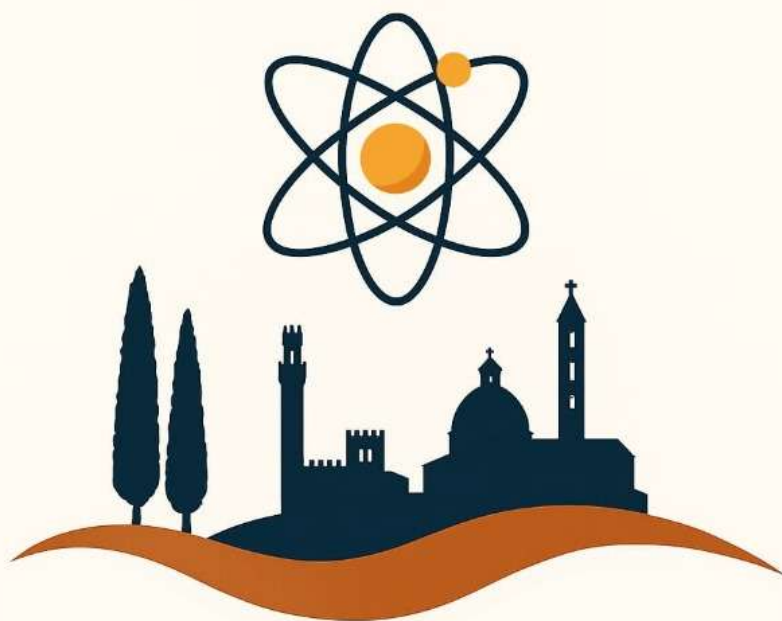


CTB12

12th Conference on Molecular Frontiers in Bioinspired Chemistry

Siena, 6–9 July 2026



CTB12

Book of Abstracts



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Welcome Message

The International Scientific Board and the Local Organizing Committee are pleased to welcome you to the 12th edition of the Chemistry towards Biology (CTB12) conference, held in Siena from July 6th to 9th, 2026.

Siena is a city with deep historical roots, known for its rich artistic heritage and the Palio, a traditional horse race that continues to define the identity of the city's contrade. Alongside its cultural history, Siena possesses a long-standing academic tradition. Founded in 1240, the University of Siena is one of the oldest in the world, with a historical focus on disciplines such as Law, Medicine, and the Natural Sciences.

We have chosen the Accademia dei Fisiocritici as our venue to reflect this commitment to academic inquiry. Established in 1691, the Academy has been a center for scientific study for centuries. By hosting CTB12 within this historic setting, we aim to connect this tradition of research with the scientific challenges of the present.

This year's edition, "New Frontiers in Bioinspired Chemistry", focuses on these evolving fields, featuring the following topics:

- Bioinorganic and Metal-Based Systems
- Chemical Biology and Synthetic Biosystems
- Biophysical and Spectroscopic Methods
- Enzyme Mechanisms and Biocatalysis
- Metabolomics and Systems-Level Molecular Profiling
- Drug Discovery and Molecular Therapeutics
- Biomaterials and Nanostructures for Health and Environment
- AI-Driven Biomolecular Simulation and Spectroscopic Prediction
- Food Chemistry and Bioactive Natural Compounds

We hope the combination of this program and the unique atmosphere of Siena will foster productive discussions and new professional connections. We look forward to a successful conference.

The Organizing Committee

Scientific Programme

6 July 2026

17:00 – 18:30 Opening Ceremony PL1 · PL2

7 July 2026

09:00 – 11:00 Bioinspired Antimicrobial Strategies PL3 · IL01 · OC01 · OC02 · OC03

11:30 – 13:00 Metal-Based Therapeutics and Redox Chemistry IL02 · IL03 · OC04 · OC05

14:30 – 16:15 Nano-Bio Systems and Drug Delivery IL04 · IL05 · OC06 · OC07 · OC08

16:15 – 16:30 Flash Poster Presentation 1st Session — fPP1 · fPP2 · fPP3

17:00 – 17:30 Flash Poster Presentation 2nd Session — fPP4 · fPP5 · fPP6 · fPP7 · fPP8 · fPP9

17:30 – 19:00 Poster Session

8 July 2026

09:00 – 11:00 Protein Dysfunction and Translational Approaches PL04 · IL06 · OC09 · OC10 · OC11

11:30 – 13:00 Neurochemical Frontiers in Metal Biology IL07 · IL08 · OC12 · OC13

14:30 – 16:30 Bioinspired Therapeutic Approaches IL09 · IL10 · OC14 · OC15 · OC16 · OC17

9 July 2026

09:00 – 11:00 Molecular Engineering for Biomedical Innovation PL5 · IL11 · OC18 · OC19 · OC20

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Venue

CTB12 is hosted by the Accademia dei Fisiocritici. Founded in 1691, this historic institution is a landmark for the dissemination of scientific culture and research.



Plenary Lectures

Plenary Lecture — PL1 · Paola Turano

Ferritin: The Golden Cage

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In Nature, ferritin is responsible for storing iron in the form of ferric oxide to build reserves and prevent Fenton reactions. Depending on the tissue, the cytosolic protein contains varying ratios of catalytic (H) and nucleation (L) subunits. When administered exogenously, ferritin can be internalized by cells via the TfR1 receptor—which is overexpressed in cancer cells—thanks to its specific interaction with H-type subunits. This has promoted the use of homopolymeric recombinant human H-type ferritin (HuHf) as a drug delivery vehicle targeting cancer cells.

In this contribution, I will illustrate how HuHf is particularly suitable for transporting gold(I)-based drugs and how effective it is at maintaining their activity at the intracellular level. The results obtained are based on a structural biology approach that integrates ESI MS, ¹⁹F NMR on fluorine-labeled HuHf, cryo-EM, molecular dynamics, and site-directed mutagenesis to demonstrate the high affinity of the cage's extensive outer surface for Au(I) compounds. This affinity is attributed to the presence of two solvent-exposed cysteine residues on each subunit, as well as the selective filtering action for cavity access exerted by the C3 ion channels [1-4].

Acknowledgements

Funded by AIRC, IG 2021 ID 26169.

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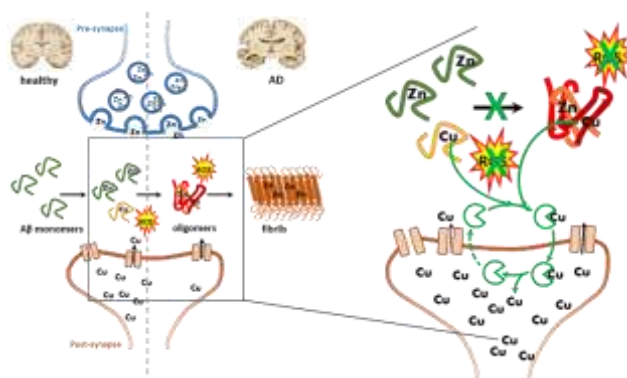
Copper and zinc in Alzheimer's disease: their role in the amyloid cascade and as therapeutic targets

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Alzheimer's disease (AD) is characterized by the extracellular deposits made of supramolecular assemblies of the amyloid- β (A β s) peptides, in which up to mM level of metal ions, mainly copper and zinc has been found. Two events related to the interaction of A β s with metal ions are involved in the etiology of AD: (i) the production of reactive oxygen species (ROS) by Cu(A β) species [1,2] and (ii) the assembly of the A β s leading to various kind of aggregates, including amyloids.[3,4] During the talk, I will show a brief overview on our contribution to the elucidation of the mechanism of Cu(A β)-induced ROS production and of Cu and Zn-modulated A β s self-assembly. Therapeutic approaches we have set up to counteract the two processes [5,6] will then be illustrated with recent examples.



Acknowledgements

C.H. warmly thank all the students and collaborators who contributed to the results that will be presented during the talk, as well as the ERC StG 638712, the ANR-21-CE06-0030 for financial supports.

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Metal-triggered folding meets retro-inverso design: reprogramming peptide function from AMPs to GLP-1 analogues

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Antimicrobial peptides (AMPs) are not limited by their sequences – their function can be reprogrammed through precise structural control. We show that combining metal coordination with retro-inverso (RI) design provides a powerful and transferable strategy to enhance peptide function.

In shrimp-derived AMPs, Cu(II) binding enforces helix formation and directly translates into increased antibacterial potency, effectively switching the peptide into a highly active state [1]. In contrast, shepherdins exploit Zn(II)-driven fibrillation, resulting in a strong enhancement of antifungal activity. These systems highlight two distinct metal-controlled mechanisms: folding versus self-assembly [2].

Retro-inverso design further elevates peptide performance by improving stability in solution, while preserving (and often enhancing) biological activity. Importantly, this concept extends beyond AMPs. In GLP-1 analogues, multiple D-amino acid substitutions lead to significantly improved stability and superior activity, with a seven-substitution liraglutide-based analogue outperforming the native hormone.

Together, these results demonstrate that metal-triggered structural control combined with RI design enables a general strategy to reprogram peptide function, from antimicrobial systems to metabolically active peptides.

Acknowledgements

The project is funded with the support of the National Science Centre (2023/51/ST5/01098).

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Reprogramming Amyloid Assembly with Cyclic D,L- α -Peptides: From Molecular Design to Alzheimer's Diagnosis and Therapy

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Alzheimer's disease (AD) is an incurable neurodegenerative disease, leading to cognitive impairment and progressive synaptic damage accompanied by neuronal loss. The misfolding and aggregation of A β and tau proteins are key hallmarks of AD. To address the need for early detection and treatment, innovative diagnostic and therapeutic strategies were developed utilizing self-assembled cyclic D,L- α -peptides [1,2]. These abiotic peptides, composed of even number of alternative D- and L- α -amino acids, self-assemble into nanotubes that replicate the hydrogen-bonding pattern of amyloid cross- β -sheets and mimic amyloid structure and function. The discovered cyclic D,L- α -peptide hexamers exhibit cross-reactivity with A β , inhibiting its aggregation and toxicity, as shown by biophysical and biochemical studies. In a transgenic AD mouse model, PET imaging with cyclic D,L- α -peptide radiotracers enabled early oligomer detection in pre-symptomatic 5xFAD mice, outperforming ¹¹C-PIB. Early AD diagnosis was also demonstrated using peptide-liposome conjugates and fluorescence imaging [3]. These peptides also improved memory and behavioral deficits by reducing A β oligomers, preserving microglial morphology, decreasing inflammation, and enhancing autophagy-mediated A β clearance.

Acknowledgements

This work was supported by the Israel Science Foundation under grant # 2926/21 and 3526/25.

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Gene editing across three domains of life

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Structural biology enabled substantial advances on understanding how gene editing machineries operate across the three domains of life. In this talk, I will present cryo electron microscopy (cryo EM) studies of key gene editing machines, highlighting how conformational heterogeneity and intrinsic molecular flexibility underpin their biological function and engineering potential.

Early cryo EM analyses revealed that NHEJ factors populate ensembles of dynamic states that enable recognition and processing of DNA ends [1-3]. Later work on CRISPR effector complexes also highlighted pronounced structural plasticity, with macromolecular complexes captured as coexisting conformations rather than single static structures [4-6]. For Φ C31 integrase, high resolution cryo EM structures illuminate large scale domain rearrangements that drive unidirectional, site specific recombination, providing a structural framework for understanding specificity and irreversibility [7].

These insights have been enabled by recent advances in cryo EM hardware as well as by developments in image processing and heterogeneity analysis software that allow continuous flexibility and transient intermediates to be resolved. Together, these studies demonstrate how embracing conformational variability, rather than averaging it out, is essential for mechanistic understanding of gene editing systems and for the rational development of next generation genome engineering tools across all domains of life.

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Invited Lectures

Invited Lecture — IL01 · Fabio Zobi

Rhenium Complexes as Antimicrobial Agents

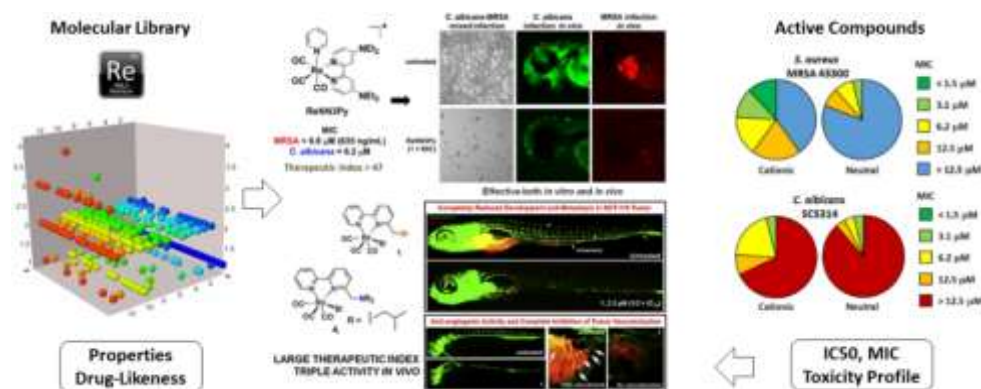
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We report on recent advances in the development of rhenium-based antimicrobial agents. This contribution presents the design, synthesis, biological evaluation and mechanistic investigations of carbonyl, diarene and oxo rhenium complexes as antibacterial and antifungal compounds. Particular emphasis is placed on rational molecular design aimed at optimizing stability and target engagement while maintaining low *in vivo* toxicity [1-2]. Our studies already allowed the identification of potent and non-toxic complexes active *in vivo* against *S. aureus* infections at MIC doses as low as 300 ng/mL [3-4], as well as against *C. albicans*-MRSA mixed co-infection, with no sign of cardio-, hepato-, hematotoxicity or teratogenicity.



Acknowledgements

Financial support from the Swiss National Science Foundation, the Fonds de recherche du Centenaire de l'Université de Fribourg and Ministry of Education, Science and Technological Development of the Republic of Serbia is gratefully acknowledged.

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Heme and metalloporphyrinoids as regulators of ROS in neurodegenerative diseases

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Heme serves as a vital prosthetic group for proteins involved in oxygen transport, electron transfer, and oxygen-dependent enzymatic activities [1]. It also functions as a critical intracellular signaling molecule [2]. However, unbound heme is cytotoxic due to its oxidative and nitrative potential. Consequently, the disruption of heme homeostasis is linked to various pathologies, including cancer, vascular disorders, and neurodegeneration, and is particularly significant in cases of severe hemolysis or traumatic brain injury [3]. We explored the binding and reactivity of hemin with peptide fragments associated with neurodegenerative diseases. Specifically, histidine residues in tau, prion proteins, and amyloid- β ($A\beta$) allow hemin coordination [4-8]. We also examined the peroxidase-like activity and aggregation induced by these hemin-peptide complexes. Given the role of reactive nitrogen species (RNS) in neurodegeneration, we investigated the nitrative reactions catalyzed by the heme- $A\beta$ 16 complex [9]. Furthermore, we are expanding our research to include metal-macrocyclic complexes (metalloporphyrinoids), specifically manganese texaphyrins, which demonstrate the ability to neutralize ROS and RNS. Preliminary results suggest that Mn-texaphyrin significantly mitigates $A\beta$ oxidation and nitration [10], highlighting its potential as a therapeutic agent for oxidative stress-related diseases.

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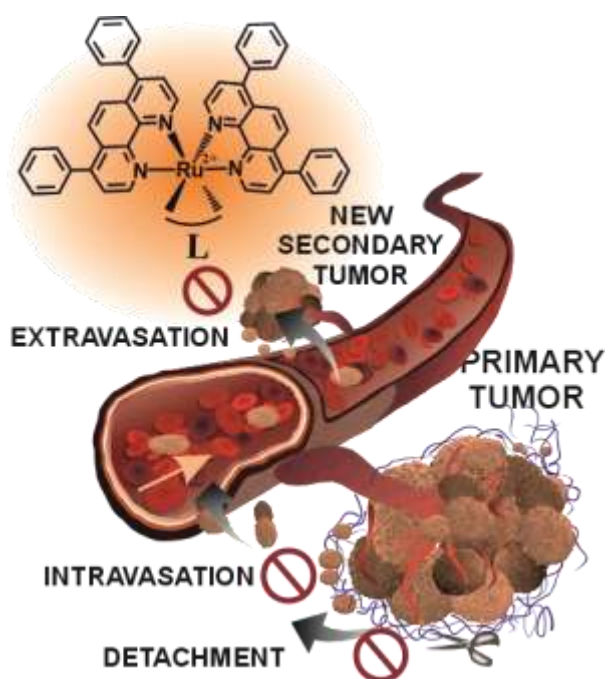
Polypyridyl Ru(II) complexes as metastasis inhibitors - *in vitro* functional assays and molecular mechanisms

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Metastasis is a complex, multistep process, and each stage can be targeted therapeutically. It is common and accounts for over 90% of cancer-related deaths. For many years, polypyridyl Ru(II) complexes have been investigated as potential anticancer agents, and *in vitro* studies largely focusing on cytotoxicity. Here, we show that beyond cytotoxic effect, selected $[\text{Ru}(\text{dip})_2\text{L}]^{2+}$ complexes (dip is 4,7-diphenyl-1,10-phenantroline and L is a 2,2'-bipyridine derivative) also modulate cell-adhesion properties, leading to inhibition of cancer-cell detachment, migration, and invasion [1,2]. The compounds are cytotoxic in the low-micromolar range, while at non-toxic doses they still affect migration and adhesion. Molecular studies indicate that these Ru(II) polypyridyl complexes alter the function of selected integrins. They also upregulate focal-adhesion components such as vinculin and paxillin, increasing the number of focal-adhesion sites. Moreover, we observed increased cell elasticity, consistent with effects on the cytoskeleton, where the highest intracellular accumulation of Ru(II) complexes was detected. Finally, the complexes inhibited metalloproteinase activity in both secreted and membrane-bound forms. Overall, our results suggest that this class of Ru complexes is a promising scaffold for agents combining cytotoxic and anti-metastatic activity, and we identify potential molecular targets underlying the observed functional changes in cancer cells.



