XV International Symposium on Inorganic Biochemistry

New Arrivals on Stage

Book of Abstracts

10-13 September 2025
Wrocław, Poland

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XV International Symposium on Inorganic Biochemistry
Book of Abstracts

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Bartosz Orzeł
Julianna Wojdyła-Parat

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Bartosz Orzeł

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2025 Wrocław

Dear Friends and Colleagues,

It is our great pleasure to welcome you to Wrocław for the XV International Symposium on Inorganic Biochemistry (ISIB). The 2025 edition proudly continues the tradition of the previous symposia, which began 40 years ago in Karpacz, thanks to the vision and efforts of Professor Henryk Kozłowski.

Over the years, the fourteen preceding meetings have been a remarkable success, bringing together leading researchers from around the world. They have focused on key areas at the intersection of inorganic, coordination, and bioinorganic chemistry with biology and medicine. Among the central topics have been chemical structure and thermodynamics, solution equilibria and metal–biomolecule interactions, transport, homeostasis and toxicity of metals in disease, as well as the development of metal-based therapies and diagnostics.

This year's symposium continues this mission, offering a forum for valuable discussions on recent advances in these areas. Alongside plenary and invited lectures, participants will have the opportunity to share their work through flash presentations and poster session. We believe that the scale of this year's meeting—around 80 participants, reflecting the 80th birthday of Professor Henryk Kozłowski—will encourage informal and stimulating exchanges between experienced scientists and younger colleagues. Promoting new collaborations among researchers with complementary expertise and goals is one of our key ambitions.

This year, under the subtitle "New Arrivals on Stage", we are delighted to welcome a number of first-time speakers to the ISIB community, while also expressing our gratitude to our long-standing friends whose continuous support has sustained the symposium for many years.

We are truly happy to host you in Wrocław.
Let us enjoy the science together!

The Organizers

Elżbieta Gumienna-Kontecka – Chair
Sławomir Potocki – Conference Secretary
Małgorzata Ostrowska
Kamila Stokowa-Sołtys
Joanna Wątły
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The conference is held under the honorary patronage of the Rector of the University of Wrocław and the Mayor of Wrocław.

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ISIB XV PROGRAMME

Wednesday, 10th September **Main Building of the University of Wroclaw**,1 University Square	
15:00-17:00	**Registration**
Session I	Chair – **GUMIENNA-KONTECKA Elżbieta**
17:00 – 17:30	**Opening Ceremony** Aula Leopoldina, Main Building of the University of Wroclaw
17:30 – 18:10	**PL1** My Bioinorganic Secrets ***KOZŁOWSKI Henryk**, University of Opole, Opole, Poland*
18:10 – 18:50	**PL2** Metal-Organic non-Trivial Structures: Synthesis and Applications ***TRABOLSI Ali**, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates*
19:00	**Welcome Reception** Oratorium Marianum, Main Building of the University of Wroclaw
Thursday, 11th September **Main Library of the University of Wroclaw**, 12 Joliot-Curie Street	
Session II	Chair – **ROWIŃSKA-ŻYREK Magdalena**
9:00-9:40	**PL 3** Metal Ions in Infectious Diseases: Bridging Pharmacy and Chemistry in the Fight Against Infectious Diseases ***CARVER Peg**, University of Michigan, Ann Arbor, USA*
9:40-10:00	**IL 1** Metal Ions in Microbial Survival and Viral Entry: Coordination Chemistry, Mechanisms, and Therapeutic Opportunities ***PEANA Massimiliano**, University of Sassari, Sassari, Italy*
10:00-10:20	**IL 2** Ferritins: Nanocages for Iron Storage/Detoxification ***LE BRUN Nick**, University of East Anglia, Norwich, United Kingdom*
10:20-10:40	**IL 3** Superoxide Dismutases as Model Systems for Studying the Evolution of Metal Preference of Metalloenzymes ***WALDRON Kevin**, Polish Academy of Sciences, Warsaw, Poland*
10:40-11:00	**IL 4** Chemical Speciation Studies in, and of, Biological Fluids: Critical Aspects in the Evaluation of Chelants Performances ***MILEA Demetrio**, University of Messina, Messina, Italy*
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Session III	Chair – **VALENSIN Daniela**
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11:50-12:10	**IL 6** NMR Spectroscopy and Complex Formation - What Can We Observe ***NOWAKOWSKI Michał**, University of Warsaw, Warsaw, Poland*
12:10-12:25	***OC 1** NMR Studies of Multidomain Snail Metallothioneins: Metalation and Metal Selectivity* ***ZERBE Olivier**, University of Zurich, Zurich, Switzerland*
12:25-12:40	**OC 2** Clavanin C Analogue Complexes with Cu^{2+} and Zn^{2+}: A Strategy Against MRSA ***GAWŁOWSKI Jakub**, University of Wroclaw, Wroclaw, Poland*
12:40-12:55	**OC 3** Microplusin: a Strong Copper-chelating and Effective Antimicrobial, Natural Peptide ***LEVERARO Silvia**, University of Ferrara, Ferrara, Italy*
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Session IV	Chair – **RODZIEWICZ-MOTOWIDŁO Sylwia**
14:30-15:10	**PL 4** Siderophores, Transporters, and Survival: The Iron Playbook of *P. aeruginosa* ***SCHALK Isabelle**, University of Strasbourg, Illkirch, France*
15:10-15:40	**KL 1** Fungal Siderophore Uptake and Its Translational Potential ***HAAS Hubertus**, Medical University Innsbruck, Innsbruck, Austria*
15:40-16:10	**KL 2** Siderophores as Scaffold for Molecular Imaging applications ***DECRISTOFORO Clemens**, Medical University Innsbruck, Innsbruck, Austria*
16:10-16:30	**IL 7** Radiolabelled Siderophores for Imaging Bacterial Infections ***PETRIK Milos**, Palacky University, Olomouc, Czech Republic*
16:30-16:45	**OC 4** Targeting Bacterial Metalloproteinases: Peptide-Based Strategies for Inhibiting Virulence Factors ***ZAWADA Martyna**, University of Wroclaw, Wroclaw, Poland*
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17:30-17:50	**IL 8** Gaining Insight into Mn(II) and Fe(II) Binding: From Model Systems to Transporter Fragments ***OSTROWSKA Małgorzata**, University of Wroclaw, Wroclaw, Poland*
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18:10-18:30	**IL10** Metalloenzyme Engineering with Non-canonical Amino Acid Incorporation ***DELIZ LIANG Alexandria**, University of Zurich, Zurich, Switzerland*
18:30-18:50	**IL 11** Designing Artificial Proteins and Metalloproteins using the Spy Construct ***TEGONI Matteo**, University of Parma, Parma, Italy*
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Session VI	Chair – **PEANA Massimiliano**
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9:40-10:00	**IL 12** PACT4EYE: Innovative Photoactivated Ruthenium Chemotherapy to Treat Eye Cancer ***BONNET Sylvestre**, Leiden University, Leiden, Netherlands*
10:00-10:20	**IL 13** Metalloporphyrinoids for Phototheranostics: From Molecular Design to Targeted Imaging and Therapy in Advanced Biological Models ***DĄBROWSKI Janusz**, Jagiellonian University in Krakow, Krakow, Poland*
10:20-10:40	**IL 14** Engineered Hemoproteins for Selective C–N and C–O Bond Formation via Abiological Mechanisms ***FASAN Rudi**, University of Texas at Dallas, Richardson, USA*
10:40-11:00	**IL 15** Chemical Biology for the Detection of Pathogens and Reactive Oxygen Species ***VAUZEILLES Boris**, Paris-Saclay University, Gif-sur-Yvette, France*
11:00-11:30	Coffee break

Session VII	Chair – **BARBA-BEHRENS Norah**
11:30-12:00	**KL 3** Re Compounds as Coordinate Covalent Inhibitors Enzymatic Activity ***COHEN Seth**, University of California San Diego, La Jolla, USA*
12:00-12:15	**OC 6** Targeting the Zinc Active Sites of MMP-1 and MMP-14: Binding Characteristics of Novel Peptide Inhibitors ***POTOK Paulina**, University of Wroclaw, Wroclaw, Poland*
12:15-12:30	**OC 7** Commercial Bisphosphonate Drugs for Targeting Pt(II)-complexes to Bone Tumors and Metastases ***BARBANENTE Alessandra**, University of Bari Aldo Moro, Bari, Italy*
12:30-13:00	**KL 4** Metalloproteins in the Immune Response and Medicine ***EBRAHIMI Kourosh**, King's College London, London, United Kingdom*
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14:50-15:05	**OC 8** Diversity of Aβ Isoforms: Toward Novel Biomarkers for Diagnosis of Alzheimer's Disease ***WEZYNFELD Nina**, Warsaw University of Technology, Warsaw, Poland*
15:05-15:20	**OC 9** Metalloporphyrinoids in Neurodegeneration: NMR-Based Interaction and Protective Effects on SH-SY5Y Cells ***KOLA Arian**, University of Siena, Siena, Italy*
15:20-15:35	**OC 10** Development of Mn(II)-Based MRI Probes with Improved Physicochemical Properties for Liver Targeting ***TIRCSÓ Gyula**, University of Debrecen, Debrecen, Hungary*
15:35-15:50	**OC 11** Structural Insights into Metal Ion Coordination by Aryloxazoline and Arylthiazoline Siderophores and Their Analogues ***SZCZERBA Klaudia**, University of Wroclaw, Wroclaw, Poland*
16:00-17:00	Flash presentations
17:00-18:30	Coffee break & Poster Session

Saturday, 13th September
Main Library of the University of Wroclaw, 12 Joliot-Curie Street

Session IX	Chair – **REMELLI Maurizio**
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10:20-10:40	**IL 18** Metalloproteome Remodeling in Calprotectin-stressed *Acinetobacter Baumannii* using Chemoproteomics ***GIEDROC David**, Indiana University, Bloomington, USA*
10:40-11:00	**IL 19** Selective Recognition of As(III) and Sb(III) by AfArsR, a Transcriptional Metalloid Regulatory Protein ***JANCSÓ Attila**, University of Szeged, Szeged, Hungary*
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12:00-12:15	**OC 12** Antioxidant Mimetic Activity of 1H-Pyrazole Metal Complexes Modulated by Polyamine Chain Length in [1 + 1] Condensation Azamacrocycles ***BONASTRE-SABATER Irene**, University of Valencia, Valencia, Spain*
12:15-12:30	**OC 13** Biologically Active Tinidazole-derived Metal Complexes: From Design to Elucidation of the Mechanism of Action ***CASTRO-RAMIREZ Rodrigo**, National Autonomous University of Mexico, Mexico City, Mexico*
12:30-12:45	**OC 14** Copper(II) Complexes of Pyridine-carboxamide Based Histidine Conjugates ***LIHI Norbert**, University of Debrecen, Debrecen, Hungary*
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14:45-15:00	**OC 17** Influence of Amino Acid Sequence in Calmodulin Binding Site on Plutonium ***FAUX Emilien**, French Alternative Energies and Atomic Energy Commission, Bagnols-sur-Cèze, France*
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15:35-16:00	Awards & Conference Closing
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FP2	Assessing the Antitumor Activity of Dinuclear Platinum–bisphosphonate Agents for Selective Treatment of Bone Metastases by *in vitro* and *ex vivo* Models	**DI COSOLA** Anna Maria
FP3	Dual Amino Acid Substitution in a MUC7-Derived Peptide Enhances Antimicrobial Resistance and Modulates Zn(II)/Cu(II) Binding Stability	**KŁOPOTOWSKA** Klaudia
FP4	Insights Into the Coordination Chemistry of Bacterial Iron Transporters	**ORZEŁ** Bartosz
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FP6	Anderson POM Hybrids with Cr and Co: A Platform for Thermoemissive and Electrofunctional Materials	**PRĘTKA** Dominika
FP7	Insights Into the Formation of Complexes in the Cu(II)/Tyrosine and Cu(II)/Tyrosine/ATP systems	**SADOWSKA** Patrycja
FP8	Study of the Catalytic Behavior of Copper(II) Complexes with Potential Catechol Oxidase Activity	**SIMON** Fruzsina
FP9	Peptidomimetics Based on MUC7 Fragment From Human Saliva: Comparison of Coordination, Structure, Stability and Antimicrobial Activity	**SZARSZOŃ** Klaudia
FP10	Enhancing Antimicrobial Activity of Salivary Proline-rich Peptides via Metal Coordination and D-Amino Acid Substitution	**ŚLUSARCZYK** Anna

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P2	Topical Application of Iron Chelators to Reduce Inflammation and Promote Chronic Wound Healing	**BUDA** Weronika
P3	Exploring the Metal Specificity of the Bacterial Mn(II) Transporters	**CHARKOT** Jakub
P4	Characteristics of Zn2+ and Cu2+ Complexes of the Active Site of M4 Family Metallopeptidase and its Inhibitors	**GŁADYSZ** Klaudia
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P6	Dual Amino Acid Substitution in a MUC7-Derived Peptide Enhances Antimicrobial Resistance and Modulates Zn(II)/Cu(II) Binding Stability	**KACHNOWICZ** Jan
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P8	Coordination Properties of a Peptidic Fragment of the Bacterial Metal Ion Transporter IroT	**OLEŚNIEWICZ** Nina
P9	New Triorganotin(IV) Syringates as Potential Antitumoral Agent: From Chemical Synthesis to Biochemical Effects	**PELLERITO** Claudia
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PLENARY LECTURES

PL 1

My Bioinorganic Secrets

Gabriela POTOCZNIAK, [a] Kinga GARSTKA-LITWIN, [a] Aleksandra HECEL, [a] Elżbieta GUMIENNA-KONTECKA, [a] Arian KOLA, [b] Daniela VALENSIN, [b] Magdalena ROWIŃSKA-ŻYREK, [c] Henryk KOZŁOWSKI, [a], [c]

[a] Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland
[b] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via A. Moro 2, 53100 Siena, Italy
[c] Institute of Health Sciences, University of Opole, 68 Katowicka Street, 45-061 Opole, Poland; henryk.kozlowski@uwr.edu.pl

The rising threat of antimicrobial resistance makes it urgent to develop therapies that hit pathogen-specific pathways. We study how pathogens acquire and transport metal ions and use these insights to strengthen host nutritional immunity. Focusing on Fe(III), Cu(II), Zn(II), and Ni(II), we carried out coordinated thermodynamic, structural, and microbiological studies of metal-metallophore complexes and their interactions with transport proteins, defining the metal-binding behavior of natural and biomimetic metallophores and the factors that determine their stability, selectivity, and efficiency. [1, 2] We show that some metallophores form very stable complexes with their target metal ions, often outperforming host systems that sequester metals. We also identify distinct interaction patterns between metallophore–metal complexes and microbial transporters, revealing molecular features that drive selective recognition. Microbiological tests confirmed that blocking metallophore-mediated metal uptake weakens pathogen growth and virulence. [3] Taken together, these results highlight metal transport as a strong, still underused antimicrobial target and point toward pathogen-specific imaging and therapeutic tools, offering fresh avenues against antimicrobial resistance. [4]

References:

[1] Garstka, K.; Hecel, A.; Kozłowski, H.; Rowińska-Żyrek, M., Specific Zn(II)-binding site in the C-terminus of Aspf2, a zincophore from *Aspergillus fumigatus*. *Metallomics*, **2022**, 14, mfac042

[2] Garstka, K.; Potoczniak, G.; H. Kozłowski, M. Rowińska-Żyrek, Aspergillus fumigatus ZrfC Zn(II) transporter scavengers zincophore-bound Zn(II). *Dalton Transactions*, **2024**, 53, 2848-2858

[3] Mular, A,; Piasta, K.; Jedyńczuk, A.; Kamińska, K.; Olshvang, E.; Metzler Nolte, N., Wojaczyńska, E.; Kozłowski, H.; Gumienna-Kontecka, E., The diversity and utility of arylthiazoline and aryloxazoline siderophores: Challenges of coordination chemistry, biological activity and selected applications. *Coordination Chemistry Reviews,* **2024**, 501, 215551/1-215551/40

[4] Garstka, K.; Hecel, A.; Kozłowski, H.; Domínguez-Martín, A. Szewczyk, K.; Rowińska-Żyrek, M.; AdcA lipoprotein involved in Zn(ii) transport in Streptococcus mutans – is it as metal-specific as expected? *Dalton Transactions*, **2025**, 1-12

PL 2

Metal-Organic non-Trivial Structures: Synthesis and Applications

Ali Trabolsi, [a)]

[a)] *Science Division, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates; Ali.trabolsi@nyu.edu*

Metal-organic complexes have always been attractive candidates for medicinal chemistry. However, synthetic metal-organic non-trivial structures such as knots and links[1] have never been considered for biological applications. In 2019, the Trabolsi group reported an unprecedented study using a set of five metal-organic trefoil knots (M-TK, M = Zn^{2+}, Cu^{2+}, Cd^{2+}, Fe^{2+} and Mn^{2+}) as efficient chemotherapeutics. The unique structural features of these 3D structures including multiple metal ions and dynamic bonds, yielded greater efficacy than cisplatin at lower doses which could potentially diminish chemotherapy side effects and increase patient comfort.[2]

In order to develop a structure-activity relationship, we extended our work to a library of homologous metal-organic non-trivial structures with different molecular topologies and number of metal centers (two to four) using optimized synthetic procedures. These structures are: [2]catenanes and Solomon links.[1] Prior to that, we found that the trefoil knots can host a variety of mono-charged anions in its cavity, in solution and the solid state. Such host-guest associations enhance the thermal stability of the trefoil knot in solution.[3] Additionally, the knots can be used to catalyze the hydrolysis of C-Br bonds.[4] Finally, the zinc complexes were proven to inhibit *in vitro* aggregation of β2-microglobulin protein responsible for systemic amyloidosis, commonly involved in pathologies such as Alzheimer's, Parkinson's diseases, and diabetes type II.

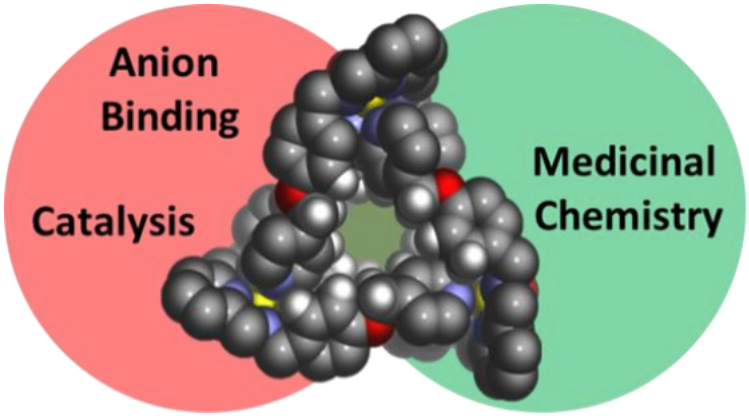

Figure 1. Single crystal structure of a metal-organic knot and an overview of their applications.

References:

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PL 3

Metal Ions in Infectious Diseases: Bridging Pharmacy and Chemistry in the Fight Against Infectious Diseases

Peg CARVER, [a)]

[a)] *University of Michigan College of Pharmacy; peg@umich.edu*

Trace elements (TEs) are essential for both humans and pathogens. Although human metal ion homeostasis is tightly regulated, pathogens have evolved strategies to evade TE sequestration. Pathogens use mechanisms like siderophore biosynthesis and high-affinity metal-ion transporters to sense, acquire, store, and export metal ions. Significant correlations exist between levels of iron, selenium, and zinc in plasma, serum, or tissues and the prevention or treatment of infections, while fewer data exist for copper, chromium, and manganese. Selenium shows benefits for sepsis patients, and zinc aids in preventing infections. Both deficiency and overload of metals can disrupt cellular function or cause damage. During infections, divalent metal homeostasis subtly shifts to either deprive pathogens of metals or use toxic metal accumulation to combat them.

Iron is an essential micronutrient for bacteria, fungi, and humans, with specialized systems for extracellular iron acquisition. Humans often sequester iron successfully, but pathogens can circumvent this. Clinically, controversy continues whether iron overload or administration of iron results in an increased risk of infection. The administration of iron chelating agents and siderophore- conjugate drugs to infected hosts seems a biologically plausible approach as adjunctive therapy in the treatment of infections caused by pathogens dependent on host iron supply (e.g. tuberculosis, malaria, and many bacterial and fungal pathogens); however, thus far, studies in humans have proved unsuccessful.

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PL 4

Siderophores, Transporters, and Survival: The Iron Playbook of *P. aeruginosa*

Isabelle J. SCHALK, [a)]

[a)] *UMR 7242, Biotechnologie et Signalisation Cellulaire, University of Strasbourg – CNRS, 300, Bd Sebastien Brant, F67412 Illkirch Cedex, France; schalk@unistra.fr*

Iron is a critical micronutrient for bacterial growth and virulence, yet it is scarcely available in most environments. *Pseudomonas aeruginosa*, a human opportunistic pathogen, has evolved a remarkable ability to adapt to iron-limited conditions by expressing a vast arsenal of at least 18 iron uptake systems. Most of these rely on siderophores—small, high-affinity iron-chelating molecules—either synthesized by *P. aeruginosa* or hijacked from other microorganisms. This impressive flexibility depends on the expression of specific TonB-dependent transporters (TBDTs) in the outer membrane, each tailored to import the ferric form of distinct siderophores.

We have identified ActA as the TBDT responsible for the uptake of the α-carboxylate-type siderophores rhizoferrin and staphyloferrin A, produced by *Rhizopus* species and *Staphylococcus aureus*, respectively. ChtA mediates the uptake of aerobactin (produced by Enterobacteriaceae), schizokinen (*Bacillus megaterium*), and arthrobactin (*Arthrobacter* species) [1]. A complementary study revealed that nine hydroxamate-type siderophores—mostly from fungi—also support *P. aeruginosa* growth under iron-limited conditions. Their uptake involves a complex interplay of TBDTs, primarily FpvB, in conjunction with FoxA and FiuA, depending on the siderophore [2].

In parallel, we have shown that aeruginoic acid and dihydroaeruginoic acid—degradation products of pyochelin, one of the two siderophores produced by *P. aeruginosa*—can themselves act as siderophores, with the TBDT FemA mediating their uptake [3].

This strategy demonstrates that *P. aeruginosa* can even transform degradation products into new opportunities for iron acquisition. Altogether, these findings highlight the remarkable adaptability of *P. aeruginosa* to iron scarcity and reveal the intricate binding specificities of TBDTs that mediate the uptake of structurally diverse ferri-siderophore complexes across the outer membrane.

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PL 5

Is Gold the New Platinum in Medicinal Chemistry?

Angela CASINI, [a)]

[a)] *Chair of Medicinal and Bioinorganic Chemistry, Department of Chemistry, School of Natural Sciences, Technical University of Munich; angela.casini@tum.de*

The discovery of the medicinal properties of gold complexes has fueled the design and synthesis of new anticancer metallodrugs, which have received special attention due to their unique reactivity and modes of action. Current research in the development of anticancer gold compounds is predominantly focused on the molecular design of drug leads with superior pharmacological activities, e.g., by exploiting their catalytic properties, or on the use of tailored drug delivery systems [1].

This lecture summarizes recent findings from our group on Au(III)-catalyzed reductive elimination in aqueous media and provides the proof-of-concept for the use of cyclometalated Au(III) C^N complexes to achieve efficient modification of proteins through C-atom transfer, enabling chemoproteomic studies (e.g. profiling of cysteine/selenocysteine residues) and novel therapeutic approaches [2]. Recently, the peculiar reactivity of Au(III) C^N complexes, enabling covalent cysteine-arylation in a gold-templated two-step mechanism, was exploited to achieve the first covalent gold-based PROTAC (**AuPROTAC**) (Figure 1) [3]. The degradome of the covalent **AuPROTAC** was characterized by establishing a cycloheximide chase assay, which was performed in a non-proliferative steady-state HL-60 cell culture system that maintained a static protein turnover and efficiently decoupled protein degradation from down-regulation. The method was verified with the known SMARCA2 and PBRM1-degrader ACBI2. Interestingly, amongst the possible targets, **AuPROTAC** could degrade the oncogenic tyrosine kinase MERTK and the thioredoxin-like 1 protein TXNL1, while their degradation was successfully rescued by proteasome inhibition.

When imaging applications are concerned, not all the radionuclides available in the Periodic Table of the Elements which are potentially suitable for imaging and/or therapy, can be bound to classical bifunctional chelators to incorporate them into targeted compounds. Here, organogold chemistry is invaluable to provide alternative strategies to trap radioactive isotopes. We showed that N-heterocyclic carbene (NHC) ligands can stabilize and target ^{198}Au(I) ions for theranostics.

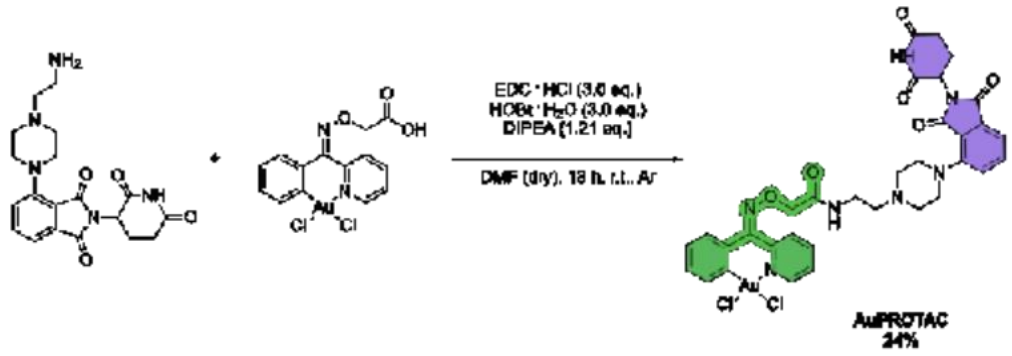

Figure 1: Schematic of the AuPROTAC design and synthesis.

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PL 6

The fascinating bioinorganic chemistry of transmembrane transition metal transporters

Gabriele MELONI, [a)]

[a)] *Department of Chemistry and Biochemistry, The University of Texas at Dallas, Richardson, TX 75080, USA; Gabriele.Meloni@utdallas.edu*

In all living organisms, the directional transport of essential and toxic transition metals across cellular membranes is controlled by transmembrane metal transporters, pumps, and associated donor/acceptor metallochaperones. These proteins act as key regulatory hubs, selectively controlling vectorial metal uptake and extrusion between the extracellular environment and cytosol, as well as regulating metal distribution within organelles. Although insights into the chemical and structural factors governing metal cargo selectivity and transport mechanisms are gradually emerging, our understanding regarding how coordination chemistry, energy transduction, translocation kinetics, and transport pathways have evolved to fulfil mechanistic requirements for cellular metal homeostasis remains limited.

In this presentation I will discuss examples showcasing our multidisciplinary strategy aimed at understanding the principles underlying cargo recognition, coordination, and translocation in different metal transporter families at the atomic level. We developed and applied real-time and quantitative methods to study metal transport events in proteoliposomes using sensor probes responsive to diverse stimuli, including metals, membrane potential, and co-transported ions. These approaches allow us to provide highlights on the kinetics, thermodynamics, transport mechanisms, and selectivity of metal transporter classes featuring diverse energetics and transport schemes. The work is unravelling how the interplay between the bioinorganic chemistry and energy-dependent/-independent translocation processes determines substrate selection and transport across lipid bilayers by transmembrane metals transporters.

