how newly synthesized LRP2 avoids premature ligand binding during its trafficking through the secretory pathway. LRPAP1, an endoplasmic reticulum (ER) chaperone of the LDLR family, is hypothesized to prevent ligand interactions during secretion. Yet, the precise mechanism governing its interaction with LRP2 and its dissociation before reaching the apical membrane remains elusive. In this study, we combined cryoelectron microscopy (cryo-EM), crosslinking mass spectrometry, and biochemical analysis to characterize the interaction landscape and stoichiometry of LRPAP1 with human LRP2. Our findings reveal that at least four copies of LRPAP1 associate with LRP2, overlapping with its ligand binding sites, suggesting a competitive inhibition mechanism. While LRPAP1 typically interacts with LDLR family members via positively charged residues binding to complement-type(LA) repeats, we identified one LRPAP1 copy engaging LRP2 through an alternative, previously uncharacterized binding mode. Additionally, we propose a pH-dependent release model in which acidic conditions induce a conformational change in LRP2, leading to the autoinhibition of LRPAP1 binding. Finally, these results allow us to propose a different binding stoechiometry for LRP2's closest homologue LRP1. Our study provides new biochemical evidence for the role of LRPAP1 as a ligand modulator of LRP2.

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ChemBioStruct26

Structural Basis of ATM Activation by MRN and DNA

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The Ser/Thr kinase ATM (ataxia-telangiectasia mutated) orchestrates the cellular response to DNA double-strand breaks (DSBs) in humans. In this process, ATM is activated by DNA and the heterotrimeric MRN complex (MRE11, RAD50, and NBS1), yet the underlying molecular mechanism is unclear. We reconstituted *in vitro* the DNA damage signalling complex comprising full-length MRN, dsDNA, and full-length ATM and determined its structures using cryo-EM [1].

One of the structures of the complex revealed that ATM, which exists as a homodimer under basal condition, transforms into to a monomer upon binding to the MRN-DNA complex, providing structural evidence for the first time for a long-standing proposal of ATM activation by MRN/DNA raised more than twenty years ago [2]. Remarkably, we also observed ATM/p53 retaining its homodimeric state complexed to MRN/DNA, providing structural evidence for a previously unrecognised MRN/DNA-mediated mechanism of ATM activation [1].

Reconstitution of the complex was leveraged on an ATM mutant, bearing mutations within a conserved domain, which binds MRN more strongly than the wild-type ATM [3]. Structural characterisation of the mutant revealed that it transitions to its active-state conformation while maintaining its homodimeric state. Likewise, oxidation also activates ATM as a homodimer [4]. In contrast, MRN/DNA-mediated activation accompanies dimer and monomer, demonstrating previously unnoticed structural plasticity in ATM that is inducible to various active-state conformations subject to different cellular contexts.

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ChemBioStruct26

Structure-Guided Development of Selective Pyrazine-Based CK1δ Inhibitors

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Casein kinase 1δ (CK1δ) is a Ser/Thr kinase involved in neuronal homeostasis and altered in neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). [1] Developing CK1δ inhibitors is challenging because of its similarity to related isoforms, especially CK1ε [2].

Starting from an in-house screening hit, a series of pyrazine-based CK1δ inhibitors was developed through a systematic SAR study. Initial optimization focused on the position 3 of the pyrazine led to the identification of nanomolar inhibitors, while subsequent exploration of the position 6 further defined the structural requirements for potency (Figure 1A). Lead compounds were extensively characterized using complementary biochemical, biophysical, and structural approaches. Kinase activity assays were used to determine the inhibitory activity, ATP competition assays demonstrated, and thermal shift assays confirmed strong target engagement, while Grating-Coupled Interferometry revealed prolonged residence times and tight-binding behavior for the most promising derivatives. To investigate the effect of kinase activation on ligand recognition, the phosphorylated and unphosphorylated CK1δ forms at Thr220 were produced, and comparatively evaluated with reference and new inhibitors. Their co-crystal structures were solved, providing insights into the molecular interactions responsible for high affinity (Figure 2B) and leading to the identification of structural frameworks for their optimization. Lead compounds displayed high selectivity for CK1δ, showing minimal off-target activity toward the closely related α and ε isoforms. In cellular and Drosophila models of ALS, they significantly reduced TDP-43 phosphorylation, a specific hallmark of this disease. Overall, this study expands the SAR of pyrazine-based CK1δ inhibitors and identifies positions 3 and 5 of the scaffold as promising sites for the development of potent and especially isoform- selective next-generation inhibitors, providing valuable chemical probes to investigate CK1δ biology, particularly in neurodegenerative diseases.

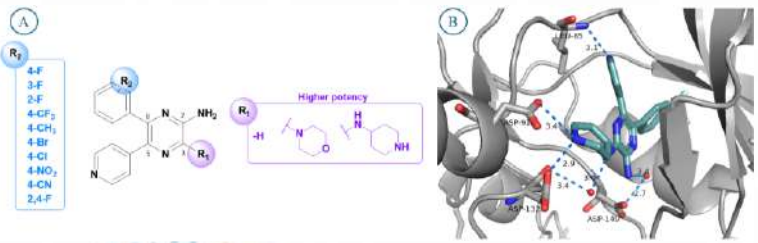

Figure 1: A) Developed inhibitors. B) Co-crystal structure of representative inhibitor-CK1δ.