Acknowledgements

Financial support from the National Science Center (DEC-2016/21/B/NZ7/01081) is acknowledged.

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Tuning Chitosan- and Cyclodextrin-Based Nanostructures to Enhance Drug Stability and Bioavailability

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Chitosan- and cyclodextrin-based nanostructures have long been explored as drug delivery platforms; however, their effective translation relies on the rational tuning of molecular architecture to achieve meaningful biological outcomes. In this context, chemical modification of chitosan and cyclodextrins, such as quaternarization and thiolation, plays a pivotal role in controlling drug stability, bioavailability, and overall biological performance.

Through systematic modulation of molecular weight, charge density, and functionalization, quaternary ammonium chitosan derivatives and cyclodextrin-conjugated systems can be engineered as multifunctional excipients, acting either as macromolecular carriers or as nanocarriers depending on their supramolecular organization. Modified chitosans and thiolated cyclodextrins enhance mucoadhesion and strengthen interactions with biological interfaces, while providing effective protection against chemical and enzymatic degradation.

These tailored systems enable the stabilization and delivery of several compounds, including corticosteroids, labile peptides, essential oils, and natural extracts, across multiple mucus-related administration routes such as ocular, oral, and pulmonary pathways. Comparative studies demonstrate that improved biological performance is primarily governed by interfacial interactions rather than by the mere presence of a nanoscale structure.

Overall, this approach highlights how the rational tuning of polysaccharide-based systems enhances drug stability and bioavailability by linking molecular design to functional performance.

From Cage Architecture to Biomaterials: The Role of Multifunctional Silsesquioxanes

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Polyhedral oligomeric silsesquioxanes (POSS), double-decker silsesquioxanes (DDSQ), and incompletely condensed POSS cages (IC-POSS) are highly attractive platforms in modern materials chemistry. Their unique cage-like silicon–oxygen frameworks combine the stability of an inorganic core with the tunability of organic substituents, offering broad possibilities for structural and functional design in advanced biomaterials [1].

In our research, we develop synthetic and post-functionalization strategies for POSS, DDSQ, and IC-POSS derivatives to obtain tailored systems for specific applications. This molecular-level design supports not only biomaterials development, but also expands the use of these compounds in supramolecular chemistry, nanotechnology, and smart surface engineering.

In my talk, I will show how these organosilicon architectures can be translated into practical solutions at the interface of chemistry, biology, and materials science. Their applications include cell-supporting substrates, implant-related materials and coatings, controlled-release carriers, molecular machines, and versatile building blocks for organic and coordination chemistry [2–10].

So, POSS, DDSQ, and IC-POSS should be viewed not only as elegant chemical structures, but as functional platforms shaping the future of modern biomaterials.

Acknowledgements

The presented research was funded by the National Science Centre, Poland (OPUS, grant nos. 2016/23/B/ST5/01480 and 2020/39/B/ST4/00910).

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Evolution Did Not Eliminate Aggregation: Functional Amyloids Enable Hormone Storage and Signaling

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Members of the PACAP-glucagon hormone family play prominent pleiotropic roles in human physiology, affecting metabolic, nervous system, endocrine, and developmental processes. Consequently, their synthetic derivatives, such as today's blockbuster GLP-1 agonists, are of outstanding importance to the pharmaceutical industry. However, the synthetic production of peptide-based active ingredients from the PACAP-glucagon family is significantly hindered by their strong intrinsic tendency to aggregate, as these peptides readily form functional amyloids. Following post-translational modification, mature hormones awaiting secretion exist in a self-assembled state within acidic secretory granules. This transient amyloid state is thought to function as a compartmentalized reservoir system, enabling selective storage of polypeptides in a dense, phase-separated, and stabilized form. In this presentation, I demonstrate that aggregation propensity has remained a conserved trait throughout approximately 600 million years of vertebrate evolution, whereas specific GPCR receptor-ligand interactions have undergone substantial plasticity. I provide structural insights into amyloid architecture based on more than two dozen amyloid-like crystal structures of aggregation-promoting segments, as well as full-length amyloid fibrils determined in our laboratory over recent years. These data reveal that the same amino acid side-chain interactions that catalyze amyloid aggregation and stabilize the resulting assemblies are also responsible for establishing specific GPCR receptor-ligand interactions. Furthermore, I show how distinct structural elements, such as pH-sensitive, charged gatekeeper residues conserved within the family-regulate aggregation propensity. Based on the results of hundreds of systematic screening experiments, I further detail how environmental parameters, including pH, NaCl concentration, and the presence of glycosaminoglycans, modulate amyloid aggregation processes. Together, our results highlight that aggregation propensity and biological activity are intrinsically linked, and that eliminating aggregation can only be achieved at the expense of biological activity [1-3].

Acknowledgements

This work was supported by Project no. 2018-1.2.1-NKP-2018-00005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2018-1.2.1-NKP funding scheme. Project no. RRF-2.3.1-21-2022-00015 has been implemented with the support provided by the European Union. HUN-REN-ELTE Protein Modelling Research Group. Project no. 2025-3.1.1-ED-2025-00013 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2025-3.1.1-ED-NKP funding scheme.

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Insulin degrading enzyme, metals and neuropeptides in amyloid diseases: Insights into the role, mechanisms and potential therapeutics

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Neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) are characterized by deposits of amyloid proteins. The homeostasis of metal ions is crucial for the normal biological functions in the brain. However, in AD and PD, the imbalance of metal ions leads to formation of amyloid deposits. Metals could bind early-stage amyloid oligomers and increase polymorphism [1-2] In the last decades, it has been extensive efforts to design compounds that can chelate metal ions with the aim of preventing the formation of amyloid deposits. But so far these compounds have not exhibited successful candidates to be used in clinical trials. Moreover, the amyloid deposits are also produced in the absence of metal ions.

This lecture will introduce the bifunctional activity of Neuropeptides (NPs) and insulin degrading enzyme (IDE) to prevent and inhibit formation of amyloid deposits in absence and in presence of metal ions. NPs are produced and released by neurons. It has been shown that neuropeptides have neuroprotective effects in the brain and reduce the formation of amyloid deposits. NPs inhibits amyloid aggregation by binding to metals [3-4] and early-stage oligomers [5-6]. IDE has been detected in the cerebrospinal fluid media and plays a role in encapsulating and degrading amyloid monomers, thus regulated the levels of amyloids. IDE shows different conformations [7] encapsulates early-stage oligomers and serve as a 'dead-end' chaperone in a non-proteolytic manner in absence [8] and in presence of metal ions [9]. The molecular mechanisms by which NPs and IDE prevent and inhibit formation of early-stage amyloid aggregates had been investigated using extensive molecular modeling tools and molecular dynamics simulations.

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Menkes Disease: metallothioneins as biomarkers?

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Menkes disease is a rare inherited disorder of copper metabolism that leads to severe neurological deterioration, connective tissue defects, characteristic hair abnormalities, and early death. Current therapies, mainly based on copper–histidine injections, have shown limited effectiveness.

Recent research has suggested that the copper complex copper–elesclomol may offer a promising alternative, as it facilitates copper delivery to mitochondria and improves key enzymatic activities in animal models. In this context, the authors explore whether metallothioneins, small proteins involved in metal homeostasis and stress responses, could serve as reliable blood biomarkers to monitor copper levels in patients receiving this new treatment.

Although metallothioneins are present in nearly all organisms and their levels increase in several diseases, including Menkes disease, no routine clinical method currently exists to measure them in human fluids. The research team presents different analytical approaches adapted to the very limited blood volumes obtainable from young patients. Initial clinical application of copper–elesclomol in a young child with Menkes disease has shown remarkable long-term improvement, and additional patients enrolled in the program are also experiencing clear health benefits, reinforcing the importance of careful copper monitoring during treatment [1-3].

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Exploiting a druggable E2 ligase for PROTAC development in Leishmaniasis therapy

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Leishmaniasis are neglected tropical diseases endemic in low- and middle-income countries, affecting up to one million people annually according to the World Health Organization. Visceral leishmaniasis, caused by *Leishmania infantum*, leads to hepatosplenomegaly and, if untreated, is often fatal. Current therapies suffer from limited efficacy, high toxicity, and emerging resistance [1]. *Leishmania* parasites survive the oxidative stress of host macrophages through a unique redox system based on trypanothione, maintained in its reduced state by trypanothione reductase (TR), a FAD-dependent enzyme. TR is an attractive drug target due to its essentiality, selectivity, and druggability. Despite extensive efforts, no TR inhibitors have progressed to clinical trials because of insufficient efficacy or toxicity issues, highlighting the need for alternative strategies [2]. Targeted protein degradation has emerged as a powerful therapeutic approach, based on bifunctional molecules such as PROTACs (PROteolysis Targeting Chimeras), which induce ubiquitination and proteasome-mediated degradation of target proteins [3]. We previously developed PROTACs aimed at TR degradation via recruitment of a putative E3 ligase in *L. infantum*, but their activity was limited and the E3 ligase remains poorly characterized. To overcome this limitation, we propose E2-recruiting PROTACs targeting TR, bypassing the need for an E3 ligase. For this purpose, we characterize an ubiquitin-conjugating enzyme (E2) from *Leishmania major* through structural and functional studies. We performed crystallographic fragment screening to identify potential druggable sites and designed E2-binding fragments using DeepFrag-based expansion and AutoDock Vina docking. These results provide a basis for the rational development of E2-recruiting PROTACs against Leishmaniasis.

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Targeting the bacterial SOS response: from structural and mechanistic investigations to inhibitor development

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The bacterial SOS response is a conserved stress-response pathway triggered by DNA damage and regulated by the DNA-damage sensor RecA and the transcriptional repressor LexA. Under genotoxic stress, RecA oligomerizes on ssDNA and induces LexA autoproteolysis, derepressing the SOS genes that encode error-prone polymerases, recombinases, virulence factors, and antibiotic resistance determinants; our work aims to elucidate the molecular events governing the RecA–LexA interplay and to develop potent inhibitors that suppress SOS signaling as a strategy to counter evolvable and virulence traits. We solved the atomic structures of the *Pseudomonas aeruginosa* LexA autoproteolytic domain, the oligomeric RecA filament, and their complex by X-ray crystallography and cryo-EM, identifying crucial requisites for LexA proteolysis [1]. Anti-LexA nanobodies were generated via llama immunization and phage display; LexA autoproteolysis assays demonstrated potent inhibition, and surface plasmon resonance quantified high-affinity binding. The SOS suppression was functionally validated in antibiotic-treated cultures by RT-PCR and GFP-reporter assays of select SOS genes [2]. Building on these structural and biophysical insights, we rationally engineered nanobody variants with improved affinity and activity, which are currently under characterization, collectively establishing a framework for anti-SOS strategies as a novel antimicrobial approach.

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Chirality-Driven Pathways Between Chemistry and Biology: From Biomimetics to Biosensing

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Chirality is a fundamental, ubiquitous, and defining feature of most molecules relevant to biology, governing molecular recognition, function, and structural complexity. Translating this intrinsic property of biomolecules into synthetic systems offers powerful opportunities to bridge chemistry and biology through the design of functional, adaptive, and responsive architectures.

In this lecture, I will present recent advances in the development of inherently chiral and conformationally dynamic supramolecular hosts, including prismarenes, pillararenes, calixarenes, and related macrocycles, as platforms to encode, transfer, and amplify chiral information [1-4]. Particular emphasis will be placed on structural features and how different elements of chirality (point, axial, planar, and helical) interplay within a single system to generate unique chiroptical properties and highly selective recognition behavior.

These concepts enable the rational design of biomimetic systems that reproduce key aspects of biological recognition, such as the stereoselective binding of amino acids and other biologically relevant molecules.

Overall, this presentation highlights chirality not only as a structural feature, but also as a dynamic and programmable element that drives the evolution of chemical systems toward biological complexity and function.

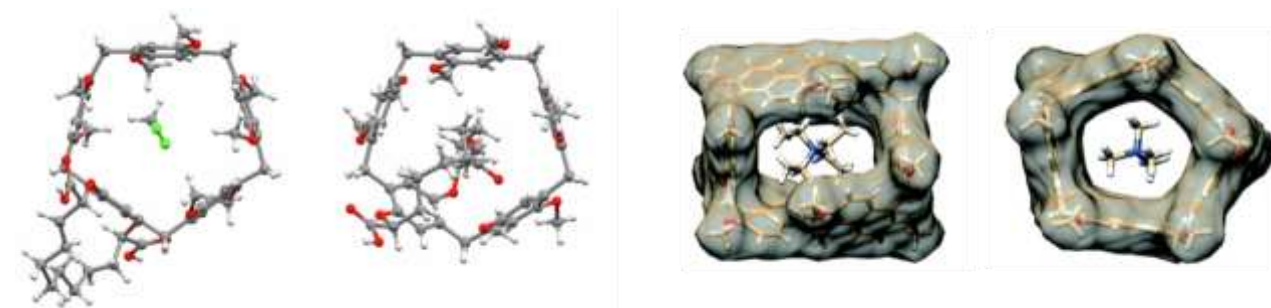


Figure 1. Representative macrocyclic structures: a pseudo-catenane and prismarenes

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Light-Driven Dissipative Replication and Protein-Catalyzed Selection in Biomimetic Systems

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Understanding how chemical systems acquire life-like behavior is a central challenge at the chemistry–biology interface [1]. Here, we present a light-driven dissipative replication in foldamer-based biomimetic systems, where continuous energy input enables non-equilibrium self-replication and dynamic selection [2]. Sequence-dependent templating and competing formation and breakdown pathways give rise to adaptive replicator populations shaped by light intensity. To extend these systems toward biological relevance, we introduced protein-catalyzed selection [3]. A promiscuous protein catalyst preferentially amplifies selected replicator cycles through binding and catalytic turnover. This catalyst-limited regime enables competitive selection reminiscent of early evolutionary processes. Together, these results demonstrate how coupling energy dissipation, self-replication, and protein catalysis leads to emergent adaptive behavior, providing a minimal model for chemical evolution and a step toward functionally evolving synthetic systems.

Acknowledgements

This research was funded by National Research, Development and Innovation Office of Hungary, PharmaLab (RRF-2.3.1-21-2022-00015 and 2020–1.1.6-JÖVŐ-2021-00004 (G.M.K.), NKFIH K135150 (G.M.K.), 2018–1.2.1-NKP-2018-00005 (HunProtExc) financed under the 2018– 1.2.1-NKP funding Scheme. Project no TKP2021-EGA-32 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme (T.A.M.). Project number RRF-2.3.1-21-2022-00015 is implemented with the support of the European Union's Recovery and Resilience Instrument.

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Spy protein: a new technology for artificial metalloenzymes

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In recent years, the demand for sustainable approaches in chemical synthesis and energy production has driven the development of artificial metalloproteins. The Spy system consists of SpyCatcher (SC), a small β -sandwich protein, and SpyTag (ST), a ~15-residue peptide [1]. A covalent isopeptide bond forms between an aspartate on ST and a lysine on SC. Here, we introduced metal-binding sites into SpyTag to generate artificial metalloproteins upon recombination with SC (Figure 1). We recently reported the first Spy construct with metalloenzyme reactive features. We report a Cu(His–His) site engineered on SpyTag and positioned near the SpyCatcher scaffold after reconstitution (Figure 1A). In parallel, a Fe–mimochrome [2] system was grafted onto SpyTag and conjugated to SC (Figure 1B). The former probes second-sphere effects on enantioselectivity in DOPA oxidation, while the latter represents a step toward redox catalysis at cell surfaces.

We also investigated photostimulated hydrogen production using Co(II/III) ATCUN motifs on SpyTag (Figure 1C). The influence of lysine residues at positions 1 and 4 on Co coordination and hydrogen evolution was examined in the presence of $[\text{Ru}(\text{bpy})_3]^{2+}$ and ascorbate under visible light.