KEYNOTE LECTURES

KL 1

Fungal siderophore uptake and its translational potential

Hubertus HAAS, [a] Isidor HAPPACHER, [a] Milos PETRIK, [b), c), d)] Clemens DECRISTOFORO, [e]

[a] Institute of Molecular Biology, Biocenter, Medical University Innsbruck, Innsbruck, Austria [b] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic [c] Institute of Molecular and Translational Medicine, University Hospital, Olomouc, Czech Republic [d] Czech Advanced Technology and Research Institute, Palacký University, Olomouc, Czech Republic
[e] Department of Nuclear Medicine, Medical University Innsbruck, Innsbruck, Austria

Fungi, including the opportunistic human pathogen *Aspergillus fumigatus*, have evolved systems that facilitate the uptake of iron - an essential micronutrient - with high affinity and specificity. These systems include reductive iron assimilation and siderophore-mediated iron acquisition [1]. Siderophores are low-molecular-mass chelators that specifically complex ferric iron. This mould produces two fusarinine-type (fusarinine C and triacetylfusarinine C (TAFC)) and two ferrichrome-type (ferricrocin and hydroxyferricrocin) siderophores to acquire and store iron. Siderophores play a crucial role in the virulence of fungal pathogens that infect animals or plants and they have high potential for use as biomarkers and for imaging fungal infections via ^{68}Ga-siderophore chelate-mediated positron emission tomography (PET) [1,2]. Molecular studies have identified and characterized the substrate specificities of the four *A. fumigatus* siderophore transporters: Sit1, Sit2, MirB, and MirD [3,4,5]. Sit1 and Sit2 exhibit both overlapping and unique substrate specificities with regard to various ferrichrome-type siderophores, including ferrirhodin, ferrichrome A, and ferricrocin. Sit1 and Sit2 both exhibit weak affinity for coprogen-type siderophores. Sit1 transports bacterial ferrioxamine-type xenosiderophores mediate and mediates the uptake of the novel antifungal drug VL-2397. MirB transports TAFC, while MirD transports fusarinine C. MirB was the only siderophore transporter to affect the virulence of *A. fumigatus* in murine aspergillosis models, demonstrating that TAFC-mediated iron uptake plays a dominant role during infection [5]. Phylogenetic analysis of siderophore transporter protein sequences enabled the prediction of the siderophores utilized by various fungal species. This could potentially allow for the imaging of fungal infections in a species-specific manner.

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KL 2

Siderophores as scaffold for Molecular Imaging applications

Clemens DECRISTOFORO, [a] Adriana KNOLL, [a] Giacomo GARIGLIO, [a] Isidor HAPPACHER, [b] Hubertus HAAS, [b] Milos PETRIK, [c], [d], [e] Elzbieta GUMIENNA-KONTECKA, [f]

[a] Department of Nuclear Medicine, Medical University Innsbruck, Innsbruck, Austria
[b] Institute of Molecular Biology, Biocenter, Medical University Innsbruck, Innsbruck, Austria
[c] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic [d] Institute of Molecular and Translational Medicine, University Hospital, Olomouc, Czech Republic [e] Czech Advanced Technology and Research Institute, Palacký University, Olomouc, Czech Republic
[f] Faculty of Chemistry, University of Wrocław, 50-383 Wrocław, Poland;

Siderophores are low molecular weight chelators produced by bacteria and fungi to scavenge essential iron. Research on these molecules has a continuing history over the past 50 years. Many biomedical applications have been developed, most prominently the use of the siderophore desferrioxamine B (DFO-B) to tackle iron overload related diseases. Also, DFO is utilized as the currently most widely used bifunctional chelator for Zr-89 utilized for Positron Emission Tomography (PET) in particular for antibody imaging.

In recent years we focussed on using cyclic hydoxamate based siderophores, mainly fusarinine C (FSC), as scaffold for a variety of applications involving PET, optical imaging and theranostics [1]. First applications using the acetylated variant triacetylfusarinine C (TAFC) revealed highly stable coordination of Gallium, allowing PET imaging using the generator radionuclide ^{68}Ga of invasive fungal infections, that was recently translated into first clinical testing [2]. By attachment of targeting vectors, peptides to small proteins, trimeric variants of FSC were developed targeting a variety of different receptors and other proteins overexpressed in cancer cells allowing labelling both with ^{68}Ga and ^{89}Zr[3] with improved targeting properties as compared to respective monomers. Furthermore, pre-targeting vectors, overcoming pharmacokinetic limitations of high molecular weight vectors, and heterodimers, targeting two different receptors with the aim to overcome tumour heterogeneity, were prepared revealing promising results. Diacetylated FSC variants allowed specific fungal targeting for bimodal vectors combining fluorescent imaging with PET or opening theranostic approaches [4]. Also, for tumour targeting development resulted in compounds with excellent properties for radioguided surgery utilizing hydrophilic and shielded near infrared dyes targeting the Fibroblast Activation Protein in the tumour microenvironment.

Most recently artificial, cyclic variants of ferrioxamines have been investigated [5], with first results showing the possibility to fine-tune microbial targeting by structural modifications of natural siderophores. Such artificial variants of natural siderophores also hold potential for widening applications in oncological targeting.

This work was funded by the Austrian Science Fund (FWF), grant DOI: 10.55776/I6613.

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KL 3

Re Compounds as Coordinate Covalent Inhibitors Enzymatic Activity

Seth M. COHEN, [a)]

[a)] *Department of Chemistry and Chemical Biology, U.C. San Diego, La Jolla, California, U.S.A.; scohen@ucsd.edu*

Covalent inhibitors have become an important tool in the development of new therapeutics. Covalent inhibitors rely on an organic chemical moiety that reacts with amino acid residues in a protein target, such as cysteine or serine. Metal complexes offer a rich alternative for creating compounds that have the capacity to selectively modify amino acid residues to produce 'coordinate covalent' inhibitors. In this presentation, the development of Re compounds that can site specifically modify enzymes will be described. These compounds can exhibit good selectivity against important enzymatic targets [1], as demonstrated by the antiviral activity (against COVID-19) of some complexes [2]. Importantly, enantiomers of chiral Re complexes show differential inhibitory activity [2], which is a hallmark of druglike, targeted interactions. In addition, it is shown that increasing the reactivity of the Re complexes leads to reduced specificity [3], but may provide a route to using these complexes in activity-based protein profiling (ABPP) [4] or new therapeutic applications [5].

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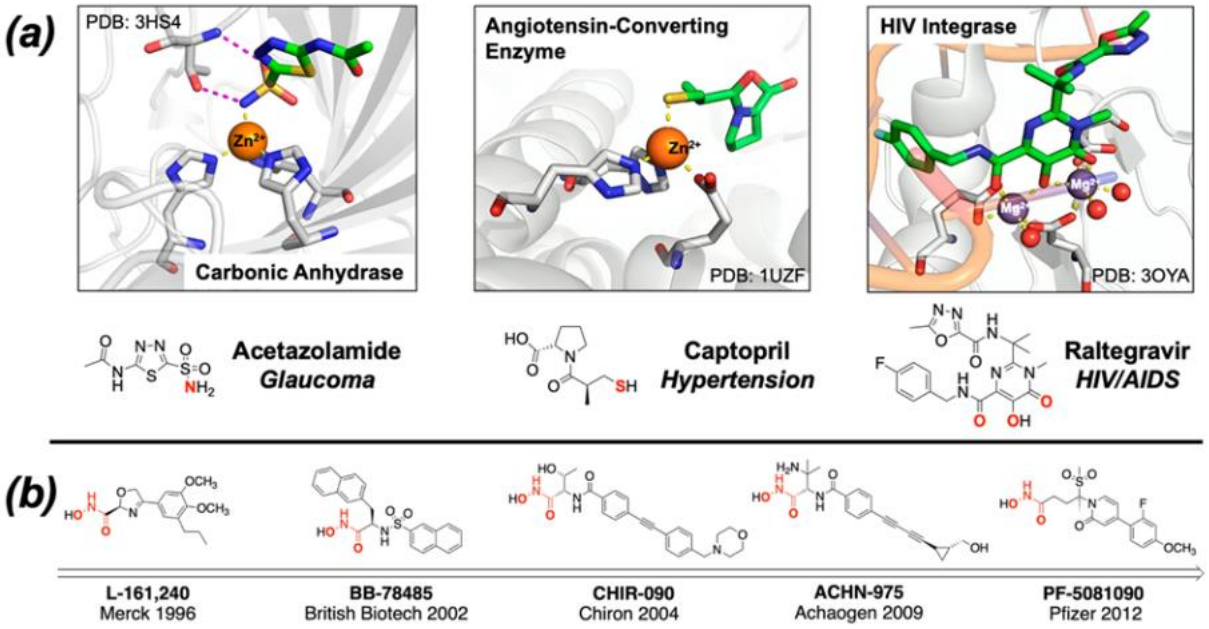

(a)
PDB: 3HS4
Carbonic Anhydrase
Angiotensin-Converting Enzyme
PDB: 1UZF
HIV Integrase
PDB: 3OYA
Acetazolamide
Glaucoma
Captopril
Hypertension
Raltegravir
HIV/AIDS
(b)
L-161,240
Merck 1996
BB-78485
British Biotech 2002
CHIR-090
Chiron 2004
ACHN-975
Achaogen 2009
PF-5081090
Pfizer 2012

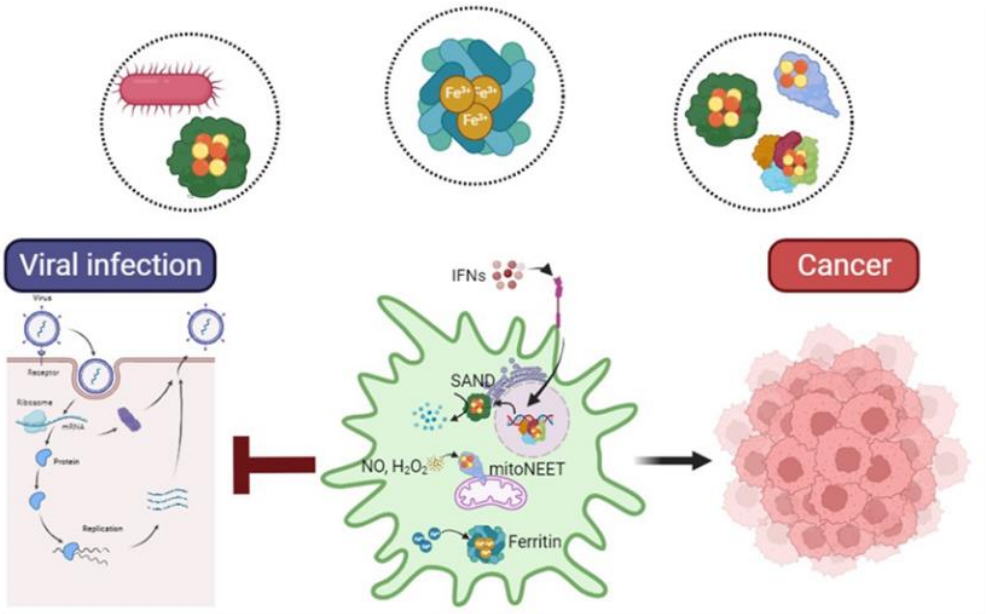
KL 4

Metalloproteins in the immune response and medicine

Nghi Thao HOANG, [a),b)] Deborah GRIFAGNI, [c)] Meritxell WU-LU, [d)] Yujie SHENG, [a)] Mengdi WU, [a)] Theo SITUMORANG, [a)] Pei-Hsin TAI, [a)] Zheng CHEN, [a)] Astrid MALUTA, [a)] Mohammed HAKIL, [a)] Bianca SUSINI, [c)] Hannah FLORANCE, [e)] Peter-Leon HAGEDOORN, [f)] Maria-Andrea MROGINSKI, [g)] Simone CIOFI-BAFFONI, [h)] Kourosh H. EBRAHIMI, [a)]

[a)] Institute of Pharmaceutical Science, King's College London, London, UK
[b)] Department of Pharmacy, Da Nang University of Medical Technology and Pharmacy, Da Nang, Viet Nam
[c)] Magnetic Resonance Center and Department of Chemistry, University of Florence, Sesto Fiorentino, Italy
[d)] Department of Chemistry, Technical University of Berlin, Berlin, Germany
[e)] Agilent Technologies, Cheadle, UK
[f)] Department of Biotechnology, Delft University of Technology, Delft, the Netherlands;
Kourosh.ebrahimi@kcl.ac.uk

Metalloproteins play fundamental roles in cellular life. Their functions in the immune response are just emerging [1]. They are gaining growing interest in biotechnological applications such as the synthesis of antivirals or antimicrobials [2], nitrogen fixation [3], and CO_2 removal [4]. I will briefly discuss a recent story from our lab that focuses on elucidating the emerging functions of metalloproteins containing iron-sulfur (FeS) clusters in the immune response. I will describe the discovery of an electron transfer pathway that activates antiviral defense in humans. These discoveries and advances have only been possible through multidisciplinary collaboration spanning the boundaries of bioinorganic chemistry, immunology, computational biochemistry, spectroscopy, and structural biology. This synergistic approach is made possible by support from the European Cooperation in Science and Technology (COST).



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KL 5

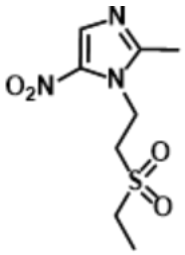
Reactivity and biological activity driven by the versatility of the nitro and sulfone groups in imidazole coordination compounds

Walter FUGAROLAS, [a] Rodrigo CASTRO-RAMÍREZ, [a] Rubí NAVARRO-PEÑALOZA, [a] Norah BARBA-BEHRENS, [a]

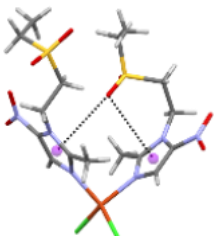
[a] Facultad de Química, Universidad Nacional Autónoma de México, C.U. Coyoacán 04510, Ciudad de México, México; norah@unam.mx

The biological activity of coordination compounds with azoles and nitroimidazoles have been extensively studied. Nevertheless, for most of them the structure-activity relationship has been overlooked. Complexes with a wide range of transition metal ions, with different coordination modes and geometries, as the relevance of intra and intermolecular non-covalent interactions in these compounds, such as lone pair···π, π···π, σ-hole, C-H···π, Cl···Cl and hydrogen bonding, will be discussed. The understanding of the coordination environment, geometry, electronic properties and weak interactions may contribute to the rational design of new compounds with potential applications in bioinorganic and medicinal inorganic chemistry [1].

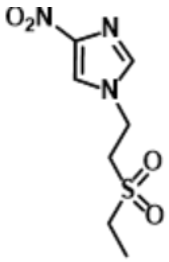
In this context, we have been interested to investigate the contribution of different substituents on the coordination chemistry and electronic properties of transition metal compounds with 5- and 4-nitroimidazole derivatives, as well as on their antiparasitic, antimicrobial or antineoplasic activity.



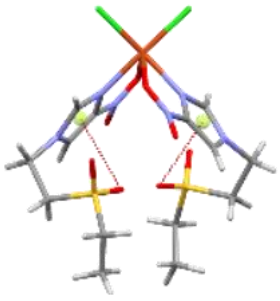
5-tnz



$[Cu(5\text{-tnz})_2Cl_2]$ Tetrahedral
Antihelmintic acidity, $EC_{50} = 0.05$ µM



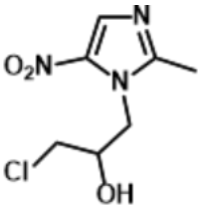
4-tnz

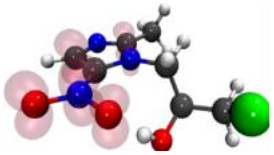


$[Cu(4\text{-tnz})_2Cl_2]$ Octahedral

The sulfone group plays a significant role through lone pair···π intramolecular interactions.

Cyclic voltammetry studies of the free 5-nitroimidazole ligands, revealed characteristic reduction peaks corresponding to the formation of nitro radical anion and hydroxylamine species, processes associated with biological activity.





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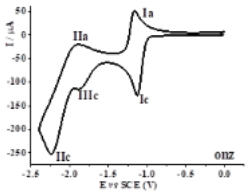

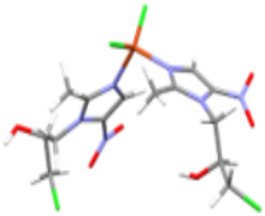

$[Cu(5\text{-ornz})_2Cl_2]$
Antitoxoplasma activity, $IC_{50} = 0.10\mu M$

The tetrahedral 5-ornz copper(II) compounds $[Cu(onz)_2Cl_2]$ and $[Cu(onz)_2Br_2]$ present significant shifts in the nitro group reduction potentials and low stabilization energies for their conformers, correlating with their high antitoxoplasma activity. Their mechanism of action is related to the nitro and the Cu(II)/Cu(I) redox processes. On the other hand, the zinc compounds, with promising antineoplastic activity, showed minimal nitro radical stabilization, thus redox processes are not contributing to their activity in cancer cell lines.

The relationship between group substitution, structural properties (covalent and weak interactions), geometry, redox properties and biological activity will be discussed.

Acknowledgements:
Financial support by DGAPA IN206922 (UNAM) and PAIP-5000-9183 (FQ, UNAM) are acknowledged.

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INVITED LECTURES

IL 1

Metal Ions in Microbial Survival and Viral Entry: Coordination Chemistry, Mechanisms, and Therapeutic Opportunities

Massimiliano F. PEANA, [a)]

[a)] *Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Italy; peana@uniss.it*

Metal ions such as iron, manganese, zinc, and copper are essential yet tightly regulated elements in microbial and viral systems, where they act as critical modulators of enzymatic function, oxidative stress response, and host interaction. In bacteria, selective metal uptake systems, such as the FeoB transporter in *Escherichia coli*, demonstrate how coordination preferences for Fe(II), Mn(II), and Zn(II) govern competition for essential micronutrients, directly impacting survival in hostile environments [1,2].
Deinococcus radiodurans, an extremophile renowned for its extraordinary resistance to ionizing and UV radiation, serves as a compelling model of Mn-based oxidative stress defence. This organism hyperaccumulates Mn(II)-antioxidant complexes, which provide global protection to cellular proteins, including those essential for DNA repair, from ROS-induced damage. Mn coordination in *D. radiodurans* offers a mechanistic framework for oxidative stress mitigation, with implications for radioprotection, aging, and cancer therapy [3-5].
In the viral domain, metal ions are increasingly recognized as modulators of host-virus interactions. The ACE2 receptor, a critical entry point for SARS-CoV-2, contains metal-binding residues capable of coordinating Zn(II) and Cu(II), potentially altering its conformation and affecting viral binding and infectivity. These insights open new avenues for antiviral intervention based on targeted metal coordination [6].
This lecture will explore the coordination chemistry underlying microbial metal acquisition, oxidative stress resistance, and viral entry, highlighting how bioinorganic insights can be leveraged toward antimicrobial, antiviral, and therapeutic innovation.

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[1] Orzel, B.; Pelucelli, A.; Ostrowska, M.; Potocki, S.; Kozlowski, H.; Peana, M.; Gumienna-Kontecka, E., Fe(II), Mn(II), and Zn(II) Binding to the C-Terminal Region of FeoB Protein: An Insight into the Coordination Chemistry and Specificity of the Escherichia coli Fe(II) Transporter. *Inorg. Chem.* **2023**, 62 (45), 18607–18624.
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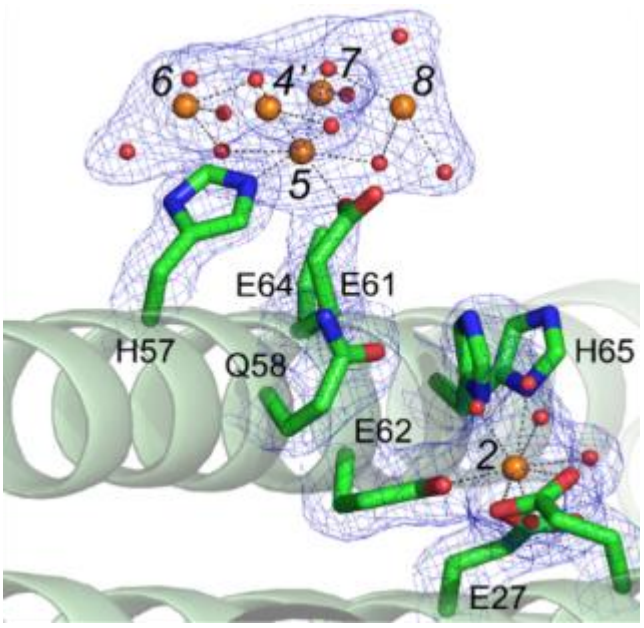
IL 2

Ferritins: nanocages for iron storage/detoxification and biotechnology

Nick LE BRUN, [a)]

[a)] *Centre for Molecular and Structural Biochemistry, School of Chemistry, Pharmacy and Pharmacology, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK; n.le-brun@uea.ac.uk*



Ferritins play a crucial role in iron homeostasis and detoxification in organisms from all kingdoms of life. They are composed of 24 α-helical subunits arranged around a hollow interior cavity in which an iron-containing mineral can be reversibly stored [1,2]. Bacteria often contain more than one type of ferritin, while animal cells contain a cytosolic ferritin, which is composed of a tissue-dependent mixture of two different subunit types, H- and L-chains. H-chain contains a catalytic site, called the ferroxidase centre, which drives Fe^{2+} oxidation, while L-chain lacks the ferroxidase centre, but contains a nucleation site for the synthesis of the mineral core.

Animal cells with high metabolic activity express another ferritin, composed of H-chain-type subunits, that is targeted to mitochondria [3]. Each subunit contains a ferroxidase centre highly related to that of the cytosolic ferritin [4], and a presumed, but undefined, nucleation site for mineral core formation.

Here, several aspects of ferritins will be discussed, including recent mechanistic studies of mitochondrial ferritin, uncovering thus far unique features, and high-resolution time-resolved X-ray crystal structures that map out the mineralization process for mitochondrial ferritin, revealing a ferrihydrite-like hydrated iron-oxo cluster containing five iron ions, representative of the nascent native mineral core of ferritin (see the Figure). Finally, the biotechnological potential of ferritins for applications in therapeutics and diagnostics, catalysis and biorecovery will be highlighted.

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IL 3

Superoxide dismutases as model systems for studying the evolution of metal preference of metalloenzymes

Lorna NIKOLIĆ, [a] Rafał MAZGAJ, [a] Mariam ESMAEELI, [a] Swati DAS, [a] Natalia KWIATOS, [a] Jana KEHL-FIE, [b] Thomas KEHL-FIE, [b] Kevin WALDRON, [a]

[a] Institute of Biochemistry and Biophysics, Polish Academy of Science, Pawinskiego 5a, Warsaw, Poland
[b] Department of Microbiology & Immunology, University of Iowa, Iowa City, IA, 52242, USA; kwaldron@ibb.waw.pl

Unlike its staphylococcal relatives, *Staphylococcus aureus* possesses not one, but two isozymes of superoxide dismutase (SOD). This pair of enzymes is derived from a gene duplication event that occurred in the ancestral lineage of *S. aureus*, *S. argenteus* and *S. schweitzerei*. Consistent with this recent origin, both *S. aureus* SODs remain highly homologous in sequence and in structure as members of the iron or manganese SOD superfamily (SodFM). And yet they have remarkably divergent biochemical properties. We previously demonstrated that the metal-preference of their catalysis is different, and that the distinct metal specificity of the SOD unique to the *S. aureus* lineage enables this pathogen to overcome manganese starvation conditions that it experiences within the host [1], through the action of the host protein complex calprotectin [2]. But the two proteins are also distinguished by another biochemical property, their differing chemical and thermal stability, despite their overall structures being essentially identical [3].

The *S. aureus* SODs have provided a window into the evolution of the crucial biochemical parameter, namely the metal-preference of catalysis, in this widely distributed family of metalloenzymes [4]. Data will be presented on the evolutionary relationship and distinct biochemical properties of these two remarkable enzymes, and how this relates to the wider evolutionary history of the SodFM family. It will be shown how computational and empirical analyses have enabled us to demonstrate that numerous switches have occurred in the metal-preference of catalysis of SodFM enzymes across the phylogenetic tree.

Furthermore, the close sequence relationship between the pair of SODs from *S. aureus*, yet distinct biochemical properties, makes them an outstanding model with which to study the structure/function relationship in this metalloenzyme family that play a crucial role in oxidative stress defence in a wide variety of organisms. Current studies that are dissecting the precise mechanisms by which these properties have been adapted through evolution will also be covered, including biophysical structure/function studies, mutagenesis to identify which residues control metal-preference and its evolution, and determination of the relationship between monomers within the homo- and heterodimeric structure of SodFMs.

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IL 4

Chemical Speciation Studies *in*, and *of*, Biological Fluids: Critical Aspects in the Evaluation of Chelants Performances

Clemente BRETTI, [a] Rosita CAPPAI, [b] Concetta DE STEFANO, [a] Sofia GAMA, [c] Demetrio MILEA [a]

[a] Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali (CHIBIOFARAM), Università degli Studi di Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy; [b] Dipartimento di Scienze Chimiche, Fisiche, Matematiche e Naturali, Università degli Studi di Sassari, via Vienna 2, Sassari, 07100, Italy; [c] Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10 (km 139.7), 2695-066, Bobadela LRS, Portugal; dmilea@unime.it

It is well known that the environmental impact, the biological activity, the general behaviour and many other properties of any chemicals are strictly dependent on the chemical form(s) in which they occur in a system of interest, *i.e.*, on their *Chemical Speciation* (*CS*; its definition and the guidelines related to the correct meaning and use of this and related terms are given by IUPAC [1]). As such, *CS* studies *in* and *of* biological fluids represent, nowadays, an essential step in many branches of chemistry, biology and medicine, since they are fundamental for a thorough understanding of biological cycles, the toxicity of elements and compounds, the performances of drugs, as well as eventual relationships between a particular species and some diseases.

One of the most effective approaches to these studies is through equilibrium analysis by thermodynamic data (*e.g.*, stability constants) aimed at defining the network of interactions between various components present in a target system. This approach is generally preferred to the direct determination of single species (which is not always straightforward), because it may also allow the modelling and the prediction of the *CS* in variable environments and conditions, as it often happens for biological fluids [2]. In fact, from a chemical-physical point of view, these fluids are multicomponent aqueous solutions of very different composition, in which several inorganic ions, as well as low, medium and high molecular weight organic molecules occur in various concentrations and in diverse conditions of temperature, ionic strength, pH [3].

However, the complexity of biological fluids is also responsible for the main difficulties in performing accurate *CS* studies and providing reliable *CS* models able to thoroughly interpret other phenomena in these media. A typical example is represented by the assessment of the performances of chelating agents in sequestering metal ions through the formation of strong complexes in solution, with particular reference to comparisons between the selectivity and the sequestering ability of the same chelant towards different metal ions, and/or different chelants towards a given cation.

Beside the intrinsic difficulties in the experimental determination of the nature and stability of strong metal complexes even in simple aqueous systems, other critical aspects are related to:

1. different speciation schemes of different systems;
2. occurrence of competing reactions (especially in real systems);

3. extrapolations to different conditions (e.g., other temperatures, ionic strengths);
4. incorrect use of common parameters for the quantification of the sequestering ability.

In this contribution, these and other aspects will be discussed, with the aim of identifying main drawbacks arising during the determination and use of thermodynamic data for the evaluation of chelants performances.

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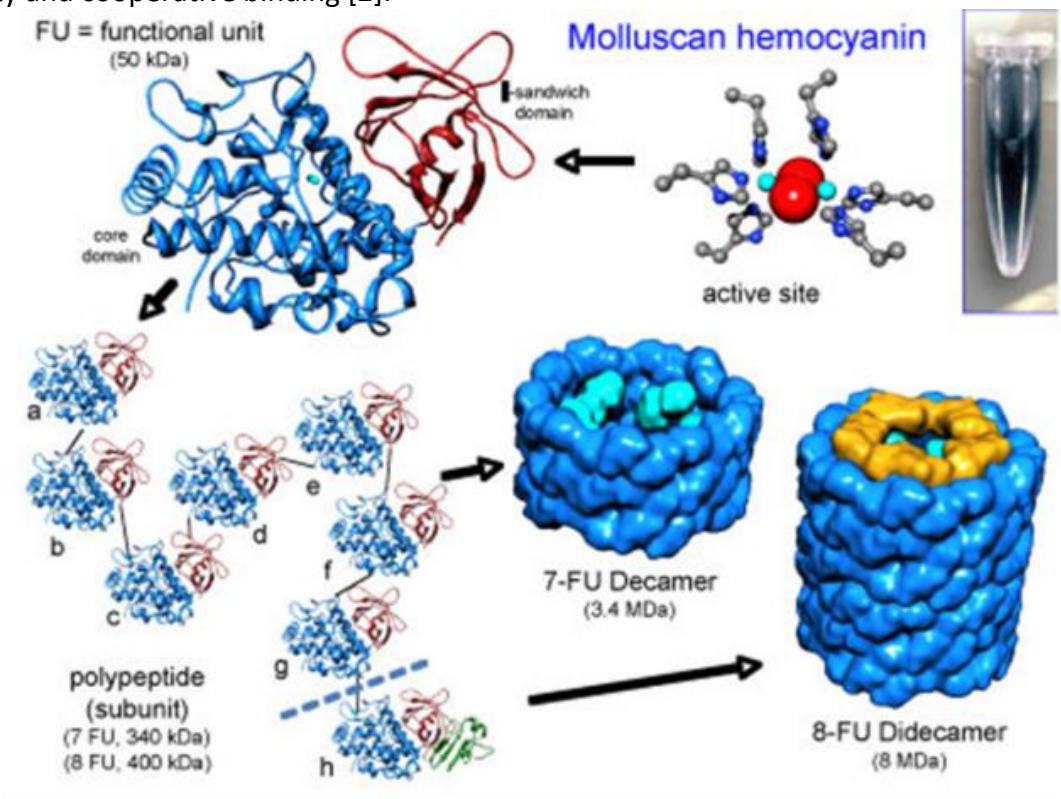
IL 5

Hemocyanin and other snail stories

Arnau PONS, [a] Mercè CAPDEVILA, [a] Òscar PALACIOS,[a]

[a] *Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallés (Barcelona, Spain); oscar.palacios@uab.cat*

Hemocyanin is a copper-containing respiratory protein found in the hemolymph of many arthropods and mollusks. It plays a key role in oxygen transport and immune defense. Unlike hemoglobin, hemocyanin binds oxygen reversibly using copper ions, imparting a distinctive blue color when oxygenated [1].
They exhibit structural diversity, functional mechanisms, and an evolutionary significance across invertebrate taxa. Recent advances in structural biology have revealed complex quaternary architectures and allosteric regulation mechanisms that underpin its high oxygen affinity and cooperative binding [2].



