19th Naples Workshop on Bioactive Peptides

A Never-Ending Tale

ABSTRACT BOOK

NAPLES - JUNE 25th - 27th 2026

Centro Congressi of University of Naples "Federico II"

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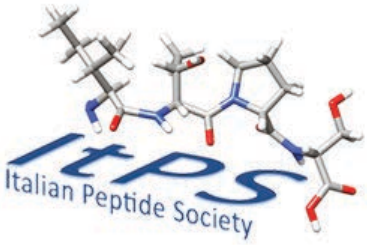

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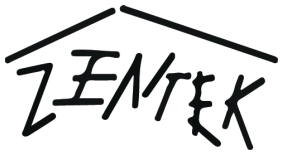
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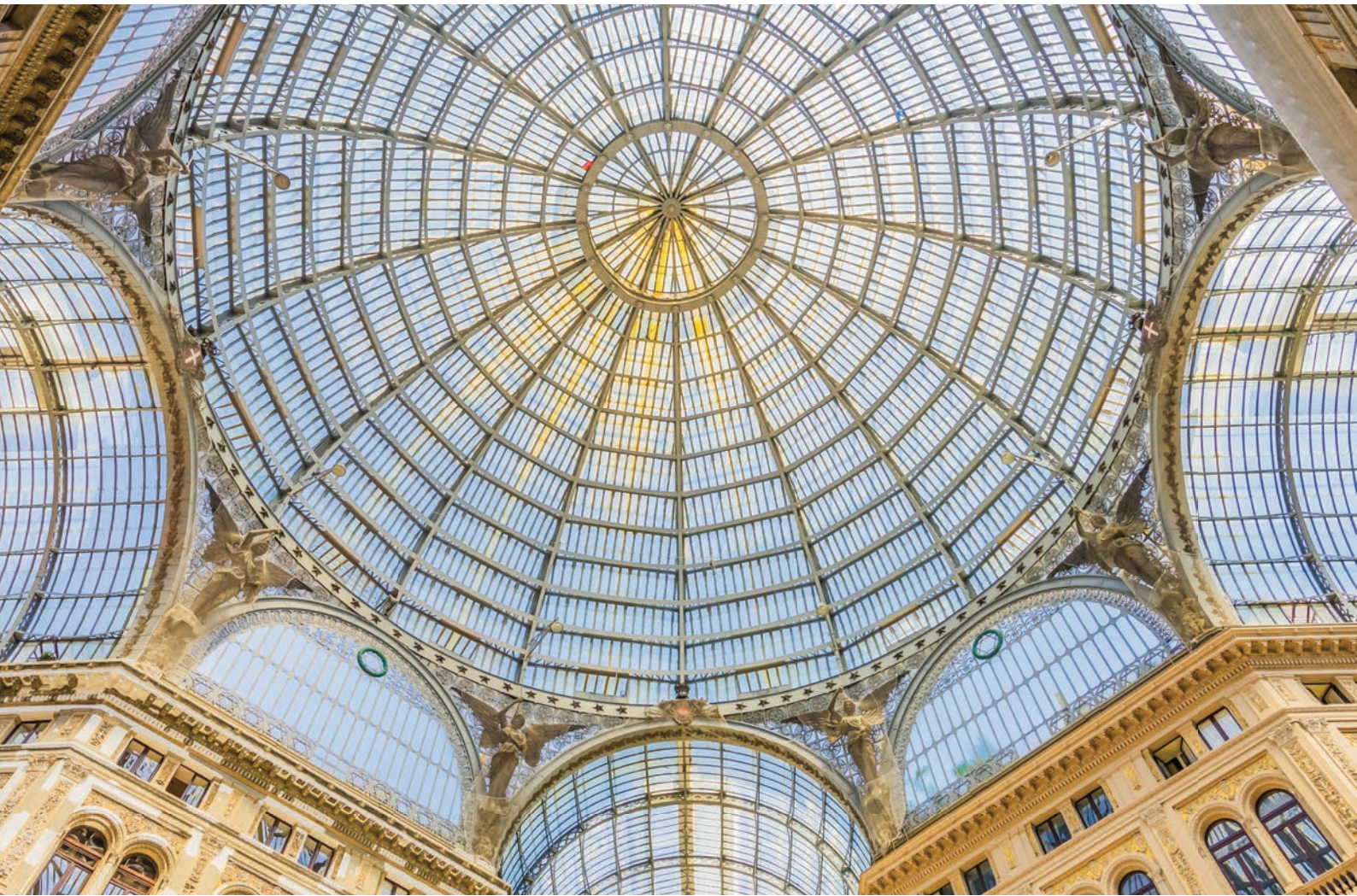