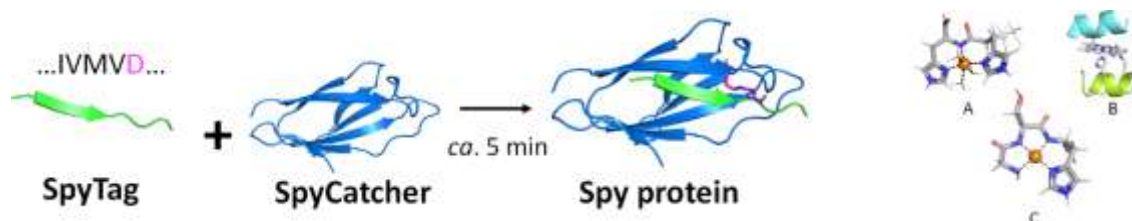


Figure 1. Left: reconstitution of the Spy construct. Right: representation of the metal sites.

Acknowledgements

Project “Artificial enzymes for the photocatalytic production of hydrogen in photosynthetic bacteria” National Recovery and Resilience Plan (NRRP), M2 C2 Inv. 3.5 funded by the European Union – NextGenerationEU. Project RSH2A_000009, C.D. 445 29/12/2022 Italian Ministry of Environment and Energy Security.

Oral Contributions

Oral Contribution — OC01 · Bela Gyurcsik

Metal ion selectivity of the AfArsR model peptide

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Certain proteins recognize metal ions – that are either toxic at low concentrations or at increased concentration in cells – through specific binding and structural changes initiating gene transcription and thereby, expression of proteins for detoxification purpose. Arsenic is a toxic metalloid, recognized by the ArsR bacterial As(III)-sensing protein family. The As(III)-binding site of the studied AfArsR protein consists of three cysteine thiolates in close vicinity with each other in the amino acid sequence of the protein. This provides an opportunity to select a short peptide sequence as the model of the protein. The same cysteine thiolates can also bind heavy metals, such as Hg(II), extremely strongly. Nevertheless, the Hg(II)-bound protein is not functional, so it can not activate the downstream biological processes.

Our results on this topic revealed that the transcriptional regulatory function of the AfArsR protein is initiated within a short oligopeptide segment. Although this As(III) binding site is very flexible, it becomes operative via different responses towards As(III) and the divalent metal ions. This work presents a circular dichroism (CD) spectroscopic study, monitoring the formation of the metal(loid) complexes and structural changes in the designed model peptide solution, supplemented by molecular dynamics simulations [1]. The results revealed that the interaction with As(III) induces changes in the peptide structure from a random-coil to a well-defined structure. The AsS3 binding site is close to be identical to that observed in the crystal structure of the native protein. On the contrary, Hg(II) binding leads to a peptide structure that is more flexible. Our investigation also highlights the weaknesses of CD predicting algorithms, relying on CD spectra of proteins with well-established crystal structures, when extended to small metal ion binding oligopeptides.

Acknowledgements

We acknowledge the financial support of National Research, Development and Innovation Office, Hungary (No.: 150330), of the Hungarian Academy of Sciences and Japan Society for the Promotion of Science (JSPS NKM2024-15/2024).

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Beyond Proteolysis: Metal-Mediated Antimicrobial Activity of Salivary-Derived D-Amino Acid Peptidomimetics

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Salivary antimicrobial proteins and peptides, such as mucin MUC7, cathelicidin LL-37, histatins, chemokine CCL-28 play a key role in host defense. These molecules (or their hydrolysis products) are promising antimicrobial agents, particularly when complexed with metal ions, but their clinical application is limited by low proteolytic stability [1-2].

To overcome proteolytic degradation, we developed a strategy for designing enzymatically stable peptidomimetics with activity enhanced by metal-ion coordination. Stereochemical modifications (e.g., D-amino acids, including the *retro-inverso* strategy) improve resistance to degradation while modulating coordination properties and biological activity. Using MUC7-derived fragments, we show that both full and site-selective modifications affect protonation equilibria as well as the geometry and stability of Cu(II) and Zn(II) complexes [3-5]. The structural changes lead to enhanced antimicrobial activity, a broader activity spectrum, and high stability under biological conditions with low cytotoxicity. Notably, a Zn(II) complex of a 12-residue D-amino acid analogue of the LL-37 C-terminal fragment retains antifungal activity against *Candida albicans* comparable to the native peptide.

Overall, combining chiral modifications with metal coordination is an effective strategy for designing stable peptidomimetics with therapeutic potential against oral pathogens and biofilm-associated infections

Acknowledgements

We thank the National Science Centre (grant UMO-2021/41/B/ST4/02654, J.W.) for financial support.

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Histatin 5 Peptidomimetics: Cu(II) and Zn(II) Coordination, Secondary Structure, and Antimicrobial Activity

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Histatins, histidine-rich salivary antimicrobial peptides, play a central role in oral defense [1]. In particular, histatin 5 exhibits potent activity against *C. albicans* and *S. aureus* at physiological conditions [2,3]. However, its therapeutic potential is limited by further enzymatic degradation. To address this, a native histatin 5 (Table 1) was modified by introducing D- and β -amino acids at the cleavage-prone site, as well as by generating an all-D analogue.

Three peptide modifications were evaluated to identify the variant with the greatest improvement in enzymatic stability (HPLC experiments) and to assess the effects of metal coordination on thermodynamic and structural properties using physicochemical techniques (potentiometry, spectroscopic methods, mass spectrometry), as well as antimicrobial activity (MIC values and cytotoxicity tests), compared to the native sequence. Obtained results support the design of effective Hst-5-based peptidomimetics enhanced by metal ions.

Native histatin 5	DSHAKRHHGYKRKFHEKHSHRGY
D-aa histatin 5	dshakrhgykrkfhekhhshrgy
2D-aa histatin 5	DSHAKRHHGYK rk FHEKHSHRGY
2 β -aa histatin 5	DSHAKRHHGYK β R β KFHEKHSHRGY

Table 1. Amino acid sequences of native and modified histatin 5, where the lowercase letter denotes a D-amino acid and the symbol ' β ' denotes a β -amino acid: β -arginine and β -lysine.

Acknowledgements

The work was supported by the National Science Centre (Grant UMO-2021/41/B/ST4/02654 to J.W.).

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Green ligands for enhanced platinum drugs

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Platinum-based chemotherapeutics remain a cornerstone of modern oncology, with cisplatin, carboplatin, and oxaliplatin still widely used for the treatment of multiple tumor types. However, their clinical utility is limited by several major drawbacks, including dose-limiting systemic toxicity, poor selectivity toward malignant tissue, and intrinsic or acquired resistance.

The growing influence of green chemistry and its guiding principles is increasingly shaping pharmaceutical research and encouraging the redesign of metal-based drugs [1–2]. This presentation will provide selected examples in which the replacement of conventional ligands with green, biocompatible, low-toxicity, and bio-inspired alternatives can actively improve the pharmacological profile and biological behavior of platinum complexes. These findings indicate that rational ligand design can reduce undesired reactivity toward specific biological substrates, improve the balance between stability and activation, and modulate transport toward tumor cells or intracellular targets [3].

Attention has been devoted to monofunctional platinum(II) complexes in which the ligand sphere is engineered to provide more than passive coordination support. Likewise, bio-inspired platinum complexes may redirect platinum reactivity beyond classical DNA cross-linking, promoting alternative mechanisms associated with specific biological targets and other noncanonical pathways [4–7].

Overall, this strategy offers a compelling route to reconcile two central goals of contemporary medicinal inorganic chemistry: the development of platinum drugs with improved efficacy and selectivity, and the design of safer and more sophisticated platinum-based anticancer agents.

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Reactive Adduct Intermediates and Solvent-Cage Effects in Azo-Initiated Oxidation of Cinnamic Acid Derivatives

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Radical-mediated oxidation is central to processes spanning oxidative stress in biological systems, cellular redox imbalance, and antioxidant evaluation. Among the most widely used model systems, azo initiators such as 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) are generally assumed to generate a radical flux that directly follows initiator decomposition, while antioxidant activity is interpreted as a simple radical-scavenging event. Yet the molecular steps linking radical generation to substrate oxidation remain poorly resolved, particularly for bioactive natural products.

Here, we combine quantitative solution-state NMR spectroscopy with LC-MS to establish a mechanistic framework for AAPH-induced oxidation of rosmarinic acid, a plant-derived natural product with reported antioxidant and neuroprotective activities. Time-resolved measurements reveal that classical single-step scavenging models fail to reproduce the observed concentration-dependent kinetics. Instead, oxidation proceeds through the accumulation of multiple covalent radical adducts that remain kinetically active and participate in subsequent reaction pathways.

Using complementary NMR, high-resolution mass spectrometry, and LC-MS/MS, we directly characterize these transient species and identify imidazolidine-type heterocyclic adducts arising from radical addition to the α,β -unsaturated side chain.

By globally analyzing initiator decomposition, substrate consumption, and intermediate evolution, we directly determine the solvent-cage escape efficiency of AAPH-derived radicals ($f \approx 0.1$), demonstrating that the effective radical flux is strongly decoupled from the intrinsic rate of initiator thermolysis. These results establish AAPH-mediated oxidation of cinnamic acid derivatives as a flux-controlled, multistep radical process governed by solvent-cage dynamics and intermediate reactivity.

More broadly, this work provides a quantitative framework for understanding the oxidative fate of natural products in biologically relevant environments, with implications for antioxidant assays, oxidative stress models, and screening platforms used at the chemistry–biology interface.

Multifunctional Polymer–Drug Nanoplatform with Polydopamine Shell for Folate-Targeted Synergistic Therapy

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Chemotherapy is limited by premature drug degradation, severe extratumoral toxicity, poor tumor selectivity, and rapid systemic clearance, all of which reduce therapeutic efficacy and patient compliance. Conventional administration of highly potent cytotoxic agents, such as paclitaxel (PTX), often requires high systemic doses to achieve therapeutic concentrations at the tumor site, resulting in severe side effects including neurotoxicity, myelosuppression, and the development of multidrug resistance. Consequently, the design of multifunctional nanocarrier systems capable of protecting labile drugs, enabling controlled release, and enhancing tumor-specific accumulation has become a central focus of interest in modern oncological nanomedicine. In this study, we report the development of multifunctional poly(ethylene brassylate-co-squaric acid) (PEBSA_Brij)-based polymeric nanoemulsions designed for targeted and controlled anticancer delivery. The copolymer matrix integrates hydrophobic domains for PTX encapsulation and polar squaric acid moieties to enhance intermolecular interactions and structural tunability. Surface functionalization with polydopamine (PDA) allowed for subsequent conjugation of folic acid (FA) to promote receptor-mediated uptake in folate receptor-overexpressing breast cancer cells [1-4].

Acknowledgements

This research is carried out and financed within the framework of the second Swiss Contribution MAPS (project code: F-RO-CH-2024-0081).

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Continuous Flow Manufacturing of Hybrid CuS–ICG Nanoparticles: From Multistage Synthesis to Enhanced Biocidal Performance

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Hybrid polymeric nanoparticles co-encapsulating indocyanine green (ICG) and decorated with copper sulfide (Cu_{2-x}S) were developed using semi-continuous micro- and millifluidic platforms. Continuous microfluidic synthesis enabled enhanced ICG loading compared to conventional batch methods, while preserving precise control over particle size, surface charge, and copper incorporation. Computational fluid dynamics analysis of the micromixer highlighted the key role of flow regimes and shear forces in achieving homogeneous nanoprecipitation. An integrated downstream process based on hollow-fiber tangential flow filtration was implemented for efficient solvent exchange and nanoparticle conditioning under continuous flow. Scale-out production was achieved using a millifluidic reactor, increasing throughput without compromising reproducibility. Photodynamic activity, evaluated using DHR 123 and SOSG probes, demonstrated efficient reactive oxygen species generation, predominantly via type I mechanisms, with 50 ppm ICG identified as the optimal formulation. Both micro- and millifluidic systems showed consistent performance. Antibacterial studies revealed strong efficacy against planktonic and biofilm-forming *Staphylococcus aureus* ATCC 25923, achieving superior log reductions compared to batch-produced nanoparticles, attributed to improved photosensitizer encapsulation. Cytocompatibility assays with human dermal fibroblasts confirmed viability above ISO 10993-5 thresholds (>70%), with continuous-flow formulations showing reduced copper-associated toxicity. This semi-continuous approach provides a scalable and intensified platform for the production of multifunctional antibacterial nanovectors with improved performance and biocompatibility [1].

Acknowledgements

V.S acknowledges support from the Spanish Ministry of Science and Innovation (grant (PID2021-127847OB-I00, PDC2022-133866-I00 and PID2024-160339OB-I00), CIBER-BBN, ELECMI and NANBIOSIS ICTSs. This work was also financially supported by MICIN/AEI (PDI2021-1261132NB-I00 and PID2023-146091OB-I00).

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Nanovesicle-Driven Enhancement of Redaporfin-Based Photodynamic Therapy and Immune Activation

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Photodynamic therapy (PDT) can induce antitumor immunity and long-term immune memory [1,2]. The encapsulation of redaporfin in Pluronic® P123 micelles significantly enhances its photodynamic and immunogenic performance. The micellar formulation increased reactive oxygen species generation and cytotoxicity against CT26 colon carcinoma cells, despite lower intracellular uptake, indicating the key role of photochemical efficiency and subcellular localization. Redaporfin/P123 preferentially accumulated in the endoplasmic reticulum, promoting immunogenic cell death. This led to improved activation of macrophages, confirming stimulation of innate immune responses. In vivo studies showed efficient tumor accumulation and retention of the formulation. PDT combined with immune checkpoint inhibitors (anti-PD-1 and/or anti-PD-L1) significantly improved therapeutic outcomes, resulting in long-term tumor eradication. This effect was associated with systemic immune activation, reflected by increased IL-1 α , IL-2, and IFN- γ levels, alongside decreased IL-4, IL-6, and IL-17.

Acknowledgements

This work was financially supported by the National Science Centre–Poland (project no 2020/37/B/NZ7/04157 given to J.M.D.) and by a grant from the Faculty of Chemistry under the Strategic Programme Excellence Initiative.

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Detecting and Modulating Proteins with Multifunctional Molecular Probes

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Protein surfaces play central roles in many biological processes, yet effectively targeting them with synthetic agents remains a major challenge. To address this, we have developed multifunctional molecular probes capable of engaging in proximity-induced, nonspecific interactions with protein surfaces. These probes can interact with diverse proteins and can be applied to multiplexed protein sensing as well as to mediate unnatural protein–protein or cell–cell interactions. The first part of the talk will focus on ID-probes, a class of fluorescent probes that mimic the function of the olfactory system [1-4]. Unlike conventional probes designed to detect a single protein, ID-probes generate distinct identification (ID) fingerprints for multiple proteins and their combinations. This capability enables the characterization of protein subpopulations in complex mixtures and in living cells; tasks that are difficult to achieve using conventional chemical “nose/tongue” approaches. In the second part of the presentation, we will describe a strategy for reversibly decorating bacteria with artificial, self-assembled synthetic receptors [5-6]. This supramolecular modification dynamically functionalizes the bacterial surface without genetic manipulation, effectively endowing the cells with new recognition capabilities. By installing synthetic receptor assemblies on bacterial membranes, we created bacterial probes (B-probes) that selectively interact with mammalian cells. These engineered bacteria function as living sensing platforms capable of distinguishing between different cancer cell types, highlighting the potential of combining synthetic chemistry with living systems to develop adaptable and programmable diagnostic tools.

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Unraveling the Nucleation Landscape of Amyloidogenic Proteins: An NMR-Driven Mechanistic Study

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Misfolded protein aggregation is associated with disorders such as Alzheimer's disease and Agel amyloidosis. Increasing evidence indicates that toxicity arises primarily from transient, soluble oligomeric intermediates rather than mature fibrils, highlighting the need to characterize early aggregation processes [1].

Solution NMR spectroscopy enables real-time investigation of these heterogeneous systems without perturbing self-assembly. In particular, ¹H Diffusion-Ordered NMR Spectroscopy (DOSY), combined with inverse Laplace transform (ILT), enables quantitative resolution of the size distribution of rapidly interconverting species, allowing detection and characterization of oligomeric populations formed at early stages of aggregation.

The DOSY-ILT strategy has been applied to the A β 40 peptide [2] and the G2-D187N gelsolin domain [3], which are associated with Alzheimer's and AGel diseases, respectively. Analysis of the evolving oligomer distribution uncovered common features in their early oligomerization processes, thereby providing a mechanistic basis for the rational design of inhibitors targeting amyloidogenic processes.

Acknowledgements

This work was supported by Fondazione Telethon ETS-Italy (Grant no. GMR24T1097). K.P., H. M. and L.R acknowledge support from Fondazione Antonio De Marco.