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Site-Selective Targeting of *Cryptosporidium parvum* Thioredoxin Reductase by Gold Compounds: Structural and Mechanistic Insights

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Cryptosporidium spp. are apicomplexan parasites responsible for cryptosporidiosis, a severe diarrheal disease that disproportionately affects young children and immunocompromisedindividuals. Therapeutic options remain limited as nitazoxanide, the only approved drug, shows reduced efficacy in immunocompromised patients [1]. Cryptosporidium lacks a canonical Glutathione Reductase and relies on the Thioredoxin reductase/Thioredoxin (TrxR/Trx) system for glutathione reduction and redox homeostasis. This unique dependency creates a critical metabolic bottleneck and positions TrxR from *C. parvum* (CpTrxR) as a high-value drug target [2], a conclusion further supported by genetic studies demonstrating its essentiality for parasite proliferation and sexual development [3]. CpTrxR is a type II TrxR featuring two distinct cysteine-based redox centers: a conserved N-terminal CXXXXC motif proximal to the FAD cofactor, and a C-terminal CGGGGKCG motif arranged within a β-hairpin-like region. Notably, this C-terminal redox architecture is unique to apicomplexan TrxRs, offering a rare opportunity for selective inhibition. Our crystallographic studies capture three previously unobserved catalytic intermediates of the CpTrxR C-terminal tail, revealing pronounced conformational plasticity and providing new insights into the enzyme's catalytic cycle and druggability. Building on this structural framework, we investigated the interaction of CpTrxR with six gold-containing compounds, including the clinically approved drugs Auranofin and Aurothiomalate, as well as gold(III) and gold-carbene complexes. Gold compounds are known to perturb redox systems in parasites [4], yet the molecular determinants governing their selectivity in apicomplexan TrxRs have remained unclear. By integrating X-ray crystallography with mass spectrometry and inhibition assays, we demonstrate that these compounds exhibit distinct, site-selective reactivity toward CpTrxR. Their chemical properties enable preferential targeting of either the N-terminal redox center or the apicomplexan-specific C-terminal motif, highlighting the role of local chemical environment, solvent accessibility, and conformational dynamics in dictating inhibitor specificity. This study defines a mechanistic framework for the rational design of type II TrxR inhibitors targeting apicomplexan parasites, addressing a critical unmet need in cryptosporidiosis and positioning gold-based scaffolds as a promising starting point.

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Looking for the next target: The story of a 70-year-old *Shigella sonnei* mutant

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Gram-negative bacteria are responsible for various severe infections, including gastroenteritis or pneumonia. Their outer membrane is covered by lipopolysaccharides in about 75- 80%, a group of molecules that contributes to integrity and antibiotic resistance. While lipopolysaccharides share conserved general features, their outer region is highly variable and able to respond to the environment, enabling bacteria to adapt to changing conditions.

Lipopolysaccharide research is not limited to identifying a new antibacterial target, compounds that interfere with lipopolysaccharide biosynthesis may reduce bacterial fitness or increase antibiotic susceptibility of other materials, making them beneficial in combined antibiotic therapies.

In this study, I examined a more than 70-year-old Shigella sonnei mutant, generated by random mutagenesis by a Hungarian research group. The mutant exhibits a very short lipopolysaccharide structure [1,2], increased susceptibility to certain antibiotics, growth abnormalities, and an inability to grow in elevated temperatures. Through genetic analysis, I identified RfaD (ADP-L- glycero-D-manno-heptose 6-epimerase) as the enzyme responsible for the altered lipopolysaccharide phenotype [3,4], revealing an underexposed vulnerability in the biosynthetic pathway.

These findings led me to propose RfaD as a potential target for chemical inhibition, with the aim of reproducing the phenotype of the now-known mutant Shigella sonnei strain. As a first step toward this approach, I performed structure-based virtual screening against the RfaD active site to find small-molecule modulators of the pathway. Several promising candidates were identified and are now prioritized for further computational and experimental validation.

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A Modular Synthetic Strategy for Late-Stage Functionalisation of *Staphylococcus aureus* Capsular Polysaccharide Type 1 Oligosaccharides

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Synthetic fragments of bacterial capsular polysaccharides are valuable tools for investigating molecular recognition and for developing well-defined glycoconjugate vaccine candidates. *Staphylococcus aureus* capsular polysaccharide type 1 (CP1) is a particularly interesting target due to the presence of two N-acetylgalactosaminouronic acid (GalNAcA) residues and an N- acetylfucosamine (FucNAc) residue in its repeating unit, with taurine substitution adding further structural diversity [1]. However, introducing such modifications at an early stage of the synthesis process can restrict the flexibility needed to access structurally diverse CP1 fragments.

We therefore developed a modular synthetic strategy that preserves flexibility for late-stage functionalisation of the CP1 trisaccharide repeat unit. The approach combines orthogonal protecting groups with post glycosylation modification of the GalNAc residues. Stereoselective glycosylation was achieved using azido-functionalised galactose donors that were equipped with a di-tert-butylsilylene (DTBS) protecting group. A p-methoxybenzyl (PMB) group at the non- reducing end provided a handle for subsequent chain elongation. The resulting trisaccharide bearing orthogonal C-6 protecting groups was obtained through regioselective protection of the disaccharide, glycosylation and reduction of the azide to the acetamide [2]. This architecture enables the site of taurine incorporation to be selected at a later stage of the synthesis. The developed chemistry offers a flexible method for generating CP1 fragments of various lengths and substitution patterns. This will facilitate future research into carbohydrate-protein recognition, antigen mapping and the design of synthetic vaccines.

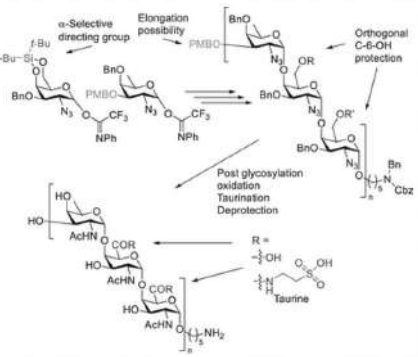

Figure 1. Protecting group strategy for CP1 building blocks and possible taurine pattern.

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Flash Presentations

Genetic manipulation of *Chlamydia trachomatis* to investigate immune escaping strategies

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Chlamydia trachomatis is an obligate intracellular pathogen that infects epithelial cells and it is the most frequent cause of bacterial sexually transmitted diseases.
Due to the pathogen's immune escaping strategies, infections frequently remain asymptomatic and, if untreated, lead to the development of severe chronic conditions like pelvic inflammatory disease, ectopic pregnancy and infertility.
During *Chlamydia trachomatis* infections, the first immune cells that are recruited are neutrophils. However, it has been observed that the pathogen attenuates the neutrophil (PMN) response via different mechanisms, some of which remain to be clarified.
The best approach to understand the mechanisms that allow the bacteria to evade the host defenses is to genetically manipulate the pathogen to produce gene-specific knockout mutants. However, mutagenesis of *Chlamydia trachomatis* has always been challenging because of its unique biphasic life cycle and its intracellular nature. In this study Fluorescence Reported Allelic Exchange Mutagenesis (FRAEM, Mueller KE, Wolf K, Fields KA, 2016) was successfully applied to obtain mutant strains of *Chlamydia trachomatis*.
In the second part of the study, neutrophils were isolated from human blood and infected with the generated mutant strains. Preliminary results suggest that one of the genes under investigation may play a role in attenuating the neutrophil response to *Chlamydia* infection.
These findings provide further insights into the complex interactions between *Chlamydia trachomatis* and the host immune system, offering potential new avenues for future research.