Beyond respiration, hemocyanin exhibits antimicrobial properties and immunostimulatory effects, positioning it as a promising candidate in biomedical research, including vaccine adjuvants and cancer immunotherapy.
Quantification and chemical characterization of hemocyanin are essential for understanding its structure, function, and potential applications. But poor information can be obtained regarding their chemical properties and quantification methodologies. They are mainly based

on spectroscopic methodologies: UV-vis, Bradford or BCA assays, as well as copper quantification by AAS or ICP-MS.
In this work we deepen insight the characterization of the behavior, structuration and composition of hemocyanin of the garden snail *Cantareus aspersum* by means of several chemical techniques: UV-vis and fluorescence spectroscopy, size exclusion chromatography, TEM and ICP-AES data.
Additionally, some curiosities regarding the relationship between the different life states of the snails and their diet, as well as their putative relationship with the synthesis of metallothioneins in snails, will be discussed.

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IL 6

NMR spectroscopy and complex formation – what can we observe?

Michał Nowakowski, [a)]

[a)] *University of Warsaw, Faculty of Chemistry, Biological and Chemical Research Centre, Warsaw, Poland*

A number of physico-chemical methods are often used for investigating the formation of complexes: microcalorimetry (ITC, DSC), UV-VIS absorption spectroscopy, fluorescence, circular dichroism (CD), Fourier Transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), capilary electropheresis (CE), ionization mass spectrometry (ESI-MS), solid phase microextraction, volatization and many more.

Some methods are better suited to study complex formation than others, and among the non-separation methods NMR spectroscopy is one of the most efficient and widely used. The main strength of NMR spectroscopy is the fact that nuclei with spin resonate at characteristic frequencies when placed in a magnetic field and the resonance position is extremely sensitive to environment. If a dynamic process changes environment of a given nucleus during measurement than its NMR spectrum will be affected (and the effects can be dramatic). By analysing such exchange affected spectra highly quantitative and reliable datasets describing exchange process (with atomic resolution) can be obtained. The talk will concentrate on solution state NMR methods and approaches allowing observation of complex formation.

Acknowledgements
Interdisciplinary Centre for Mathematical and Computational Modeling, University of Warsaw for computational time (Project id: 3695, Grant id G98-2084).

IL 7

Radiolabelled siderophores for imaging bacterial infections

Milos PETRIK, [a), b), c)] Katerina DVORAKOVA BENDOVA, [a)] Barbora NEUZILOVA, [a)] Kristyna KRASULOVA, [a)] Marian HAJDUCH, [a), b), c)] Hubertus HAAS, [d)] Clemens DECRISTOFORO, [e)]

[a)] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic [b)] Institute of Molecular and Translational Medicine, University Hospital, Olomouc, Czech Republic [c)] Czech Advanced Technology and Research Institute, Palacký University, Olomouc, Czech Republic [d)] Institute of Molecular Biology, Biocenter, Medical University Innsbruck, Innsbruck, Austria [e)] Department of Nuclear Medicine, Medical University Innsbruck, Innsbruck, Austria; milos.petrik@upol.cz

Invasive microbial infections are a major cause of morbidity and mortality in immunocompromised patients [1]. A dramatic increase in the incidence of hospital-acquired infections caused by opportunistic pathogens has been observed in recent years. Early and accurate diagnosis of infection is essential for effective treatment of patients and prevention of pathological complications. A number of diagnostic tests and methods are currently used in clinical practice. However, most of these methods lack sufficient specificity and/or sensitivity. The availability of a rapid and reliable tool for the diagnosis of infectious diseases represents a major unmet need in the treatment of critically ill patients.

Molecular imaging provides the potential for specific and sensitive detection and localization of infections, in particular based on radiotracers for single photon emission computerized tomography (SPECT) and positron emission tomography (PET) [2]. However, the radiotracers commonly used in clinical practice (e.g. 2-deoxy-2[^{18}F]fluoro-D-glucose, [^{67}Ga/^{68}Ga]Ga-citrate and radiolabelled white blood cells) are not optimal for imaging microbial infections. These probes are often non-specific and target predominantly secondary effects of infection, such as increased blood flow and vascular permeability, activated endothelial cells, or polymorphonuclear cell migration.

Therefore, several attempts have been made to develop more specific radiotracers for imaging microbial infections. These include radiolabelled antibodies, antibiotics, antimicrobial peptides, vitamins, aptamers, bacteriophages or different molecules employing bacterial metabolism such as sugars, sugar alcohols, D-amino acids, N-acetyl muramic acid derivates, paraaminobenzoic acid, nucleoside analogues or siderophores [3].

The siderophore-based iron acquisition system could be an interesting target for molecular imaging. Siderophores are specific iron chelators produced by many microorganisms that are recognized by specific microbial transporters. Radiolabelled siderophores could be a highly specific tool for infection imaging, considering the essential role of the siderophore system for iron acquisition and virulence of microorganisms together with its upregulation during infection, whereas they are not utilized by mammals. We have recently demonstrated that several siderophores can be radiolabelled, replacing iron without loss of bioactivity and allowing molecular imaging of microbial infections by PET [4]. In this talk, we will briefly report and summarize our work on radiolabelled siderophores for imaging bacterial infections.

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IL 8

Gaining Insight into Mn(II) and Fe(II) Binding: From Model Systems to Transporter Fragments

Malgorzata OSTROWSKA, [a] Karolina PAWLIK, [a] Bartosz ORZEL, [a] Massimiliano PEANA, [b] Elzbieta GUMIENNA-KONTECKA, [a]

[a] Faculty of Chemistry, University of Wrocław. Joliot-Curie 14, 50-383, Wrocław, POLAND [b] Department of Chemistry and Pharmacy, University of Sassari, Via Vienna 2, 07100, Sassari, ITALY; malgorzata.ostrowska3@uwr.edu.pl

Manganese has garnered considerable attention in recent years due to its pivotal role in nearly all forms of life, particularly in pathogens and the host immune system. There is an increasing amount of emerging evidence that the invading microbes utilize Mn(II) as a key micronutrient to resist the effect of host-mediated oxidative stress [1, 2]. Mn(II) plays a significant role in the adaptation of pathogenic bacteria to the human host. It is evident that the host and pathogens compete for Mn(II) during infection, and the genetic inactivation of Mn(II) homeostatic mechanisms decreases the ability of many bacterial species to successfully colonize and cause disease within multiple hosts. Although structural and biophysical studies provide general support for a simple competition model, in which the extracellular chelator calprotectin (CP) and Mn(II)-specific uptake systems compete for the same metal based on their respective affinities, there is much more to be learned about Mn(II) coordination chemistry to fully understand this process [3, 4, 5]. The main constraints on the way to understand manganese homeostasis from a bioinorganic chemist's point of view are a negligible number of papers concerning coordination, structure, stability, and mode of action of Mn(II)-peptide complexes.

In our research, we have chosen model Mn(II) ligands based on polyhistydyl sequences, and their alanine, aspartic, and glutamic acid derivatives [6], as well as parts of unstructured regions of Mn(II) and Fe(II) binding proteins and their mutated derivatives. We used a variety of physicochemical methods, such as potentiometry, mass spectrometry, NMR, EPR and CD spectroscopy to characterize the complex species formed between the chosen peptide fragments and Mn(II), Fe(II), and Zn(II) ions. Obtained results could provide insight into poorly discovered thermodynamics and coordination chemistry of Mn(II) ions with specific metal transporters.

Acknowledgments
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IL 9

Antibiotic resistance – a novel approach using peptidomimetics inspired by human salivary peptides

Joanna WĄTŁY,[a)] Klaudia SZARSZOŃ,[a)] Anna ŚLUSARCZYK,[a)] Jan KACHNOWICZ,[a)] Marta LUSZAWSKA,[a)] Fabio ZOBI,[b)] Valentina BORGHESANI,[c)] Tomasz JANEK,[d)]

[a)]Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland, [b)]Department of Chemistry, University of Fribourg, Chemin du Musée 9, 1700 Fribourg, Switzerland, [c)]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy, [d)]Wrocław University of Environmental and Life Sciences, Department of Biotechnology and Food Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland; joanna.watly2@uwr.edu.pl

Antibiotic resistance remains one of the most pressing challenges in modern medicine, necessitating the development of alternative antimicrobial strategies. This study focuses on a novel class of antimicrobial agents - peptidomimetics inspired by naturally occurring peptides found in human saliva.

Salivary antimicrobial peptides such as histatins, chemokines, cathelicidins, defensins, and mucins exhibit innate infection-fighting capabilities.[1-3] However, their therapeutic potential is limited by susceptibility to enzymatic degradation.[4] To overcome this, we employed, *e.g.* a dual-modification strategy involving the incorporation of D-amino acids and sequence inversion (*retro-inverso* approach) (Table 1), significantly enhancing metabolic stability. Furthermore, coordination of metal ions such as Zn(II) and Cu(II) increased the antimicrobial activity of these compounds.[5]

Table 1. Studied salivary-derived antimicrobial peptides and their D-amino acid-containing peptidomimetics (lowercase letters indicate D-amino acids).

ORIGIN	NATIVE PEPTIDE	PEPTIDOMIMETIC
MUCIN 7	FPNPHQPPKHPDK	FPNPHQPPkhPDK / fpnphqppkhpdk
MUCIN 7	KSHFELPHYPGL	kshfelphypgl / lgpyhplefhsk
MUCIN 7	EGRERDHELRHRRHHHQSPK	EGRERDHELRHRrhHHQSPK
HISTATIN 5	DSHAKRHHGYKRKFHEKHHSHRGY	DSHAKRHHGYKrkFHEKHHSHRGY / dshakrhhgykrkfhekhhshrgy
CHEMOKINE CCL-28	HRKKHHGKRNSNRAHQGKHETYGHKTPY	hrkkhhgkrnsnrahqgkhetyghktpy
CATHELICIDIN LL-37	DFLRNLVPRTES	dflrnlvprtes

The resulting metal-peptidomimetic complexes demonstrated high stability and broader-spectrum activity against pathogens and their biofilms compared to native peptides, while maintaining low cytotoxicity towards healthy human cells. Preliminary results indicate a clear correlation between metal-binding properties, secondary structure, and biological function.

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IL 10

Metalloenzyme engineering with non-canonical amino acid incorporation

Sandro FISCHER, [a)†] Anton Natter PERDIGUERO, [a)†], Kelvin LAU, [b)]
Alexandria DELIZ LIANG, [a)]

[a)] Department of Chemistry, University of Zurich; Winterthurerstrasse 190, 8057, Zurich, Switzerland. [b)] Protein Production and Structure Characterization Core Facility (PTPSP), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland. †These authors contributed equally*; alexandriadeliz.liang@chem.uzh.ch*

Metalloenzymes are rich targets for protein engineering. While traditional mutagenesis with the 20 canonical amino acids has enabled significant advances, its chemical scope remains limited. The incorporation of non-canonical amino acids (ncAAs) into proteins has the potential to expand the chemistry accessible through mutagenesis and e enable precise control through mutations that are more finely tuned and rationally designed than those achieved with canonical amino acid mutagenesis. To demonstrate this potential, we examined ncAA-based mutagenesis of a classical metalloenzyme: a laccase. Laccases are multicopper oxidases that catalyze the one-electron oxidation of various substrates. Fungal laccases exhibit high redox potentials, high activity, and broad substrate scope. In contrast, bacterial laccases are much easier to express, are thermally stable, and tolerate a wider range of reaction conditions. However, bacterial laccases have low activity and low redox potentials. Thus, bacterial laccases present many advantages, but their lower activity precludes them from more widespread use.

Based on fundamental chemical principles, we posited that ncAA-based protein engineering could be used to improve the activity of bacterial laccases[1]. Based on these principles, we designed and synthesized several ncAAs that we posited could improve catalysis, engineered the corresponding genetic code expansion tools (aminoacyl-tRNA synthetase/tRNA pairs) for incorporation of these ncAAs, and validated these tools in a classical model protein (GFP). Using these tools, we expressed laccases variants with site-specific incorporation of the target ncAAs and performed a comparative study to assess the changes endowed by ncAA-based mutagenesis. Our results demonstrate that rationally designed ncAA variants can significantly improve catalytic performance. Furthermore, the enhancements derived from ncAA-based mutagenesis provide a foundation for additional optimization using traditional directed evolution. Overall, this work highlights the power of ncAA-based mutagenesis to fine-tune catalytic sites beyond the capabilities of conventional approaches.

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IL 11

Designing artificial proteins and metalloproteins using the Spy construct

Valentina BORGHESANI, [a] Chiara BOTTONI, [a] Andrea Lourdes SPINOSA, [a] Marianna VESCOVI, [a] Angelo BOLCHI, [a] Gloria SPAGNOLI, [a] Camilla COTTI, [a] Davide CAVAZZINI, [a] Giovanni MAESTRI, [a] Matteo HOCH, [a] Federico DROGHETTI, [b] Mirco NATALI, [b] Matteo TEGONI, [a]

[a] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma [b] Department of Chemical and Pharmaceutical Sciences, University of Ferrara; matteo.tegoni@unipr.it

Metalloproteins promote some of the most complex biomolecular processes in nature. The design of artificial metal binding sites or catalytic sites on proteins is often challenging, especially where non-natural amino acid residues are involved. The protein we used is Spy, an artificial protein redesigned for its use as protein conjugation tool. Spy is made by two component: SpyCatcher (SC) which is a small beta sandwich protein, and SpyTag which is a peptide of ca. 15 residues.[1] The Asp residue on ST reacts with a lysine on SC to form an isopeptide bond. We devise to design metal sites on the SpyTag peptide and, by recombination with SC, to obtain an artificial metalloprotein (Figure 1). Although the use of the Spy system is large in the literature, no examples of design of artificial metal sites using Spy were reported.

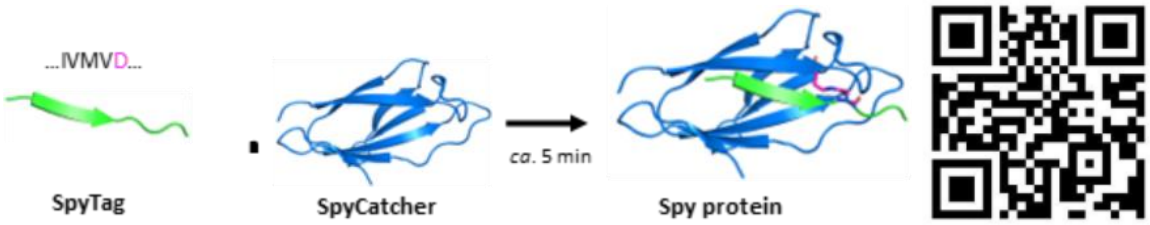

Figure 1: Representation of the Spy protein (left) along with the two components SpyTag (green) and SpyCatcher (blue). Scan the code, select [AR] in the left side menu, follow the instructions, and enjoy!

Using this *Trojan-horse* approach, we are currently developing artificial metalloenzymes which embed sever functional groups, from metal binding sites for redox catalysis to luminescent dyes for bioimaging and energy transfer.[2] In this communication we will discuss two types of artificial metalloproteins: copper-proteins for oxidation reactions of organic substrates, and Ni(II) or Co(III) metallopeptides for the photocatalytic production of hydrogen.

As for the copper oxyenzymes, the initial design of the copper(II) Spy construct involved an ATCUN (Amino Terminal Cu and Ni binding site) at the N-terminus of ST. By employing GSH and DAH triads we acquired SC/ST adducts that exhibit selective binding to Cu(II) at the ATCUN site of SpyTag. The binding at SpyCatcher occurred only upon the addition of a second equivalent of Cu(II). The subsequent copper(II) Spy construct was devised by introducing a

tandem His-His site on SpyTag (Figure 2A). $Cu(His)_2$ sites are recognized for their ability to promote the oxidation of catechols, prompting us into the study of the oxidation of both L- or D-Dopa to assess the presence of enantioselectivity in the rate of oxidation of the substrates.

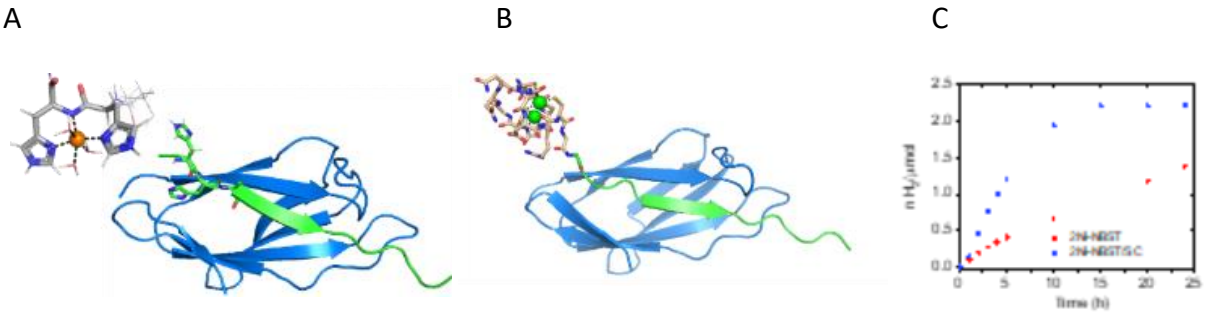

Figure 2: Representation of: A) bis-His Spy protein; B) di-nickel NBST/SC Spy protein. C) plot of µmol of produced H_2 as a function of irradiation time (at 460 nm) for a system containing di-nickel NBST or di-nickel NBST/SC, $[Ru(bpy)_3]^{2+}$ and ascorbate.

As for the artificial metal sites for the photostimulated production of hydrogen, we have studied a system based on the minimalist two-nickel peptide developed by Timm and coworkes (NB metallopeptide).[3] To this purpose we have synthesized a SpyTag peptide which bears the NB sequence upstream the SpyTag sequence (NBST peptide). Once reacted with 2 eq. of Ni^{2+}, the obtained metallopeptide was tested for the production of hydrogen in the presence of $[Ru(bpy)_3]^{2+}$ as the photoactivatable dye and ascorbate as the sacrificial reductant. Indeed, both the di-nickel NBST peptide and the corresponding reconstituted Spy protein acted as catalyst in the photochemical production of molecular hydrogen.

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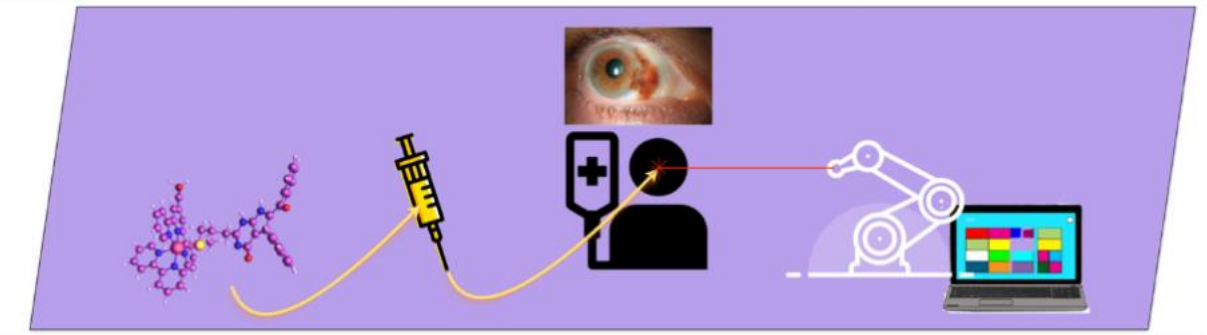
IL 12

PACT4EYE: Innovative Photoactivated Ruthenium Chemotherapy to Treat Eye Cancer

Sylvestre BONNET, [a)]

[a)] *Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333CC Leiden, The Netherlands; bonnet@chem.leidenuniv.nl*



Uveal melanoma (UM) is a rare tumor of the eye; its current treatment leads to lower quality-of-life for the patients (sight loss), 50% of whom eventually die from metastases to the liver. The PACT4EYE project aims at the first clinical development of a new technique called photo-activated chemotherapy (PACT) for the treatment of UM. It makes use of a new, patented ruthenium-containing prodrug (Ru-MTI) that must be activated by green or red light to become toxic.[1, 2]

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IL 13

Metalloporphyrinoids for Phototheranostics: From Molecular Design to Targeted Imaging and Therapy in Advanced Biological Models

Janusz M. DABROWSKI, [a)]

[a)] *Faculty of Chemistry, Jagiellonian University, Kraków, Poland; janusz.dabrowski@uj.edu.pl*

Recent developments in bioinorganic chemistry have illuminated the versatility of metal complexes as phototheranostic agents. In particular, metallated porphyrinoids—macrocyclic compounds coordinated with transition metals offer a unique opportunity to integrate targeted photodynamic therapy (PDT) with real-time fluorescence or photoacoustic imaging [1,2].

This contribution focuses on the rational design, photophysical characterization, and biological evaluation of Zn(II)-, Pd(II)-, and Pt(II)-coordinated porphyrins, phthalocyanines, and bacteriochlorins intended for the image-guided treatment of infections and solid tumors. Using time-resolved singlet oxygen phosphorescence, EPR spin trapping, and transient absorption techniques, we established structure–property relationships that link metal center identity, macrocyclic halogenation, and axial substitution to singlet oxygen yield, triplet-state lifetime, and photostability. Biological validation included both standard and advanced 3D models, ranging from MDR bacterial biofilms and infected keratinocytes to hiPSC-derived tumor organoids and murine models of wound infections and colon carcinoma (Figure 1).

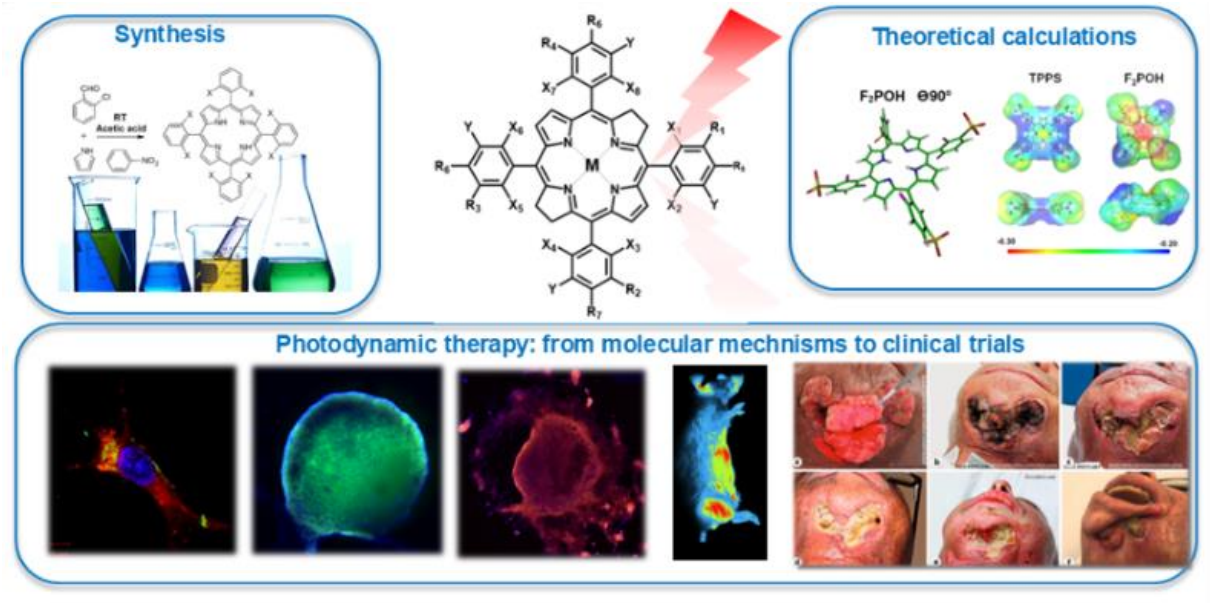

Figure 1. Schematic overview of the main areas of current research activity discussed in this work.

Porphyrin complexes bearing Zn(II) and Pd(II) centers exhibited potent antimicrobial photoinactivation, including complete eradication of *Staphylococcus aureus* biofilms and inhibition of bacterial invasion into epithelial cells [3]. Structural optimization for near-infrared (NIR) activation led to the identification of two lead compounds: $ZnSO_2tBu$ (a sulfonated Zn(II)

phthalocyanine) and F_4BMet (a fluorinated sulfonamide bacteriochlorin). These compounds combined strong NIR absorption, high singlet oxygen generation, and tumor-selective biodistribution. Notably, $ZnSO_2$tBu induced complete regression of CT26 tumors in 84% of treated mice following vascular-targeted PDT, while F_4BMet enabled durable remission in 30% of animals and allowed real-time tumor visualization [4,5].

Most recently, we developed novel tetrapyrrolic bioconjugates incorporating acetaminophen moieties for cyclooxygenase-2 (COX-2)-guided PDT. These compounds leverage overexpression of COX-2 in the tumor microenvironment to achieve selective accumulation and phototoxicity. The lead conjugate induced up to 80% complete responses in vivo and modulated key biochemical pathways, including prostaglandin and VEGF signaling.
Altogether, this work illustrates how inorganic coordination chemistry, combined with molecular pharmacology and advanced *in vitro* and *in vivo* biological models, can deliver next-generation metal-based agents for highly selective and mechanistically tailored photodynamic interventions.

Acknowledgments:
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IL 14

Engineered hemoproteins for selective C–N and C–O bond formation *via* abiological mechanisms

Rudi FASAN, [a)]

[a)] *Department of Chemistry and Biochemistry, The University of Texas at Dallas Richardson (TX), United States; rudi.fasan@utdallas.edu*

Our group is interested in expanding the reaction scope of biological catalysts beyond that encompassed by naturally occurring enzymes. This talk will describe our recent progress toward engineering heme-containing proteins and enzymes into efficient biocatalysts for mediating C—H amination reactions via nitrene transfer, a synthetically valuable transformation useful for forging new C—N bonds. Leveraging mechanistic insights on productive and unproductive pathways implicated in these non-native reactions, we will also describe the development of engineered hemoproteins for selective functionalization of an aliphatic C-H bond into a C-O bond via an unprecedent, abiological mechanism. These studies extends the reactivity of naturally occurring metalloenzymes and expands the range of synthetically valuable transformations accessible through biocatalysis for organic synthesis, medicinal chemistry, and other applications.

IL 15

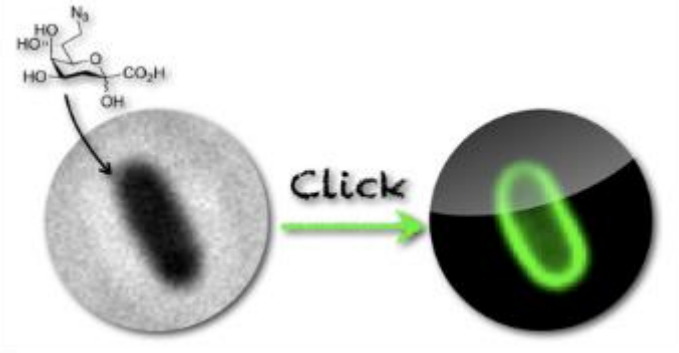
Chemical biology for the detection of pathogens and reactive oxygen species

Boris VAUZEILLES, [a)]

[a)] *Chemical Biology Department, ICSN, CNRS UPR 2301, Université Paris-Saclay, 91198 Gif-sur-Yvette (France); boris.vauzeilles@cnrs.fr*

The development and application of synthetic tools to explore biological processes, as well as the use of biological systems as a source of inspiration for the design of molecular devices, are the main focus of our research.