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Advancing graft evaluation: integrating metabolomics into prognostic models for heart transplantation

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Primary Graft Dysfunction (PGD) is the leading cause of early morbidity and mortality after heart transplantation, with a pooled PGD incidence of 20% and a related 1-year mortality of 26% [1]. Additional complications arise from the use of marginal grafts, obtained by donation after circulatory death (DCD) [2].

This study aimed to integrate tissue metabolomics in the multimodal procedure for organ evaluation: the objective was to identify new molecular markers of Ischemia Reperfusion Injury (IRI) and prognostic markers of PGD.

Endomyocardial biopsies (EMBs) were collected at the graft procurement and two weeks after implantation. Hydrophilic metabolites extracted from EMBs were quantified combining the conventional NMR metabolomics workflow [3] with Chenomx NMR Suite v26.01 (Chenomx Inc., Edmonton, AB, Canada). Statistical analyses and machine learning were performed using R (version 4.4.0).

Although direct oxidative stress quantification was not feasible, the concentration dynamics of high energy compounds (PCreatine/ATP) and the AMP/ATP ratio suggested a mitochondrial inefficiency in grafts developing severe dysfunction. A depletion of physiological taurine is observed in the best performing hearts: this calls for a closer examination of the role of this β -amino acid in the IRI contest [4]. The study also included one case of donation after circulatory death, involving the use of a normothermic perfusion system for organ transport. This unique case yielded an excellent clinical outcome, a seemingly contradictory observation for a marginal heart which prompts to further investigation about the molecular mechanisms underlying the different heart transplantation procedures

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Effect of selected mutations on sulfide binding to neuroglobin

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Discovered in 2000 in human and mouse brain and neurons, Neuroglobin (Ngb) still draws attention due to its neuroprotective role under oxidative stress conditions and neurodegenerative diseases [1]. Although featuring the classical “3-over-3 α -helical sandwich” globin fold, Ngb contains a bis-histidyl 6-coordinated heme *b* [1]. Formation of the intramolecular disulfide bond Cys46-Cys55 under oxidizing conditions weakens distal His64 coordination, increasing affinity towards exogenous ligands [1].

H₂S acts as an endogenous neuromodulator implicated in neurological, cardiovascular, and metabolic disorders [2]. Heme proteins can bind sulfide triggering redox processes. Ngb can interact with H₂S, undergoing heme reduction and inefficient conversion to S₂O₃²⁻, with the His64Ala substitution enhancing the interaction [3].

We used a combination of spectroscopic techniques (UV-Vis, Resonance Raman) and stopped flow kinetics to explore the role of key residues in the distal pocket region (His64, Lys67) in controlling H₂S binding to Ngb and the properties of the corresponding Fe(III)-S adducts. As for other heme proteins [4], formation of the Fe(III)-S Ngb adducts was followed by that of sulfheme-containing species, characterized by the covalent modification of the tetrapyrrole ring. Experimental data will be discussed in relation to the protein function and the structure of its active site.

Acknowledgements

University of Modena and Reggio Emilia FAR Mission Oriented 2022 (Cobalt substituted globins as catalysts for electrochemical hydrogen production – G.B.) funding program.

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Interaction between copper and neuronal proteins: a DOMINO effect in neurodegeneration

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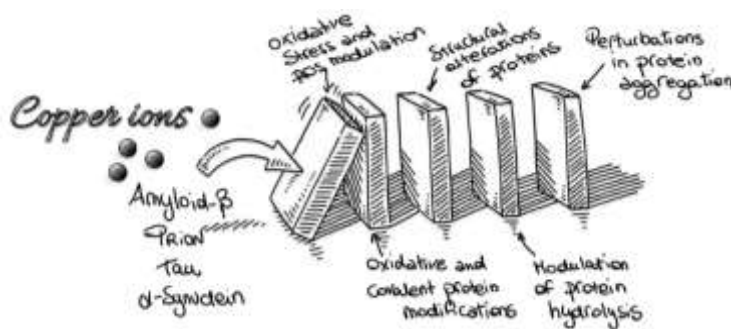
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Neurodegenerative disorders such as Alzheimer's and Parkinson's disease are characterized by protein misfolding, metal dyshomeostasis, and oxidative stress. Copper interactions with neuronal proteins, including β -amyloid, prion, tau and α -synuclein, critically modulate protein stability while driving redox cycling and ROS production. Mechanistically, copper redox activity is mainly controlled by histidine- and cysteine-based coordination sites, including ATCUN motifs in N-truncated A β and cysteine-containing regions in tau fragments [1-2]. These residues, together with the nature of substrate molecules, govern the formation of redox-active Cu species and transient Cu(I) intermediates [3]. Membrane-like environments further tune copper reactivity, preserving catalytic accessibility in Cu/A β complexes while promoting redox-inactive sequestration of Cu/PrP and Cu/ α Syn systems [1,4]. Copper can oxidatively modify proteins [5] and, concurrently, enhance peptide stability against hydrolysis [6], thus potentially favoring the accumulation of amyloidogenic species that promote fibril formation and neurotoxicity. The role of copper extends further, contributing to multiple interconnected pathways that ultimately drive a domino-like neurodegenerative progression.



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Decoding Zn(II) Capture by *Aspergillus fumigatus*: Coordination Chemistry Meets Antimicrobial Intervention

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Antibiotic resistance is one of the most pressing challenges in modern medicine. Equally concerning are fungal infections, whose treatment remains difficult due to the fundamental similarity between fungal and human cells. One notable example of this growing threat is a well-adapted saprophytic and opportunistic fungus *Aspergillus fumigatus*, which causes, among others, invasive pulmonary aspergillosis (mortality rate may reach up to 90%).

To survive in the host, *A. fumigatus* secretes an extracellular zincophore Aspf2, which is released into surrounding host cells, where it binds Zn(II). The most probable Zn(II) binding site is its C-terminal region, which binds it via a {2N_{im}, 2S} binding mode [1]. Subsequently, this protein delivers it to the fungal pathogen through physical interaction with the ZrfC transporter (Figure 1). The most probable binding site for ZrfC transporter is the MNCHFAGVEHCIGAGESESGSSQ fragment, which binds Zn(II) via the same set of donor atoms as the C-terminal fragment of the Aspf2 zincophore [2]. Nevertheless, the ZrfC fragment coordinates Zn(II) with higher affinity than the Aspf2 one. It enables efficient Zn(II) transport from the zincophore to the transporter (Figure 1) and holds a promise for the development of novel therapeutic strategies.

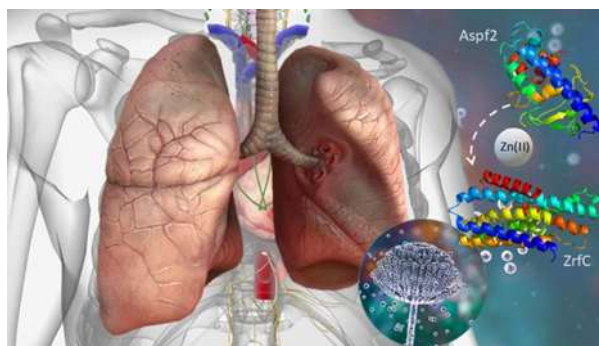


Figure 1. Schematic model of zincophore-based transport in *Aspergillus fumigatus*.

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Disarming bacterial virulence factors: rational design of peptide inhibitors for metalloproteinases

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Antibiotic resistance makes once treatable infections lethal. We urgently need new therapies. Targeting bacterial virulence factors offers a highly promising strategy [1]. Metalloproteinases (MPs) from the M4 and M10 families are key virulence factors that degrade host tissues. Their activity relies entirely on a zinc ion cofactor. This makes the catalytic metal center a prime target for inhibition [2]. Studying full enzyme structures is highly complex. Therefore, we used peptide mimetics based on the enzyme's binding sites from resistant strains. We analyzed how these models coordinate Zn(II) and Cu(II) ions. We explored the thermodynamic potential of copper to displace native zinc. Using these insights, we designed a library of peptide inhibitors. We modeled them on natural substrates to optimize flexibility and specificity (Fig. 1) [3]. We tested various metal-binding groups at different positions in the sequence to maximize affinity. We characterized these interactions using potentiometry, mass spectrometry, NMR spectroscopy, UV-Vis spectroscopy, and circular dichroism. This approach revealed the stoichiometry, stability, and donor atoms of both binary and ternary complexes. Decoding this metal coordination provides a solid foundation for rational drug design. Disrupting bacterial virulence at the molecular level opens a powerful new pathway against antimicrobial resistance.

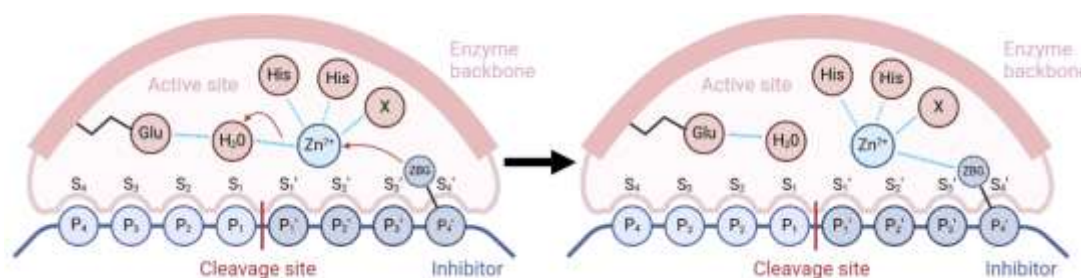


Figure 1. Mechanism of inhibition by a substrate-mimicking inhibitor. The inhibitor binds to the active site via a zinc-binding group (ZBG), preventing substrate cleavage and release [4].

Acknowledgements

Financial support by the National Science Centre, Poland (UMO-2023/50/E/ST5/00434) is gratefully acknowledged.

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Copper trafficking in bacteria – insights from metallophores to regulatory and chaperone system

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Copper homeostasis in bacteria requires a balance between its essential role and its toxicity. Although many components of copper handling have been identified, the molecular details of how copper is bound, transferred and regulated are still not fully understood [1]. Here, we combine insights from three functionally distinct systems, CopM, CopY and CusF, to examine how local sequence features influence copper handling at different stages of the pathway.

Across these systems, a consistent picture emerges. Metal binding depends not only on the presence of coordinating residues, but also on their arrangement and the surrounding chemical environment. In the CopM/OprC system, the histidine-rich MxxHH motif binds Cu(II) more strongly than the extracellular OprC site, which suggests that copper may be transported as part of a stable metallophore complex rather than as a free ion [2]. A similar dependence on sequence organization is observed in the CopY regulator, where a short cysteine-rich motif is sufficient to bind metal ions and induce structural changes. Cu(I) promotes the formation of multinuclear species, while Zn(II) stabilizes simpler complexes [3]. In CusF-derived motifs, histidine spacing also affects metal selectivity. Compact arrangements such as the -HHH- motif favor Cu(II) binding, whereas more distributed residues shift the preference toward Zn(II). This pattern appears consistently across the studied systems [4].

Taken together, these results indicate that the function of metal-binding proteins is governed not only by amino acid composition, but also by local sequence organization. Understanding these relationships provides a clearer picture of copper trafficking and may help identify new strategies to disrupt metal homeostasis in pathogenic bacteria.

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The work was supported by the National Science Centre (Grant UMO-2023/51/D/ST5/01798).

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The pH-dependent zinc sequestration by calprotectin as a driver of Nutritional Immunity in the vaginal environment

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Metrodora's interest focuses on exploring the mechanisms of nutritional immunity within the vaginal microenvironment, specifically focusing on how the environment regulates the bioavailability of essential transition metals like Zn²⁺ or Mn²⁺ and its potential impact on the vaginal microbiome. While calprotectin (CP), a 24 kDa heterodimeric protein, has well-characterized antimicrobial properties through its ability to bind and sequester Zn²⁺ and Mn²⁺, two trace metals essential for bacterial growth, it has not been well studied in the vaginal environment. Furthermore, there are contradictory reports on CP levels during shifts from a healthy, lactobacilli-dominated vaginal microbiome with pH 3.5–4.5 to the dysbiotic, polymicrobial vaginal microbiomes characteristic of bacterial vaginosis with an elevated pH >4.5 [1–2]. Additionally, CP's metal-binding activity has been characterized only from pH 5.0–7.0 [3]. We addressed these gaps by investigating CP metal-binding activity across a broader pH range (3.5–6.0) and measuring the concentrations of CP and trace metals in vaginal fluid collected from women with diverse vaginal microbiomes. Metrodora developed a protocol for extracting and processing neat, undiluted vaginal fluid from swabs, rather than using cervico-vaginal lavage, thereby enabling more precise quantification of the specimen and eliminating pH modification. We used ELISA to quantify various metal-binding proteins in vaginal fluid, ICP-MS to assess the biological availability of transition metals, and colorimetric assays to measure key bacterial metabolites such as acetate and lactate. Using recombinant CP, we demonstrated how the pH of the vaginal environment, a key variable in vaginal health, modulates CP's overall capacity for trace metal sequestration. This work illustrates the relationships among pH, calprotectin concentration and activity, and background trace metal levels that determine whether nutritional immunity is activated or silenced in the vaginal environment during health and dysbiosis.

Acknowledgements

We thank Prof. W. Chazin for the recombinant CP and Prof. M. Frankowski for his collaboration on ICP-MS analysis.

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Genetic surface modification of the human ferritin platform for biomedical applications

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Human heavy chain ferritin (HuHf) is a robust, cage-like protein scaffold ideally suited for biomedical applications [1]. Its versatility has established it as a powerful tool for the targeted delivery of anticancer agents, including metal-based compounds [2–4]. The selectivity of this protein platform stems from the specific interaction between its subunits and the Transferrin Receptor 1 (TfR1) [5], which is significantly overexpressed in cancer cells.

In this study, I report preliminary data demonstrating the overexpression of TfR1 on dendritic cells, the primary antigen-presenting cells responsible for orchestrating an immune response. To visualize this interaction, we engineered proof-of-concept fluorescent HuHf conjugates using Alexa Fluor dyes, alongside a HuHf-eGFP (enhanced Green Fluorescent Protein) chimera. These tools allowed us to monitor the internalization of ferritin into the cells in real-time. Simultaneously, a structural analysis of the chimera is underway to provide a comprehensive understanding of the molecular features of this platform.

Alongside this, we designed a specialized tumor-antigen-presenting ferritin. The NY-ESO-1 peptide, a highly immunogenic antigen prevalent in various tumors, was fused to the N-terminal of the ferritin to facilitate immunogenicity studies on dendritic cells.

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HSA-Metallo drug Bioconjugates: How Protein Binding Modulates the Cytotoxicity of Pt(II) and Au(I) complexes

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Protein-metallo drug bioconjugates are a promising class of anticancer agents that combine cytotoxic metal pharmacophores with the selectivity of protein scaffolds to reduce systemic toxicity [1].

The transport and delivery of metallo drugs via plasma proteins, particularly Human Serum Albumin (HSA), are critical yet controversial factors in determining their clinical efficacy. HSA is an ideal delivery vehicle due to key residues—such as Cys34, Trp214, and Tyr411—which form stable adducts that transform this common plasma protein into a targeted, effective transport system [2]. This study investigates the pharmacological consequences of metal-protein binding by synthesizing and characterizing bioconjugates of HSA with three standard metallo drugs: cisplatin (CIS), oxaliplatin (OXA), and auranofin (AF).

Bioconjugates were prepared by direct incubation and characterized using Electrospray Ionization Mass Spectrometry (ESI-MS) and Inductively Coupled Plasma (ICP) spectroscopy to determine metal-to-protein ratios and quantify the bound metal.

The antiproliferative effects were evaluated in A2780 (ovarian) and HCT116 (colorectal) cancer cell lines and compared with the respective free drugs. Significant differences in cellular effects were observed between the two platinum-based drugs and the gold-based drug, AF.

The nature of the metal center and its specific interaction with the HSA carrier play a crucial role in determining the final cytotoxic profile. These findings provide fundamental insights for the design of new metallo drug albumin conjugates. Specifically, they highlight the need for delivery formulations that can protect metal-based payloads from deleterious plasma interactions, ensuring their pharmacological activity remains intact upon reaching the target.

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Double Disguise: Camouflaging Photocages for Bioorthogonally Controlled Conditional Activation

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Photolabile protecting groups enable the controlled release of biologically active compounds upon light irradiation, providing high spatial and temporal control over biological processes. This principle forms the basis of photoactivated chemotherapy, in which drug activity is restored only at the irradiation site. Although light-triggered drug release is well suited to localized tumors, spatial control by light alone is insufficient for disseminated or non-localizable malignancies, necessitating additional levels of targeting.

To address this limitation, we investigated a conditional photoactivation strategy in which the photosensitivity of a visible-light-responsive photolabile protecting group is temporarily suppressed by a chemically cleavable masking motif [1]. The system is based on a rhodol-derived photocage [2], where substitution at the phenolic oxygen atom renders the molecule colorless and transiently photo-inactive by preventing the formation of the photolabile oxo form and favoring a photo-inactive leuko form at neutral pH.