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Ellagitannins as Potent Inhibitors of SGK1: New Insights for Therapeutic Applications

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Kinases are key regulators of cellular signalling and are involved in the onset and progression of several human diseases, including cancer. Serum- and glucocorticoid-regulated kinase 1 (SGK1) is a serine/threonine kinase belonging to the AGC kinase family and is involved in multiple physiological processes, including cell proliferation, survival, ion transport, and stress responses [1]. SGK1 is overexpressed in several cancer types and dysregulated in pathological conditions including hypertension, diabetes, ischaemia, and neurodegenerative disorders [2]–[4]. Moreover, SGK1 activity has been associated with tumour progression and resistance to anticancer therapies, making this kinase a promising target for drug development [5].

Using a combined ligand- and structure-based in silico approach, we screened a panel of potential SGK1 inhibitors and identified several ellagitannins, polyphenolic compounds mainly derived from pomegranate and other plant sources and known for their anti-inflammatory and anticancer properties [6]. Biochemical validation showed that several of these compounds potently inhibit SGK1, with IC50 values in the nanomolar range, and display high selectivity toward SGK1.

Mechanistic studies further indicated that the most potent compound acts as an ATP-competitive inhibitor, consistently with the binding mode predicted by the *in silico* model.

Despite their potent biochemical activity, the high hydrophilicity of ellagitannins limits their ability to cross cell membranes and reach intracellular SGK1. To overcome this limitation, selected compounds were incorporated into PEG-PEI based nanogels and delivered into cells. Nanogel internalisation was confirmed by fluorescence microscopy and flow cytometry, allowing optimisation of both nanogel concentration and incubation time. Intracellular SGK1 target engagement was subsequently evaluated using a NanoBRET-based assay in living cells.

Nanogel-mediated delivery enabled efficient intracellular activity, with the selected ellagitannin FM_3 producing almost complete SGK1 inhibition. These findings identify ellagitannins as a novel class of potent and selective SGK1 inhibitors and support nanogel-mediated delivery as an effective strategy to overcome their limited membrane permeability. Overall,this work provides a basis for the further optimisation of ellagitannin-derived compounds as pharmacological tools and potential therapeutic agents targeting SGK1.

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Monoamine Oxidases across evolution: from drug targets to biocatalysts

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Biogenic amines are pivotal players in many biological processes and amine oxidation is an important enzymatic reaction to metabolize and regulate these molecules. In mammals Monoamine Oxidases (MAOs) are membrane-bound flavoenzymes dedicated to regulate the levels of aromatic neurotransmitters and they represent drug targets for neurological diseases [1]. Highly homologous enzymes catalyzing the same reaction but on different substrates have been found in microbes as water-soluble proteins, which proved valuable biocatalytic tools for enantioselective synthesis of active pharmaceutical ingredients (APIs) [2]. We recently identified a MAO from the thermophile *Thermoanaerobacterales bacterium* (MAOTb) [3]. MAOTb showed melting temperatures of about 70 ºC in different buffers and organic solvents and can be expressed at high yields in *Escherichia coli*. MAOTb preferentially oxidizes n-alkyl monoamines but is active also on polyamines and aromatic monoamines. The crystal structure of MAOTb determined at 1.5 Å showed a high similarity with mammalian monoamine oxidases in the core of the catalytic site in front of the FAD cofactor, while the enzyme features a much more open active site cavity in proximity of the protein surface [4]. In parallel, evolutionary studies showed the presence of a different MAO gene repertoire in fish, highlighting the presence of a new family member (MAO F) in addition to the common enzyme isoform shared with mammals [5].

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ChemBioStruct26

Development of a virus-free cell assay for the evaluation of viral protease inhibitors

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Development of new antiviral strategies targeting specific viral enzymes typically begins with *in vitro* screening of compounds against recombinant proteins, followed by validation in cell-based infection assays. These approaches require high biosafety level facilities, limiting accessibility and increasing costs. To overcome this constraint, we developed a virus-free, cell-based luminescent assay to evaluate inhibitors of viral proteases, thereby reducing the number of candidate molecules to be tested in infection-based systems.

We established this approach using the SARS-CoV-2 main protease (Mpro), a key enzyme for viral replication. The system relies on NanoBiT luciferase technology, in which two subunits are fused within a single construct and separated by a flexible spacer containing a protease-specific cleavage site. Protease activity induces cleavage of the reporter, disrupting luciferase integrity and resulting in loss of luminescence, whereas inhibition restores the signal proportionally to compound potency. This behaviour was confirmed using the reference inhibitor GC376 (IC_{50} = 9.43 +- 1.69 µM) and the approved antiviral Nirmatrelvir (IC_{50} = 11.11 +- 3.48 µM) [1] [2]. The assay works in both lytic and live-cell conditions. Building on this platform, we extended this strategy to West Nile virus (WNV), a flavivirus of increasing epidemiological concern. We developed a NanoBiT-based reporter containing a cleavage site recognized by WNV NS2B/NS3. Also in this case, co-expression with the viral protease resulted in a strong reduction in luminescence (>80%), confirming that the assay efficiently reports proteolytic activity in a dose-dependent manner. To further validate the platform, we tested IRBM-Z-2, a described ZIKV and WNV NS2B/NS3 protease inhibitor [3]. IRBM-Z-2 showed dose-dependent inhibition of WNV protease activity in both lytic and live-cell conditions.

Overall, these virus-free platforms represent versatile tools for the rapid screening of viral protease inhibitors, with potential applications across multiple viral systems and significant implications for antiviral drug discovery.