PROGRAM

THURSDAY, JUNE 25TH

10:30-19:00 Registration
13:45-14:00 Welcome address

SESSION 1
Chair: M. Ruvo - P. Grieco

14:00-14:50 **Opening Lecture - Fernando Albericio, University of KwaZulu-Natal, South Africa**
Redifining Peptide Synthesis: Emerging Strategies for Sustainable Future

14:50-15:20 **PL1 - Guido Franzoso, Imperial College, UK**
Cancer-Selective targeting of the NF-κB pathway with GADD45β/MKK7 inhibitors: Clinical evidence from early-phase trial

15:20-15:50 **PL2 - Christian Becker, University of Vienna, Austria**
The impact of posttranslational modifications on chaperone function and protein degradation

15:50-16:20 **PL3 - Philip Thompson, Monash University, Australia**
Diverse cyclic peptide architectures for targeting melanocortin receptors

16:20-17:00 **Coffee Break & Poster Session**

17:00-17:30 **PL4 - David Craik, The University of Queensland, Australia**
Update on applications of cyclotides in drug design

17:30-18:00 **PL5 - Severin Schneebeli, Purdue University, United States**
Permeation-Enhancer Mediated Peptide Transport Across Biological Membranes: Mechanism, Simulation, and Emerging Single-Molecule Approaches

18:00-18:25 **KN1 - Roberto Corradini, University of Parma, Italy**
Peptide Nucleic Acids (PNAs) as scaffolds for multi-functional compounds with multi-purpose applications

18:25-18:45 **O1 - Chloé Cayrou, CEA Saclay Gif sur Yvette, France**
Engineering Therapeutic-Relevant Snake Peptide Toxins with Selenocysteine to Investigate Folding and Stability

18:45-19:05 **O2 - Michael Gütschow, University of Bonn, Germany**
Redirecting the Peptide Cleavage Causes Protease Inactivation

19:15-20:30 **Welcome Party**

PROGRAM

FRIDAY, JUNE 26TH

SESSION 2
Chair: A. De Simone – A. Del Gatto

09:00-09:30 **PL6 - Gianluigi Veglia, University of Minnesota, United States**
Molecular Details for activation and deactivation of the sarcoplasmic reticulum Ca2+-ATPase (SERCA) by membrane polypeptides

09:30-09:55 **KN2 - Xuechen Li, The University of Hong Kong**
Disrupt Peptide Aggregation for Difficult Peptide/Protein Synthesis

09:55-10:15 **O3 - Leopoldo Sitia, IRCCS San Raffaele, Italy**
Peptide-based fluorescent conjugates for targeted imaging, fluorescence-guided surgery, and phototherapy in solid tumors

10:15-10:40 **Coffee Break**

SESSION 3
Chair: M. Saviano – A. Accardo

10:40-11:10 **PL7 - Ines Neundorf, University of Cologne, Germany**
Design of cell-permeable DHHC peptides targeting S-palmitoylation dynamics

11:10-11:35 **KN3 - Nicolò Tonali, CEA Saclay, France**
From endogenous and natural peptides to functionalized pharmacological tools: synthesis, development and applications in neurodegenerative diseases and cancer

11:35-12:00 **KN4 - Marc Devocelle, Royal College of Surgeons, Ireland**
Novel antibiotic- and lipid-peptide conjugations applied to antimicrobial peptides

12:00-12:20 **O4 - Lihi Adler-Abramovich, Tel Aviv University, Israel**
Engineered Supramolecular Nanostructures for Tissue Regeneration

12:20-12:40 **O5 - Maayan Gal, Tel Aviv University, Israel**
AI-driven cyclic peptides inhibiting undruggable intracellular PPIs: application to the Calcineurin-NFAT interface for improved immunosuppressants

12:40-13:00 **O6 - Marina Sala, University of Salerno, Italy**
Towards green solid phase peptide synthesis: DBN for sustainable Fmoc removal