Chemical unmasking is achieved through a bioorthogonal inverse electron-demand Diels–Alder reaction between a trans-cyclooctene (TCO) moiety and tetrazine, followed by cleavage. This reaction restores the colored, photolabile oxo form of the photocage, enabling subsequent drug release upon green-light irradiation.

The dual-activation concept was evaluated in a cellular model by conjugating the TCO-substituted rhodol photocage to the topoisomerase I inhibitor SN-38 and treating SK-OV-3 cells. Neither light irradiation nor tetrazine treatment alone induced significant drug release; however, the combined use of chemical unmasking and light activation resulted in cytotoxicity comparable to that of free SN-38.

Overall, these results demonstrate that dual masking improves the selectivity of light-activated drug delivery systems and establish conditional photoactivation as a versatile framework for enhancing the precision of photoresponsive therapeutic platforms.

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Indole-Based Porphyrin-COX-2 Inhibitor Conjugates as Targeted Photosensitizers for Photodynamic Therapy

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Colorectal cancer (CRC) is one of the leading causes of cancer related mortality and is frequently associated with overexpression of cyclooxygenase 2 (COX 2), an enzyme implicated in inflammation, tumour progression, and resistance to apoptosis [1]. Consequently, COX 2 represents an attractive target for selective anticancer strategies. Photodynamic therapy (PDT) is a minimally invasive treatment modality based on light activated photosensitizers; however, its efficacy is often limited by insufficient tumour selectivity [2].

In this work, we report the design and synthesis of a new series of porphyrin-COX 2 inhibitor conjugates based on 1 tosylindole derived scaffolds. The obtained compounds constitute a platform for further photophysical and biological evaluation as COX 2 targeted photosensitizers for PDT [3].

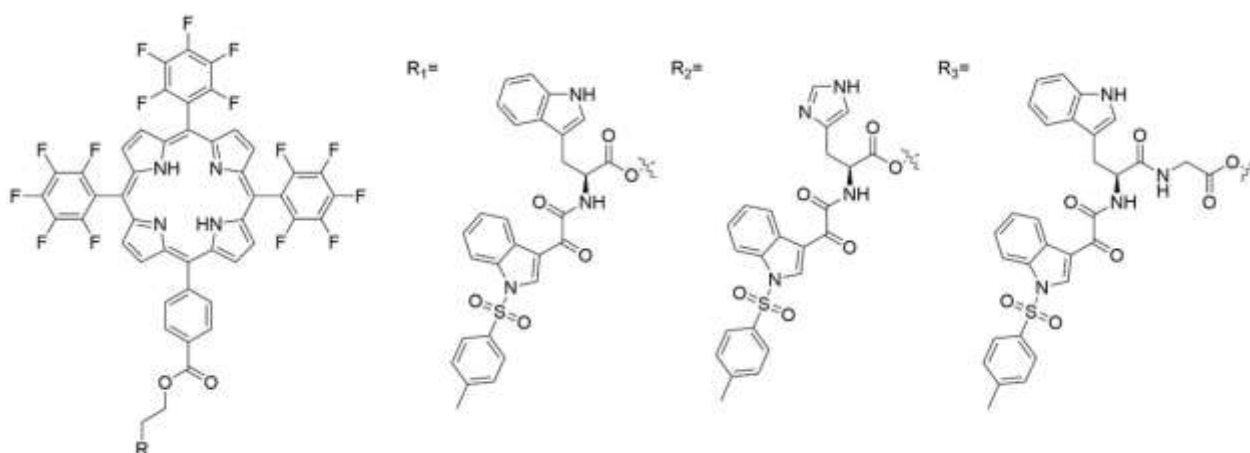


Figure 1. General chemical structures of synthesized conjugates

Acknowledgements

This research was funded by the National Science Centre (NCN), Poland, under the Opus project no. 2020/37/B/NZ7/04157 and project no. 2024/55/B/NZ7/03169.

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Microcalorimetry for the design and engineering of biocatalysts

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The stability and activity of a biocatalyst are key factors in determining its effectiveness in industrial and research applications. Differential Scanning Calorimetry (DSC) has demonstrated the ability to determine the thermal stability of biocatalysts because it can provide key information such as: reversibility of the unfolding process, enthalpy, onset Temperature (T_{onset}) of the denaturation event and melting temperature (T_m). On the other hand, Isothermal Titration Calorimetry (ITC) has been primarily used to characterize the thermodynamics of ligand binding. Nevertheless, ITC can also be used for assessing the kinetic parameters for a given biocatalyst, by measuring the heat associated with the conversion of substrate into product, as well as the stability that can be extrapolated as residual activity. Recently we have combined DSC and ITC to characterize in depth the stability and activity of a series of biocatalysts, such as flavin-containing monooxygenase [1], cytochrome P450 [2] and TMAO demethylase [3]. When combined with DSC, ITC offers the possibility to avoid lengthy and inaccurate indirect activity measurements guiding both the design and the rational engineering of biocatalysts.

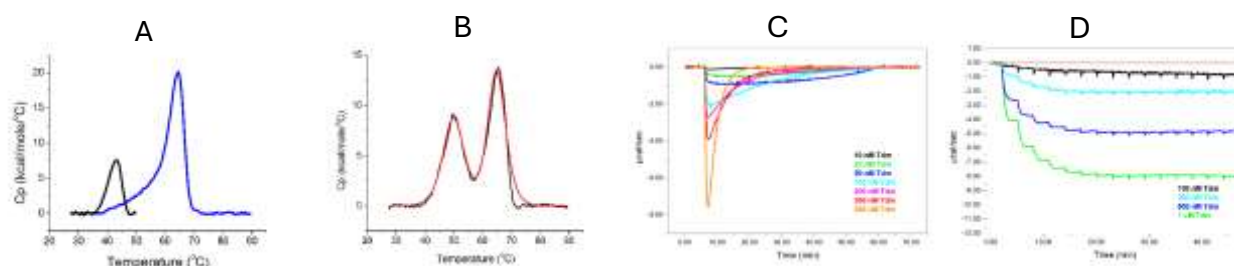


Figure 1. DSC and ITC of TMAO demethylase (Tdm). A) Denaturation in two steps (from 25°C to 50°C (black), then from 25°C to 90°C (blue)). B) Thermogram with the experimental curve (black) and fitting of the two-state denaturation model (red). C) Kinetic assay in single-injection mode from 10 to 500 nM Tdm. D) Kinetic assay in multiple-injection mode with 0.1 to 1 μM Tdm and 600 μM TMAO.

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Hypoxia Active Ir(III) and Ru(II) Photosensitizers for PDT

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Transition metal complexes possess key photophysical properties - good absorption in the visible spectrum, intense emission, and long-lived triplet states - that make them excellent photosensitisers for photodynamic therapy (PDT). Here presented are two complex families utilising the Ru(II)/Os(II) polypyridyl and Ir(III) cyclometalated frame: (A) featuring bipyridine (bpy) ligands with donor triphenylamine (TPA) and acceptor trifluorotoluene (TFT) groups, and (B) where Nile Red (NR) is conjugated to the bpy (Figure 1) [1-3]. Complexes A show a moderate affinity towards DNA and can photooxidise NADH. *In vitro* cell studies with MCF-7 cells reveal that Ru-1 and Ir-1 have moderate cytotoxicity, generate large amounts of reactive oxygen species (ROS) in the dark and after photoexcitation, and have good phototoxicity indices (PI). The NR complex family showed activity in SKBR-3 cells and further studies of [Ir-3ENR]Cl in MCF-7 cells under normoxic (O₂ 21 %) and hypoxic (O₂ 1 %) conditions revealed a remarkable PI of 179, mitochondrial accumulation and a Type-I PDT mechanism that was retained under hypoxia. The optimised [3ENR-Ru-EP]²⁺ featuring an ethynyl pyrene is non-toxic in the dark (up to 64 µM) and has nM IC₅₀ after photoexcitation, and doubles its activity under hypoxic conditions.

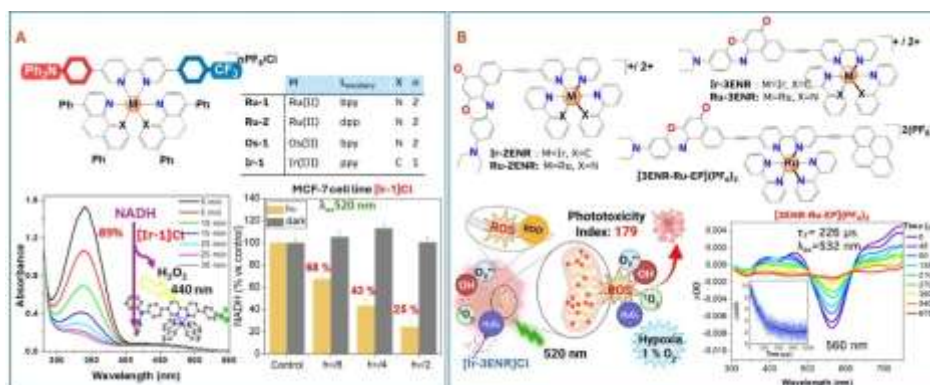


Figure 1. (A) Structures of TPA-bpy-TFT complexes and NADH photooxidation studies of Ir-1. (B) NR complexes and schematic of [Ir-3ENR]Cl *in vitro* anticancer activity and ns transient absorption spectra of [3ENR-Ru-EP]²⁺.

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Poster Contributions

Poster — P01 · Dominick Barczyk — [fPP1]

Engineering Fluorinated Tetrapyrrolic Photosensitizers: How Structural Design Controls Triplet Lifetimes and Photodynamic Activity

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Photodynamic therapy (PDT) is a therapeutic approach that combines light, photosensitizer (PS), and oxygen to generate reactive oxygen species (ROS). These species induce oxidative stress in the tumor microenvironment, leading to cancer cell death, vascular damage, and immune response activation [1].

The efficacy of PDT depends not only on efficient intersystem crossing (ISC), but critically on the lifetime of the resulting triplet excited state. Structural modifications such as fluorination or the incorporation of metal ions increase spin-orbit coupling and thereby enhance ISC. The same factors can also accelerate triplet-state decay. In particular, metal centers with open-shell character and strong metal-centered spin-orbit coupling often exhibit shortened triplet lifetimes due to enhanced non-radiative deactivation pathways. In contrast, closed-shell metal centers tend to preserve longer-lived triplet states. Importantly, even in closed-shell systems, heavier metal ions can significantly shorten triplet lifetimes as a consequence of strong spin-orbit coupling and faster spin-forbidden relaxation. Therefore, achieving an optimal balance between efficient triplet-state population and sufficient triplet lifetime is essential for effective ROS generation [2, 3].

Tetrapyrrolic photosensitizers were evaluated for their photophysical properties, including light absorption, photodegradation quantum yield, triplet-state lifetimes, ROS generation, as well as their lipophilicity and *in vitro* photodynamic performance. Preliminary *in vivo* studies indicated high stability and preferential accumulation, enabling the identification of structure-property relationships relevant to PDT.

Acknowledgements

We thank National Science Center for Opus grant no 2020/37/B/NZ7/04157 and 2024/55/B/NZ7/03169.

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SpyTag/SpyCatcher system: towards the development of a bio-inspired heme catalyst

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SpyTag/SpyCatcher (ST/SC) is a biotechnological tool constituted by a protein scaffold (SC) that is able to recognize and spontaneously bind an independent oligopeptide (ST) through an isopeptide bond between the side chains of an aspartate (belonging to ST) and a lysine (belonging to SC) [1]. This system was firstly developed by splitting the protein FbaB from *Streptococcus pyogenes* in two portions, a 13 residues peptide and the 116 residues complementary domain, that spontaneously reconstitute in few minutes [2].

In this work, the peptide sequence was designed to contain a consensus fragment, that allows the recognition between ST and SC, and two histidine residues to allow the coordination of hemin [figure 1]. The intended function of this bio-inspired system is ROS regulation, and therefore its pseudo enzymatic activity as peroxidase- and catalase-like reactivity have been tested. The assays showed that, while the ST-hemin complex has enhanced reactivity towards hydrogen peroxide, this is not the case when the STSC adduct is formed. The cofactor ability to bind ST was assessed both with the free peptide and after assembly with SC and it was observed that, while the ST-hemin binding occurs with micromolar affinity, the coordination of hemin with the reconstituted adduct is not maintained with the same strength. For this reason, ongoing studies aim to optimize the system by covalently binding the cofactor to the N-terminus of the peptide.

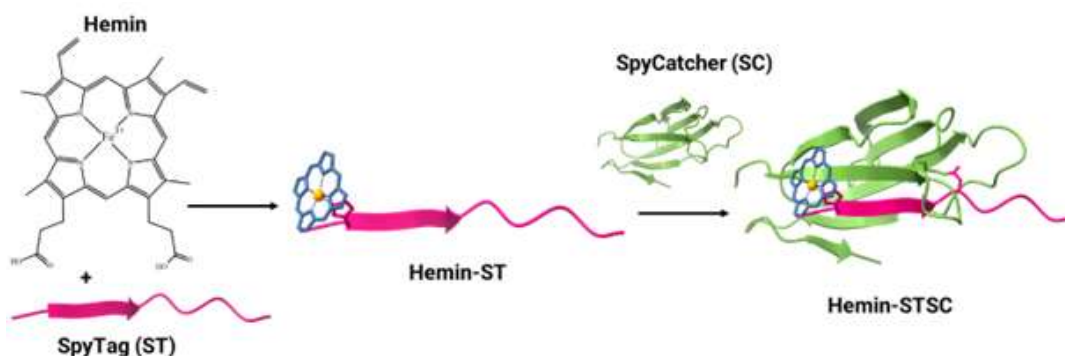


Figure 1. Scheme of the formation of the heme-ST/SC complex]

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Antimicrobial activity of copper(II) complexes with coumarin derivatives

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Coumarins (benzo- α -pyrone) belong to a broad group of flavones derivatives. These compounds are characterized by a broad spectrum of biological activity, including antibacterial, anticancer, and anti-inflammatory properties [1-3]. Many of these compounds exhibit metal-chelating properties, enabling the formation of coordination complexes that often display enhanced biological activity [4]. Copper(II), a biologically relevant transition metal, plays an essential role in numerous enzymatic and redox processes and has been extensively investigated for its antitumor potential [5].

In current studies, we analyze the antimicrobial activity of newly obtained Cu(II) complexes and their coumarin ligands. The tested complexes exhibited potent antibacterial activity, suggesting their potential as novel compounds to bolster existing antibiotics and chemotherapeutics in combating resistance.

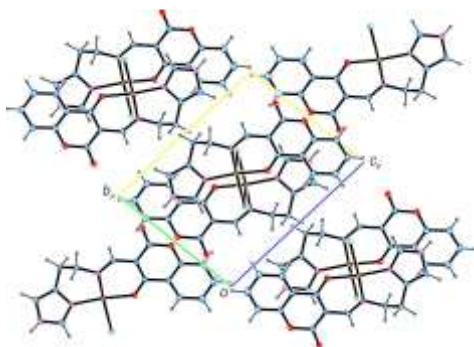


Figure 1. Structure of copper(II) complex with coumarin

Acknowledgements

This work was supported by grant No. 503/3-066-02/503-31-001 from the Medical University of Lodz, Poland.

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Integrated NMR metabolomics and deep-learning framework for evaluating the health status of differentiated SH-SY5Y cells

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Untargeted NMR metabolomics is widely applied for the analysis of the full metabolic profile and the screening of specific metabolites, acting as specific biomarkers [1]. In this study the cellular media of differentiated SH-SY5Y neuroblastoma cells were investigated by NMR spectroscopy with the aim to correlate a specific metabolic profile to cellular health condition. Researchers have utilized the SH-SY5Y cell line to investigate the impact of Alzheimer's-related proteins on neuronal health, providing crucial insights into the mechanisms underlying the disease [2]. The NMR samples were obtained by collecting the cellular media of differentiated SH-SY5Y neuroblast-like cells treated with different concentrations of several compounds, including the peptide Amyloid β and other natural compounds known to correlate with Alzheimer's disease [3]. The effects of the cellular vitality upon specific treatments were also determined by the Neutral Red Uptake (NRU) test and they were strongly dependent on the type of applied treatment. The collected NMR data were processed by using a compression technique based on convolutional autoencoders, which can perform a high dimensionality reduction in the spectral signal (up to more than 300 times), maintaining informative features (guaranteed by a reconstruction error always smaller than 5%). Moreover, before compression, an ad hoc preprocessing method was devised to remedy the scarcity of available data. The compressed spectral data were then used to train some SVM classifiers to distinguish diseased from healthy cells, achieving an accuracy close to 78%, a significantly better performance with respect to using standard PCA-compressed data [4].