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Targeting diadenosine tetraphosphate hydrolase in *Pseudomonas aeruginosa*: A structural path to antivirulence drug discovery

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Pseudomonas aeruginosa (*Pa*) is an opportunistic bacterial pathogen responsible for severe pulmonary infections, particularly in immunocompromised individuals and patients with cystic fibrosis. It is characterized by a wide array of adaptive, survival, and drug-resistance mechanisms, making *Pa* infection a major and potentially life-threatening clinical challenges [1]. Diadenosine tetraphosphate (Ap4A) is an alarmone whose intracellular concentration is strictly regulated by specific Ap4A-hydrolyzing enzymes, primarily the diadenosine tetraphosphatase hydrolase ApaH in *Pa*. Recently, *in vivo* studies have shown that ApaH-deficient cells are significantly impaired in their infectivity and persistence in different hosts, including mice [2]. Together with the absence of ApaH homologues in vertebrates, these findings identify this enzyme as an attractive target for antivirulence drug development.

In this context, we undertook a structural and functional characterization of ApaH. X-ray crystallography was integrated with site-directed mutagenesis, enzymatic characterization and *in vivo* phenotypic assays. Crucially, the *in vitro* catalytic activities of ApaH active-site mutants correlated with the virulence-associated traits observed *in vivo* [3]. Guided by structural characterization of ApaH, an *in silico* virtual screening identified candidate inhibitors whose activity was subsequently evaluated throught *in vitro* assays. Concurrently, a high-throughput crystallographic fragment screening is underway.

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Selective Electrochemical Recognition of L- and D-Citrulline at a Poly-erythrosine/TiO2@S-Doped Graphene Interface

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Amino acid metabolism has attracted considerable interest in cancer research due to its extensive range of metabolic processes [1]. While literature establishes total citrulline as a key marker of reduced colorectal cancer risk via the arginine biosynthesis pathway, traditional analytical methods fail to differentiate between L- and D-enantiomers [2]. Poly-erythrosine B functionalised TiO2@Sulfur doped graphene (poly-ERY/TiO2@S-Gr) was employed to develop a novel stochastic electrochemical sensor that is capable of selectively detecting L and D-citrulline (Figure 1). The consistent integration of polyERY and TiO2@S-Gr onto the single-walled carbon nanotube screen printed electrode (SWCNTPE) was validated by structural and morphological investigations, which enhanced the efficacy of molecular recognition and electron transfer kinetics. The electrochemical properties of the fabricated interfaces were evaluated using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), demonstrating enhanced charge-transfer capabilities and a larger electroactive surface area. The sensing interface exhibited minimal limitations of quantification, broad linear concentration ranges, and remarkable sensitivity, enabling the concurrent detection of both enantiomeric forms of citrulline in complex biological matrices, including whole blood and saliva. This work introduces a sustainable, selective, and high-performance stochastic interface for the enantioselective detection of citrulline in biological and clinical research.

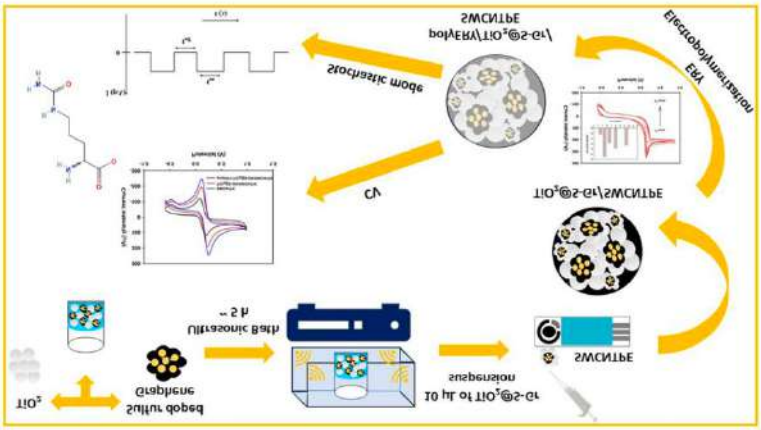

Figure 1. Graphic illustration of the functionalization process of the poly-ERY/TiO_2@S-Gr/SWCNTPE electrode

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Structural characterization of a thermophilic PETase isolated from a geothermal environment

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The growing environmental threat of polyethylene terephthalate (PET) pollution has driven scientific focus toward biodegradation, promoting a new class of enzymes called PETases [1]. However, industrial application demands thermally resilient PET hydrolases that can operate at high temperatures, close to PET glass transition temperature (65 – 85 °C). At these temperatures its polymeric chains become more flexible enhancing substrate accessibility towards the enzyme [1]. Here, we report the structural analysis of DIEGO (*Depolymerising Isolated Enzyme from Geothermal Origin*), a novel thermophilic PETase identified in geothermal metagenomes [2]. DIEGO achieves complete, one-step bioconversion of post-consumer PET into terephthalic acid (TPA) and ethylene glycol (EG) within 96 hours at 60 °C [2]. Operating at this elevated temperature boosts substrate accessibility and drives the auto-hydrolysis of MHET and BHET intermediates. In solution and crystalline structural analyses reveal that DIEGO adopts a classic α/β-hydrolase folding, consisting of a nine β-strands core flanked by ten α-helices. DIEGO also displays the conserved catalytic triad (Ser-Asp-His) and a single C-terminal disulfide bond. The enzyme exhibits high intrinsic thermostability with a melting temperature of approximately 89 °C, which is further enhanced by the presence of divalent cations as Mg^{2+} and Ca^{2+}. Through X-ray crystallography we resolved four different DIEGO structures coming from different crystallization conditions. The analysis of the crystal structures revealed the presence of PEG fragments close to the active site, mimicking PET substrate, and a magnesium-binding site that reduces the flexibility of coordinating residues. Structural comparisons with the mesophilic *Ideonella sakaiensis* PETase (*Is*PETase) [3] and the thermophilic Leaf-Branch Compost Cutinase (LCC) [4] highlight DIEGO's unique structural features. Combining high-temperature activity with a highly stable scaffold, DIEGO represents a powerful candidate for industrial PET upcycling and circular economy processes.