This lecture will present a selection of some of our work in this area, which involve the development of new labeling strategies for the detection and identification of live bacteria,[1] including *Legionella pneumophila*, a serious pathogen responsible for Legionnaires' disease, as well as the design and evaluation of new, fast-reactive hydrogen peroxide-sensitive borinate triggers for the elaboration of probes for the detection of reactive oxygen species.[2]



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IL 16

Real-Time Electrochemical and Spectroscopic Mapping of Amyloid-like Peptide Fibrillization

Justyna SAWICKA, [a] Arian KOLA, [b] Milena CZESZEJKO, [a] Zuzanna PIETRALIK-MOLINSKA, [c] Piotr HAŃCZYC, [d], [e] Paweł NIEDZIAŁKOWSKI, [a] Mateusz LEŚNIEWSKI, [a] Tomasz KULESZA, [a] Maciej KOZAK, [c] Adam LIWO, [a] Daniela VALENSIN, [b] Sylwia RODZIEWICZ-MOTOWIDŁO, [a]

[a] Faculty of Chemistry, University of Gdańsk, Poland; [b] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy; [c] Department of Macromolecular Physics, Adam Mickiewicz University, Poland; [d] Institute of Experimental Physics, University of Warsaw, Poland; [e] Center of Cellular Immunotherapies, Warsaw University of Life Sciences, Poland; s.rodziewicz-motowidlo@ug.edu.pl

The early stages of amyloid fibrillization are elusive due to their transient nature and low signal amplitudes. We propose a multimodal strategy using EIS, AFM, NMR, and enhanced ThT lasing to track fibril formation of the model peptide NH_2-QAGIVV-NH_2 [1] in real time. Gold electrodes functionalized with the peptide enabled sensitive detection of aggregation via EIS, revealing a Boltzmann-type shift in charge transfer resistance (ΔRct) with a kinetic inflection point at ~65 h. Parallel NMR experiments captured gradual emergence of new amide and aliphatic signals over 96–132 h without stable secondary structures but with strong local perturbations, particularly around Val5/Val6. ThT lasing revealed two aggregation thresholds with temperature-dependent kinetics, indicating rapid β-sheet formation. High peptide concentrations (125–250 mg/mL) accelerated the process, with mature fibrils forming after ~21 h at 37 °C and ~29 h at room temperature. AFM and TEM confirmed formation of unbranched fibrils ~7–12 nm thick and >500 nm long, consistent with amyloid-like morphology. Collectively, our data validate EIS and lasing as powerful tools for capturing early-stage fibrillization kinetics and molecular organization of short amyloidogenic peptides. Additionally, coarse-grained molecular dynamics simulations were employed to explore fibrillization kinetics. Although not directly time-correlated with experiments, simulations showed a sharp transition from transient aggregates to mature fibrils, reflecting key experimental features.

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IL 17

Structural insights and redox kinetics of ancestral cytochrome P450 reductase in nanodiscs.

Kamila STOKOWA-SOLTYS, [a), b)] Yichi ZHANG, [c)] Francisco ZARATE-PEREZ, [a)] Elizabeth M. J. GILLAM, [c)] John C HACKETT, [a)]

[a)] Department of Chemistry and Biochemistry and Biomolecular Sciences Institute, Florida International University, Miami, United States, [b)] Faculty of Chemistry, University of Wroclaw, Wroclaw, Poland, [c)] School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, Australia; kamila.stokowa-soltys@uwr.edu.pl

The stepwise supply of electrons from NADPH to cytochromes P450 (CYPs) *via* cytochrome P450 reductase (CPR) supports many oxidation reactions that play a crucial role in the metabolism of endogenous compounds and the detoxification of exogenous substances. It is the only known membrane-bound flavoprotein localized in endoplasmic reticulum. CPR contains a hydrophobic N-terminal which serves as a membrane anchoring domain and a redox active C-terminal flavoprotein domain. The latter domain shuttles electrons from NADPH to FAD, and then to FMN to finally reach the heme of CYP. The membrane provides a scaffold for protein interactions, and possibly modulates catalytic activity. Understanding the impact of the membrane on protein dynamics, ligand recognition, and catalysis present significant challenges. Therefore, we incorporated an ancestral CPR into lipoprotein nanodiscs (CPR-NDs) including POPC and POPG lipids or liver lipids. Importantly, both ancestral CPR and CPR-NDs readily reduced cytochrome c. Low resolution solution structures were determined by size exclusion chromatography-small-angle X-ray scattering (SEC-SAXS) and a hybrid modeling approach. Furthermore, the influence of lipid composition on the kinetics of flavin reduction by NADPH was studied using the stopped-flow UV-vis and fluorescence measurements. The NDs ensure both catalytically active reductase and afford NADPH binding measurements in a native-like membrane environment. In summary, CPR-NDs are catalytically active models that are readily interrogated by small angle scattering methods to reveal the functional orientation in and possible interactions with membranes.

Acknowledgments:
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IL 18

Metalloproteome remodeling in calprotectin-stressed *Acinetobacter baumannii* using chemoproteomics

Maximillian K. OSTERBERG, [a] Daniel W. BAK, [b] Claudia ANDREINI, [c] Jeanette M. CRITCHLOW, [d] Walter J. CHAZIN, [e] Eric P. SKAAR, [d] Eranthie WEERAPANA, [b] and David P. GIEDROC, [a]

[a] Department of Chemistry, Indiana University, Bloomington, IN 47405-7102 USA [b]Department of Chemistry, Boston College, Chestnut Hill, MA 02467-3860 USA [c] Magnetic Resonance Centre (CERM), University of Florence, Florence, Italy [d] Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232, USA; [e] Departments of Biochemistry and of Chemistry, Vanderbilt University, Nashville, TN 37240, USA; giedroc@iu.edu

The growth of bacterial pathogens is limited by "nutritional immunity", where the infected host deploys metal scavenging proteins to starve the pathogen of essential transition metals. An important transition metal-sequestering proteins is the Ca^{2+}-activated S100A8-S100A9 heterotetramer, calprotectin (CP). Prior work shows that CP induces a significant Fe- and Zn-starvation response in the opportunistic pathogen, *Acinetobacter baumannii*, in liquid culture [1-2]. Here, we employ a quantitative thiol-specific chemoproteomics platform to pinpoint changes in abundance-corrected cysteine reactivity, and by extension cellular metal occupancy in metalloenzymes, that occur when *A. baumannii* is incubated with physiological CP in liquid culture relative to an untreated WT control. Changes in protein abundance with CP stress reveals a pronounced Zn-limitation and Fe-starvation response and reciprocal regulation of three enzymes of central carbon metabolism, including aconitase. A majority of 2645 quantifiable Cys-containing peptides that show an increase in abundance-corrected Cys reactivity (150) are derived from known Zn- and Fe-S-cluster proteins, revealing a significant decrease in metal occupancy in the proteome. Myriad cell processes are compromised by undermetalation of the metalloproteome, including enzymes that function in the TCA cycle and respiration, GTP metabolism [1,3], ribosome remodeling, tRNA charging, and proteostasis. A direct comparison of a strain lacking the candidate metallochaperone ZigA [4] (ΔzigA) with the wild-type strain reveals that the loss of ZigA is effectively silent in this assay. We conclude that CP induces a widespread, negative impact on the metalation status of the metalloproteome that results in a significant nutrient limitation response. Supported by grants from the US National Institutes of Health (R35 GM118157, R01 AI101171, T32 GM131994).

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IL 19

Selective recognition of As^{III} and Sb^{III} by AfArsR, a transcriptional metalloid regulatory protein

Annamária TÓTH,[a] Béla GYURCSIK,[a] Lars HEMMINGSEN,[b] Bálint HAJDU,[a,b] Éva HUNYADI-GULYÁS,[c] Zeyad H. NAFAEE,[a] Juliana SCHELL,[d] Atsushi KAWAGUCHI,[e] Attila JANCSÓ [a]

[a] Department of Molecular and Analytical Chemistry, University of Szeged, Hungary; [b] Department of Chemistry, University of Copenhagen, Denmark; [c] Laboratory of Proteomics Research, Biological Research Centre, Hungarian Research Network (HUN-REN), Hungary; [d] European Organization for Nuclear Research (CERN), Switzerland; [e] Department of Infection Biology, Faculty of Medicine, University of Tsukuba, Japan;
jancso@chem.u-szeged.hu

Transcriptional metalloregulators are highly sensitive and metal ion selective proteins that regulate bacterial metal ion and metalloid homeostasis, initiated by the binding of a cognate metal(loid) ion to their effector binding site.[1] A very important question regarding the operation of these sensory proteins is how they recognise the correct metal or metalloid ions even if other ions may potentially bind just as efficiently, or even more strongly to their binding sites.

AfArsR is a bacterial transcription factor from *Acidithiobacillus ferrooxidans* that regulates metalloid detoxification by responding selectively to As^{III} and Sb^{III}. The homodimeric protein coordinates these metalloids at its metalloid binding site (MBS) consisting of three cysteine residues located at the flexible C-terminal region in each monomer chain.[2] A recent solution spectroscopic study on a 10-mer oligopeptide, probing the MBS of the AfArsR protein, allowed us to propose some of the possible structural factors leading to the recognition of the cognate metalloids.[3] Nevertheless, investigation of the whole protein is essential to get a more comprehensive insight into the derepression processes, approached by experiments both under *in vitro* and cellular conditions.

UV-monitored titrations of the AfArsR protein by the cognate metalloids As^{III} and Sb^{III}, as well as by other non-cognate metal ions, e.g. Hg^{II}, Pb^{II}, Cd^{II} and Zn^{II}, indicated that all these ions are bound tightly by the protein. The effect of these ions on the interaction of the protein with the regulated specific DNA was investigated by electrophoretic mobility shift assays (EMSA). The obtained data reflected that only the metalloids can promote the unbinding of the protein from the DNA, however, competition studies also showed that the presence of Hg^{II} can prevent this by the presumable displacement of the protein-coordinated metalloids. The influence of these ions on the DNA-protein interaction was also followed by a cellular assay (I-Block [4]), reflecting the functional metalloid selectivity of this protein in the derepression process inside the cells.

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IL 20

Understanding bacterial metal-binding proteins to disrupt ion homeostasis and antimicrobial resistance

Aleksandra HECEL, [a] Arian KOLA, [b] Daniela VALENSIN, [b] Danuta WITKOWSKA, [c] Henryk KOZŁOWSKI, [a), c)] Magdalena ROWIŃSKA-ŻYREK, [a]

[a] Faculty of Chemistry, University of Wroclaw, 50-383 Wroclaw, Poland; [b] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, 53100 Siena, Italy; [c] Institute of Health Sciences, University of Opole, 45-060 Opole, Poland; aleksandra.hecel2@uwr.edu.pl

Transition metal ions are indispensable for many microorganisms, as they support key biological functions and contribute to their pathogenic potential. Pathogens secrete specialized metal-binding molecules called metallophores, which have drawn considerable attention due to their effectiveness in capturing and delivering essential metals [1].

Bacterial zinc balance is controlled by systems like the Zn(II)-specific transporter ZnuABCD, responsible for regulating zinc import, distribution, and removal. Histidine residues play a central role in zinc coordination within proteins. For example, histidine-rich loops found in proteins such as ZnuA [2] and ZnuD act as “metal catchers”, capturing zinc ions and delivering them to specific protein regions. Binding of metal ions to ZnuD also causes structural rearrangements within the protein [3].

Nickel is toxic to bacteria, posing a challenge that is addressed by proteins like HypB, a chaperone that inserts nickel into [NiFe]-hydrogenases. In *E. coli*, the metal-binding capacity of HypB’s N-terminal segment surpasses that of its GTPase domain, with a particular preference for Ni(II) ions over other metal-binding proteins such as SlyD [4].

While copper import in bacteria has been less studied, the outer membrane transporter OprC has been identified as a key player in this process. OprC facilitates copper uptake through a unique CxxxM-HxM metal-binding site. The interaction between metallophore CopM and OprC has been extensively studied. We identified two metal-binding sites in CopM, with MxxHH and MHxxH motifs, both capable of binding Cu(II). At pH 7, the MxxHH motif shows the highest affinity for Cu(II), suggesting that it binds copper more tightly than the CxxxMHxM site in OprC. This indicates that CopM likely transports copper into the cell alongside the metal through OprC [5].

Elucidating these metal transport pathways in bacteria offers promising strategies to disrupt bacterial physiology and could play a significant role in overcoming antimicrobial resistance (AMR) by targeting microbial metal uptake systems with new therapeutic interventions.

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ORAL COMMUNICATIONS

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OC 1

NMR Studies of Multidomain Snail Metallothioneins: Metalation and Metal Selectivity

Oliver ZERBE, [a] **Pengfei LIU**, [a] **Christian BAUMANN**, [a] **Renato VALSECCHI**, [a]

[a] *Department of Chemistry, University of Zurich, 8057 Zurich, Switzerland*

Metallothioneins (MTs) are metalloproteins rich in cysteine residues, that coordinate to mono- or divalent metal ions via these cysteine residues [1]. Snail MTs play important biological role in heavy metal detoxification [2,3]. A single snail MT can immobilize up to 6 divalent or 12 monovalent metal ions. MTs proteins are unfolded in the metal-free (apo) state but assume a distinct structure upon coordinating to metal ions.

MT from the roman snail *Helix pomatia* (HpMT) was the first to be discovered that exists in Cd/Zn and Cu-selective isoforms[4]. We recently determined the structure of HpMT in the Zn- and Cd-bound states[5]. By reconstructing the phylogenetic tree and expressing the such-determined proteins we could demonstrate that heteronuclear 2D is very suitable to investigate conformity of sequences to binding a specific metal[6]. Metal-selective isoforms exist in a well defined state resulting in high-quality NMR spectra, unselective forms reveals signatures of conformational exchange.

Heteronuclear NMR can also be used to study metalation of apo-MT by their respective metal ions. To this end we have added metal ions to the apo form *Littorina Littorea* MT (LlMT), a 3-domain MT [7]. We could demonstrate that metal ions are preferably integrated into the C-terminal domain at sub-stoichiometric metal concentrations. We could also show that metal ions can be transferred from metalated to apo protein, and many MTs can serve as metal donors. Importantly, metal transfer between either apo and metalated forms or between two differently metalated MTs only occurs when proteins can form direct contacts.

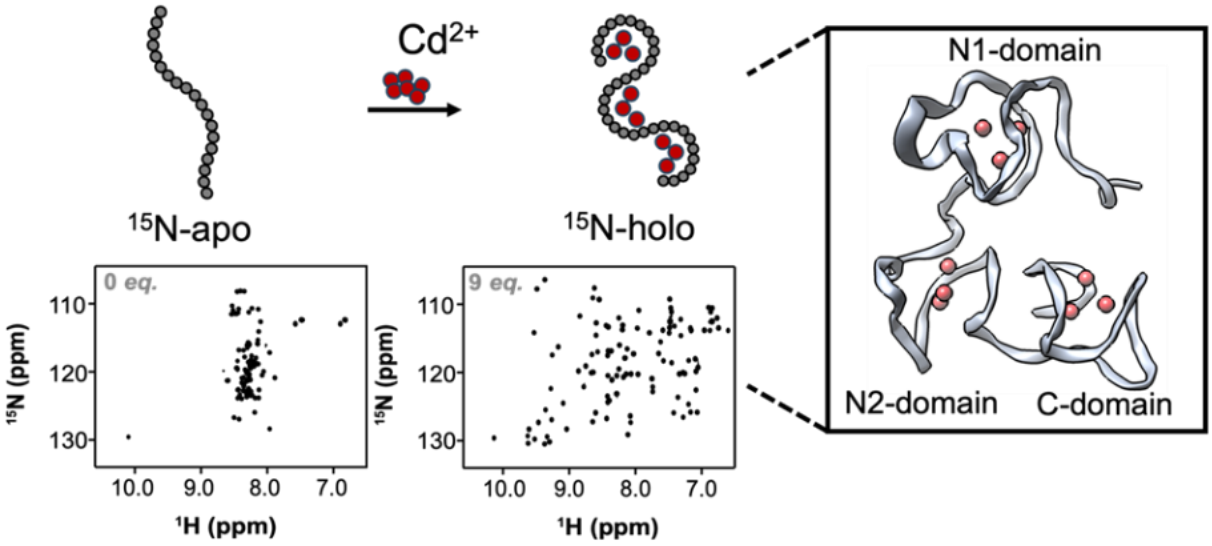

Figure: Addition of Cd ions into the three domains of apo-LlMT is followed by [^{15}N,^{1}H]-HSQC spectra

Some of the snails live in deep sea and are hence exposed to high aquatic pressures. To study how metalloproteins, and in particular MTs, react to high pressure, and to compare their behavior to non-metal binding globular proteins we have studied a deep see MT under high pressure in the NMR tube. Surprisingly, these proteins do not denature even at 3 kbar of pressure. However, we could demonstrate that hydrogen bonds are destabilized. I will reveal how NMR parameter in these high-pressure experiments are related to the presence of cavities and to proximity to metal-binding sites.

Finally, I will reveal our recent data on Cu-metallothioneins. We have compared Cu- and Cd/Zn-selective *HpMT* isoforms. We have attempted to swap metal selectivity of these isoforms with a minimal number of mutations. The work has been very insightful for us to understand the molecular determinants of metal selectivity.

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OC 2

Clavanin C Analogue Complexes with Cu^{2+} and Zn^{2+}: A Strategy Against MRSA

Jakub GAWŁOWSKI, [a] Mariusz DZIADAS, [a] Agnieszka MATERA-WITKIEWICZ, [b] Daniela VALENSIN, [c] Magdalena ROWIŃSKA-ŻYREK, [a]

[a] Faculty of Chemistry, University of Wroclaw, ul. F. Joliot-Curie 14, 50-383 Wroclaw, Poland.
[b] Faculty of Pharmacy, Wroclaw Medical University, ul. Borowska 211a, 50-556 Wroclaw, Poland.
[c] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy; Jakub.gawlowski@uwr.edu.pl

Antibiotic resistance is a growing global threat. According to projections by the World Health Organization (WHO), multidrug-resistant bacterial infections could be responsible for up to 10 million deaths annually by 2050. Among the most promising candidates for combating this crisis are antimicrobial peptides (AMPs), which exhibit strong antimicrobial properties [1].

The coordination of divalent metal ions can significantly enhance the antimicrobial efficacy of AMPs. For instance, clavanin C - a marine AMP isolated from *Styela clava* - forms complexes with Cu^{2+} and Zn^{2+} ions that demonstrate potent activity against various pathogens [2]. However, the clinical application of AMPs is often hampered by their low enzymatic stability.

One strategy to improve the resistance of peptides to enzymatic degradation is enantiomeric substitution of the native peptide. A particularly innovative approach in the field of bioinorganic chemistry is the retro-inverso modification. This technique involves both the reversal of the peptide sequence and the substitution of L-amino acids with their D-enantiomers (Figure 1), thereby enhancing both stability and bioactivity.

Native clavanin C	VFHLLGKIIHHVGNFVYGFSHVF
D-clavanin C	vfhllgkiihhvgnfvygfshvf
Retro-inverso clavanin C	fvhsfgyvfngvfhiikgllhfv

Figure 1. Amino acid sequences of investigated peptides.

Potentiometric titration, mass spectrometry and spectroscopic methods (UV-Vis, CD and Far-UV CD) allowed us to determine coordination spheres of modified clavanins complexes and learn their secondary structures. We also used the HPLC method to evaluate the effect of enantiomeric exchange on enzymatic stability of peptides.

In our study, we investigated the coordination properties and antimicrobial activity of modified clavanin C peptides. Furthermore, we explored the impact of enantiomeric substitution strategies on the enzymatic stability of clavanin C.

Acknowledgements:
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OC 3

Microplusin: a strong copper-chelating and effective antimicrobial, natural peptide

Silvia LEVERARO, [a] Denise BELLOTTI, [a] Klaudia SZARSZOŃ, [b] Magdalena ROWIŃSKA-ŻYREK, [b] Maurizio REMELLI, [a]

[a] Department of Chemical, Pharmaceutical and Agricultural Sciences, Università degli Studi di Ferrara, via Luigi Borsari 46, 44121 Ferrara, Italy
[b] Faculty of Chemistry, University of Wroclaw, ul. F. Joliot Curie 14, 50-383 Wroclaw, Poland; silvia.leveraro@unife.it

Nowadays, the phenomenon of antimicrobial resistance is a serious public health challenge. The emergence of strong resistance mechanisms among bacteria is rendering many diseases difficult to treat, consequently diminishing the efficacy of previously effective drugs. One of the main aims of researchers is to identify and design novel drugs effective against pathogens. Among potential candidates, antimicrobial peptides (AMPs) represent a very interesting substrate for drug design due to their broad spectrum of activity, different mechanisms of action and low propensity to induce antimicrobial resistance. Natural AMPs are abundant across all kingdoms of life; microplusin is a representative example.

Microplusin is an antimicrobial peptide isolated from the thick *Rhipicephalus microplus*. Structurally, microplusin consists of five α-helices with disordered N- and C-termini. It contains six cysteine residues forming three disulfide bonds and a histidine-rich region in both the N- and C-terminal domains [1]. This peptide shows a broad spectrum of activity, being efficacy against Gram-positive and Gram-negative bacteria, fungi and yeast at micromolar and submicromolar concentrations [2].

Different studies indicate that while microplusin does not disrupt bacterial membranes, it acts as a potent chelator of copper ions. The ability of an AMP to chelate metal ions can confer an antimicrobial effect by sequestrating these essential micronutrients from the surrounding environment, thereby depriving pathogens of nutrients for their survival and growth. This mechanism is known as “nutritional immunity”. Furthermore, microplusin has been shown to negatively affect cellular respiration in *Micrococcus luteus,* most likely through the removal of copper ions from heme-copper terminal oxidases [3].

We decided to study short fragments of this protein, corresponding to the N-terminal (HHQEL) and C-terminal (DPEAHHEHDH) domains. Both terminal sequences are unfolded and rich in histidine residues and lie on the same side of the structure. Previous NMR studies suggest a preferred binding site in the N-terminus, however an unequivocably determination of the binding residues has not been obtained and the bioinorganic chemistry of microplusin still remains unravelled. By means of different experimental techniques (mass spectrometry, potentiometry, UV-Vis spectrophotometry and circular dichroism) we have characterized the formed copper complexes with the selected fragments and compared the Cu(II) binding behavior of the two putative N- and C- terminal sequences of microplusin.

Financial support of the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.1 – NextGenerationEU (grant PRIN PNRR 2022-P2022EMY52; CUP F53D23008960001; project title: “EnzyMime – Biocompatible and sustainable enzyme mimics

based on metal complexes with peptide derivatives: synthesis, characterization and potential biological applications") is gratefully acknowledge.

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OC 4

Targeting Bacterial Metalloproteinases: Peptide-Based Strategies for Inhibiting Virulence Factors

Martyna ZAWADA, [a] Paulina POTOK, [a] Sławomir POTOCKI, [a]

[a] Faculty of Chemistry, University of Wroclaw, 50-383 Wroclaw, Poland; martyna.zawada@uwr.edu.pl

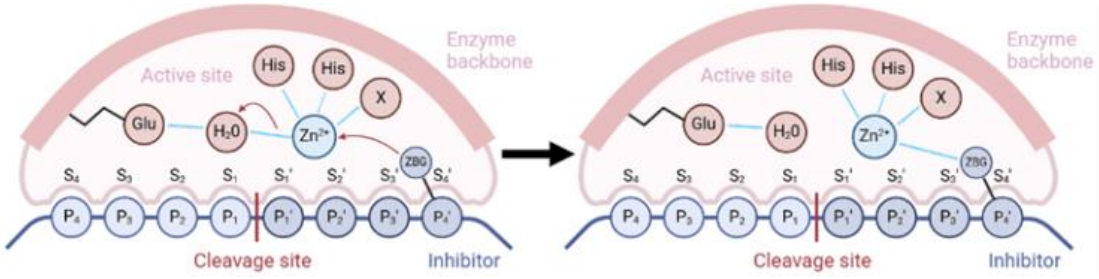

Figure 1: Mechanism of inhibition by a substrate-mimicking inhibitor. The inhibitor binds to the active site via a zinc-binding group (ZBG), preventing substrate cleavage and release [1].

The growing threat of antibiotic resistance has turned once-manageable infections into serious health challenges. As traditional antibiotics become less effective, the need for alternative therapeutic strategies is becoming increasingly urgent [2]. Among the key virulence factors produced by pathogenic bacteria are metalloproteinases (MPs) from the M4 and M10 families. These enzymes contribute to pathogenesis by breaking down host tissues and evading immune defenses. Their catalytic activity relies on the coordination of Zn(II) at the active site, making the zinc ion both essential for function and a promising target for inhibition [3].

In our work, we analyzed MPs from antibiotic-resistant bacterial strains, focusing on how their active sites interact with Zn(II) and Cu(II). We investigated the coordination properties of these metal ions to assess whether copper could competitively bind and displace zinc, thereby inhibiting enzymatic activity. To pinpoint the residues most critical for metal binding, we conducted point mutations on selected amino acids within the active site [4].

We also developed substrate-mimicking peptide inhibitors designed to resemble the natural substrates of these enzymes. These peptides aim to improve specificity and pharmacological behavior compared to conventional inhibitors (Fig. 1) [5]. Their interactions with both metal ions and enzyme active sites were thoroughly examined.

A combination of physico-chemical methods including mass spectrometry, NMR, UV–Vis spectroscopy, and circular dichroism was employed to characterize the binding affinities and structural changes associated with metal coordination and inhibitor binding. These studies provided detailed insight into the differing behaviors of zinc and copper and their influence on enzyme-inhibitor interactions.

By dissecting the coordination chemistry at the heart of bacterial metalloproteinase function, our study lays the groundwork for designing selective inhibitors. This approach offers a novel direction for weakening bacterial virulence and contributes to the ongoing search for effective strategies to combat antibiotic resistance.

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OC 5

MCT1- targeting copper complex in nuclear medicine: a preliminary investigation for theranostic applications.

Matteo MARI, [a] Carol IMBRIANO, [b] Silvia BELLUTI, [b] Mattia ASTI, [c] Veronique PATINEC, [d] Raphäel TRIPIER, [d] Alfonso ZAMBON, [a] Erika FERRARI, [a]

[a] Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia, via G. Campi 103, 41125, Modena, Italy.
[b] Department of Life Sciences - University of Modena and Reggio Emilia, via Campi, 213/d, 41125, Modena, Italy;
[c] Radiopharmaceutical Chemistry Section, Nuclear Medicine Unit, Azienda USL-IRCCS Reggio Emilia, via Amendola 2, 42122, Reggio Emilia, Italy.
[d] Univ de Brest, UMR-CNRS 6521 CEMCA, 6 avenue Victor le Gorgeu, F-29200 Brest, France; matteo.mari@unimore.it

MCT1 is a membrane protein responsible for the transport of lactate normally expressed in healthy tissues. However, in some cancer lines its expression results upregulated and allows the establishment of the "Warburg effect". This mechanism explains the aggressive character of specific tumoral types and allows MCT1 to be a potential target for theranostic applications in oncology [1]. To date, only few molecules acting as inhibitors for MCT1 are known [2], and the interaction of some of them with MCT1's pocket has been elucidated by X ray crystal data [1]. One of the recently developed MCT1 inhibitor (7ACC2), that reached clinical trials, is a coumarin derivative [2]. Coumarins are natural phyto-compounds known since the 19th century, their scaffold has attracted the attention of synthetic and medicinal chemists for decades and showed a large variety of biological activities [3]. The structure of 3-carboxy,7-hydroxycoumarin (7ACC2) was modified to insert a bifunctional chelator (BFC) to obtain a platform that can be labelled with a suitable radiometal.

Copper shows a triplet of isotopes useful for both diagnosis and therapy (^{61}Cu, ^{64}Cu, ^{67}Cu). No3py is a well-known polyazamacrocycle chelator that quickly forms a stable complex with copper (II) at room temperature [4], encouraging its application for radiolabelling.

The bifunctional version of no3py (no3pyCOOH) was conjugated to the coumarin targeting vector using a PEG chain long enough to reduce the impact of the metal complex on MCT. The obtained molecule is intended to have high affinity for MCT1 and deliver the appropriate radioactivity to the tumoral site.