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A battering ram for new antibacterial delivery systems: the depolymerase from bacteriophage ϕ BO1E

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Over the last century, the advent of antibiotics turned bacterial infections from fatal diseases to curable ailments. However, the landscape is changing due to the increase in Antimicrobial Resistance (AMR), which is becoming the primary concern for the World Health Organization. A new paradigm to fight bacterial infections may arise from the enzymatic tools of bacteriophages, viruses that are able to breach bacterial defenses and replicate within bacterial cells.

The bacterial capsule is an important virulence factor of *Klebsiella pneumoniae*, one of the most threatening Gram-negative bacteria. Recent studies aim at taking advantage of the specificity of phage-derived depolymerase enzymes cleaving the capsular polysaccharide (CPS), the main component of the capsule.

The phage ϕ BO1E [1] infects the *K. pneumoniae* strain KPB-1, clade II of Clonal Group 258 [2], one of the main lineages responsible for the global pandemic dissemination of multidrug-resistant *K. pneumoniae*. Its depolymerase was produced as a recombinant protein, and its thermal stability and enzymatic activity were evaluated under different pH and ionic strength conditions. Structural analysis revealed a trimeric enzyme featuring an N-terminal β -helical domain and a C-terminal jelly-roll domain (Figure 1).

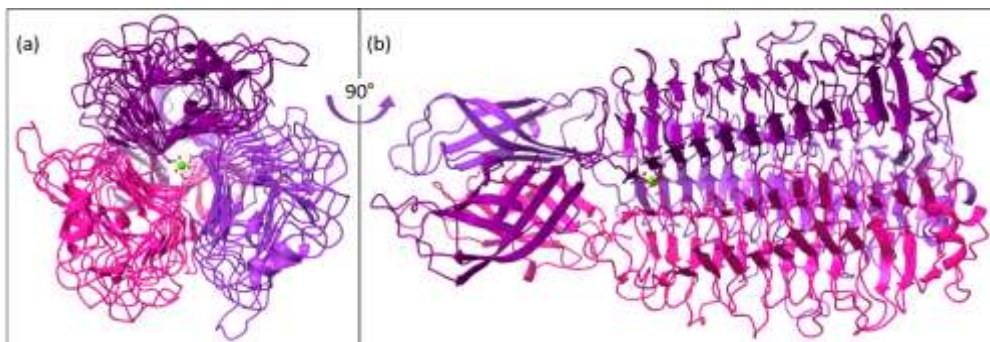


Figure 1. Crystal structure of the endoglycosidase from phage ϕ BO1E

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The role of cellular reducing agents in the protection of zinc finger proteins against toxic metal ions

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Recently, there has been an increasing interest in use of silver in medicine, especially in the form of silver nanoparticles (AgNPs). Medical devices, bone implants, products for everyday use, including cosmetics, bedding, sportswear, protective equipment and food containers, are coated with AgNPs for their antibacterial properties [1]. The spread of AgNPs increases the environment's and also the population's exposure to Ag(I) ions. The intracellular dissolution of AgNP is followed by the mass formation of Ag-S bonds, therefore thiol groups in proteins have been identified as cellular targets of Ag(I) ions. Similarly, Hg(II) is an extremely toxic metal ion mostly attributed to its interaction with S-donor atoms of biomolecules, thereby altering their structure.

In cell culture experiments, inhibition of zinc finger proteins (ZFPs) has been identified as one of the mechanisms of toxicity of these metal ions [2]. The zinc finger (ZF) motifs of ZFPs are responsible for molecular recognition. A ZFP can specifically recognize the DNA molecule only if Zn(II) coordinates to the protein and stabilizes its secondary structure. Therefore, potential competition with foreign metal ions may change the DNA-binding property of ZFPs and their original function [3].

At the same time, cysteine-containing peptides or proteins present in the cells may prevent the binding of these metal ions to the ZF motifs through competition due to their soft character. In our research, the effect glutathione as a potential coordinating agent towards these toxic ions was investigated. The main goal of this research was to quantify the metal ion interaction with an artificial Cys2His2-type zinc finger protein (1MEY#) in the absence and presence of DNA. We also monitored the DNA recognition ability of 1MEY# in the absence and presence of interfering agents. The results to be presented suggest that the intracellular environment is well equipped for the defence of the cell against the studied toxic heavy metal ions.

Acknowledgements

This research work was supported by the bilateral cooperation project of the Polish and Hungarian Academy of Sciences (NKM2023-38). It was also supported by the National Research, Development and Innovation Fund (No.: 150330).

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Cu²⁺ and Zn²⁺ complexes of *retro-inverso* clavanin C as potent antimicrobial agents

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Antimicrobial resistance, particularly in methicillin-resistant *Staphylococcus aureus* (MRSA), remains a major therapeutic challenge [1]. Antimicrobial peptides (AMPs) are promising due to their broad-spectrum activity, which can be further enhanced by coordination with metal ions such as Cu²⁺ and Zn²⁺ [2]. However, their susceptibility to proteolytic degradation limits clinical application.

In this study, we examine *retro-inverso* analogues of clavanin C, designed to improve enzymatic stability while preserving biological function. Their metal-binding properties, structure, and antimicrobial activity were evaluated [3-4].

The modified peptides exhibit increased resistance to enzymatic degradation while maintaining or enhancing antimicrobial efficacy. Notably, both the *retro-inverso* peptides and their metal complexes show improved activity against MRSA compared to the native peptide.

These findings highlight *retro-inverso* design combined with metal coordination as a promising strategy for developing stable antimicrobial agents.

Acknowledgements

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The influence of a histidine mutation on the properties of the arsenic(III)-sensing AfArsR protein

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Arsenic(III) and its compounds pose significant environmental and health risks, making their detection an important analytical task. Although instrumental methods are accurate and sensitive, their use is costly, laboratory-bound, and often requires complex sample preparation. Metalloid-regulatory proteins, such as the arsenic-sensitive members of the ArsR protein family, detect the presence of the recognized metalloid, As(III), at molecular level. This occurs through the activation of regulated genes, which, via the generation of a measurable response signal in a designed assay system, enables the detection of As(III) while also allowing for the study of the metalloid-regulating proteins.

In this work, we investigated the As(III) sensitivity and selectivity of the AfArsR protein using a variant containing the H97D point mutation *in vitro* and *in vivo*, with an aim to contribute to a better understanding and design of the function and potential applications of metalloid-sensing proteins. We studied the interactions of the mutant protein with recognized arsenic(III) as well as with divalent metal ions. The I-Block system based on intracellular gene regulation [1], as well as *in vitro* spectrometric methods served to study the properties of the protein, with particular attention to its selectivity toward metal(loids) ions. Mass spectrometry measurements confirmed the variant's ability to bind metal(loids) ions, while circular dichroism spectroscopy revealed that the effect of metal(loids) ions is negligible on the secondary structure of the protein.

According to the I-Block assays, the H97D AfArsR protein retained its specific DNA-binding and regulatory capabilities; however, compared to the wild-type protein, higher concentrations of As(III) were required to achieve the same effect. This was also supported by electrophoretic mobility shift assays, as the dissociation of the DNA-protein complex occurred at higher As(III) concentrations compared to the wild-type. Based on a comparison of the effects of different metal ions, the system is As(III)-selective. Our results show that the H97D mutation primarily modifies the conditions for the response to arsenic(III) in AfArsR function.

Acknowledgements

We acknowledge the financial support of National Research, Development and Innovation Office, Hungary (No.: 150330), of the Hungarian Academy of Sciences and Japan Society for the Promotion of Science (JSPS NKM2024-15/2024).

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GroEL1: A Multifunctional Chaperonin Involved in Mycobacterial Metal Homeostasis and Biofilm Maturation

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Due to the growing challenge of antibiotic resistance, it is crucial to understand the bacterial mechanisms that provide protection against the host immune system and drug treatments. In *Mycobacteria*, the GroEL heat shock proteins exist in two forms: GroEL1 and GroEL2. GroEL2 is an essential chaperonin responsible for protein folding and bacterial survival, whereas GroEL1 features a unique, unstructured histidine-rich C-terminal (HRCT) tail suggesting a specialized role in metal coordination. This may be linked to bacterial resistance against metal-induced stress, encountered within the macrophage phagosome [1-2].

Furthermore, GroEL1 is involved in the synthesis of cell wall lipids required for phagosomal escape and drug resistance. It also plays a key role in the synthesis of mycolic acids necessary for biofilm maturation [3]. In view of the multi-functionality of GroEL1 and its HRCT tails, we investigated the complexes formed between HRCT sequences from various bacterial species and metal ions such as Zn(II) and Cu(II). These complexes were characterized using a range of methods, including potentiometry, UV-Vis, and circular dichroism (CD). Understanding these bacterial defense mechanisms is crucial for advancing scientific knowledge and may contribute to the development of novel therapeutic strategies.

Bacterial Species	C-terminal Sequence
<i>Mycobacterium lepromatosis</i>	Ac-KTEEH ^{red} DHHGHAH ^{green}
<i>Mycobacterium ulcerans</i>	Ac-EDDGHGHHGHAH ^{green}
<i>Mycobacterium smegmatis</i>	Ac-EHGHGHHHGHHAH ^{green}

Table 1. Comparison of C-terminal Motifs Across Selected Mycobacterium Species.

Acknowledgements

Financial support by the National Science Centre, Poland (UMO-2023/50/E/ST5/00434) is gratefully acknowledged.

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Disrupting α -Synuclein Aggregation via azaGly-Engineered Cyclic D,L- α -Peptides: A Disease-Modifying Strategy for Parkinson's Disease

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Protein misfolding and aggregation into amyloid fibrils are central to Parkinson's disease (PD) pathology. α -Synuclein (α -syn) aggregation into cross β -sheet-rich structures forms toxic oligomers and Lewy bodies, leading to dopaminergic neurodegeneration. Current treatments remain symptomatic and do not target the underlying aggregation process. Here, we present aza-glycine (azaGly)-modified cyclic D,L- α -peptides as a novel strategy to inhibit α -syn aggregation. Building on the activity of cyclic D,L- α -peptide CP-2 on Alzheimer's disease-associated amyloid β [1] and α -syn [2] incorporation of azaGly residues enhances hydrogen bonding, structural stability, and interactions with low molecular weight oligomers. Few azaGly analogs of CP-2 were evaluated using biophysical aggregation assays and evaluated for their protecting activity *in vitro* neuronal cells [3], and transgenic *Caenorhabditis elegans* models of PD. Additionally, the ability of the cyclic peptides to disrupt cross-amyloid interactions between α -syn and amyloid β was studied using the Thioflavin T assay. These findings highlight azaGly-engineered cyclic peptides as promising disease-modifying agents targeting protein aggregation in PD.

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Outsmarting Bacteria: Bi(III) Meets Desferrioxamine B and E

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Bismuth compounds have been used in medicine for centuries and currently play an important role in the eradication of *Helicobacter pylori*, as well as in restoring antibiotic sensitivity in multidrug-resistant strains. Their efficacy is associated with a “Trojan horse” mechanism, in which Bi(III) ions enter bacterial cells by mimicking iron ions, enabling them to disrupt key metabolic pathways [1-2]. However, one of the main therapeutic limitations of bismuth is its low bioavailability [3], which may be improved through the use of siderophores.

In this work, we focused on determining the complexation properties of two siderophores, desferrioxamine B (DFOB) and desferrioxamine E (FOX E), toward Bi(III) ions. In particular, the stoichiometry, thermodynamic stability, and speciation of the resulting complexes were determined over a wide pH range. The studies were carried out using mass spectrometry (ESI-MS), potentiometric titrations, and UV–Vis spectroscopy.

The applied approach allowed us to overcome the challenges associated with the aqueous chemistry of Bi(III), including its extensive hydrolysis and the limited availability of spectroscopic data [4]. The proposed models indicate the formation of mononuclear bi- and trihydroxamate complexes for both ligands. The results obtained demonstrate that FOX E exhibits stronger binding properties compared to DFOB.

Based on the determined stability constants and calculated pBi values, both siderophores were identified as efficient chelators of Bi(III) ions [5]. This suggests their potential application in targeted delivery of bismuth as an antimicrobial agent, as well as candidates for radiolabeling with the ²¹³Bi radioisotope in advanced infection therapies.

Acknowledgements

This work was conducted as part of the NCN-funded project (UMO-2022/47/I/ST4/02354).

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Identification of phenolic compounds in *Centaurea cyanus* L. petals and their synergistic biological potential

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Our study, together with previous ones, indicates that extracts from different species of *Centaurea* L. may be of significant importance in various fields, including pharmacology, medicine, and cosmetology, due to their diverse activities: anticancer, antidiabetic, anti-inflammatory, antidepressant, antioxidant, and antimicrobial [1-3].

Phenolic compounds were identified in six differentially prepared extracts of *Centaurea cyanus* L. flowers using UPLC-PDA-Q/TOF-MS technique. Thirty-five individual phenolics were confirmed, comprising hydroxycinnamic acids, flavonols, flavones, anthocyanins, and N-p-coumaroyl spermidine derivatives. The ethanolic extract obtained from *Centaurea cyanus* petals, following chloroform defatting, was found to contain a chemically diverse and quantitatively rich profile of phenolic compounds. The total content of identified phenolics reached 42.18 mg/g DW in the extract, which is consistent with the view that cornflower petals represent one of the most phenolic-rich floral materials within Asteraceae. The ethanolic extract was dominated by flavones, apigenin derivatives, which accounted for more than half of the total phenolic fraction. Apigenin-O-hexoside-O-glucuronide and apigenin-3-O-glucoside-(6"-acetyl)glucoside were the most abundant constituents. 3-Caffeoylquinic acid, quercetin glucoside, and N1,N5,N10-tri-p-(E,E,E)-coumaroyl spermidine, also dominated the polyphenol-rich profile. The extract exhibited notably high antioxidant capacity, as measured by DPPH (240.34 $\mu\text{mol TE/g}$), ABTS (170.53 $\mu\text{mol TE/g}$), and FRAP (189.93 $\mu\text{mol TE/g}$). In the present study, the cytotoxic properties of the *Centaurea cyanus* L. extract against reference mouse L929 fibroblasts and human Hs68 fibroblasts, as well as its antibacterial and antifungal activities, were also investigated.

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Heme-Thiolate Mediated Formation of NO-Ferroheme and S-Nitrosothiols

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S-nitrosothiols (RSNOs) and NO-ferroheme species are recognized as important signaling intermediates, particularly in nitric oxide (NO) transport within the vascular system [1]. Although both species are known to form concurrently from NO and thiols in the presence of ferric heme complexes, the detailed mechanisms of their *in vivo* formation remain unresolved. Herein, we investigate the kinetic and spectroscopic properties of ferric TMPS (TMPS = meso-tetrakis(sulfonatomesityl)porphyrinato-iron(III)) complexes with thiolate ligands (GSH, cysteine, and N-acetylcysteine) and their reactivity towards nitric oxide. The study revealed the initial coordination of NO to the heme-thiolate complex, yielding ferric nitrosyl intermediate, [Fe(TMPS)(SR)(NO)]. In a subsequent, slower step, this intermediate is converted to a NO-ferroheme through the direct attack of a second NO molecule on the coordinated thiolate, accompanied by concomitant release of the corresponding S-nitrosothiol (RSNO) (Figure 1) [2].

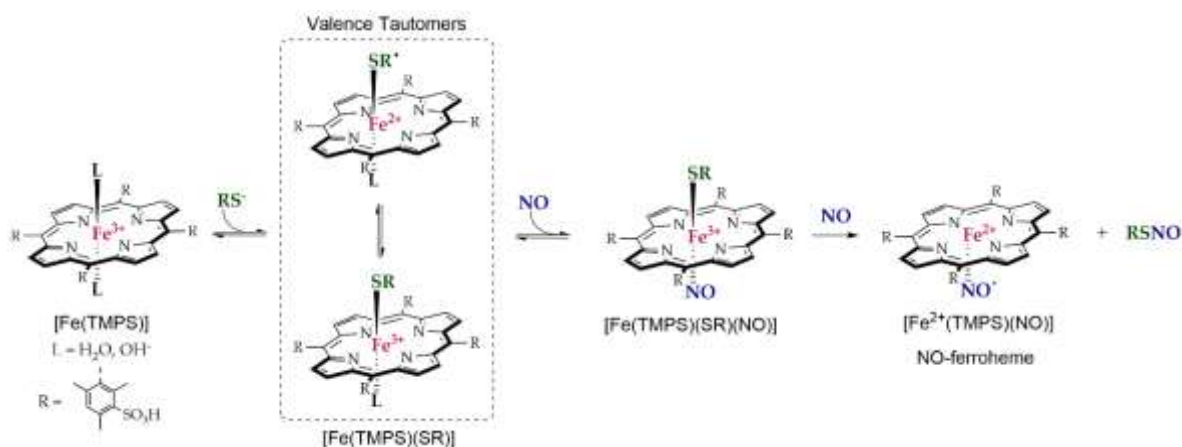


Figure 1. Proposed mechanism for TMPS-mediated NO-ferroheme and RSNO generation.