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A Structural Biology Facility at Elettra 2.0

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Elettra-Sincrotrone Trieste is a multidisciplinary research center of excellence for materials and life sciences, specialized in managing a high-quality synchrotron light source, a FEL and several support laboratories. One of such labs is the Structural Biology Laboratory (SB Lab), which comprises research
groups working on structure-based discovery of new drugs and on the structural analysis of protein-nucleic acid interactions.
The synchrotron ring, since 2025, is undergoing a substantial upgrade with the building of an entirely new source - Elettra 2.0 - with will significantly enhance the brilliance and coherence of the X-ray source, surpassing the capabilities of the Elettra ring. In this framework, the SB Lab, exploiting the Next Generation EU fundings (PNRR - PRP@CERIC) underwent a substantial expansion and upgrade. This action occurs within a joint project among Elettra, IOM and IC-CNR outstations, and AREA Science Park that points to establish an integrated environment for structural biology in the Basovizza science site, combining synchrotron-based techniques and advanced laboratory facilities. In this context, the SB Lab will play the role of central core facility contributing the gene to structure workflow, to support the upgraded micro-crystallography (μXRD/MX) beamline, the renewed high-flux SAXS beamline and the
newly installed Cryo-EM facility. New personnel and instrumentation have been acquired, in the SB Lab allowing an increased output and new and better experiments. The implemented upgrades not only benefit the internal research groups, but with a facility-like approach, offer services to external users spanning from protein production and characterization, to protein crystallization and the solution of the tridimensional structure. The objective is to facilitate the access to the structural biology field and provide a more complete structural biology workflow at Elettra Sincrotrone Trieste. Examples of the running research activities and the recent results will be presented.

Capturing protein dynamics through room temperature and time-resolved serial crystallography on ID29 at the ESRF

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Understanding the intrinsic flexibility and dynamics of macromolecules at the atomic level is central to modern mechanistic research and structure-based drug design. Conventional cryogenic macromolecular crystallography generally provides static macromolecular structures at 100K, where cryocooling enables a single macrocrystal to tolerate a higher accumulated radiation dose [1]. However, cryocooling can also alter conformational ensembles and suppress physiologically relevant structural dynamics [2]. Serial macromolecular crystallography (SMX), whether at an XFEL or synchrotron, is a technique whereby a complete dataset is collated from single diffraction patterns, gathered from many individual microcrystals. By distributing the radiation dose across many crystals, SMX enables efficient room temperature data collection, thus allowing macromolecular structures to be studied in a more physiologically relevant state than by cryogenic crystallography [3],[4]. Importantly, SMX can also be coupled to a defined physical or chemical trigger, such as light exposure or ligand mixing, enabling structural dynamics to be captured in a time-resolved manner [5].

The European Synchrotron Radiation Facility (ESRF) is the first fourth-generation synchrotron to construct a beamline (ID29) dedicated to SMX experiments for the collection of static or time-resolved structures at room temperature. ID29 can deliver microsecond X-ray pulses down to 10 µs with a high repetition rate (up to 925 Hz), and benefits from a larger bandwidth (1%), tuneable energy range (10-35 keV) and a charge-integrating Jungfrau 4M detector [5]. To enable a wide variety of room temperature experiments, ID29 has a high-viscosity extruder, fixed target scaffolds and a tape drive system available for routine collection of ground state structures.

Additionally, ID29 can cater for different sample delivery systems, including viscous injectors, flow cells, microfluidics *etc*. For time-resolved experiments, the aforementioned sample delivery systems can be coupled to a synchronised nanosecond laser for pump-probe microsecond experiments, while the tape drive setup can be used in conjunction with a mixing nozzle to perform ligand mixing experiments on a millisecond timescale.

The combination of these capabilities provides a versatile beamline for investigating macromolecular dynamics and ligand binding at room temperature. In particular, the ability to initiate and resolve structural changes across microsecond-to-second timescales offers opportunities to capture transient structural states inaccessible to conventional cryogenic crystallography, with applications ranging from fundamental mechanistic studies to modern structure-based drug discovery [3].

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Structural Insights into RBP3 and Its Potential Interaction with GLUT1

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Retinol Binding Protein 3 (RBP3) is a glycoprotein localized in the interphotoreceptor matrix (IPM). Although its role in the IPM is still under debate, RBP3 is known to shuttle retinoids between photoreceptors and the Retinal Pigmented Epithelium (RPE) [1]. Mammalian RBP3 is a single glycosylated polypeptide chain of around 1325 amino acids organized into four homologous modules of ~300 amino acids, originating from a gene quadruplication event [2]. The high flexibility of RBP3 represents a major obstacle to full-length, high-resolution structure determination. So far, X-ray structures are available only for individual modules, while the full-length protein has been characterized by Cryo-Electron Microscopy (CryoEM) [3,4]. Here, we combine crystallization studies of RBP3 module 4 (RBP3M4), CryoEM, and Small Angle X-Ray Scattering (SAXS) to investigate its structural organization and ligand-induced conformational changes [4]. Beyond its role in retinoid transport, RBP3 has been implicated in the regulation of retinal glucose uptake and VEGF signaling, suggesting a functional relationship with the glucose transporter GLUT1 [5]. However, the molecular basis of this relationship remains unresolved. Our structural characterization of RBP3 provides a framework for investigating its potential interaction with GLUT1 and for understanding how RBP3 structural organization may relate to its emerging metabolic and protective functions in the retina.