Efforts are now devoted to the design and synthesis of new no3py derivatives bearing negative charged moieties in order to tune the polarity and the net charge of the resulting copper (II) complexes. This would influence the interaction with MCT1 as well as the whole pharmacokinetic profile of the obtained drug.

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OC 6

Targeting the Zinc Active Sites of MMP-1 and MMP-14: Binding Characteristics of Novel Peptide Inhibitors

Paulina POTOK, [a] Robert WIECZOREK, [a] Oscar PALACIOS, [b] Merce CAPDEVILA, [b] Elżbieta GUMIENNA-KONTECKA, [a] Sławomir POTOCKI, [a]

[a] Faculty of Chemistry, University of Wroclaw, Joliot-Curie 14, 50-383 Wroclaw, Poland; [b] Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain; paulina.potok@uwr.edu.pl

According to the International Agency for Research on Cancer, cancer caused 9.7 million deaths worldwide in 2022. By 2050, the number of new cases is expected to reach 35 million, a 77% increase [1]. Despite advances in targeted therapies, cancer metastasis remains the main cause of cancer-related deaths. To address this problem, this study focuses on two matrix metalloproteinases, MMP-1 and MMP-14, zinc-dependent enzymes strongly linked to tumour progression [2]. Metalloproteinases are a diverse group of enzymes present in both bacteria and humans, where they participate in physiological processes as well as in pathogenic mechanisms [3, 4]. The main goal is to identify peptide-based inhibitors and characterise their interactions with Zn(II) in the MMPs' active sites using peptide models.

Two strategies were used to select and design inhibitors. Peptide sequences identified by phage display screening in previous studies were chosen as MMP-14 inhibitors [2]. For MMP-1, inhibitors were designed based on the amino acid sequences of natural substrates. The binary complexes of Zn(II) with each inhibitor and the ternary ones formed between the inhibitor and the Zn(II)-MMP-1 or Zn(II)-MMP-14's active site were analysed using potentiometric titrations, mass spectrometry, nuclear magnetic resonance spectroscopy, and density functional theory calculations. These methods were applied to determine their stoichiometry, coordination modes, and thermodynamic stability. The results showed that despite differences in design strategy and target enzyme, the most stable inhibitors in each group shared a common structural feature: the zinc-binding group (ZBG) was located near the N-terminal region of the inhibitor sequence. For MMP-14, the phage display-derived Inhibitor with the sequence SDMAHSLPGHSH demonstrated the highest thermodynamic stability in ternary complexes. This peptide coordinated Zn(II) through aspartic acid residues and formed cooperative hydrogen-bonding networks, resembling interactions observed in natural tissue inhibitors of metalloproteinases [2]. Among the MMP-1 inhibitors, inhibitor with the sequence CPQGLGR showed the strongest preference for ternary complex formation, as indicated by positive ΔlogK and percent relative stabilisation values.

These findings highlight that the spatial arrangement of the ZBG in inhibitor play a key role in determining the stability and coordination properties of the complexes. The observed thermodynamic parameters support the selection of these peptides as promising candidates for further development as selective MMP inhibitors.

The financial support from the Polish National Science Centre (NCN, UMO-2023/49/N/ST5/03831) is gratefully acknowledged.

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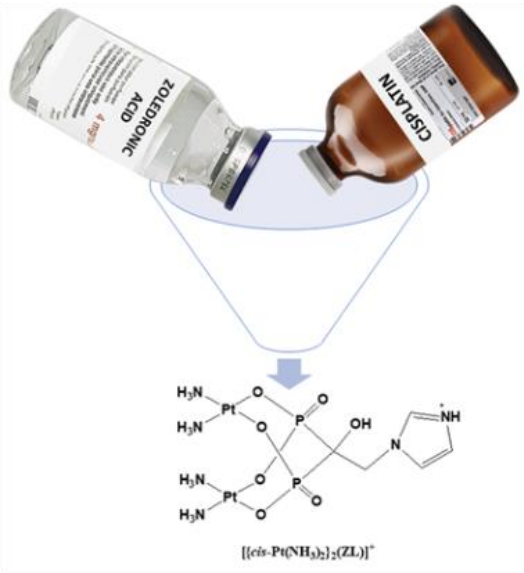
OC 7

Commercial bisphosphonate drugs for targeting Pt(II)-complexes to bone tumors and metastases.

Alessandra BARBANENTE, [a] Anna Maria DI COSOLA, [a] Mauro NISO, [b] Nicola MARGIOTTA, [a]

[a] Dipartimento di Chimica. Università degli Studi di Bari Aldo Moro. Via E. Orabona, 4. 70125 Bari (Italy). [b] Dipartimento di Farmacia-Scienze del Farmaco. Università degli Studi di Bari Aldo Moro. Via E. Orabona, 4. 70125 Bari (Italy); alessandra.barbanente@uniba.it

Bone cancer and bone metastases, often stemming from primary cancers such as breast and prostate cancer, are extremely painful and challenging to be treated, typically by surgery, radiotherapy, chemotherapy and analgesics.[1] Platinum-based drugs are effective against bone cancers and metastases but are limited by severe systemic side effects due to poor specificity. In order to improve drug targeting towards bone tumors and metastases and to reduce systemic toxicity, commercial bisphosphonate (BP) ligands, such as zoledronate and pamidronate, which selectively accumulate in bone tissue, have been conjugated with clinically used Pt-based drugs such as cisplatin and oxaliplatin [2,3]. We show here the synthesis and full characterization of a series of dinuclear Pt(II)-BP complexes obtained by the combination of the commercial drugs, that were also tested for their stability under physiological and acidic conditions, for their reactivity with DNA models, and for their cytotoxicity against a panel of human tumor cell lines.



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OC 8

Diversity of Aβ Isoforms: Toward Novel Biomarkers for Diagnosis of Alzheimer's Disease

Aleksandra TOBOLSKA, [a] Klaudia GŁOWACZ, [a] Katarzyna BIERNAT, [a] Aleksandra DĄBAŁA, [a] Wojciech WRÓBLEWSKI, [a] Patrycja CIOSEK-SKIBIŃSKA, [a] Nina E. WEZYNFELD, [a]

[a] Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland; nina.wezynfeld@pw.edu.pl

Alzheimer's disease (AD) is the most prevalent form of neurodegenerative disorder, marked by a gradual decline in memory and cognitive function. Among the molecular mechanisms associated with AD, amyloid-beta (Aβ) aggregation is the most thoroughly studied. Diagnostic efforts often rely on measuring the ratio of Aβ(1-42) to Aβ(1-40) in cerebrospinal fluid.
However, the Aβ family encompasses a wide range of peptide variants, also truncated at the N-terminus. For instance, levels of N-terminally truncated Aβ(4-x) peptides in the brain can exceed those of the full-length Aβ(1-x) peptides. The pyroglutamated Aβ isoforms, Aβ(pyr3-x) and Aβ(pyr11-x), were found to trigger the nucleation of Aβ aggregates. The Aβ(5-x) amount is rising during clinical trials for AD with BACE1 inhibitors. This diversity could also be extended with post-translational modifications, such as phosphorylation. Our research has shown that N-truncations and modifications could significantly alter the ability of Cu(II)-Aβ complexes to generate reactive oxygen species (ROS), a known pathological hallmark of AD.
Such variety in the redox activity of Cu(II)/Aβ could also be employed in the recognition of Aβ isoforms. To explore it, we designed libraries of Aβ analogues and recorded the electrochemical signals of their Cu(II) complexes. They were further analysed using Principal Component Analysis (PCA), enabling effective recognition of single substitutions in the peptide sequences [1] and the position and number of phosphorylated residues.
An alternative strategy involves using quantum dot–Aβ peptide interactions, which generate distinctive fluorescence signals. The chemometric analysis of these fluorescence patterns provided robust discrimination among different Aβ peptides [2].
Our focus is on combining electrochemical and spectroscopic signal analysis with machine learning models to create a sensitive diagnostic platform for early AD detection and detailed profiling of Aβ-related pathological changes.

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OC 9

Metalloporphyrinoids in Neurodegeneration: NMR-Based Interaction and Protective Effects on SH-SY5Y Cells

Arian KOLA, [a), b)] Valerio CICCONE, [a)] Matteo TEGONI, [c)] Simone DELL'ACQUA, [d)] Lucia MORBIDELLI, [a)] Daniela VALENSIN, [b)]

[a)] Department of Life Science, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy
[b)] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy
[c)] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy
[d)] Dipartiment of Chemistry, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy; arian.kola@unisi.it

In higher organisms, reactive oxygen and nitrogen species (ROS/RNS) serve essential roles in cellular signaling, immune defense, and the regulation of physiological functions at both the cellular and organ level [1, 2]. The modulation of these species is largely mediated by metal cofactors, among which, heme stands out as a versatile system capable of facilitating both dioxygen activation and the scavenging of ROS/RNS. Over the past decade, research on heme and its synthetic analogues has expanded to include structurally modified macrocycles such as contracted (corroles, COR) and expanded (texaphyrins, TEX) porphyrinoids [3-8]. Metal complexes of these macrocycles display distinctive redox and catalytic behaviors toward ROS and RNS, influenced by the ligand architecture and the metal center's coordination environment.

In this study newly synthesized COR and TEX metal complexes were investigated to explore their application as modulators or suppressors of ROS with potential in therapeutic strategies targeting Alzheimer's Disease. A set of water-soluble COR- and TEX-based metal complexes were evaluated for their interactions with neurotoxic peptides such as amyloid beta (Aβ), whose aggregation has been shown to be mitigated by complexes like Mn-TEX and Fe-COR [6]. Finally, their neuroprotective effects were also evaluated in SH-SY5Y cells under amyloid-induced stress through NMR-based metabolomics. The results highlight specific interactions and suggest potential roles for these compounds as protective agents in neurodegenerative disease models.

Acknowledgments

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OC 10

Development of Mn(II)-Based MRI Probes with Improved Physicochemical Properties for Liver Targeting

Alexis Cabahug ACHACOSO, [a), b)] Sajtos Gergő ZOLTÁN, [a), b)] Váradi BALÁZS, [a), b)] Garda ZOLTÁN, [a), c)] Gyula TIRCSÓ, [a)]

[a)] Department of Physical Chemistry, Faculty of Science and Technology University of Debrecen, Hungary, [b)] Doctoral School of Chemistry University of Debrecen, Hungary and [c)] Center of Molecular Biophysics, CNRS, University of Orléans, France; Gyula.tircso@science.unideb.hu

Introduction: Molecular probes capable of targeting specific organs or tissues, such as the liver, spleen, or lymph nodes can be of particular interest for the non-invasive detection of diseases, metabolic disorders etc. The use of hepatobiliary-specific agents in liver imaging can improve the detection of lesions in the liver, allowing for the differentiation of hepatocellular and nonhepatocellular lesions, and provide specific characterization of some hepatocellular lesions, such as focal nodular hyperplasia.[1] Therefore, liver-specific probes can provide relevant information which in turn may be used to evaluate the anatomy and function of the biliary tree and liver. Recently, it was reported that 3,6-PC2A-9-EOB is a potential liver-specific probe.[2] The given probe was shown to accumulate in the liver, but its inertness is questionable. Therefore, in the present work, we investigated the possibility of improving the physicochemical properties (stability, inertness and relaxivity response of the Mn(II)-based liver-specific probe by applying 3,9-PC2A ligand platform during the ligand design, which has been identified as one of the best candidates for chelating Mn(II).[3]

Methods: The ligands were synthesized using standard chemical synthetic techniques, while the metal complexes were isolated and characterized by high-pressure liquid chromatography (HPLC), mass spectrometry (MS), and ^{1}H relaxometry. The thermodynamic stability of the Mn(II) complexes were determined by the combination of pH-potentiometric and ^{1}H relaxometric techniques, while solvent exchange kinetics was studied via variable temperature ^{17}O NMR method. Dissociation kinetics of the complexes were accessed by studying metal exchange reactions with essential metal ions (Cu(II) and Zn(II)) and their serum stability was also evaluated by using commercially available human blood serum.

Results: The 3,9-PC2A-9-EOB ligand forms a less thermodynamically stable Mn(II) complex ($\log K_{MnL}$=15.42, pMn=8.35) than that observed for the parent macrocycle ($\log K_{MnL}$=17.09, pMn=8.64) which is the consequence of lower ligand basicity caused by the electron withdrawing effect of the EOB moiety attached to the parent 3,9-PC2A ligand platform. Kinetic studies showed that the inertness of the [Mn(3,9-PC2A-6-EOB)] complex improved significantly as compared to its isomeric form, [Mn(3,6-PC2A-9-EOB)] ($t_{1/2}$=2.93 h at pH=6.0 vs. 0.13 h at pH=7.0, 25 eq. Zn(II) ion at 37 °C), and this was also true for the relaxivity data (r_{1p}/r_{2p}=3.50/7.37 vs. 2.83/5.37 at 37 °C, 1.41 T). Furthermore, the inertness of the [Mn(3,9-PC2A-6-EOB)] complex and relaxivity data (r_{1p}/r_{2p}=4.70/10.37 at 25 °C, 1.41 T) were significantly improved as compared to the values observed for the parent [Mn(3,9-PC2A)] chelate ($t_{1/2}$=0.35 h at pH=6.0, r_{1p}/r_{2p}=2.43/5.38 at 25 °C, 1.41 T).

Discussion: The use of an ethoxybenzyl (EOB) sidearm attached to the pyclen macrocycle (3,9-PC2A) slightly decreased the thermodynamic stability of the Mn(II)-based liver-specific probe, but it has notably improved the relaxation and dissociation kinetics parameters. In addition, the attachment of the EOB moiety at the N6 position of the macrocycle made the Mn(II) complex of the 3,9-PC2A-6-EOB significantly better candidate in terms of relaxation and kinetic parameters than the isomeric 3,6-PC2A-9-EOB ligand reported previously.

Conclusion: The relaxivity response and inertness of the potential Mn(II)-based liver specific imaging probes can be tuned by the proper selection and arrangement of the metal binding pendant arms attached to the macrocyclic platform (i.e. ligand topology).

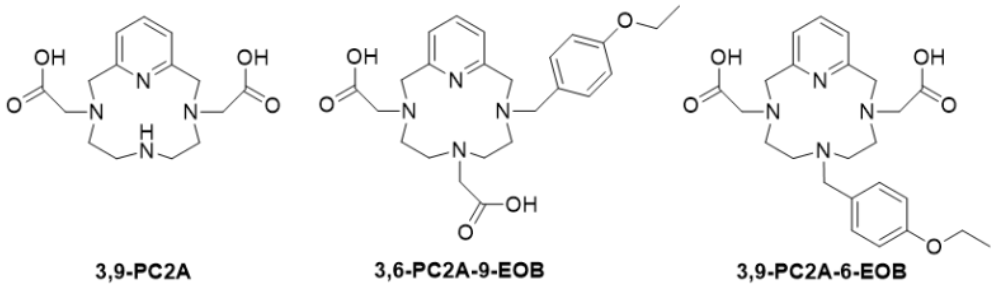

Figure 1. Formulae of the 3,9-PC2A, 3,6-PC2A-9-EOB and 3,9-PC2A-6-EOB ligands.

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OC 11

Structural Insights into Metal Ion Coordination by Aryloxazoline and Arylthiazoline Siderophores and Their Analogues

Klaudia SZCZERBA, [a)] Andrzej MULAR, [a)] Karolina PIASTA, [a)] Karolina KAMIŃSKA, [a), b)] Elżbieta WOJACZYŃSKA, [b)] Henryk KOZŁOWSKI, [c)] Òscar PALACIOS, [d)] Mercè CAPDEVILA, [d)] Elżbieta GUMIENNA-KONTECKA, [a)]

[a)] Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland; [b)] Faculty of Chemistry, Wrocław University of Science and Technology, Norwida 4/6, 50-373 Wrocław, Poland; [c)] Institute of Health Sciences, University of Opole, Pl. Kopernika 11A, 45-040 Opole, Poland; [d)] Departament de Química, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Spain; klaudia.szczerba@uwr.edu.pl

Siderophores, which are low molecular weight compounds, are secreted by microorganisms to scavenge, uptake and control iron levels [1]. Such complexes must first be recognized by appropriate membrane receptors and transporters, translocated into the cell, and finally the Fe(III) ion may be released, often through reduction to Fe(II) ion [2]. Interestingly, siderophores are not recognized by animal organisms which predisposes them to be used in medicine, e.g. as probes or therapeutic substances to pathogenic cells [3]. Moreover, they are also able to bind other metal ions as part of the nutritional passivation and the protection against host macrophages [4-5]. Unfortunately, there is limited information available on the coordination chemistry and thermodynamic stability constants of aryloxazoline and arylthiazoline siderophores complexes with Fe(III) and other metal ions [1].

In this research, we focus on the determination of the coordination properties of aryloxazoline and arylthiazoline siderophores, which comprise phenol/catechol and oxazoline/thiazoline heterocycles together with other binding units, e.g., prepseudomonine (Fig. 1) [1].

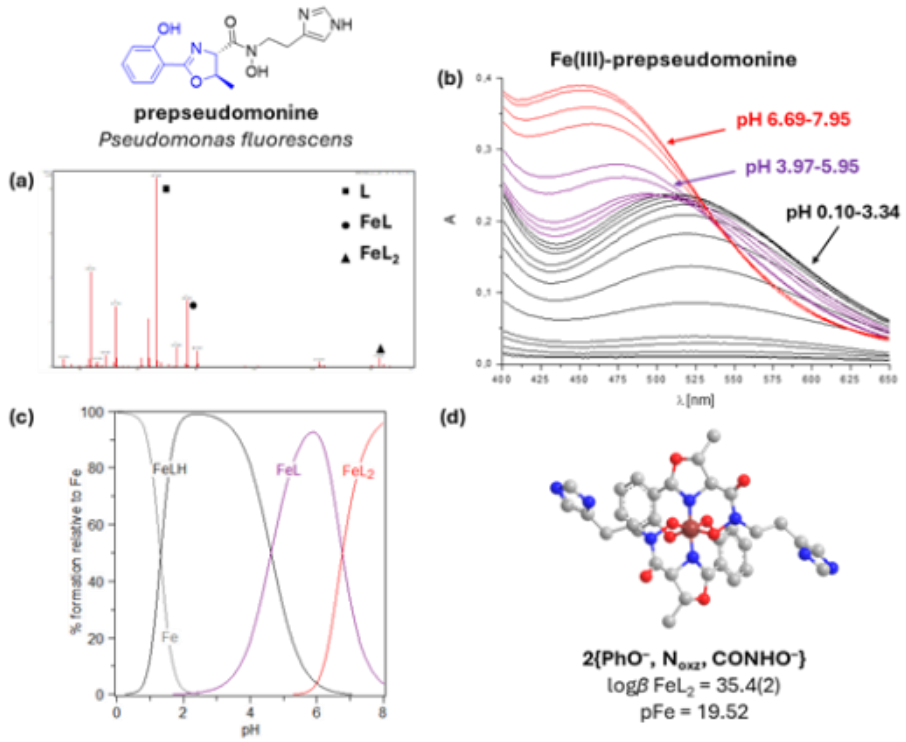

Figure 1. Mass spectra (a), UV-Vis absorption spectra (b), the pH-dependent distribution of complex species (c), and the proposed structure in solution (d) of the Fe(III)-prepseudomonine complex.

We aim at understanding how structural changes affect the stability of the formed complexes with Fe(III) and other metal ions, and learn which siderophore binding groups are crucial for an efficient chelation. The next step in our future research will focus on the biological recognition of analogues by bacterial receptors.

Acknowledgements:
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OC 12

Antioxidant Mimetic Activity of 1*H*-Pyrazole Metal Complexes Modulated by Polyamine Chain Length in [1 + 1] Condensation Azamacrocycles

Irene BONASTRE-SABATER, [a] **Alberto LOPERA-LÓPEZ,** [a] **Álvaro MARTÍNEZ-CAMARENA,** [a), b)] **Salvador BLASCO,** [a] **Antonio DOMÉNECH-CARBÓ,** [c] **Hermas R. JIMÉNEZ,** [d] **Begoña VERDEJO,** [a] **M Paz CLARES,** [a] **Enrique GARCÍA-ESPAÑA,** [a]

[a] Instituto de Ciencia Molecular (ICMol), University of Valencia, C/Catedrático José Beltrán 2, 46980, Paterna (Spain) [b] Departamento de Química Inorgánica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, avda. Complutense s/n. 28040 Madrid (Spain) [c] Departamento de Química Analítica, Universidad de Valencia, Calle Dr. Moliner s/n. 46100 Burjassot, Valencia (Spain) [d] Departamento de Química Inorgánica, Universidad de Valencia, Calle Doctor Moliner s/n, 46100 Burjassot, Valencia (Spain); irene.bonastre@uv.es

A mimetic system of the active site of a metalloprotein can be a simple metal complex able to reproduce, at least in part, the structure, properties, or function of its active centre. Such systems are particularly relevant in the context of reactive oxygen species (ROS) which are essential for maintaining the physiological functions in the organism. However, an imbalance in oxygen metabolism shifted toward pro-oxidant species can lead to a condition known as oxidative stress, which has been linked to neurodegenerative disorders, such as Parkinson's, Alzheimer's, Huntington's diseases.[1] Against ROS the first biological line of defense is the family of metalloenzymes called superoxide dismutase (SOD) [1] but is the family of catalase enzymes the ones finishing the cell detoxification process.

In this work we purpose the design, synthesis and characterization of three [1+1] azacyclophane macrocycles using 1*H*-pyrazole as the aromatic spacer and three polyamines with different chain lengths, the pentaamine 1,5,8,11,15-pentaazadecane (L1) and the hexaamines 1,5,8,12,15,19-hexaazanonadecane (L2) and 1,5,9,13,17,21-hexaazaheneicosane (L3). We evaluated the SOD-like activity using the McCord–Fridovich enzymatic assay [2], while catalase and peroxidase activity were assessed using three different methods: the xylenol orange [3], ABTS [4], and amplex red assay [5]. We observed among the tested systems, that L2 and L3 binuclear cooper complexes have one on the highest antioxidant activities so far reported. [6]

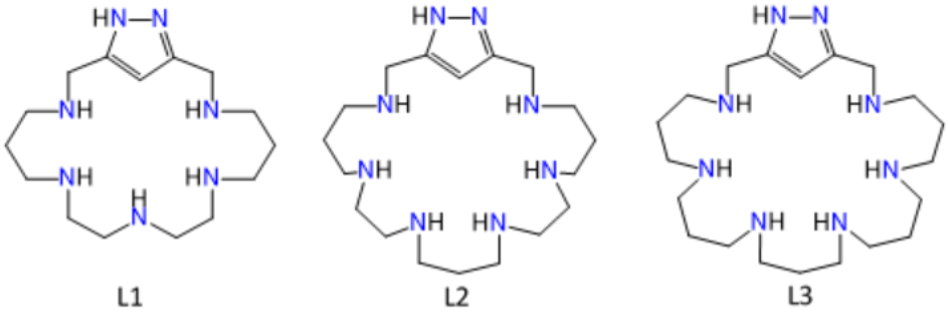

Figure 1: Ligand drawing

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OC 13

Biologically active tinidazole-derived metal complexes: From design to elucidation of the mechanism of action

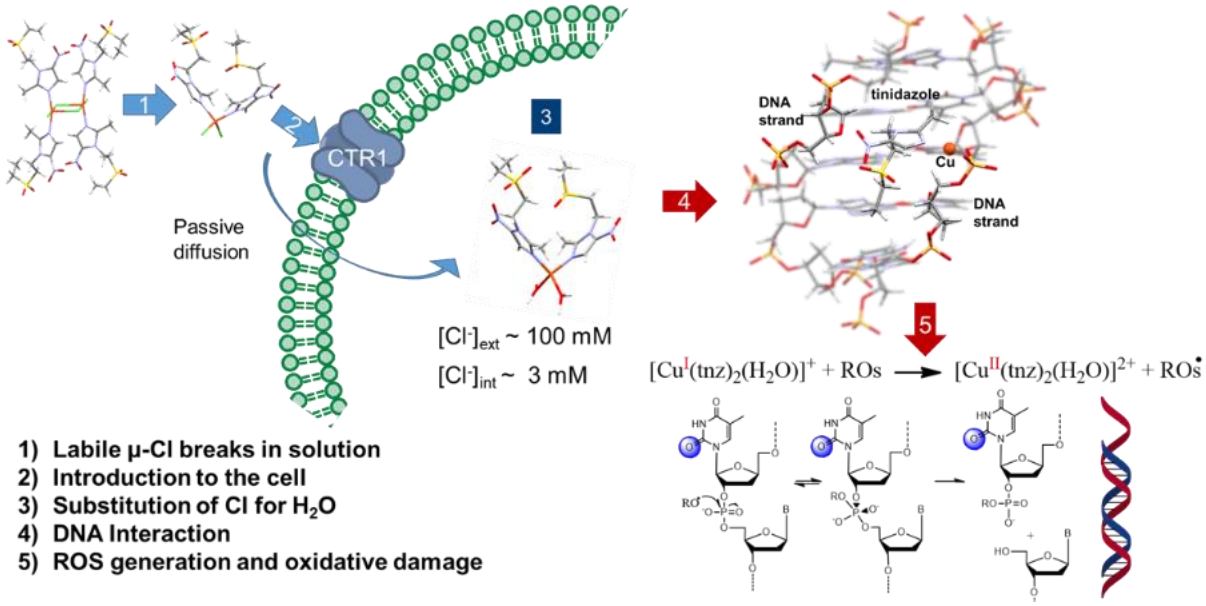
Rodrigo CASTRO-RAMÍREZ, [a] **Francisco SANCHEZ-BARTÉZ,** [b] **Isabel GRACIA-MORA,** [b] **Norah BARBA-BEHRENS,** [a]

[a] *Facultad de Química, UNAM, P. C. 04510, México.* [b] *UNIPREC, Facultad de Química, UNAM, P. C. 04510, México*; *rozurigokas@gmail.com*

We have been interested in investigating the potential therapeutic properties of novel coordination compounds using bioavailable metal ions (i. e. Co, Ni, Cu, Zn,) and a biologically active, 5-nitroimidazole derivative, tinidazole (tnz). A series of distorted tetrahedral complexes with these metal ions were obtained and fully characterised. From them, the complex $[Cu(tnz)_2Br_2]$ proved to be the most active against *dactylogyrids monogeneans*, an helmint that infects the gills of fish of human consumption. Although this copper complex was metabolised whilst administered through intubation, the method of adding the compound directly to the tank holding the fishes, proved to be highly efficient, with upwards of 95% antihelmintic activity after 8 hrs of exposure. Structurally, these complexes showed to be quite versatile. The $[Cu(tnz)_2Cl_2]$ was crystallised in two different conformations, a green compound (kinetic conformer) depicting an open conformation and a yellow compound (thermodynamic conformer) in a bent conformation. This change in conformation was possible due to a novel weak interaction known as lone pair$\cdots\pi$. Where the highly polarized imidazole ring shows an electron deficiency in the centre that is neutralised by a pair of electrons located in the $O_{sulphone}$ [1]. Using various computational and topological techniques we were able to prove this to be an attractive, localised and directional interaction, that appears in tinidazole metal complexes, regardless of the metal ion [2].

Upon these excellent results, we expanded our study on the potential biological activity of these complexes by testing them as anticancer agents. A series of copper(II) complexes with tnz were evaluated against different cell lines with the dinuclear $[Cu(tnz)_2(\mu\text{-}Cl)Cl]_2$ and the $[Cu(tnz)_2Br_2]$ compounds showing the highest activity against most of the cell lines tested. Additionally, doing a comparative study using healthy (MCF-10A) and cancerous (MCF-7) breast cells, these two complexes showed selectivity towards cancer cell with $IC_{50/MCF\text{-}7}$ = 6.9 µM and $IC_{50/MCF\text{-}10A}$ = 28.6 µM for $[Cu(tnz)_2(\mu\text{-}Cl)Cl]_2$ and $IC_{50/MCF\text{-}7}$ = 14.9 µM and $IC_{50/MC10\text{-}A}$ = 33.1 µM for $[Cu(tnz)_2Br_2]$ [4]. We have also performed a series of studies, both computational and experimental, to elucidate the mechanism of action of these complexes. UV-Vis DNA titration and ethiduim bromide displacement studies were performed with the cytotoxic copper-tnz compounds. From these studies, we observed complex-DNA interactions *via* electrostatic contacts or minor groove binding with good K_b and K_{SV} (e. g. $2.6x10^6$ and $3.36x10^3$ for $[Cu(tnz)_2(\mu\text{-}Cl)Cl]_2$). Additionally, given the metal ion of these complexes and its II/I oxidation states, we studied the potential oxidative damage towards DNA through a Fenton-like reaction. The gel electrophoresis results for these compounds showed that at 4 µM of [complex] most DNA has been damaged [3]. To further assess these properties, a molecular dynamics (MD) study was done using optimised geometries from single X-ray diffraction structures of the Cu-tnz complexes and the *Dickerson-Drew* DNA dodecamer. From the MD

results we observed that the DNA-complex adducts with the highest E_{int} presented at least one tnz molecule intercalated in the minor groove, parallel to both DNA strands. A QSAR assisted study using previously reported Cu(II) complexes from different research groups allowed us to calculate the $Cu^{II/I}$ redox potential of the DNA-complex adducts. The interaction of the Cu-tnz complexes with DNA shifted its redox potential to a more positive value, making it more accessible for it to oxidatively damage DNA, suggesting a synergistic effect with the groove-binding interactions [4]. With these theoretical/experimental approach we have been able to propose the mechanism of action for Cu-tnz complexes depicted in the figure bellow.