Acknowledgements

The study was supported by National Science Centre, Poland, under the grant 2019/35/B/ST4/04266. The author acknowledges financial support for participation in this conference from the Excellence Initiative - Research University (ID.UJ) programme at the Jagiellonian University.

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Copper(II) Complexation with Monensin A: Solution Studies Reveal a Unique Interaction

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As a natural polyether antibiotic, Monensin A features a quasi-cyclic structure formed by hydrogen bonding between its terminal ends. Structurally, it possesses a lipophilic outer surface due to externally oriented alkyl groups, enclosing a hydrophilic inner cavity. The chiral nature of the molecule allows its coordination properties in solution to be analyzed by circular dichroism (CD) spectroscopy. These data revealed that the metal-to-ligand ratio determines the formation of two specific complexes with divalent metal ions. A ligand excess leads to the neutral $[M(\text{Mon})_2(\text{H}_2\text{O})_2]$ biscomplex, whereas an excess of the metal ions promotes the formation of a positively charged monocomplex. Earlier studies detailed the UV-CD analysis of these species, alongside VIS-NIR CD investigations of the complexation with Co(II) and Ni(II) [1].

Here we investigated the distinct interactions of Cu(II) with monensinate A by with Cu(II) perchlorate and chloride in methanolic solution. Compared to other M(II) cations, Cu(II) exhibits unique complexation behavior. Spectral changes in reaction mixtures, with Cu(II) to Mon[−] ratios ranging from ~ 1:10 to 5:1, were followed by using VIS/NIR-CD, VIS absorption, and EPR spectroscopies. An additional complex compared to the other divalent metal ions, was found by matrix rank analysis of the data. ESI-MS was utilized to identify the formed complexes, and their conditional stability constants were calculated by the PSEQUAD program [2]. The supplementary nature of EPR and CD spectra suggested that a dinuclear Cu(II) complex is formed with monomer-like EPR properties.

Acknowledgements

This research work was supported by the bilateral cooperation project of the Czech and Hungarian Academy of Sciences (NKM2025-83) and by the Erasmus+ mobility program.

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Impact of natural alkaloids on amyloid- β induced cellular toxicity

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder for which no curative therapy is currently available. Among the therapeutic approaches in use, acetylcholinesterase (AChE) inhibitors represent a well-established strategy for the early stages of the disease. In this context, two natural alkaloids derived from plants of the *Amaryllidaceae* family, galantamine (GAL), approved by the FDA in 2001, and lycorine (LYC), have shown promising neuroprotective and anti-amyloidogenic properties.

The present study investigated the molecular interactions of GAL and LYC with the pathogenetic peptide A β 40, combining spectroscopic techniques (NMR, CD, UV-Vis) with cellular assays on differentiated SH-SY5Y cells, used as a neuronal model. NMR experiments (NOESY and TOCSY) demonstrated that both alkaloids directly interact with A β 40, causing a slowdown in the molecular motion of the alkaloids in solution and selective changes in the peptide resonances. CD spectra further revealed that the addition of GAL and LYC induces conformational changes in the secondary structure of both A β 40 and A β 42 isoforms, with LYC additionally causing a shift of the absorption minimum from 218 to 221 nm.

From an antioxidant standpoint, both alkaloids proved capable of delaying ascorbate oxidation in copper/ascorbate systems, reducing the production of reactive oxygen species (ROS) normally catalyzed by the Cu-A β complex.

Cellular results confirmed the protective ability of GAL and LYC against A β -induced cytotoxicity. Particularly noteworthy is the observation that LYC, even when used at significantly lower doses than GAL, exerts a comparable cytoprotective activity, consistent with the molecular data supporting its interaction with the amyloid peptide.

Taken together, these findings suggest that GAL and LYC may exert a multimodal neuroprotective effect, through inhibition of amyloid aggregation, modulation of A β secondary structure, and reduction of oxidative stress, representing promising candidates for the development of novel therapeutic strategies against Alzheimer's disease [1].

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Polyphenol-functionalized Au/Ag nanostructures as sustainable biomaterials for biomedical applications

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Bimetallic nanostructures (Au/Ag NPs) are promising platforms for nanomedicine due to versatile and tunable properties, along with their often-underestimated biological activity. In particular, their functionalization with naturally derived polyphenols provides a sustainable approach to designing biocompatible and bioactive materials, with potential to modulate cellular responses and support tissue regeneration [1-2].

This study focuses on the development of green synthetic protocols of the Au/Ag nanoparticles functionalized with polyphenolic compounds. Synthesis parameters were optimized using aqueous polymer-based systems and naturally derived reducing/stabilizing agents, in line with sustainable chemistry principles. Preliminary biological evaluation on an *in vivo* zebrafish larval model indicated low cytotoxicity within the tested concentration range, encouraging further investigation of these materials as candidate of potent biomaterials.

Future work will focus on more precise evaluation of the developed nanomaterials, particularly in the context of generated oxidative stress and their potential role in tissue regeneration.

Acknowledgements

The research was funded by the strategic program “Initiative of Excellence” at Jagiellonian University - Research Support Module (RSM) #5 (project no. U1U/W20/NS/28.06).

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Enzyme activatable bioorthogonal iminosydnone (ImSyd) platforms

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Bioorthogonal chemistry offers powerful tools for selective reactions in biological environments; however, precise control over reactivity remains a significant challenge. Iminosydnone (ImSyd) are emerging 1,3-dipoles that undergo rapid [3+2] cycloaddition with strained alkynes such as dibenzocyclooctyne (DBCO), affording pyrazole derivatives along with the release of an amine by-product [1]. Their reactivity critically depends on deprotonation of the exocyclic 6-NH, which enables dipole formation. Substitution at this position significantly suppresses cycloaddition, providing a convenient chemical switch to modulate reactivity.

Here, we develop enzyme-activated iminosydnone as a platform for triggered bioorthogonal chemistry. Our approach relies on the introduction of a nitroreductase (NTR)-cleavable protecting group at the 6-N position, generating a ‘caged’ (6N blocked) and unreactive state. Upon enzymatic reduction by NTR, the protecting group is removed, restoring the free 6-NH and enabling cycloaddition with DBCO.

This system is designed for selective activation under hypoxic conditions, where NTR activity is increased, enabling precise spatiotemporal control of bioorthogonal reactivity. Overall, this work establishes enzyme-responsive iminosydnone as promising tools for targeted activation and chemical transformations in complex biological systems.

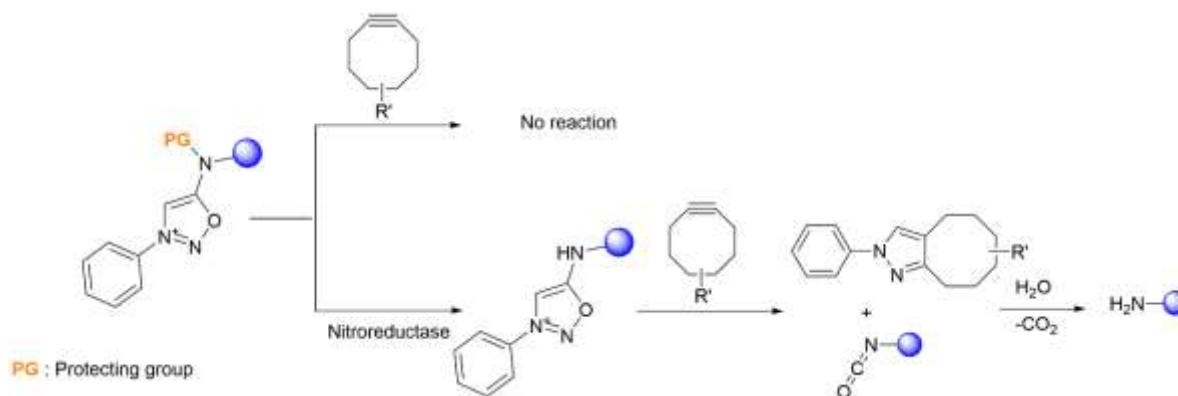


Figure 1. Representation of the ImSyd bioorthogonal reactivity

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Photodynamic antimicrobial chemotherapy, using porphyrinoids metalated to a ruthenium (II) complex for the treatment of antimicrobial resistant bacterial strains

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Porphyrinoids are a class of organic compounds that can serve as potential photosensitisers due to their strong visible absorption and tunable chemical properties, which can be further enhanced through metal coordination. This study explores the use of porphyrins in photodynamic antimicrobial chemotherapy (PACT), a light-activated strategy that generates reactive oxygen species to combat antimicrobial-resistant (AMR) bacteria [1-2]. We report the synthesis and characterisation of 9 *meso*-substituted porphyrins as precursors for ruthenium(II) complexes. The porphyrins were prepared via acid-catalysed condensation and purified by column chromatography [3]. Their structures and purity were confirmed using UV–Visible, FTIR, and NMR spectroscopy, yield obtained was 16-53%. These ligands were further metalated to form porphyrin-Ru (CO) complexes. Ongoing work focuses on the evaluation of their photodynamic antimicrobial activity against AMR bacterial strains [4+]. This study establishes a synthetic and structural foundation for the development of ruthenium–porphyrin photosensitisers as potential candidates for PACT applications.

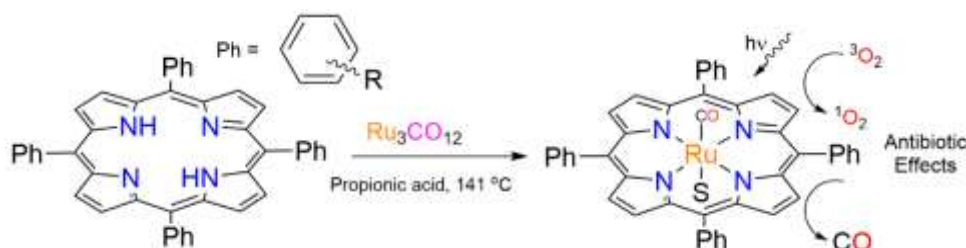


Figure 1: Metalation of a ruthenium carbonyl into a porphyrin molecule, and antibiotic photoproducts obtained from light excitation

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The interaction of NO-ferroheme and low-molecular-weight S-nitrosothiols with human serum albumin: Implications for nitric oxide signaling

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Nitric oxide (NO) is a crucial physiological mediator involved in the regulation of the cardiovascular, nervous, and immune systems. Its most recognized functions include vasodilation, modulation of neurotransmission and stimulation of angiogenesis. Due to the high reactivity and short half-life of free NO, its role as a direct activator of soluble guanylate cyclase (sGC) remains under discussion. Recent literature reports suggests that the nitrosylated heme complex (NO-ferroheme), rather than free NO, may be responsible for the efficient activation of sGC, thereby revising the classical concept of the NO–sGC–cGMP signaling pathway [1-2]. Another important component of indirect NO signaling is represented by S-nitrosothiols (RSNOs), such as S-nitrosoglutathione (GSNO) and S-nitrosocysteine (CysNO), which act as carriers and reservoirs of NO through transnitrosation mechanisms. RSNOs may also be formed in processes involving nitrosylated heme complexes [3-4]. Human serum albumin (HSA), the major plasma protein responsible for the transport of a wide range of ligands, plays an important role in heme scavenging and bioavailability of RSNOs, and NO. In this study, the physicochemical interactions of NO-ferroheme/heme, GSNO/GSH and CysNO/Cys with HSA were characterized with particular emphasis on their binding processes and their impact on protein properties. Fluorescence quenching of HSA's tryptophan, analyzed using the Stern–Volmer model, demonstrated strong heme binding and a markedly higher affinity of RSNOs relative to free thiols. Protein melting temperature measurements revealed enhanced thermal stability of HSA in the presence of both heme and NO-ferroheme, while TRIC analysis enabled the determination of the corresponding dissociation constants.

Acknowledgements

Research was funded by grant 2019/35/B/ST4/0426 under NCN project OPUS 18 and was carried out using equipment purchased under the ATOMIN project (grant no. POIG.02.01.00-12-023/08).

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Induced circular dichroism of thioflavin T in amyloid deposits rationalized by time-dependent density functional theory

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The combined induced circular dichroism (ICD) / time-dependent density functional theory (TDDFT) approach for structural identification has been successfully applied to find the conformation of ligands bound to native proteins [1]. Here, we show that this method can be extended to amyloid systems. Thioflavin T (ThT) entrapment in amyloid deposits leads to optically active conformers detectable by ICD via their monosignate or bisignate bands [2]. We apply B3LYP/6-311++G(d,p) calculations to identify the molecular form (monomer or dimer) responsible for the ICD of ThT in two amyloid deposit constituents, insulin fibrils and heparin. The spectral signature of the monomer is analysed with respect to the torsion angle θ between the molecular fragments. Dimer structures are generated, and the effects of the i) relative monomer orientation, ii) interchromophoric distance, and iii) degree of intramolecular rotation on the CD features are systematically documented. Comparison of the simulated and experimental spectra (Figure 1) reveals that insulin fibrils interact with monomeric ThT, while heparin binds two distinct ThT dimers. These results recommend the ICD/TDDFT method as a reliable basis for the interpretation of ICD signals of amyloid dyes.

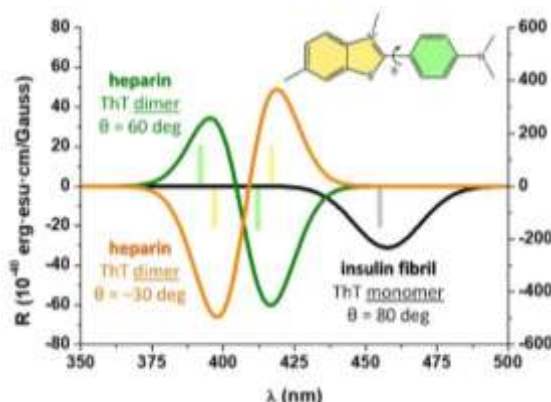


Figure 1. Calculated vs. experimental (as bars) CD of ThT bound to insulin and heparin.

Acknowledgements

This work was supported by the Ministry of Research, Innovation and Digitization, CNCS-UEFISCDI, contract number 93TE/3.01.2025, project code PN-IV-P2-2.1-TE-2023-0189, within PNCDI IV.

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Revised EFSA Guidance for Novel food applications: Focus on product characterization

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The European Commission requested EFSA to update the scientific Guidance for the preparation of applications for the authorisation of novel foods, previously developed following the adoption of Regulation (EU) 2015/2283 on novel foods [1]. This Guidance document [2] provides advice on the scientific information needed to be submitted by applicants / interested business operators towards demonstrating the safety of the novel food.

Requirements pertain to the description of the novel food, production process, compositional data, specifications, proposed uses and use levels, and anticipated intake of the novel food. Furthermore, the Guidance indicates the information needed to assess the history of use of the novel food and/or its source, absorption, distribution, metabolism, excretion, toxicological information, nutritional information, and allergenicity.

The applicants should integrate and interpret the data presented in the different sections to provide their overall considerations on how the information supports the safety of the novel food under the proposed conditions of use. Where potential safety hazards were identified, they are to be disclosed and discussed in relation to the anticipated intake of the novel food and the proposed target populations. Based on the information provided, EFSA assesses the safety of the novel food under the proposed conditions of use.

The updated guidance reflects:

- The latest EU regulatory changes,
- Advancements in food research and technologies,
- EFSA's knowhow,
- Methodological innovation and new digital tools,
- Feedback from academia, consumers groups, authorities, and industry

The main scope of the Guidance is assisting the relevant stakeholders in the preparation of the NF applications.

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The anticancer activity, molecular docking and structural characterization of copper(II) coumarin–pyridine complexes

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Coumarin-based compounds and their copper(II) complexes have raised considerable interest as potential anticancer agents [1]. Copper(II) coordination may enhance the biological activity of coumarin derivatives through mechanisms including DNA interaction, ROS generation, and apoptosis induction, while their metal-chelating properties support the formation of complexes with improved antiproliferative activity [2–4].

In this study, novel coumarin derivatives bearing pyridine substituents and their corresponding copper(II) complexes were synthesized and evaluated for anticancer activity against five human cancer cell lines. The breast cancer panel included hormone receptor–positive MCF-7 cells and triple-negative breast cancer cell lines MDA-MB-231 and HCC38, as well as two endometrial carcinoma cell lines, Ishikawa and Hec-1A. Structural characterization of the synthesized compounds was performed using crystallographic X-ray analysis, and molecular docking studies were conducted to investigate potential molecular mechanisms and ligand–target interactions.

Acknowledgements

This work was supported by Grant No. 503/3-066-02/503-31-001 from the Medical University of Lodz, Poland.