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ChemBioStruct26

Green-engineered nanostructured electrochemical interface for stochastic recognition of IL-6 and IL-8 in complex biological matrices

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Interleukin-6 (IL-6) and interleukin-8 (IL-8) are key pro-inflammatory mediators implicated in colorectal cancer progression and represent relevant molecular targets for sensitive bioanalytical detection [1,2]. In this study, a nanostructured stochastic electrochemical interface was engineered by integrating silver nanospheres (AgNSs) with green-synthesized cerium dioxide nanoparticles (CeO_2NPs), reduced graphene oxide (rGO), and palladium (Pd) within a screen-printed carbon electrode (SPCE) architecture (Figure 1). CeO_2 nanoparticles were synthesized using tea extract as a sustainable synthesis medium, providing an environmentally responsible route for the preparation of the sensing material. The resulting AgNSs/CeO_2NPs–rGO@Pd interface was characterized by complementary structural, morphological, and electrochemical techniques, confirming the successful assembly of the nanocomposite and favorable interfacial electron-transfer characteristics. The stochastic sensor exhibited broad analytical ranges from 4.00×10^{-18} to 1.00×10^{-6} g mL^{-1} for IL-6 and from 1.00×10^{-20} to 1.00×10^{-6} g mL^{-1} for IL-8, together with ultralow limits of quantification. The analytical performance was further validated in complex biological matrices, including tumor tissue, urine, whole blood, and saliva, with recoveries above 98% and relative standard deviation (RSD) values below 0.2%. These results demonstrate the potential of combining green nanomaterial synthesis with stochastic electrochemical recognition for highly sensitive cytokine determination in biologically relevant samples. The developed AgNSs/CeO_2NPs–rGO@Pd/SPCE platform provides a sustainable and high-performance approach for the sensitive determination of IL-6 and IL-8, with potential applications in multiplexed cytokine assessment and early cancer-related biomarker screening.

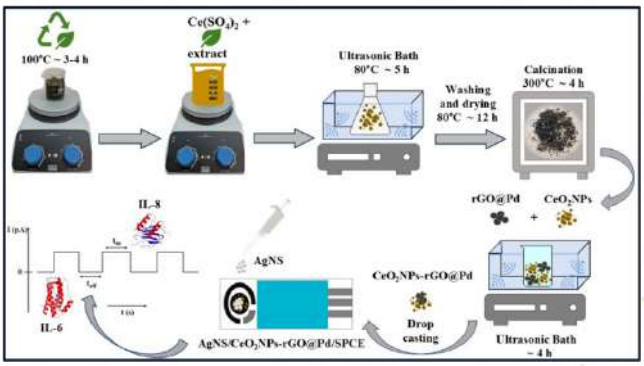

Figure 1. Schematic representation of the fabrication of the AgNSs/CeO_2NPs–rGO@Pd/SPCE stochastic sensor.

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ChemBioStruct26

Structural and functional insights into peptidoglycan hydrolases involved in Gram-positive cell division

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Enterococcus faecium is a leading cause of multidrug-resistant healthcare-associated infections and is currently classified by the World Health Organization as a high-priority pathogen. Secreted antigen A (SagA), a major peptidoglycan hydrolase involved in cell division and cell wall remodeling, has emerged as a promising vaccine candidate. However, the molecular mechanisms regulating its activity and the structural basis of antibody recognition remain poorly understood. Given the need of a strong regulation of bacterial peptidoglycan hydrolases [1], which are potentially suicidal, we combined structural modelling, site-directed mutagenesis and biophysical binding analyses to identify residues regulating substrate access to investigate its regulatory mechanism. In parallel, we investigated the structural basis of SagA recognition by a monoclonal antibody with strong opsonic activity against *E. faecium*. [2] Overall, this study provides new structural and functional insights into our understanding of the molecular mechanisms underlying cell division regulation and antibody recognition of multidrug-resistant *E. faecium*.

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ChemBioStruct26

NAT2 mediated acetylation of vorinostat

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Arylamine N-acetyltransferase 2 (NAT2) is a key metabolic enzyme responsible for the acetylation of amines and is primarily involved in xenobiotic metabolism. NAT2 activity significantly influences the pharmacokinetics and toxicity of several clinically used drugs, including procainamide, clonazepam, and sulfasalazine, as well as numerous aromatic amines, hydroxylamines, and hydrazines. Although NAT2 is well established as an amine-acetylating enzyme, its ability to modify other functional groups remains underexplored.

In our recent study, we investigated the involvement of NAT2 in the metabolism of histone deacetylase (HDAC) inhibitors containing a hydroxamic acid moiety [1], including vorinostat, an FDA-approved drug for the treatment of T-cell lymphoma. Vorinostat has also shown promising anticancer activity in other malignancies and has been evaluated in clinical trials [2], although its broader clinical application has been limited due to toxicity. We demonstrated that vorinostat exhibits NAT2-dependent toxicity in colorectal cancer cell models. This finding was intriguing from a chemical perspective as NAT2 is classically associated with N-acetylation of amines, whereas hydroxamic acids are generally considered to undergo preferential O-acetylation [3]. We therefore sought to elucidate the structure of enzymatic acetylation of vorinostat and to characterize the resulting metabolite.

Using a combination of enzymatic and chemical synthesis, ^{15}N-HMBC NMR spectroscopy, and UHPLC-HRMS, we confirmed the NAT2-mediated O-acetylation of vorinostat. Our findings highlight the potential role of NAT2 genotype in modulating the activity and toxicity of selected anticancer drugs, providing structural insight into the formation of a toxic vorinostat metabolite. These findings provide a potential mechanistic link between NAT2-mediated metabolism and the toxicity of vorinostat.

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ChemBioStruct26

Structural diversity of O-polysaccharides from plant-pathogenic bacteria

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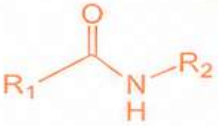
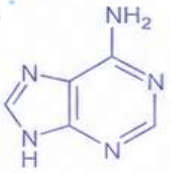


Bacterial phytopathogens are important causal agents of crop diseases, contributing to tissue damage, impaired plant growth, significant yield losses, and, in severe cases, plant death. Among the economically relevant bacterial pathogens of plants are species of the genera *Pectobacterium* and *Dickeya*, belonging to the family *Pectobacteriaceae*. These bacteria are particularly important pathogens of potato, where infection can result in wilting, stem blackening, tissue maceration, and soft rot of tubers, both during cultivation and storage.

Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, consists of three structurally distinct regions: lipid A, the core oligosaccharide, and the O-specific polysaccharide (OPS). As the most distal region of LPS, the OPS is composed of repeating oligosaccharide units displaying considerable structural variability. Due to its surface exposure and structural diversity, the OPS is involved in interactions between bacterial cells and their environment and may contribute to host-pathogen interactions.