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OC 14

Copper(II) complexes of pyridine-carboxamide based histidine conjugates

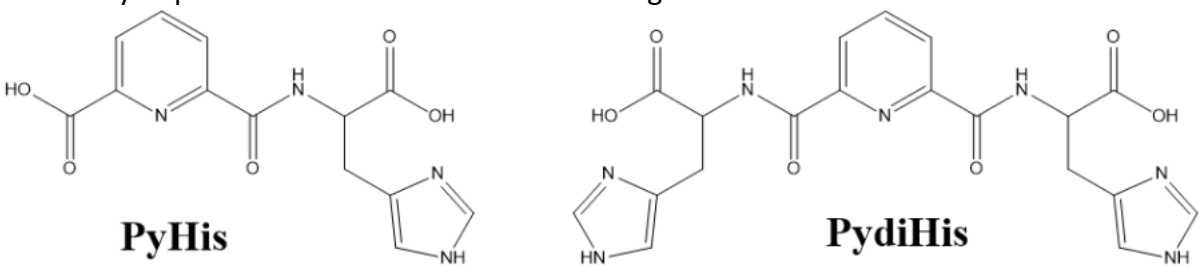
Róbert DIÓSZEGI, [a] Nóra V. MAY, [b] István FÁBIÁN, [a] Norbert LIHI, [a]

[a] HUN-REN–UD Mechanisms of Complex Homogeneous and Heterogeneous Chemical Reactions Research Group, University of Debrecen, Debrecen, Hungary
[b] Centre for Structural Science, Research Centre for Natural Sciences, Hungarian Research Network (HUN-REN), Budapest, Hungary; lihi.norbert@science.unideb.hu

Superoxide anion is formed during the incomplete reduction of molecular oxygen in biological systems. Elevated level of superoxide anion may lead to oxidative stress and cause DNA damage, inflammatory diseases or development of human cancer. [1,2] Biological systems release antioxidant enzymes to control the level of reactive oxygen species and to protect against the growing levels of oxidative damage. One of the enzymes is called superoxide dismutase (SOD). SODs are involved in the decomposition of superoxide anion radical to molecular oxygen and hydrogen peroxide. [3] Low-molecular weight transition-metal complexes are capable to assist the decomposition of superoxide anion, thus, they can be applied as potential drugs in the treatment of various diseases associated with elevated ROS level or as antiaging agents. Since the direct administration of SOD enzymes is not feasible due to their large size and limited cell permeability, considerable attention has been focused to obtain SOD mimics as alternatives of SOD enzymes to overcome some of these limitations.

In this presentation, we discuss the synthesis and characterization of two ligands containing histidine residues (Scheme 1) and its thermodynamic and spectroscopic studies with copper(II). The SOD activity of the complexes formed at physiological pH was tested via the xanthine/xanthine-oxidase/NBT assay and dedicated stopped-flow methods developed in our laboratory.

The ligands exhibit high affinity to bind copper(II) and demonstrate excellent SOD activity. To explore the biological relevance of these findings, the antioxidant activity of the complexes was studied on the mycelial growth of *Aspergillus niger*. The complexes showed the ability to protect cellular metabolism and mitigate the adverse effect of oxidative stress.



Scheme 1. Structural formulae of the ligands.

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Culture and Innovation from the source of the National Research, Development and Innovation Fund and the University of Debrecen Scientific Research Bridging Fund (DETKA).

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OC 15

Polyamine cryptand complexes for targeting non-canonical DNA G-quadruplexes

Laura MULET-RIVERO, [a] María Amparo LOPO-MARCH, [a] Ariadna GIL-MARTÍNEZ, [a] Estefanía DELGADO-PINAR, [a] Jorge GONZÁLEZ-GARCÍA, [a] Enrique GARCÍA-ESPAÑA, [a]

[a] University of Valencia, Institute of Molecular Science, C./ Catedrático José Beltrán 2, Paterna 46980, Spain; laura.mulet@uv.es

Non-canonical nucleic acid structures have attracted considerable attention in many science fields, including chemistry, biology, physics, materials and nanotechnology. They include triplexes, *i*-motifs, three/four-way junctions or G-quadruplexes (G4). G4 structures are formed in guanine-rich sequences, in which four guanine bases are held together by Hoogsteen hydrogen bonds to form a coplanar G-quartet, and then two or more G-quartets stack to form the G-quadruplex structure retaining sodium or potassium ions in a central core channel [1,2]. Strikingly, many putative G-quadruplex forming sequences have been identified in the genomes of human and viruses, and evidences suggest their pivotal role in key biological processes such as ageing, neurodegenerative diseases and cancer [1]. Therefore, these G4 structures have been proposed as potential targets by small molecules for therapeutic intervention.

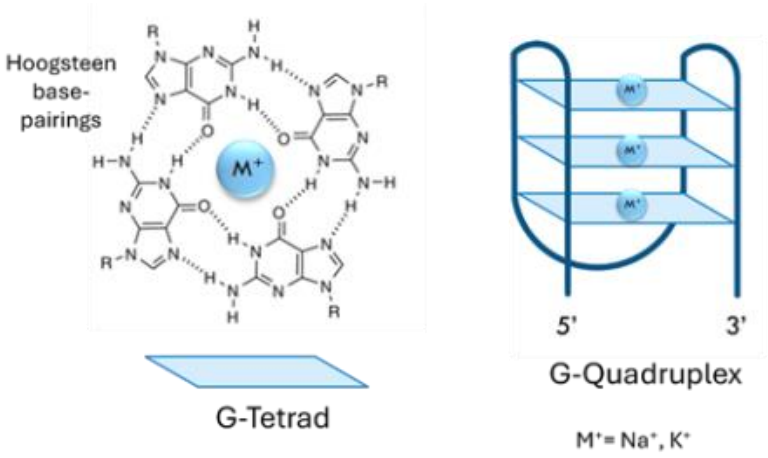

Figure 1. G-quadruplex structure.

In this work, we present various molecules containing polyamines linked by triphenylamine moieties with cryptand-like structures, as well as their corresponding polynuclear copper complexes. The number of amines in their structures, which confers a positive net charge, along with their aromatic core, enables efficient binding to G-quadruplex DNA structures. First, we studied the acid-base behavior of the ligands and their polynuclear copper complexes to elucidate the species present at physiological pH through potentiometric and UV-Vis experiments. Then, we assessed their interaction with G4 DNAs by using UV-Vis/fluorimetric titrations, circular dichroism, fluorescence resonance energy transfer melting assays, and gel electrophoresis. The results point out the importance of copper coordination

to efficiently interact with G4 structures and open a new approach of these cryptand molecules for future applications as catalysts and therapeutics.

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OC 16

Hemopeptides Conjugates as Versatile Ligands for the Selective Binding of G-Quadruplexes

Leen MASSALHA, [a] Adiel RICHTER-LEVIN, [a] Nurit ADIRAM-FILIBA, [a] Eyal GOLUB, [a]

[a] Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel; eyal.golub@biu.ac.il

The design of topology- and sequence-specific GQ ligands has greatly benefited from conjugatingmultiple binding elements targeting distinct structural motifs. A particularly effective approachinvolves conjugating aromatic molecules with short peptide chains, where the former binds external tetrads and the latter interacts with loops/grooves. We repurposed microperoxidase-11 (MP-11), a semi-natural hemopeptide with an 11-mer peptide tethered to a heme group, as a versatile GQ ligand. Similar to heme, MP-11 preferentially binds parallel GQs with minimal dsDNA interaction.

MP-11's subcomponents synergistically enhance GQ binding: the heme moiety directs specificity to parallel GQs, while the peptide chain increases affinity via non-specific interactions. This enables MP-11 to achieve sequence specificity through unique mechanisms: (i) selectively uncaging a GQ sequence in dsDNA encoding c-MYC, and (ii) binding hybrid GQs and converting them to parallel topologies, favoring less stable sequences.[1]

Accordingly, MP-11 represents an elaborate platform for exploring specificity mechanisms for GQs by designed ligands. Moreover, MP-11 is a versatile ligand that can be adapted to a specific purpose as it can be further manipulated by either varying the metal ion at the center of the porphyrin ring or mutating the residues at the GQ sequence.

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OC 17

Influence of amino acid sequence in calmodulin binding site on plutonium redox properties

Emilien FAUX, [a] Diego MORENO MARTINEZ, [a] Sandrine SAUGE-MERLE, [b] Catherine BERTHOMIEU, [b] Claude BERTHON, [a] Laurence BERTHON, [a]

[a] CEA, DES, ISEC, DMRC, Montpellier Univ., Marcoule, France [b] Aix-Marseille Univ., CEA, CNRS, BIAM, Saint Paul-Lez-Durance, France; emilien.faux@cea.fr

Actinides are radioactive metals of high importance in civil and military nuclear activities. Most actinides being alpha emitters, it is essential to investigate their behavior within the human body. In solution, actinides may be found under various oxidation states. At the oxidation states V and VI, they form molecular cations AnO_2^+ and AnO_2^{2+}. These actinyls are the most stable form of uranium and neptunium under physiological conditions.[1] Their linear geometry can greatly affect the affinity of biological ligands towards them.

In this work, site I of calmodulin (CaM) was chosen as template of the EF-hand binding motif, which is widely present in calcium-binding proteins. It has been shown that shortening the complexation loop of calmodulin site I from 12 to 10 amino acids increased its affinity for uranyl. The shortened loop in CaMΔ variant fits five oxygen ligands in the equatorial plane of the linear cation, resulting in a 100-fold increase of the affinity for uranyl, reaching the sub-nanomolar range (log K = 9,55 ± 0,11).[2] Because of this strong affinity, CaMΔ was chosen to investigate its interaction with the plutonyl and neptunyl cations in medium buffered at pH 6. Absorption spectroscopy confirmed the interaction of CaMΔ with plutonyl cation. Nevertheless, after complexation, a gradual reduction of $Pu(VI)O_2^{2+}$ to $Pu(V)O_2^+$ was observed.

In order to understand the origin of plutonium reduction, this work focused on designing calmodulin variants with different amino acid sequences derived from the binding site of CaMΔ (Figure 1). The corresponding CaM-$Pu(VI)O_2^{2+}$ complexes were analyzed using molecular dynamics simulations to explore the plutonium coordination environment. Key parameters such as plutonium-oxygen distance, exchange dynamics, number of coordinated water molecules, and binding free energies were determined and compared. Based on these results, two promising variants were selected for experimental investigation (CaMΔWT and CaMΔ11). The interactions between $Pu(VI)O_2^{2+}$ and these calmodulin variants were characterized using visible and X-ray absorption spectroscopies. Experimental data were compared with the simulations. In the case of CaMΔWT, which exhibited the lowest exchange dynamics of the coordination sphere, the reduction of plutonyl was approximatively eight times slower than with CaMΔ.

Variant	Sequence in site I
CaMΔ	D G D G Y I T A A E
CaMΔWT	D G D G Y I T **T K** E
CaMΔ4	D G D G Y I T A **E** E
CaMΔ5	D **P** D G Y I T A A E
CaMΔ6	D G D G Y I **K** A A E
CaMΔ7	D G **E** G Y I T A A **D**
CaMΔ8	D G **E** G Y I T **T K D**
CaMΔ9	D G D G Y I **E** A A **T**
CaMΔ10	D G D G Y I **E** A A **S**
CaMΔ11	D G D G Y I **E** A A E
CaMΔ12	D G D G Y I **E** A **K** E
CaMΔ13	D G D G Y I **E T K** E

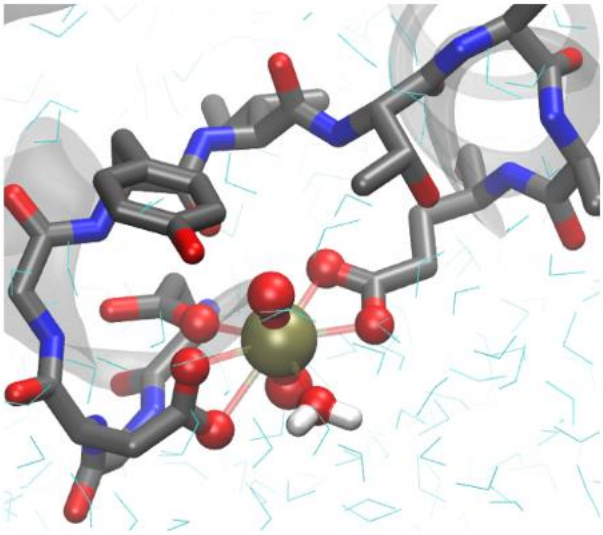

Figure 1 – Left: table listing all CaMΔ-derived variants investigated by molecular dynamics. The bold font indicates the mutated amino acids. Right: representative snapshot of a molecular dynamics simulation of a CaMΔ - Pu(VI) complex.

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OC 18

Histidine Mutations Drive Changes in Mn(II), Fe(II), and Zn(II) Binding to Calprotectin Fragments

Karolina PAWLIK, [a] Małgorzata OSTROWSKA, [a] Massimiliano PEANA, [b] Elżbieta GUMIENNA-KONTECKA, [a]

[a] Faculty of Chemistry, University of Wroclaw, Wroclaw, Poland [b] Department of Chemical, Physical, Mathematical and Natural Science, University of Sassari, Italy; karolina.pawlik@uwr.edu.pl

Manganese is an essential trace element for a diverse number of organisms. It serves as a cofactor for indispensable enzymes such as pyruvate carboxylase, arginase or ribonucleotide reductase. It also shields against oxidative stress as complexes with both organic and inorganic ligands, serves as a cofactor in superoxide dismutase, or replace more susceptible to Fenton chemistry Fe(II) ions in proteins [1, 2, 3].

The manganese present in human body can be used as nutrient by many pathogenic bacteria to resist host-induced oxidative stress. This is why one of immune system response to bacterial infection is nutritional immunity. The process consists of efflux of essential metal ions from the site of infection, with simultaneous influx of metal ions toxic to pathogens. To combat those effects bacteria possesses its own Mn(II) transporters in cell membrane, that compete with host metal ion chelators. The impoverishment of infection site in Fe(II), Zn(II) and Mn(II) ions is carried out by extracellular metal chelating proteins – the S100 family [4, 5]. One of them – calprotectin is a unique Mn(II) chelator. Calprotectin possesses two ligand binding sites, of which one is extraordinary as is consists of six histidine residues and no oxygen donors. Most of known Mn(II) chelators possess a coordination sphere that consists of both nitrogen and oxygen donors. There is no other known Mn(II) chelating protein with such binding site [5, 6].

Here we present our recent results regarding thermodynamic studies of Mn(II), Fe(II) and Zn(II) complexes with various model peptides based on Mn(II) chelating proteins found in nature. Our results focus on influence of the number and topology of various amino acids such as histidine, aspartic acid and alanine on thermodynamic stability and stoichiometry of formed complexes.

Acknowledgements
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FLASH PRESENTATIONS

FP 1

Can Phosphorylation Modulate the Redox Activity of Copper–Peptide Complexes?

Katarzyna BIERNAT, [a] Aleksandra DĄBAŁA, [a] Aleksandra TOBOLSKA, [a] Nina E. WEZYNFELD, [a]

[a] Warsaw University of Technology, Faculty of Chemistry, Chair of Medical Biotechnology, Noakowskiego 3, 00-664 Warsaw, Poland; katarzyna.biernat.dokt@pw.edu.pl

Phosphorylation is the most prevalent post-translational modification, playing a pivotal role in regulating protein activity and their interactions with other biomolecules. This process influences numerous essential cellular functions, including energy metabolism, cell signalling, synaptic plasticity, and memory formation. Phosphorylation is also critical for proper neuronal function, and its dysregulation can lead to protein destabilization and pathological aggregation. A well-known example is the hyperphosphorylation of the intracellular tau protein, a hallmark molecular mechanism in Alzheimer's disease (AD) [1].
It has also been shown that extracellular amyloid-β (Aβ) peptides undergo phosphorylation. However, the impact of this modification on molecular processes involving Aβ peptides and their complexes with Cu(II) ions remains poorly understood. Notably, Cu(II)–Aβ complexes play a key role in neurodegeneration by catalyzing the formation of reactive oxygen species (ROS), thereby disrupting cellular redox homeostasis. Given the diversity of Aβ peptide forms present in the brain and the lack of literature data on how phosphorylation affects the redox properties of Cu(II)–peptide complexes, we focused on the analogue of Aβ5-x, whose copper complexes are known to undergo both reduction and oxidation processes [2].
The aim of this study was to investigate the influence of phosphorylation on the redox behavior of Cu(II)–peptide complexes using a library of synthetic peptides containing the His-2 motif. We examined both the unmodified peptide and its phosphorylated analogues, differing in the number and positions of phosphorylated serine residues.
Our findings demonstrate that phosphorylation markedly alters the electrochemical signals recorded by cyclic voltammetry and differential pulse voltammetry. The reduction potentials and signal intensities for Cu(II) reduction in phosphorylated peptide complexes were significantly different from those observed for the unmodified sequence. These effects depended strongly on both the location and the number of phosphorylated serine residues.
The results suggest that phosphorylation may play a crucial role in modulating the redox properties of Cu(II)–peptide complexes, which could be important for understanding the molecular mechanisms underlying neurodegenerative diseases.

Acknowledgments: This research was supported by the National Science Centre, Poland, under Sonata project no. 2023/51/D/ST4/00401.

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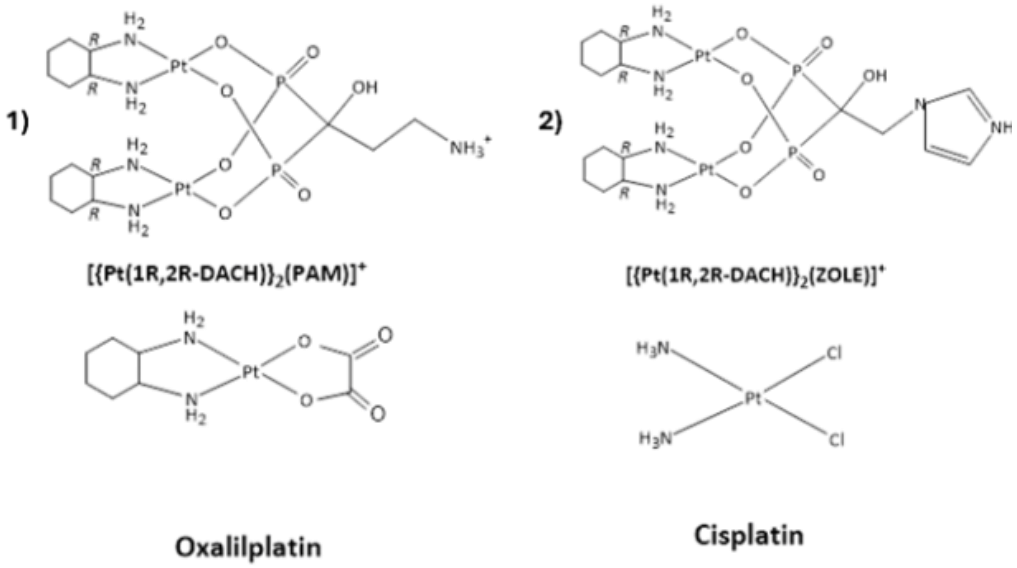
FP 2

Assessing the antitumor activity of dinuclear platinum–bisphosphonate agents for selective treatment of bone metastases by *in vitro* and *ex vivo* models

Anna Maria DI COSOLA, [a] Alessandra BARBANENTE, [a] Karim TIDJANI, [b] Jeroen J. J. P. VAN DEN BEUCKEN, [b] Nicola MARGIOTTA, [a]

[a] Dipartimento di Chimica, Università degli Studi di Bari Aldo Moro, Via E. Orabona, 4, 70125 Bari, Italy;
[b] Department of Dentistry- Regenerative Biomaterials, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Philips van Leydenlaan 25, 6525 EX Nijmegen, The Netherlands; anna.dicosola@uniba.it

Bone metastases are a frequent and severe complication in advanced-stage cancers, especially prostate cancer [1]. While platinum-based drugs remain a fundamental approach of chemotherapy, their application is limited by a poor tumor selectivity and systemic toxicity [2]. In this study, we investigated the anticancer potential of two novel dinuclear platinum-bisphosphonate compounds, $[\{Pt(1R,2R\text{-DACH})\}_2(PAM)]^+$ (**1**; DACH = diaminocyclohexane; PAM = pamidronate), $[\{Pt(1R,2R\text{-DACH})\}_2(ZOLE)]^+$ (**2**; ZOLE = zoledronate) [3], designed to merge the cytotoxic effects of platinum with the bone-targeting capability of bisphosphonates. Their performance was compared to the clinically used drugs oxaliplatin and cisplatin as well as to untreated controls. Initial *in vitro* evaluation was performed on human PC3 prostate cancer cells using 2D cultures. Cells were exposed to both compounds under continuous exposure and 24-hour treatment regimes at two key concentrations (25 and 100 µM). Cell viability was measured via AlamarBlue™ assay, supplemented by DAPI nuclear staining for morphological analysis. Compound **2** demonstrated the most pronounced and reproducible cytotoxic activity, competing with that of cisplatin. To better reflect the tumor microenvironment, we developed an *ex vivo* model using standardized human bone discs with 2 mm defects seeded with PC3 cells, simulating bone metastatic lesions. Drug efficacy was monitored over 10 days using bioluminescence imaging (BLI).



Scheme 1. *Sketches of [{Pt(1R,2R-DACH)}$_2$(PAM)]$^+$ (**1**), [{Pt(1R,2R-DACH)}$_2$(ZOLE)]$^+$ (**2**), oxalilplatin and cisplatin.*

The results confirmed significant viability reduction of PC3 cells within bone tissue for both compounds but **2** exhibited stronger responses in the *ex vivo* bone environment. The parallelism observed between *in vitro* and *ex vivo* data supports the potential of these dinuclear Pt-bisphosphonate complexes as candidates for targeted therapy against bone metastases from prostate cancer. Their dual-action mechanism (platinum-mediated cytotoxicity and bisphosphonate-driven bone affinity) offers a promising strategy to improve drug localization at metastatic sites while minimizing systemic side effects.

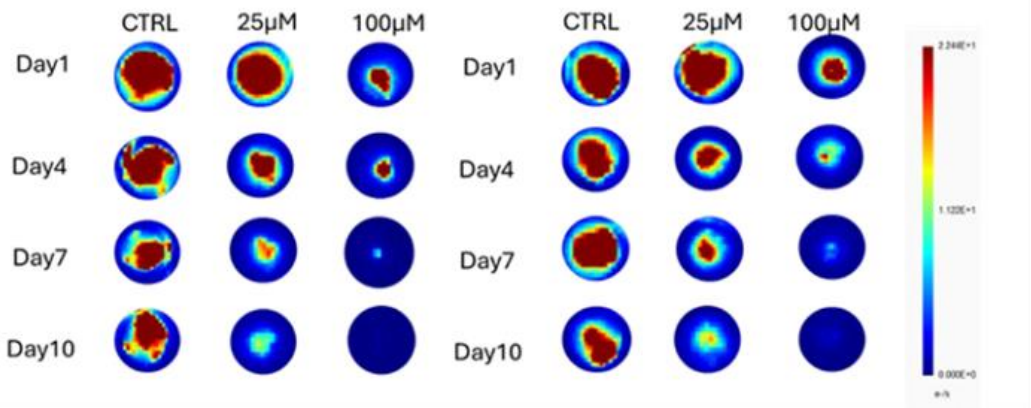


Figure 1: Representative ex vivo bioluminescent images of bone discs + PC3 cells treated with ***1*** *(A)* and ***2*** *(B)* over 10 days of continuous exposure at two different concentrations (25 and 100 μM).

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FP 3

Histidine-Rich Motifs Under Pressure - CopT and Copper Homeostasis in *S. solfataricus* and *S. tokodaii.*

Klaudia KŁOPOTOWSKA, [a] Natalia KARKOWSKA, [a] Aleksandra HECEL, [a]

[a] Faculty of Chemistry, University of Wrocław; 332086@uwr.edu.pl

Antibiotic resistance is one of the biggest challenges in modern medicine, creating an urgent need to develop new strategies for treating bacterial infections. Since bacteria require transition metals for survival, a thorough understanding of their metal uptake mechanisms may provide innovative avenues for designing novel therapeutic approaches. Pathogenic bacteria have evolved efficient metal ion transport systems based on (i) chaperone proteins and (ii) transmembrane transporters, which interact with chaperones to facilitate the import of metal ions into the cell [1].

The CopT protein, identified in both Saccharolobus solfataricus [2] and Sulfurisphaera tokodaii [3], plays a key role in copper homeostasis and detoxification. CopT is part of the CopRTA system, where it functions as a copper-binding buffer, sequestering excess Cu(I) ions and reducing their toxicity. This protein contains a conserved C-terminal domain rich in histidine residues (GPKGMPHGH in S. solfataricus and GPKGMPGEEGHH in S. tokodaii), which are crucial for binding transition metal ions such as Cu(I), Cu(II), and Zn(II). Its periplasmic and extracellular localization suggests a possible role as a metallophore, involved in copper acquisition or trafficking [4].

It was essential to investigate the stability, geometry, and overall structure of the metal complexes formed with two C-terminal domain of CopT protein over a broad pH range. To achieve this, a combination of advanced physicochemical techniques was employed, including potentiometric titrations, UV-Vis spectroscopy, circular dichroism (CD), electron paramagnetic resonance (EPR) spectroscopy, and mass spectrometry.

The results obtained provide valuable insights into the ways bacterial proteins bind metal ions, allowing for a better understanding of copper homeostasis mechanisms and potentially serving as a foundation for further studies on their therapeutic applications.

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

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FP 4

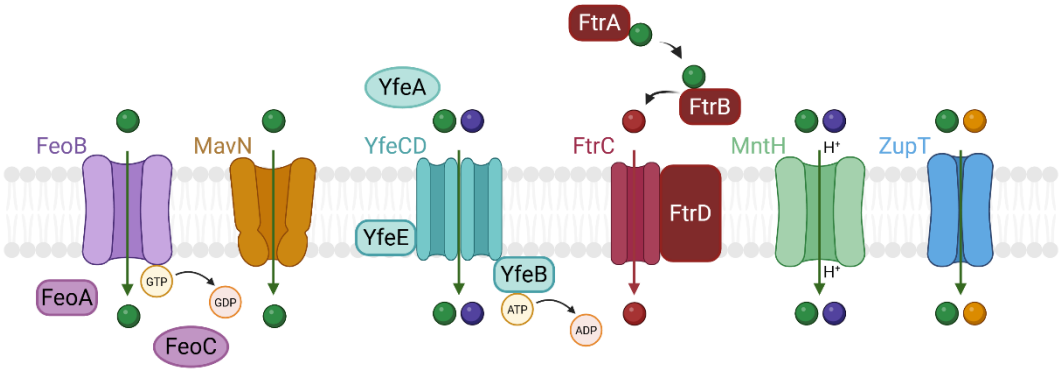
Insights into the coordination chemistry of bacterial iron transporters

Bartosz ORZEŁ, [a] Alessio PELUCELLI, [b] Małgorzata OSTROWSKA, [a] Sławomir POTOCKI, [a] Henryk KOZŁOWSKI, [a], [c] Massimiliano PEANA, [b] Elżbieta GUMIENNA-KONTECKA, [a]

[a] Faculty of Chemistry, University of Wroclaw, 50-383 Wroclaw, Poland [b] Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Via Vienna 2, 07100 Sassari, Italy [c] Institute of Health Sciences, University of Opole, 45-060 Opole, Poland; bartosz.orzel@uwr.edu.pl

Iron is an essential element for bacterial survival. During infection, pathogens must efficiently uptake iron ions from the host. Under anaerobic conditions, Fe(II) is the main source of available iron, and its efficient assimilation is often crucial for bacterial survival under oxygen-limited conditions [1].