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Combining the Spy System and Mimochromes to develop a novel class of Biocatalysts

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Mimochromes are miniaturized metalloproteins based on a deuteroporphyrin IX scaffold flanked by two α -helices, designed to mimic the structural environment of heme in natural hemoproteins. These systems are able to reproduce the key interactions required for metalloporphyrins to perform distinct functions depending on the coordinated metal. In particular, Fe-MC6*a has been shown to exhibit significant peroxidase activity [1]. The SpyTag/SpyCatcher system can be used as protein conjugation platform, based on the spontaneous formation of a covalent isopeptide bond between a short peptide (SpyTag) and its protein partner (SpyCatcher), ensuring high specificity and stability [2].

In this project, Fe-MC6*a is conjugated to the SpyTag through a SPAAC click reaction to obtain a catalytic system able to bind SpyCatcher, allowing its immobilization on the bacterial surface. Spectrophotometric kinetic studies of peroxidation have been carried out on the Spy-conjugated system and will also be performed on the bacterium-anchored system, in order to evaluate how immobilization affects the catalytic activity.

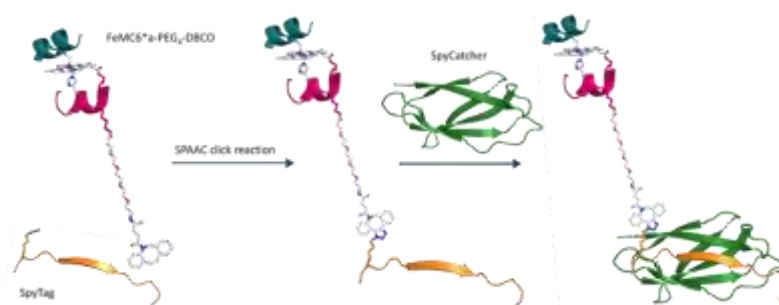


Figure 1. Schematic representation of the SC/ST-FeMC6*a complex formation.

Acknowledgements

Project “Artificial enzymes for the photocatalytic production of hydrogen in photosynthetic bacteria” National Recovery and Resilience Plan (NRRP), M2 C2 Inv. 3.5 funded by the European Union – NextGenerationEU. Project RSH2A_000009, C.D. 445 29/12/2022 Italian Ministry of Environment and Energy Security.

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Structural study on a new class of HIV-1 protease inhibitors

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HIV-1 protease (PR) is an essential enzyme in viral maturation and a pharmacological target for antiretroviral therapy; however, resistance-associated mutations can significantly reduce the efficacy of clinically available protease inhibitors [1-4].

The development of novel inhibitors capable of retaining activity against drug-resistant PR variants therefore remains a priority.

We tested a library of newly designed compounds, developed at the University of Basilicata, as inhibitors of HIV-1 PR using enzymatic inhibition assays on the wild-type (WT) enzyme and on three clinically relevant mutant variants (V32I, V82A, V82S). Inhibition experiments performed with a fluorescence-based FRET assay revealed that several compounds effectively inhibit PR activity. In particular, two compounds displayed inhibitory potencies comparable to, or exceeding, that of the reference drug darunavir, a clinically approved protease inhibitor. The lead compound exhibited sub-nanomolar IC₅₀ values against the WT enzyme and against the V82A and V82S mutants, while reduced potency was observed for the V32I variant, indicating a mutation-dependent effect on inhibitor efficacy. Other compounds in the library showed weaker or negligible inhibitory activity.

To investigate the mechanism of inhibition, we employed experimental and computational approaches. X-ray crystallography revealed isomorphous PR-inhibitor complexes (space group P2₁2₁2), showing a conserved binding mode characterised by strong interactions with the catalytic aspartates [5] and an extensive network of hydrophobic contacts. Molecular docking was used to model complexes not resolved crystallographically. Docking reproduced the overall placement of the inhibitor within the active site; however, differences in specific interaction patterns were observed, with the ligand adopting an extended conformation rotated by approximately 180° relative to the crystallographic pose.

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^{7D}Liraglutide: proteolytic stability and metal ion coordination

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Glucagon like peptide-1 (GLP-1) is an peptide hormone indirectly responsible of blood glucose level control. GLP-1 and GIP are accountable of incretin effect, which lack is first symptom of type 2 diabetes mellitus [1-2].

The newly designed ^{7D}liraglutide is an answer to fast digestion of GLP-1 and its analogues by proteolytic enzymes – neprilysin (NEP) and dipeptide peptidase IV (DPP 4) [3-4].

The applied enantiomeric exchange of amino acids made ^{7D}liraglutide resistant to proteolysis significantly increasing its half-life. Furthermore, we analysed coordination properties of GLP-1, liraglutide and ^{7D}liraglutide (Fig. 1) with Zn(II) (which high concentrations are present in pancreas [5]) and Cu(II) ions.

All peptides have an α -helical structure which is unbothered by metal ion addition. Both Zn(II) and Cu(II) bind to the studied peptides in a histamine-like binding mode.



Figure 1. Sequences (i) of GLP-1 with sites susceptible to enzymatic cleavage highlighted, and amino acids critical for the activity (ii) liraglutide; (iii) ^{7D}liraglutide with amino acids that have undergone enantiomeric exchange highlighted.

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The project is funded with the support of the National Science Centre (2023/51/ST5/01098).

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Integrating *in silico* and biophysical approaches to characterize protein-ligand binding

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Protein–ligand interactions underlie nearly all cellular processes. The strength of these interactions, commonly expressed by the dissociation constant (K_D), provides key insight into molecular recognition and is essential for the rational design of biologically active compounds [1]. Recent advancements in biophysical instrumentation have broadened the experimental toolkit with the introduction of techniques such as Nano Differential Scanning Fluorimetry (nanoDSF) and Temperature-Related Intensity Change (TRIC). These technologies, available in NanoTemper instrumentation, have emerged as sensitive and versatile tools for high throughput screening and affinity ranking under close to native conditions [2]. Integrating such biophysical techniques with computational approaches, such as molecular docking, enables a multiscale understanding of binding mechanisms. In this presentation, we illustrate the value of combining these methods to interrogate protein–ligand interactions across several systems.

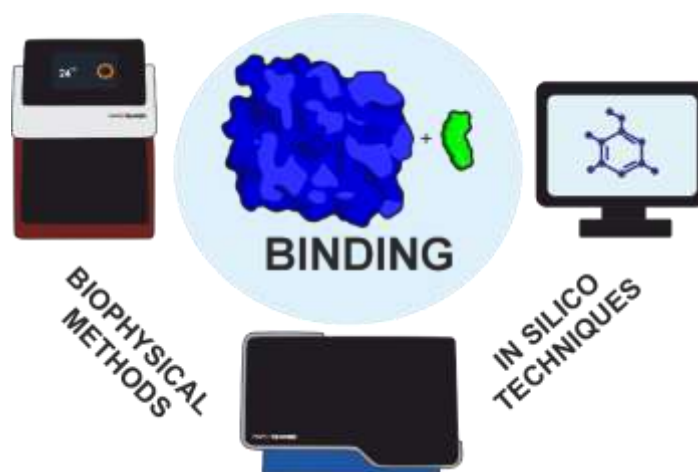


Figure 1. Protein-ligand binding studied by biophysical and *in silico* methods.

Acknowledgements

This work was co-financed by funds from the state budget, granted by the Ministry of Science and Higher Education (Poland), within the programme entitled “Perły Nauki II” (PN/02/0143/2023).

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Modulatory effect of resveratrol on amyloid-based albumin hydrogel

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Amyloid-based hydrogels represent a significant class of functional biomaterials used for a variety of applications, including controlled drug delivery and tissue engineering [1-2]. The study evaluates the influence of resveratrol (RESV), a polyphenolic compound, on bovine serum albumin (BSA) thermal stability using calorimetric and spectroscopic methods. The results are further used to prepare two amyloid-based albumin hydrogels noted by BSA025+RSV015 and BSA050+RSV025.

Differential scanning calorimetry results show a concentration-dependent effect of RESV on protein thermal stability. Circular dichroism data indicate slight stabilization of the protein α -helix structure under native conditions, but increased β -sheet content upon thermal incubation. The red shift observed in Congo Red absorbance assay confirms the formation of β -sheet-rich aggregates organized into fibrillar structures. Thioflavin T assay reveals temperature-dependent fibrillation without a lag phase, with RESV slowing aggregation kinetics but not inhibiting fibril formation. Dynamic light scattering measurements support a multi-pathway aggregation mechanism, showing the transition from small oligomers to larger aggregates upon thermal treatment; RESV influences aggregate size distribution. The rheological measurements on the amyloid-based BSA hydrogels show that both systems exhibit gel-like behavior with dominant elasticity and shear-thinning flow, while BSA025+RSV015 displays the highest viscoelastic moduli and viscosity, indicating optimal synergistic interactions for a strong and stable gel network. Furthermore, the diffusion-controlled release behavior of these hydrogels highlights their potential for applications in controlled delivery systems.

Acknowledgements

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS-UEFISCDI, contract number 93TE/03.01.2025, project code PN-IV-P2-2.1-TE-2023-0189, within PNCDI IV.

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Synthesis and biological evaluation of tridentate azido-ligand rhenium (I) complexes

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The increasing emergence of antimicrobial resistance and the reduced efficacy of conventional antibiotics have created an urgent need for new metal-based therapeutic strategies [1]. Due to their favorable photophysical properties and biological potential, rhenium(I) tricarbonyl complexes have emerged as promising candidates for the development of novel antimicrobial and antiviral agents [2]. In this study, a tridentate azido-functionalized ligand was synthesized via copper-catalyzed azide-alkyne cycloaddition (CuAAC) between zidovudine and a terminal alkyne. Then, the ligand coordinated to the fac-[Re(CO)₃] core to obtain the corresponding rhenium(I) complex. This synthetic approach is based on methodologies previously reported for rhenium bisquinoline tricarbonyl complexes and 1,2,3-triazole-functionalized thymidine derivatives obtained through CuAAC cycloaddition reactions [2-3]. The resulting complexes were characterized using ¹H NMR, FTIR, and MS analyses, which confirmed successful ligand coordination and complex formation. Preliminary docking studies suggest that this class of rhenium(I) complexes may represent promising MurG targeting agents with potential antimicrobial activity.

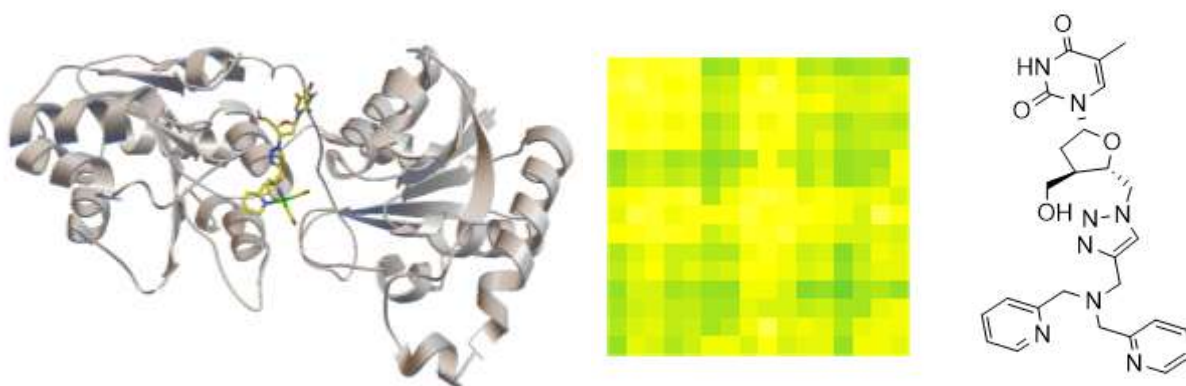


Figure 1. Left: calculated pose of Re complex in MurG enzyme ions; Right: structure of the investigated ligand and heat map of different derivatives of the same where the green regions in the heat map correspond to areas of high docking score the with MurG enzymes.

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Self-assembled nanogels based on modified chitosan and sericin as versatile platforms for potential medical applications

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Nanogels based on natural polymers are becoming increasingly popular for biomedical applications due to their biocompatibility and potential in drug delivery applications [1-3]. In this study, self-assembled nanogels based on maleoyl chitosan (MAC) and sericin (Ser) were developed and investigated as potential carriers for amoxicillin (Amox), an antibiotic used to treat skin infections. This work aims to evaluate the influence of polymer concentration on nanogel formation, as well as their suitability for loading with Amox. Nanogels were prepared at different concentrations and with varying ratios of the compounds, and were preliminarily characterised in terms of particle size and distribution using dynamic light scattering (DLS). Preliminary DLS results suggests the formation of nanoscale structures with relatively homogeneous size distributions, while variations dependent on concentration suggest changes in intermolecular interactions within the network. Other investigations focused on structural and morphological characterization (Fourier Transform Infrared (FTIR) spectroscopy, Scanning transmission electron microscopy (STEM)), as well as on the loading and release of Amox. These studies aim to assess the potential of the developed chitosan/sericin-based nanogels as versatile platforms for biomedical applications.

Acknowledgements

This research is carried out and financed within the framework of the second Swiss Contribution MAPS (project code: F-RO-CH-2024-0081).

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Flip the Peptide, Boost the Power: Metal-Driven Stability and Activity in Salivary AMPs Rich in Prolines

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Antimicrobial peptides (AMPs), derived from salivary proteins, are crucial elements of innate immunity, offering comprehensive protection against bacteria and fungi. We investigate the role of metal ion coordination and enantiomeric exchange in modulating the stability and antimicrobial activity of salivary proline-rich peptides, key components of innate immunity. Metal ions such as Cu(II) and Zn(II) can both limit microbial access to essential metals and enhance peptide activity by altering their charge and conformation, although natural peptides are prone to rapid proteolytic degradation [1-2]. Here, we examine mucin-7 fragment alongside designed peptidomimetics, including *retro* and *retro-inverso* analogues, as a strategy to improve their stability and biological efficacy (Figure 1).

Enantiomeric modification significantly enhances resistance to proteolytic degradation, thereby improving the overall stability of the peptides. These findings underscore the value of stereochemical and sequence inversion approaches as effective tools for modulating peptide function and durability in biologically relevant environments.

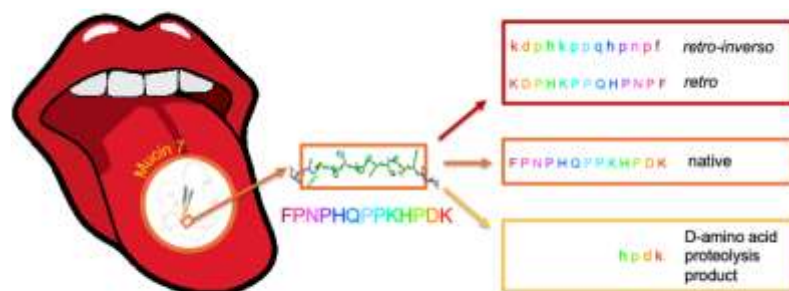


Figure 1. Native fragment of salivary antimicrobial peptide and corresponding peptidomimetics.

Acknowledgements

We thank the support of the National Science Centre (Grant UMO-2021/41/B/ST4/02654 to J.W.)

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Turning zinc against metalloproteinases: design of substrate-mimicking peptide inhibitors

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Metalloproteinases are metal-dependent enzymes that hydrolyze peptide bonds. They are expressed in both bacteria (MPs), where they contribute to virulence, and in humans (MMPs), where they play a key role in cancer progression [1-2]. Among them, MMP-1 is a Zn(II)-dependent enzyme responsible for extracellular matrix (ECM) degradation -

a process essential for tumor growth, migration, and invasion. Due to their elevated expression in many cancers, MMPs are considered important therapeutic targets [2-3].

This study focuses on the interaction between the MMP-1 catalytic domain, Zn(II) and designed substrate-mimicking peptide inhibitors: H-CPQGLRG-OH (Inh-1), H-PQGLRGC-OH (Inh-2). Potentiometric titration allowed us to obtain the stability constants for the binary and ternary complexes and to determine the distribution of their speciation as a function of pH. Complex stoichiometry was confirmed by mass spectrometry, while UV-Vis spectroscopy assessed catalytic properties.

The MMP-1 peptide model reproduces the native Zn(II) coordination environment, consisting of three histidine residues and one water molecule, as in the enzyme's catalytic site. Despite minor structural differences (position of cysteine residue), the inhibitors exhibit distinct coordination behavior. Inh-1, with cysteine at the N-terminus, forms a stronger binary complex with Zn(II) than Inh-2, which has cysteine located at the C-terminus. Moreover, ternary complexes are more stable than binary systems, with the highest stability observed for the MMP-1-Zn(II)-Inh-1 complex.

These results suggest that Inh-1 can effectively block substrate access to the catalytic Zn(II) ion, thereby inhibiting MMP-1 activity. Overall, this study provides detailed thermodynamic and structural insight into Zn(II)-mediated interactions in MMP-1 systems and highlights the importance of cysteine positioning in the rational design of selective peptide inhibitors with potential anticancer applications.

Acknowledgements

Financial support by the National Science Centre, Poland (UMO-2023/50/E/ST5/00434) is gratefully acknowledged.

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