The aim of this study was to characterize the chemical structures of OPSs isolated from selected strains of the genera *Dickeya* and *Pectobacterium*. Structural elucidation was performed using a combination of complementary analytical approaches, including sugar composition and methylation analyses, determination of absolute configurations, NMR, and MS [1-3]. The obtained structural data contribute to a better understanding of the chemical diversity of OPSs within the family *Pectobacteriaceae*. Such knowledge may provide further insights into the molecular basis of host-pathogen interactions and the structural features associated with bacterial adaptation and pathogenicity. Moreover, OPS structural profiles may serve as valuable chemotaxonomic markers, supporting the differentiation and identification of closely related bacterial taxa.



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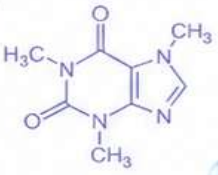

ChemBioStruct26

Bio-Inspired Stable {Co4O4} Molecular Catalyst for the Oxygen Evolution Reaction

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The development of efficient and durable water oxidation catalysts based on polynuclear metal-oxo clusters remains a significant challenge and opportunity in the context of converting renewable energy into chemical fuels. Among these, cubic {Co_4O_4} clusters have garnered considerable attention as molecular water oxidation catalysts, owing to their unique combination of heterogeneous and homogeneous catalytic properties, as well as their structural resemblance to biological systems [1-3]. However, their practical application in the oxygen evolution reaction (OER) is limited by stability concerns, necessitating the development of effective stabilization strategies.

Inspired by the architecture of the oxygen-evolving complex in photosystem II (PSII),4 we report a polymer-hybrid approach for immobilizing {Co_4O_4} cubane oxo clusters to enhance their performance as OER catalysts. This strategy involves the design of asymmetric cubane structures and their incorporation into a conjugated polypyrrole matrix, aiming to improve catalytic activity while suppressing intermolecular aggregation during operation. The asymmetric architecture contributes to improved stability by mitigating decomposition pathways and enhances catalytic efficiency through the presentation of a directed cofacial dihydroxide motif at the active site. Beyond providing structural stabilization, polypyrrole functions as a p-type conducting polymer, facilitating hole transport during the OER and resulting in significantly increased turnover frequencies (TOFs) compared to both unmodified {Co_4O_4} clusters and conventional heterogeneous cobalt oxide catalysts. Combined experimental and computational analyses suggest the presence of multiple oxygen evolution pathways, modulated by intramolecular hydrogen bonding interactions. Overall, this work presents a rational design strategy for enhancing the efficiency and durability of {Co_4O_4} cubane oxo clusters, demonstrating their potential as accessible and highly adaptable catalysts for water oxidation.

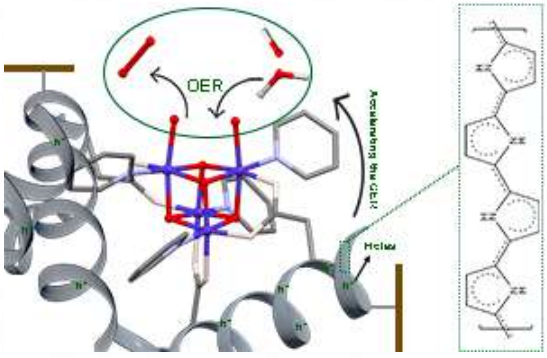

Figure 1. Bio-inspired {Co_4O_4} stabilized by asymmetric coordinated ligands and Ppy polymer for OER.

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AI-guided identification of novel phosphoethanolamine transferase inhibitors as potential colistin adjuvants against antimicrobial resistance

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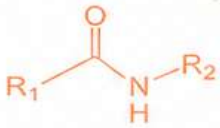

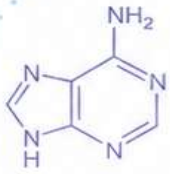

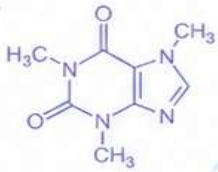

Antimicrobial-resistant pathogens pose a serious threat to human and animal health. The World Health Organization (WHO) considers colistin a last-resort antibiotic against multidrug-resistant Gram-negative bacteria such as *Acinetobacter baumannii* and *Klebsiella pneumoniae*. However, transferable resistance to colistin has emerged, making it a critical public health concern. A key resistance mechanism involves modification of lipid A in the bacterial outer membrane through the addition of phosphoethanolamine (PetN), which reduces negative charge and weakens colistin binding. This process is catalyzed by PetN transferases such as PmrC.

Previous work identified a small molecule, s-Phen, capable of binding PmrC with micromolar affinity, highlighting this enzyme as a promising drug target and supporting the development of colistin adjuvants. Leveraging advances in artificial intelligence (AI), this study applies structure-based drug design to identify new PmrC inhibitors. A panel of candidate compounds was generated using AI tools developed at the Helmholtz Centre in Munich, synthesized at the University of Siena, and tested for binding via microscale thermophoresis. Two compounds demonstrated measurable affinity for PmrC. Subsequent microbiological

assays, conducted with the University of Naples Federico II, showed a significant reduction in the minimum inhibitory concentration (MIC) of colistin in resistant *A. baumannii* isolates. These results provide a foundation for further optimization and support the development of effective colistin adjuvants to combat multidrug-resistant infections.

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ChemBioStruct26

Harnessing Cashew NutShell Liquid for Target-Based Sustainable Antiviral Drug Discovery via Ugi 4-C reaction