Due to its high solubility, Fe(II) is transported as a free metal ion. Fe(II) uptake occurs through various assimilation systems, such as FeoABC, MavN, and YfeABCD (Fig. 1). The key components of these systems are the transmembrane proteins, directly involved in metal ion transport across the bacterial membrane. Despite the crucial importance of Fe(II) transporters for bacterial pathogenicity, the coordination chemistry and mechanism of action of many of these transporters remain poorly understood. To characterize the coordination properties of transmembrane Fe(II) transporters, we selected peptide models of potential metal binding sites and studied their complexes with metal ions using a variety of physicochemical methods (potentiometry, mass spectrometry, NMR spectroscopy, CD, EPR) [2,3]. We present the stoichiometry of the complexes, their stability, and the Fe(II)-binding atoms as an important insight into both the metal ion binding characteristics of bacterial transporters and the Fe(II) coordination chemistry.



Acknowledgments
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FP 5

From Metal Binding to Proteolytic Stability: A Study of GLP-1 RA Their Interaction with Zn(II) and Cu(II)

Gabriela POTOCZNIAK, [a)] **Magdalena ROWIŃSKA-ŻYREK**, [a)] **Mariusz DZIADAS**, [a)]

[a)] *University of Wrocław, Faculty of Chemistry, Biologically Active Metallopeptides Research Group, 14 F. Joliot-Curie Street, 50-383 Wrocław, Poland; gabriela.potoczniak@uwr.edu.pl*

The incretin effect refers to the phenomenon of enhanced insulin secretion in response to oral glucose intake, mediated by the release of incretin hormones such as glucagon-like peptide-1 (GLP-1). This effect plays a crucial role in maintaining normoglycemia through its insulinotropic action and its influence on glucagon secretion [1–2].

The loss of the incretin effect is one of the hallmarks of type 2 diabetes mellitus. However, the therapeutic application of native GLP-1 is limited due to its rapid degradation by enzymes such as dipeptidyl peptidase IV (DPP-4) and neprilysin (NEP) [1,3].

The aim of this project is to analyze GLP-1, its therapeutic analogue liraglutide, and a modified version of liraglutide in which D-enantiomers are introduced in positions susceptible to enzymatic degradation, replacing the natural L-amino acids (Fig. 1).

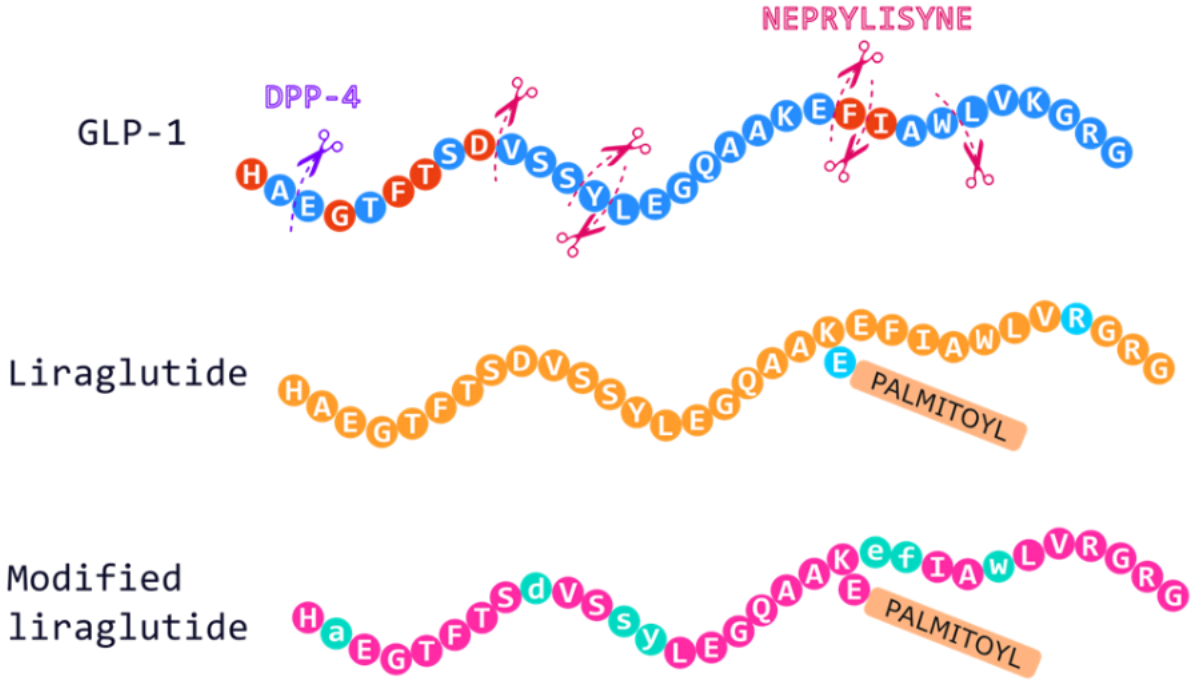

Figure 1. Sequences of GLP-1 (with cleavage sites for DPP-4 and NEP marked, as well as amino acids involved in receptor interaction), liraglutide (with modifications relative to GLP-1 highlighted), and modified liraglutide (with highlighted D-amino acids).

We focused on determining the coordination mode of Zn(II) ions—associated with type 2 diabetes [4]—as well as Cu(II) ions, and on assessing the proteolytic stability of the modified analogue. We stablished that at near-physiological pH, both GLP-1 and liraglutide exhibit histamine-like coordination with Zn(II) and Cu(II) ions, and we highlighted the differences in proteolytic stability between GLP-1 and its analogues.

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FP 6

Anderson POM Hybrids with Cr and Co: A Platform for Thermoemissive and Electrofunctional Materials

Dominika PRĘTKA, [a] Cristian HERNANDEZ, [a] Violetta PATRONIAK, [a] Marcin RUNOWSKI, [a] Adam GORCZYŃSKI, [a] Nahir VADRA, [a] *

[a] Adam Mickiewicz University, Faculty of Chemistry, Uniwersytetu Poznańskiego 8, 61-614 Poznań, Poland; dominika.pretka@amu.edu.pl

Polyoxometalates (POMs) - anionic metal-oxide clusters, represent a class of supramolecular assemblies with high tunable activity and rich structural diversity, due to the possibility of combining different components. This relates both to the type of metal ions in the {MO_x} polyhedral units and in the central cavity, as well as to their potential for introducing organic ligands to form hybrid materials. Hybrid organic–inorganic systems based on POMs are emerging as promising functional materials for stimuli-responsive applications with tailored properties, including redox activity, luminescence, and ionic mobility [1-3]. Liquid crystals (LCs), on the other hand, are soft matter system characterized by long-range order and properties of intermediate states between a solid and a liquid. The responsive to external stimuli of LCs and tunable optical properties make them promising candidates for integration with emissive components [4]. In this work, a new family of Anderson-type POM hybrids has been synthesized using Co(III) or Cr(III) as the central atom and fully characterized (Figure 1). These POMs were encapsulated by tailored organic ligands designed with one to three aliphatic chains of varying length, promoting supramolecular organization and potential liquid crystalline behavior. The ligands also include coordination pockets capable of hosting additional metal ions, offering the possibility of dual emission.

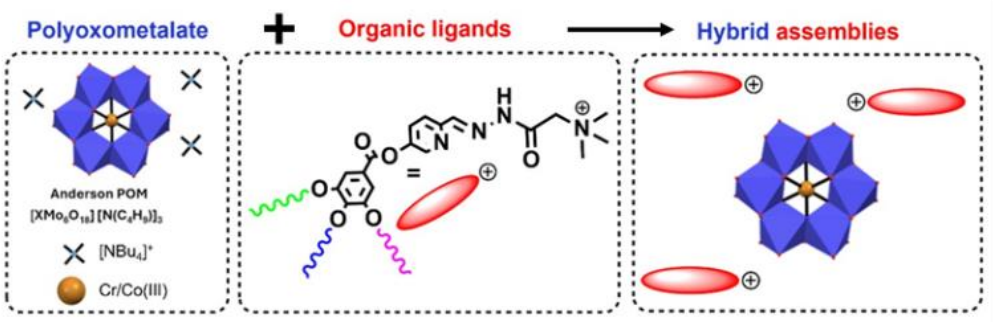

Figure 1. Schematic representation of the synthesis of hybrid assemblies (POM-organic ligands).

Photophysical characterization shows that Cr-based precursors exhibit strong red luminescence with a marked temperature dependence, validating their potential application as thermal sensors (Figure 2). Differences in chain length and substitution patterns are currently being correlated with self-assembly and optical response.

Many POMs—including Anderson types—exhibit several discrete redox states within narrow potential windows. The combination with these tailored organic ligands offers the possibility of potential advantages for resistive memory applications due to improved processability and supramolecular influence on stable redox states.

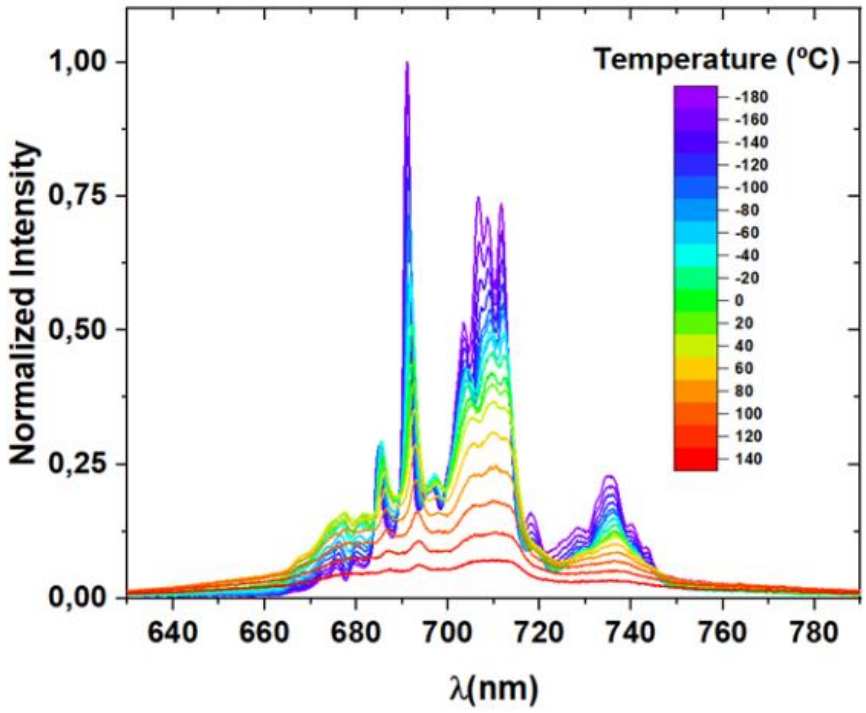

Figure 2. Anderson Cr precursor emission spectrum as a function of temperature.

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FP 7

Insights into the formation of complexes in the Cu(II)/Tyrosine and Cu(II)/Tyrosine/ATP systems

Patrycja SADOWSKA, [a] Romualda BREGIER-JARZĘBOWSKA, [a] Wojciech JANKOWSKI, [a] Renata JASTRZĄB, [a]

[a] Adam Mickiewicz University, Faculty of Chemistry, Uniwersytetu Poznańskiego 8, 61-614, Poznań, Poland; patrycja.sadowska@amu.edu.pl

Transition metal ion complexes with biologically relevant ligands are crucial for understanding numerous physiological and biochemical processes [1]. This study examines model systems involving copper(II) ions and two important biomolecules: tyrosine (Tyr) and adenosine-5'-triphosphate (ATP). Tyrosine participates in redox reactions and forms biologically active complexes [2], while ATP is central to cellular energy transfer and signaling pathways [3]. Investigating Cu(II) complexes with these ligands offers valuable insights into metal–ligand interactions.

Complexation reactions were studied in aqueous solution using potentiometric and spectroscopic (Vis, EPR) methods, as well as DFT studies (Figure 1). Based on the results of our investigations, we determined the mode of coordination in the complexes formed in the model Cu(II)/Tyrosine and Cu(II)/Tyrosine/ATP systems.

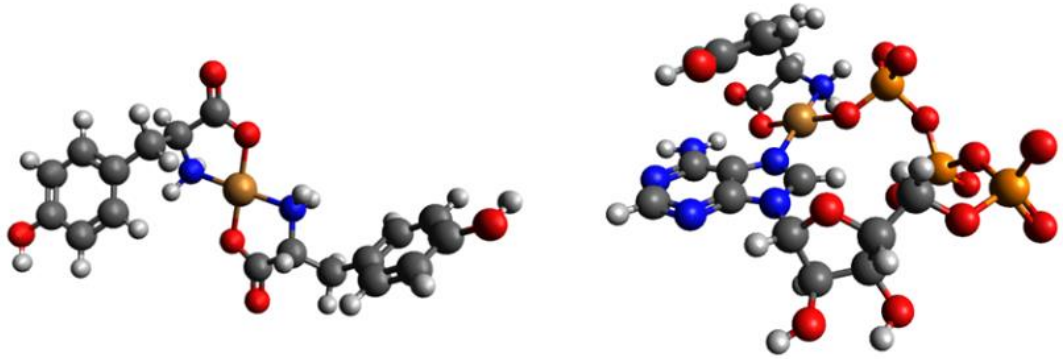

Figure 1. Structures of the complexes with the strongest interaction between Cu(II) and ligands molecules, obtained by molecular modelling and DFT studies.

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FP 8

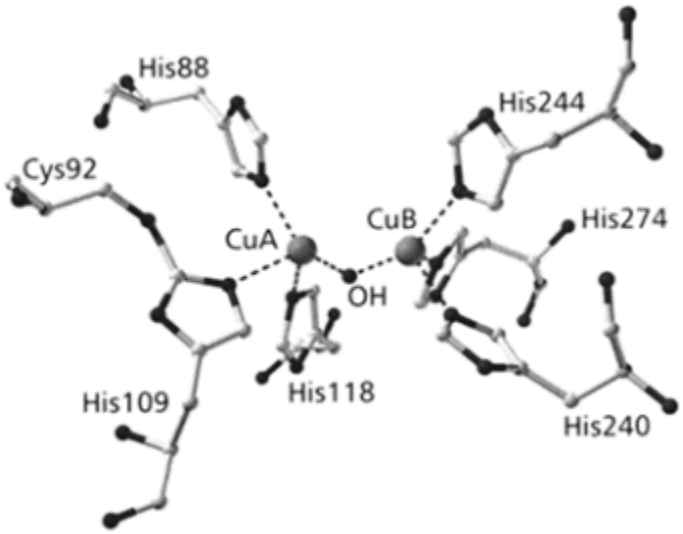
Study of the catalytic behavior of copper(II) complexes with potential catechol oxidase activity

Fruzsina SIMON, [a] Dóra Vargáné SZALÓKI, [a] István FÁBIÁN, [a] Norbert LIHI, [a]

[a] HUN-REN–UD Mechanisms of Complex Homogeneous and Heterogeneous Chemical Reactions Research Group, Department of Inorganic and Analytical Chemistry, University of Debrecen, Debrecen, Hungary; simon.fruzsina@science.unideb.hu

The chemical reactions catalyzed by metal-centered enzymes in living organisms serve as inspiration for the design of new functional metal complexes due to their selectivity and efficiency. These compounds are often referred to as biomimetics. The aim of biomimetic synthesis is to replicate the structural and functional features of enzyme active sites using synthetically accessible model compounds, thereby advancing our understanding of natural catalytic mechanisms and enabling new applications in the field of chemistry. [1]

Copper-containing enzymes play key catalytic roles in various biochemical processes, including electron transport, oxidation reactions, and oxygen binding and activation. Hemocyanin (oxygen transport), tyrosinase (hydroxylation of monophenols and oxidation of catechols), and catechol oxidase (oxidation of catechols) belong to the group of type-3 copper proteins. These enzymes possess magnetically coupled dinuclear copper(II) centers at their active sites. [2]



Scheme 1. The structure of the catechol oxidase active site.

Among these enzymes, catechol oxidase is of particular significance, as it catalyzes the oxidation of phenolic compounds under physiological conditions in the presence of molecular oxygen. Following oxygen activation at the dinuclear copper(II) active site, the substrate undergoes oxidation. During this process, catechol-type compounds are converted into quinone derivatives, thus, these reactions possess important biological and industrial relevance. [3]

Traditional oxidation methods often require stoichiometric amounts of inorganic oxidants that are toxic and environmentally harmful. Consequently, the development of catalytic systems capable of activating molecular oxygen with minimal environmental impact is gaining increasing attention. The challenge of activating molecular oxygen lies primarily in its kinetic inertness. Under standard conditions, molecular oxygen does not react readily with organic substrates. Furthermore, a major issue in using oxygen in chemical reactions is its poorly controllable reactivity, which often results in low selectivity or overoxidation. [4–7]

The aim of this work is to synthesize and investigate new copper(II)-containing metal complexes that, through their structure and function, may serve as biomimetics of the catechol oxidase enzyme. Given that these enzymes typically possess multiple metal-binding sites, our goal was to prepare and study a novel ligand containing multiple pyridine subunits capable of simultaneously coordinating at least two metal ions. In addition to synthesis, we aimed to examine the complex formation reactions between the ligand and copper(II) ions. The stability constants and the coordination modes of the corresponding copper(II) complexes were determined by pH-potentiometric titrations and great variety of spectroscopic techniques including UV-Vis, MS and EPR spectroscopies. The reactivity of the complexes formed at physiological pH was also studied in the reaction with ortho-dihydroxyphenol-type substrate compounds. These substrates exhibit structural features similar to molecules oxidized by naturally occurring catechol oxidase.

Acknowledgement:
The authors are grateful for the financial support of the Hungarian National Research, Development and Innovation Office (OTKA K-139140).

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FP 9

Peptidomimetics Based on Muc7 Fragment From Human Saliva: Comparison of Coordination, Structure, Stability and Antimicrobial Activity

Klaudia SZARSZOŃ, [a] Fabio ZOBI, [b] Tomasz JANEK, [c] Robert WIECZOREK, [a] Arian KOLA, [d] Daniela VALENSIN, [d] Joanna WĄTŁY, [a]

[a] Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland
[b] Department of Chemistry, Fribourg University, Chemin Du Musée 9, 1700 Fribourg, Switzerland
[c] Department of Biotechnology and Food Microbiology, Wrocław University of Environmental and Life Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland
[d] Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via A. Moro 2, 53100 Siena, Italy; klaudia.szarszon@uwr.edu.pl

Salivary mucin, MUC7, involved in the body's innate immune defense, is naturally cleaved into peptide fragments in saliva, some of which exhibit potent antimicrobial activity.[1] However, their therapeutic potential is limited by rapid enzymatic degradation. To overcome this challenge, a native MUC7-derived peptide was chemically modified using D-amino acids and a *retro-inverso* strategy to enhance its stability without compromising its function.[2]

Figure 1: Amino acid sequence of native MUC7 fragment (L1) and its peptidomimetic modifications (L2 and L3).

Considering the established influence of metal ions on antimicrobial peptides [3], we investigated the coordination behavior and biological properties of both native and modified MUC7 fragments in the presence of Cu(II) and Zn(II) ions. A comprehensive suite of experimental and computational techniques, including potentiometric titrations, UV-Vis, circular dichroism (CD), EPR, NMR spectroscopy, mass spectrometry, and DFT calculations, was employed to characterize metal binding, peptide and complex structure, and thermodynamic stability of complexes.

Our findings reveal that the standard substitution of L-amino acids with D-amino acids, as well as the modifications introduced by the *retro-inverso* strategy, preserve the peptides' secondary structure and antimicrobial efficacy, while significantly altering the thermodynamic stability of the metal complexes.[4,5]

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

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FP 10

Enhancing Antimicrobial Activity of Salivary Proline-rich Peptides *via* Metal Coordination and D-Amino Acid Substitution

Anna ŚLUSARCZYK, [a] Tomasz JANEK, [b] Denise BELLOTTI, [c] Joanna WĄTŁY, [a] Valentina BORGHESANI, [d]

[a] Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland.
[b] Faculty of Biotechnology and Food Sciences, Wrocław University of Life Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland.
[c] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Via Luigi Borsari 46, 44121 Ferrara, Ferrara, Italy.
[d] Department of Chemistry, Life Sciences, and Environmental Sustainability, University of Parma, Parco Areadelle Scienze 11 A, 43124 Parma, Italy; anna.slusarczyk@uwr.edu.pl

Overcoming the challenge of antibiotic resistance requires innovative strategies such as stable, metal-enhanced AMPs [1]. Human salivary antimicrobial peptides are key components of the innate immune defense, exhibiting natural antimicrobial properties against a broad spectrum of pathogens. The antimicrobial activity of these peptides is significantly enhanced upon coordination with metal ions such as copper or zinc, stabilizing their structure and increasing biological efficacy [2]. This metal-dependent activation mechanism is particularly relevant for proline-rich sequences, which often lack a defined secondary structure yet display potent activity through specific molecular interactions. Despite their advantages, the therapeutic application of AMPs is often limited by their susceptibility to enzymatic degradation in a physiological environment. To address this, current research focuses on designing peptidomimetics—structurally modified analogs that retain or even enhance antimicrobial activity while exhibiting improved resistance to proteolysis [3].

In this study, we investigate the enzymatic stability, thermodynamics, coordination behavior, structural properties, and antimicrobial activity of Cu(II) and Zn(II) complexes with salivary proline-rich peptides and their D-amino acid-substituted analogs (**Figure**).

A comprehensive analytical approach was employed to investigate the coordination behavior and stability of metal–peptide and metal–peptidomimetic complexes. This included potentiometric titration, a range of spectroscopic techniques (UV-Vis, circular dichroism, and electron paramagnetic resonance), mass spectrometry, and high-performance liquid chromatography (HPLC). The antimicrobial potential of the studied compounds was evaluated through biological assays.

Our findings reveal that metal coordination preferences vary depending on the applied modifications. Enantiomeric substitution of amino acids significantly enhances the thermodynamic stability of Cu(II) and Zn(II) complexes, while the enzymatic stability of partially modified peptides remains unchanged. In contrast, the fully D-amino acid analog exhibits exceptional resistance to proteolysis.

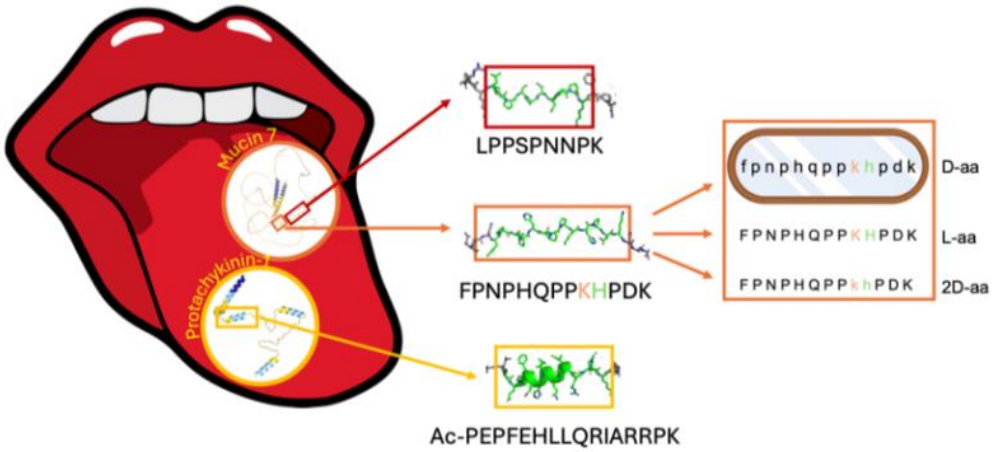

Figure: Proline-rich antimicrobial peptide fragments derived from salivary proteins Mucin-7 and Protachykinin, along with their D-amino acid-substituted mimetics (lowercase letters mean D-amino acids). Enzymatically vulnerable regions are highlighted in color.

Among all analyzed peptides, the LPPSPNNPK peptide demonstrated the strongest antimicrobial activity. It is characterized by its high proline content, which likely contributes to enhanced biological performance through increased rigidity and potential for favorable interactions with microbial membranes.

Additionally, only the protachykinin-derived fragment adopts a defined secondary structure, forming a polyproline II (PPII) helix, possibly playing a role in facilitating specific coordination geometry and improved molecular recognition or membrane interaction.

Overall, this study highlights the importance of enantiomeric substitutions and proline-rich motifs in tuning the stability and activity of antimicrobial peptides. These strategies provide a rational basis for designing next-generation antimicrobial agents with enhanced therapeutic potential.

Acknowledgments:
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POSTER PRESENTATIONS

P 1

Thermodynamic and spectroscopic analysis of peptide-based enzyme mimics containing the ATCUN site

Denise BELLOTTI, [a] Silvia LEVERARO, [a] Gianpiero ADAMI, [b] Valentina ALBANESE, [a] Silvia BERTO, [c] Riccardo BONSIGNORE, [d] Eugenio GARRIBBA, [e] Remo GUERRINI, [a] Antonello MERLINO, [f] Salvatore PACIFICO, [a] Maurizio REMELLI, [a]

[a] Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, via Luigi Borsari 46, 44121 Ferrara, Italy
[b] Department of Chemical and Pharmaceutical Sciences, University of Trieste, Via Licio Giorgieri 1, 34127 Trieste, Italy
[c] Department of Chemistry, University of Torino, via Pietro Giuria 5, 10125 Torino, Italy
[d] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze, 90128 Palermo, Italy
[e] Department of Medicine, Surgery and Pharmacy, Università di Sassari, Viale San Pietro, 07100 Sassari, Italy
[f] Department of Chemical Sciences, University of Napoli Federico II, via Cupa Nuova Cintia, 21, 80126 Napoli, Italy; blldns@unife.it

Enzyme mimics (EMs) are molecular entities that typically replicate the binding and catalytic function of natural enzymes [1]. In this work we focus on peptide-based EMs with an ATCUN (Amino-Terminal Copper and Nickel) binding site, in order to promote a multitarget antimicrobial mechanism. Our approach in designing novel EMs relies on synthetic branched peptides to generate a previously unexplored category of EMs. A biocompatible central scaffold serves as the core of the EM structure, to which various oligopeptides can be attached [2,3]. The presented work consists of a preliminary study of two selected oligopeptides: AAHAWG-NH_2 and AAHAWGELLKKLLEELKG-NH_2. The peptide ability to form stable Cu(II) complexes has been investigated, together with the ability to generate reactive oxygen species. Indeed, the presence of an ATCUN motif can lead to an increased antimicrobial power, due to copper catalytic action. Potentiometric titrations, mass spectrometry and different spectroscopic techniques (e.g. UV-Vis absorption, circular dichroism) have been employed to thoroughly study the metal interaction with the selected peptides (Figure 1).

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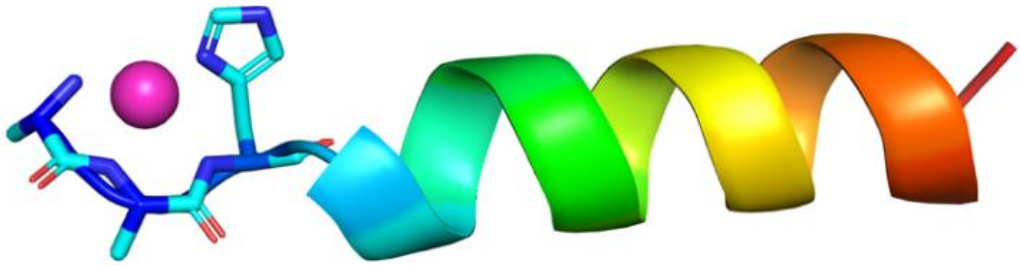

Figure 1: Coordination and structure hypothesis for the Cu(II)/AAHAWGELLKKLLEELKG-NH_2 complex.