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The use of biowaste in medicinal chemistry as source for the development of new drug candidates has gained considerable attention in recent years. Here, we envisage the use of Cashew Nutshell Liquid (CNSL) [1] as a renewable starting material for the development of novel and sustainable antiviral inhibitors. Our endeavours focus on treating infections caused by the West Nile Virus (WNV), a disease that is becoming increasingly prevalent in Western countries due to megatrends, with Italy being one of the main hotspots. In view of the rapid spreading of the virus, the absence of a medical treatment and the severe consequences of such infections, medicinal chemistry efforts are urgently needed. In this context, we pioneer the use of CNSL as starting material that is functionalized in a single step *via* the Ugi four-component (U-4C) reaction [2] to efficiently deliver a green library of antiviral small molecule inhibitors. Specifically, we are synthesizing bio-based CNSL derivatives targeting the active site of the NS2B-NS3 protein, an essential enzyme for viral replication for which crystallographic data are available [3]. Moreover, this pocket is conserved among West Nile, Dengue and Zika viruses opening the possibility of developing *pan*-antiviral small molecules. Considering that the active site consists of a catalytic triad made by serin, histidine and aspartate, we envisioned incorporating basic functional groups to enhance binding affinity toward the target. Furthermore, since most reported NS2B-NS3 protease inhibitors are substrate-derived peptidomimetics [4], we believe that adopting the Ugi-4CR reaction, which directly affords peptide-like scaffolds, represents a strategic synthetic approach. So far, we managed to optimize the synthetic route in a green chemistry fashion with one component of the Ugi-4C coming directly from CNSL, while the other three introduced in a sustainable manner. We made a subset of around 30 differently substituted derivatives featuring chemically diverse branched substituents on the scaffold. Importantly, we obtained a co-crystal structure at 1.8 Å with the most potent in the serie (NS2B- NS3 WNV, IC50 = 78 μM; NS2B-NS3 Zika, IC50 = 70). Based on these initial findings, we are enriching our library with novel derivatives to further increase the affinity toward the target following a structure-based approach. By fulfilling two of the Green chemistry principles (#2 and #7) [5], our results highlight the potential of CNSL-derived compounds as safe, effective and environmentally friendly anti-infective agents. They also promote waste valorisation and local drug production, while aligning with global sustainable development goals.

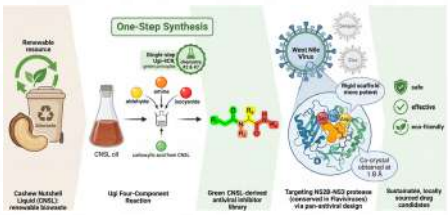

Figure 1. Design strategy for the generation of sustainable antiviral agent against the NS2B-NS3 protease.

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Deciphering the KP32 Phage Architecture: Implications for Antimicrobial Strategies against *Klebsiella pneumoniae*

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The global escalation of antimicrobial resistance (AMR) in *Klebsiella pneumoniae* has positioned this pathogen as one of the most critical threats to public health worldwide. Central to its virulence is the production of a thick capsular polysaccharide (CPS) layer that shields the bacterium from host immune responses and antibiotic penetration [1]. Nevertheless, bacteriophages have evolved specialized strategies to infect antimicrobial-resistant bacteria, representing a valuable approach to tackle the AMR emergency. As a *K. pneumoniae*-targeting phage, the podovirus KP32 possesses a sophisticated enzymatic machinery comprising depolymerases that specifically recognize, bind, and hydrolyze the CPS, thereby sensitizing the pathogen to antibiotics and immune system intervention. Understanding the molecular basis of this serotype-specificity is crucial for the development of targeted precision antimicrobials. We elucidated the structure of the first KP32 phage depolymerase, KP32gp38 [2], and provided details about its interaction with its specific substrate and the other KP32 phage depolymerases, KP32gp37 [2,3].

In this context, Artificial Intelligence (AI) is playing an increasingly transformative role in structural biology. In a previous work, building on the homology between the KP32 virus and the *E. coli* T7 phage, we used an AI-assisted integrative approach employing AlphaFold 3.0 to model the phage portal attached to a 12-fold depolymerase branching system and composed of a dodecameric KP32gp31 and a hexameric KP32gp32 [3]. Expanding this scope, we leveraged the capabilities of AlphaFold 3.0 to decode the complex structural organization of the KP32 phage, consisting of the capsid, core, DNA-ejection portal, and tails and involving over 500 proteins [4]. However, while AI-driven models significantly accelerate the elucidation of macromolecular complexes, this work emphasizes the critical necessity of experimental validation to ensure biological accuracy. Cryo-electron microscopy and X-ray crystallography offer a complementary approach to bridge the gap between AI predictions and experimental evidence and provide a roadmap to elucidate structure-related depolymerases mechanisms, which is is essential for designing effective novel antimicrobial strategies.

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The dual role of Rv3463 in *Mycobacterium tuberculosis*: bridging redox metabolism to host immune response

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According to the WHO's Global Tuberculosis Report 2024, tuberculosis (TB) has resurged as the leading cause of death from a single infectious agent, overtaking COVID19, with 8.2 million diagnosed and 1.25 million deaths. BCG is still the only vaccine approved to prevent TB, despite its limited efficacy, particularly in adults. Finding new TB antigens for the design of new subunit or multi-epitope vaccines may represent a valid approach to fight TB since they have a greater safety profile and can be given to immunocompromised individuals without risk of infection [1].
Currently, we are investigating a number of potentially effective mycobacterial antigens, as they effectively induce activation of macrophages or dendritic cells (DCs) maturation via interaction with TLR4/TLR2 receptors and possess anti-mycobacterial action [2-4]. Moreover, the identification of the most immunogenic parts of the targets allows the use of structural vaccinology as a rational guide to re-design more stable and effective antigens and adjuvants [5].
The mycobacterial protein Rv3463 has recently emerged as a potent immunomodulator capable to induces macrophage activation through the TLR2 and TLR4 pathways and phagolysosome fusion, leading to the effective clearance of intracellular mycobacteria in macrophages [6]. Here, we report the first experimental structural characterisation of Rv3463. This protein can exist as both a monomer and a dimer depending on environmentally condition. We hypothesized that while monomeric Rv3463 exists as an intracellularly inactive protein capable of modulating redox enzymes, the exposure to oxidizing extracellular environment triggers a conformational shift towards protein dimerization. Given these distinct functional characteristics, we have designated the Rv3463 protein as ps-F420-OR. Collectively, our data point to a likely dual role of ps-F420-OR as both an intracellular redox modulator and an extracellular TLR4/TLR2 agonist, offering the opportunity to explore ps-F420-OR as a novel target for TB immunotherapies.

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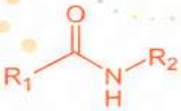

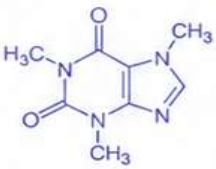

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