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P 2

Topical Application of Iron Chelators to Reduce Inflammation and Promote Chronic Wound Healing

Weronika BUDA, [a] Elżbieta GUMIENNA-KONTECKA, [a]

[a] *Uniwersytet Wrocławski, Wydział Chemii, ul. F. Joliot-Curie 14, 50-383 Wrocław; weronikabudauwr@gmail.com*

The treatment of chronic wounds, especially in patients with diabetes [1], sickle cell anemia [2], or those who have undergone radiotherapy [3,4], is particularly difficult. Rapid and effective tissue repair and minimization of inflammation are key factors in improving the patient's health and preventing necrosis of untreated and degraded tissues.

In recent years, research groups have shown increased interest in the use of iron chelators in transdermal dressing formulations. The active agent in these therapies is deferoxamine (DFO), which, in addition to its strong ability for iron chelation, is also an FDA-approved agent. In the context of chronic wounds, studies have shown that DFO effectively initiates angiogenesis, and when used prophylactically, it prevents tissue necrosis while supporting the healing of ulcers through the reduction of oxidative stress [5].

This poster summarizes the current state of knowledge on the effectiveness of topical application of iron chelators in the form of hydrogel dressings or creams in the treatment of chronic wounds. The selected studies focus on wound therapies targeting conditions caused by external factors such as radiation exposure, as well as diseases like diabetes. The discovery of a rapid and effective treatment method would represent a major advancement in modern medicine.

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P 3

Exploring the metal specificity of the bacterial Mn(II) transporters

Jakub CHARKOT, [a] Karolina PAWLIK, [a] Karolina MICHNA, [a] Dominika SZEWCZYK, [a] Elzbieta GUMIENNA-KONTECKA, [a] Malgorzata OSTROWSKA, [a]

[a] University of Wrocław, Department of Chemistry, F. Joliot-Curie 14, 50-383 Wroclaw; 347351@uwr.edu.pl

Although far less studied than iron or zinc, manganese has attracted considerable attention in recent years due to its role in nearly all forms of life, especially in pathogens and the host immune system. An increasing body of evidence now suggests that invading microbes utilize Mn(II) as a key micronutrient to resist the effects of host-mediated oxidative stress. The requirement for manganese by pathogenic bacteria necessitates the acquisition of this metal ion from the host environment, making manganese acquisition a potential therapeutic target at the host–pathogen interface [1, 2].

The capture and transport of Mn(II) into the cell are facilitated directly through Mn(II)-specific import systems. To date, two major classes of Mn(II) transporters in prokaryotes have been characterized: the natural resistance-associated macrophage protein (NRAMP) transporters (MntH) and the ATP-binding cassette (ABC) Mn(II) permeases (MntABC). Both are highly selective for Mn(II) over Fe(II) or other divalent transition metal cations, and each can accumulate millimolar concentrations of intracellular Mn(II) even when environmental levels are scarce. Structural and biophysical studies generally support a simple competition model, in which the extracellular chelator calprotectin (CP) and Mn(II)-specific uptake systems compete for the same metal based on their respective affinities. However, much more remains to be learned about Mn(II) coordination chemistry to fully understand this process [3, 4, 5].

In our research, we selected unstructured regions of Mn(II)-binding proteins and their mutated derivatives. We used a variety of physicochemical methods—such as potentiometry, mass spectrometry, NMR, EPR, and CD spectroscopy—to characterize the complex species formed between the selected peptide fragments and Mn(II), Fe(II), and Zn(II) ions. The obtained results may provide insights into the poorly understood thermodynamics and coordination chemistry of Mn(II) ions with specific metal transporters.

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P 4

Characteristics of Zn^{2+} and Cu^{2+} complexes of the active site of M4 family metallopeptidase and its inhibitors

Klaudia GŁADYSZ, [a] Martyna ZAWADA, [a] Sławomir POTOCKI, [a]

[a] Uniwersytet Wrocławski, Wydział Chemii, Fryderyka Joliot-Curie 14, 50-300 Wrocław; 323797@uwr.edu.pl

Studies focused on identifying bacterial virulence factors are crucial for developing new therapeutic strategies in response to the growing global problem of antibiotic resistance. One example of an antibiotic-resistant bacterium is *Legionella pneumophila*, the causative agent of Legionnaires' disease, which can lead to multi-organ complications and even patient death [1,2].

Among the virulence factors are metallopeptidases (MPs), which may serve as potential targets for novel therapeutic strategies. These enzymes are responsible for degrading host tissues and components of the immune system, thereby promoting the development of infection and hindering its elimination by the host organism [3,4].

In this study, the active site of an MP from the M4 family derived from *Legionella pneumophila* was analyzed, along with two potential inhibitors based on substrate sequences. Previous studies have shown that replacing the Zn(II) ion with Cu(II) results in a significant reduction of the enzyme's catalytic activity [5,6]. Combining metal ion substitution with substrate-sequence-based inhibition could therefore provide a synergistic effect, leading to stronger and more selective inhibition of metallopeptidase activity.

Using potentiometric titration, UV-Vis spectroscopy, circular dichroism, and nuclear magnetic resonance spectroscopy, the sequence of the studied domain was determined, and it was shown that the inhibitor with the sequence HNLGLARN forms more stable complexes with the studied metal ions. A key role is played by the "histamine-like binding mode" {NH_2, N_{im}}, which, according to the literature, contributes to the stability of the complex [7].

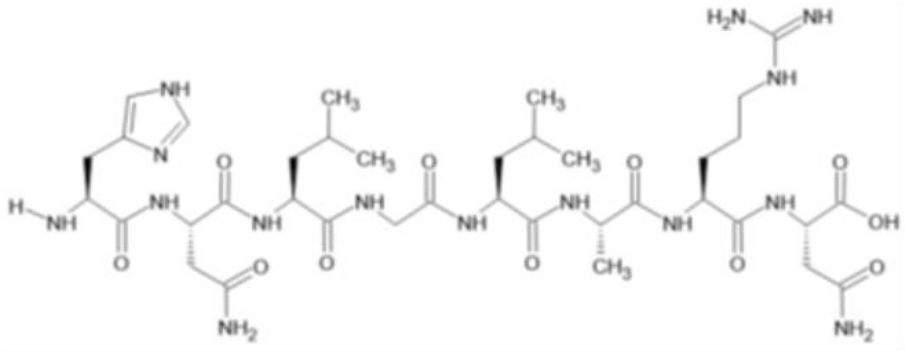

Figure 1. Structure of the HNLGLARN inhibitor. Created using ChemSketch, version 2021.1.3.

The obtained results serve as a starting point for further research on substrate sequence–based inhibitors, including HNLGLARN and its modifications, which, when

complexed with transition metal ions, may form more stable complexes and thus enhance the therapeutic potential of the studied inhibitors.

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P 5

Bi(III) Ion Complexes with Siderophores – Towards New Solutions for Combating Microorganisms

Wiktoria JOŃCZYK, [a] Klaudia SZCZERBA, [a] Elżbieta GUMIENNA-KONTECKA, [a]

[a] Uniwersytet Wrocławski, Wydział Chemii, ul. F. Joliot-Curie 14, 50-383 Wrocław; 322648@uwr.edu.pl

Bismuth compounds have been used in medicine for centuries. Initially, they were employed in gastrointestinal disorders, against syphilis, or as surgical dressings. Nowadays, bismuth compounds are most commonly used in the fight against *Helicobacter pylori*, where bismuth exhibits bactericidal activity [1]. Recent studies have shown that bismuth-based drugs can also be used against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus pumilus* [2].

Pseudomonas aeruginosa (blue pus bacillus) is a bacterium that causes nosocomial infections and exhibits high resistance to antibiotics, which poses a serious problem in the treatment of infections caused by this microorganism [3,4]. It has been shown that the use of bismuth-based drugs in combination with antibiotics increases their activity against this bacterium. This effect is due to the binding of Bi(III) ions by siderophores secreted by the bacterium—pyoverdine and pyochelin—responsible for capturing Fe(III) ions, leading to a reduction in the concentration of this metal, essential for the bacterium's survival, and ultimately to bacterial cell death [4].

Inspired by this fact, we undertook research aimed at characterizing the properties of bismuth coordination compounds with other siderophores, with a view to their further use in antibiotic therapy and combating bacterial resistance. At the conference, we will present studies carried out for desferrioxamine B (DFO), a siderophore secreted by *Streptomyces pilosus*, which is also used as a drug in cases of excessive iron accumulation in the body and aluminum poisoning [5,6].

To determine the stability constants of the complexes, potentiometric titration and pH-dependent titration using UV-Vis spectroscopy were employed. The metal-to-ligand stoichiometry in the complexes was determined by mass spectrometry. A series of competitive titrations in DFO–Bi(III)–Fe(III) systems was also carried out, confirming the binding of Bi(III) to DFO as well as the siderophore's preference for one of these metals.

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P 6

Dual Amino Acid Substitution in a MUC7-Derived Peptide Enhances Antimicrobial Resistance and Modulates Zn(II)/Cu(II) Binding Stability

Jan KACHNOWICZ, [a] Klaudia SZARSZOŃ, [a] Aneta JEZIERSKA, [a] Tomasz JANEK, [b] Joanna WĄTŁY, [a]

[a] University of Wrocław, Department of Chemistry, F. Joliot-Curie 14, 50-383 Wrocław
[b] Faculty of Biotechnology and Food Sciences, Wrocław University of Life Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland; 331999@uwr.edu.pl

The problem of increasing antibiotic resistance concerns us all. Even now, there are bacterial strains causing infections with mortality rates comparable to those from before the antibiotic era.[1] For this reason, new strategies are being sought to combat antibiotic-resistant pathogens. One such strategy involves antimicrobial peptides found in saliva and their complexes with metal ions such as Cu(II) or Zn(II).[2] An example of such a peptide is the N-terminal fragment of a glycoprotein present in human saliva, mucin 7, with the sequence EGRERDHELRHRRHHHQSPK.[3] However, this fragment is susceptible to proteolytic degradation. To create a peptide more resistant to enzymatic breakdown, the most degradation-prone site was modified by replacing two L-amino acids with their D-enantiomers (Table 1).

Table 1. Sequence of the native peptide and the peptidomimetic (lowercase letters 'rh' represent D-arginine and D-histidine).

Native sequence	Peptidomimetic sequence
EGRERDHELRHRRHHHQSPK	EGRERDHELRHRrhHHQSPK

The properties of the resulting peptide fragment were analyzed using a range of physicochemical methods and compared with the native fragment, revealing significant differences in structure and metal ion binding with Cu(II) and Zn(II).
The results showed that the peptide containing the two D-amino acids binds Cu(II) ions significantly more strongly, especially in the pH range of 7–9. This is likely due to a different mode of coordination and the spatial arrangement of D-His compared to its L-analogue. In the case of Zn(II) complexes, the efficiency and mode of metal ion binding by the native peptide and its peptidomimetic were found to be the same. An impact of the enantiomeric substitution on the secondary structure of the ligand and its complexes with both Cu(II) and Zn(II) was also observed. To gain deeper insight into these phenomena, in silico methods were employed, which produced results in good agreement with the experimental findings.
I would like to thank the National Science Centre (NCN) for financial support (NCN OPUS-21 project, no. 2021/41/B/ST4/02654, Dr. Joanna Wątły) and the Polish computing centers WCSS and Cyfronet AGH for providing computational time, software, and all their assistance.

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P 7

Lighting Up Infections: Artificial Siderophores for Selective Imaging of Infectious Bacteria

Adriana KNOLL, [a] Katerina BENDOVA, [b] Isidor HAPPACHER, [c] Isabelle J SCHALK, [d] Elzbieta GUMIENNA-KONTECKA, [e] Hubertus HAAS, [c] Milos PETRIK, [b] Clemens DECRISTOFORO, [a]

[a] Department of Nuclear Medicine, Medical University of Innsbruck, 6020 Innsbruck, Austria.
[b] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, 77900 Olomouc, Czech Republic
[c] Institute of Molecular Biology, Biocenter, Medical University Innsbruck, A-6020 Innsbruck,
[d] University of Strasbourg, Bld Sébastien Brant, 67412, Illkirch, Strasbourg, France
[e] Faculty of Chemistry, University of Wrocław, 50-383 Wrocław, Poland;
adriana.knoll@i-med.ac.at

Diagnosing bacterial infections in the human body can be challenging, as the source of infection is often difficult to detect, and rapid diagnosis is critical. PET (Positron emission tomography) imaging with bacteria-specific agents offers a promising approach to localize infection sites. This powerful tool would allow clinicians not only to find the location of infections but also to identify the causative bacterial species, or at least narrow it down. Combining both capabilities could be particularly valuable for diagnosing cryptic infections. In this study, we investigated two siderophores, the naturally occurring FOX E and FOX 2-5, a synthetic siderophore and analog of FOX E. As FOX E and FOX 2-5 show the same molecular weight of 600.7 Da, their difference is the position of the hydroxamic groups, which are arranged in reverse. Both were tested across various bacterial strains. Our in vitro and in vivo studies revealed that FOX E and FOX 2-5 possess specificities toward different bacterial strains. Our findings also indicate that the bacteria exhibit distinct uptake patterns for gallium- and iron-labeled siderophores. These findings highlight the potential of artificial siderophores as versatile agents for both the detection and characterization of bacterial infections, offering a promising step toward more precise and personalized diagnostic strategies.

P 8

Coordination properties of a peptidic fragment of the bacterial metal ion transporter IroT

Nina OLEŚNIEWICZ, [a] Bartosz ORZEŁ, [a] Elżbieta GUMIENNA-KONTECKA, [a]

[a] Faculty of Chemistry, University of Wrocław, 50-383 Wrocław, Fryderyka Joliot-Curie 14, Poland; 337503@uwr.edu.pl

IroT (also known as MavN – more regions allowing vacuolar colocalization N) is a membrane Fe^{2+} ion transporter. In in vivo studies, IroT demonstrates high specificity for Fe^{2+} ions, although under in vitro conditions it is also capable of transporting other divalent metal ions such as Mn^{2+}, Co^{2+}, and Zn^{2+} [1, 2]. Its presence has been primarily detected in bacteria of the genus Legionella, although it is also found in other obligate intracellular parasites, such as Rickettsiella [2].

The aim of this study was to characterize the coordination properties of a selected fragment of the IroT transport protein, which is present in its native form in *L. pneumophila*. IroT transports Fe^{2+} ions into the Legionella-containing vacuole (LCV) and may contribute to the increased virulence of the pathogen [3]. The research focused on a fragment derived from loop 7 of the protein, which includes the EXXE motif and histidine residues, which are commonly associated with metal ion binding. Potentiometric titration and mass spectrometry were used to determine the ligand's deprotonation constants, the stability constants of its metal complexes, and their stoichiometry. The formation of these complexes was studied across a pH range of 2–11. The analyzed fragment was shown to form stable 1:1 complexes with Fe^{2+}, Mn^{2+}, and Zn^{2+} ions. The greatest stability at pH ~6.1 (the pH found in the LCV) was observed for Zn^{2+} complexes, followed by Mn^{2+}, with Fe^{2+} forming the weakest complexes, which may result from differences in the coordination chemistry of these metals. The results provide a basis for further studies on the selectivity of IroT and its role in iron transport under in vivo conditions.

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P 9

New Triorganotin(IV) syringates as potential antitumoral agent: From chemical synthesis to biochemical effects

Claudia PELLERITO, [a] Federica AFFRANCHI, [b] Antonietta NOTARO, [b] Chiara ZICHITTELLA, [c] Salvatore BARRECA, [d] Ivan Pietro OLIVERI, [d] Tiziana FIORE, [a]

[a] Dipartimento di Fisica e Chimica - Emilio Segrè, University of Palermo, Italy [b] Dipartimento di Scienze e Tecnologie biologiche, chimiche e farmaceutiche, Plesso di Biochimica, Policlinico, University of Palermo, Italy [c] Dipartimento di Biomedicina, Neuroscienze e Diagnostica avanzata, Laboratorio di Biochimica, University of Palermo, Italy [d] Dipartimento di Scienze Chimiche, University of Catania, Italy; claudia.pellerito@unipa.it

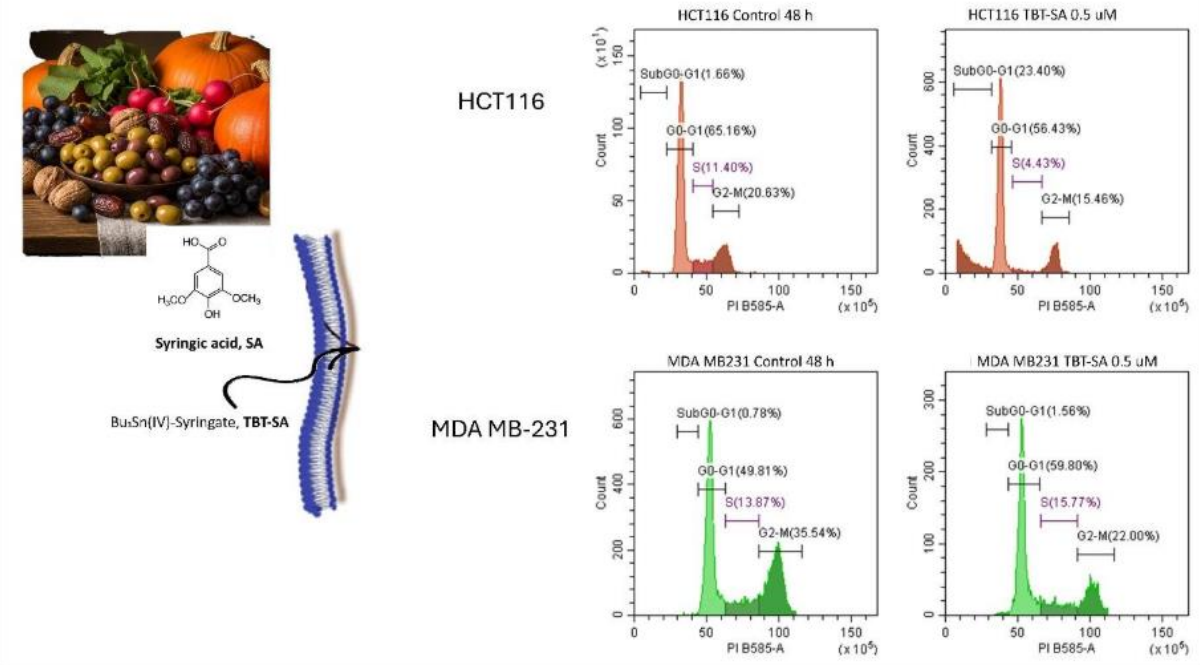
Natural bioactive compounds have been shown to alter the redox balance of cells and affect fundamental cellular processes like the cell cycle, apoptosis (programmed cell death), and inflammation. Syringic acid (SA), chemically known as O-methylated trihydroxy benzoic acid or 4-hydroxy-3,5-dimethoxy, is one of the most common phenolic acids and belongs to the class hydroxybenzoic acid. SA has a chemical structure featuring a benzene ring bonded to a hydroxyl (-OH) group, a carboxylic acid (-COOH) group, and two methoxy (-OCH_3) groups. The strategic placement of these methoxy groups at positions 3 and 5 on the aromatic ring is responsible for its beneficial therapeutic properties. SA demonstrates potent free radical scavenging properties, effectively mitigating oxidative stress markers. Its diverse therapeutic applications encompass preventing conditions such as diabetes, cardiovascular diseases (CVDs), cancer, and cerebral ischemia. Furthermore, it exhibits antioxidant, antimicrobial, anti-inflammatory, antiendotoxic, neuroprotective, and hepatoprotective activities.[1]

With the aim of improving the antitumor efficacy of SA, we synthesized new triorganotin(IV) syringates. In fact one side organotin compounds represent potential cancer therapeutics due to their pro-apoptotic action [2], the other syringic acid possess coordination potential donor atoms for organotin(IV) moiety, such carboxylate and phenolate.

The coordination environment at the tin center was investigated spectroscopically by Fourier Transform Infrared (FT-IR), Electrospray Ionization Mass Spectroscopy (ESI-MS), Nuclear Magnetic Resonance (NMR) spectroscopies. Following synthesis, chemical characterization in solid and solution state, we evaluated compounds effects in colon and breast cancer cells. In previous studies, we showed that exposure to a micromolar concentration of an organotin(IV) complex with caffeic acid, a catechol-containing polyphenol found in coffee, induces a time- and dose-dependent decrease in colorectal cancer cell viability. Additionally, we found that even nanomolar concentrations of a tributyltin(IV) complex with ferulic acid, another hydroxycinnamic acid derivative, were effective in reducing the viability of HCT116, HT-29, and Caco-2 colon cancer cells. On the other hand, both free ligands were completely inefficacious at the same treatment conditions. [3,4]

The evaluation of biological effects of the triorganotin(IV) derivatives of syringic acid evidenced that tributyltin(IV)syringate complex (TBT-SA) shows a significant cytotoxic activity in tumor cells, reducing the proliferation of colon cancer HCT116 cells of about 80% at 500 nM and of about 60% that of breast cancer MDA-MD231 cells after 48 h of treatment. At the same dose, the parent SA was completely ineffective. The analysis of cell cycle distribution

confirmed the results demonstrated the presence of a preG0-G1 peak (from 1.86% to 23.40% in treated cells) in colon cancer cells, while only a modest block in G0-G1 phase was present in breast cancer cells.



Taken together, our results strongly suggest that the combination of organotin(IV) moieties with natural polyphenols ligands represents a promising therapeutic strategy for colon cancer.

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P 10

Dissecting Copper and Zinc Binding Sites in Small Heat Shock Protein Fragments: Toward a Molecular Understanding of sHSP–Metal Interactions

Agnieszka SZEBESCZYK, [a)]

[a)] *Institute of Health Sciences, University of Opole, Katowicka str. 68, Opole, Poland; agnieszka.szebesczyk@uni.opole.pl*

Small heat shock proteins (sHSPs) are a class of molecular chaperones that are upregulated in response to elevated temperatures, playing a multifaceted role in maintaining cellular homeostasis. Among them, crystallins – members of the sHSP family – are particularly notable for their abundance in the eye lens, where they form highly ordered polymeric assemblies [1]. These structures are not only essential for maintaining lens transparency but also confer thermal stability to the tissue.

Given the structural and functional importance of crystallins, the interaction between these proteins and metal ions, such as zinc and copper, has emerged as a subject of scientific interest [2,3]. Metal ion coordination may significantly influence protein stability and function, particularly under stress conditions [4]. Therefore, elucidating the specific binding sites and the thermodynamic stability of metal-protein complexes is crucial for understanding the mechanisms underlying their protective effects.

However, the tendency of sHSPs to form large oligomers complicates direct structural studies. As a result, investigating smaller peptide fragments and their synthetic analogs offers a more tractable approach for probing metal binding properties at the molecular level.

One such protein, HSPB1, contains a conserved α-crystallin domain and exhibits sequence similarity within its disordered regions not only to crystallins but also to ferritin, the principal iron storage protein. In this study, peptide fragments derived from HSPB1, along with their alanine-substituted analogs (Figure 1), were analyzed using a combination of thermodynamic and analytical techniques. This approach enabled the identification of key amino acid residues involved in metal ion coordination.

Interestingly, different experimental strategies yielded varying results, highlighting the complexity of metal-peptide interactions. Nevertheless, the integrative application of multiple analytical methods provided a comprehensive profile of metal ion binding characteristics within selected peptide regions of HSPB1, offering valuable insights into the broader functional roles of small heat shock proteins under stress conditions.

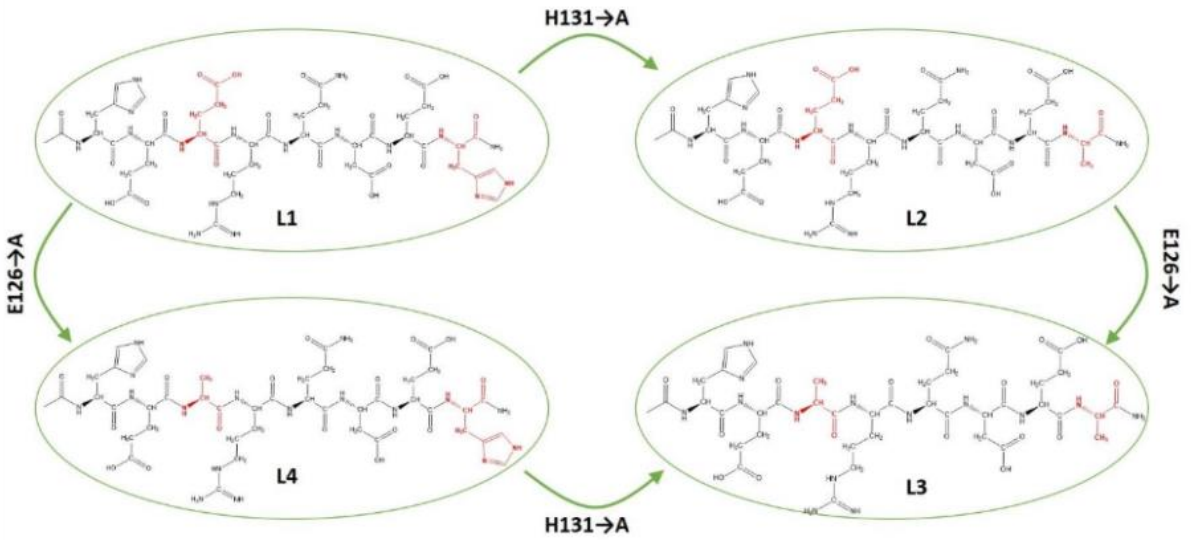

Figure 1: Schematic representation of the studied peptides with the position of the aminoacidic substitutions evidenced in red in their structure. L1 – Ac-HEERQDEH-NH_2; L2 - Ac-HEERQDEA-NH_2; L3 - Ac-HEARQDEA-NH_2; L4 - Ac-HEARQDEH-NH_2.

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P 11

Metalloproteinases and their peptide inhibitors – thermodynamic characterisation of complexes with Zn(II) ions

Wiktoria WOŹNIAK, [a)] Paulina POTOK, [a)] Sławomir POTOCKI, [a)]

[a)] Faculty of Chemistry, University of Wrocław, F. Joliot-Curie 14, 50-383 Wrocław, Poland; wiktoria.wozniak@uwr.edu.pl

Extracellular matrix metalloproteinases (MMPs) are a zinc-dependent enzymes, whose catalytic site contains a Zn(II) ion, coordinated by three histidine residues, essential for their proteolytic activity. Metalloproteinases are capable of degrading extracellular matrix components (ECM), thus playing a key role in cell proliferation, differentiation, and apoptosis. For this reason, MMPs are strongly associated with the development of cancer, as ECM proteolysis is essential for the growth, migration and invasion of cancer cells. Increased MMPs concentrations have been demonstrated in many cancers, making them attractive therapeutic targets. Consequently, there is an intensive search for new anti-cancer strategies that would involve inhibiting metalloproteinase activity by targeting therapeutics to Zn(II) ions and/or the catalytic site of MMPs [1,2].

The main objective of this study was to characterise the interaction between the MMP-1 catalytic domain, Zn(II) and designed substrate-mimicking peptide inhibitors: H-CPQGLFR-OH (Inh-1), H-PQGLGRC-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 depending on pH. The UV-Vis method was used to investigate the catalytic properties of the tested complexes. The stoichiometry of the complexes was confirmed using mass spectrometry.

We first established that the MMP-1 peptide model reproduces the native Zn(II) coordination environment, consisting of three histidines and one water molecule, as in the catalytic site of the enzyme. Kinetic studies defined K_m and V_{max} constants and the optimal pH for MMP-1's activity, which is estimated at pH 8.8. Based on the determined kinetic parameters, it was found that the tested catalytic domain exhibits significant catalytic activity, although lower than that of the native MMP-1 protein. Nevertheless, this activity is sufficient to perform the relevant measurements. Although inhibitors differ only in the position of the cysteine residue, their interactions with Zn(II) and Zn(II)-MMP-1 complex vary significantly. 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. Calculated relative stability (%R.S.) shows that the ternary complexes are more stable than binary ones, with higher stability observed for MMP-1-Zn(II)-Inh-1 system compared to MMP-1-Zn(II)-Inh-2. Therefore, formation of ternary MMP-1-Zn(II)-Inh-1 complex can sterically block the binding of the substrate at the catalytic Zn(II), thus inhibit the proteolytic activity of MMP-1.

This study expands current knowledge on the coordination properties of the Zn(II)-MMP-1 complex and provides complete physicochemical, structural and thermodynamic data on the complexes formed between MMP-1, essential Zn(II) ion, and designed inhibitors. By elucidating how cysteine positioning influences complex stability and catalytic inhibition,

these results may be used as a valuable basis for development of more selective and potent MMPs inhibitors, as potential anticancer strategies.

Acknowledgements:
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