13:00-14:30 **Lunch on site**

SESSION 4
Ind (dedicated to Companies)
Chair: G. Morelli

14:30-14:45 **O1-Ind - Thomas Bruckdorfer, Iris Biotech, Germany**
TAPS – Where to put which Tag in Tag Assisted Peptide Synthesis

PROGRAM

14:45-15:00 **O2-Ind - Antonio Ricci, Fresenius Kabi iPSUM, Italy**
Industrial strategies for peptide manufacturing: combining sustainability and cost

15:00-15:15 **O3-Ind - Stefano Pegoraro, BACHEM, Switzerland**
The New Golden Age of Peptides: Manufacturing Evolution and Emerging Therapies

15:15-15:30 **O4-Ind - Giorgio Marini, CEM, Italy**
State of the art Microwave Peptides Synthesis

SESSION 5
Dedicated to the memory of Prof. Luis Moroder
Chair: M. L. Mangoni – C. Becker

15:30-15:55 **KN5 - Lukasz Berlicki, Wroclaw University, Poland**
Miniproteins: design, synthesis, and applications

15:55-16:20 **KN6 - Daniela Kalafatovic, University of Rijeka, Croatia**
Designing peptides with generative AI

16:20-16:45 **KN7 - Tomislav Roncevic, University of Split, Croatia**
Microscale Thermophoresis as a Tool for Quantifying Peptide-Membrane Interactions in Bacteria

16:45-17:10 **KN8-A - Marta De Zotti, University of Padua, Italy**
Conjugation with Nucleobases Amplifies Peptide-Mediated Liposome Fusion
KN8-B - Marta De Zotti, University of Padua, Italy
Searching for Nanostructured or pOre fOrming Peptides for therapY COST Action

17:10-17:30 **O7 - Burkhard Bechinger, University of Strasbourg, France**
Antimicrobial peptides: Mechanism of action, synergy and mimetics

17:30-17:50 **O8 - Paolo Rovero, University of Florence, Italy**
Antiviral peptides against SARS-CoV-2

17:50-18:30 **Coffee Break & Poster Session**

SESSION 6
Young Researchers From Local Area
Chair: D. Tesauro – S. Galdiero

18:30-19:30 ***Flash Communications***
FL1 – Rosanna Culurciello
FL2 – Anastasia Ferraro
FL3 – Martina Dragone
FL4 – Carlo Diaferia
FL5 – Maria Della Valle
FL6 – Mosab Jedea
FL7 – Elena Lagreca
FL8 – Rosaria Schettini
FL9 – Anna Palma

Social Event: Ride for Panoramic view from Monte Echia
Free Evening

SATURDAY, JUNE 27TH

SESSION 7
Chair: D. Marasco – M. Devocelle

09:00-09:30 **PL8 - Meritxell Teixido, Gate2Brain, Spain**
Gate2Brain, an Entrepreneurial Journey Towards Patients

09:30-09:55 **KN9 - Luisa Ronga, University of Pau and Pays de l'Adour, France**
The role of selenoenzymes in the toxic and therapeutic activity of metallic compounds

09:55-10:15 **O9 - Ozan Fidan, Gipfel Life Sciences, Austria**
The next generation peptide sequencing with AI

10:15-10:35 **O10 - Deisy Y. R. Sarmiento, Universidad Autonoma de Bucaramanga, Colombia**
Artificial intelligence-guided design of Kisspeptin-10 analogs reveals bioactive peptides with cytotoxicity activity in breast cancer cells

10:35-11:10 **Coffee Break**

SESSION 8
Chair: R. Fattorusso – M. Teixido

11:10-11:40 **PL9 - Maurizio Pellecchia, UC Riverside, United States**
Novel covalent strategies for therapeutic peptides

11:40-12:00 **O11 - Vincenzo Amenduni, University of Bari, Italy**
A comprehensive multi-language database and conversion toolkit for macrocyclic peptides

12:00-12:20 **O12 - Chiara Falciani, University of Siena, Italy**
Branched Oncolytic Peptides (BOPs) against cancer

12:20-12:40 **O13 - Francesco Quilli, University La Sapienza, Italy**
Cashing in on Fragments: A Peptide-Based Discovery of KCASH2 Binding to MAD2A

12:40-13:00 **O14 - Katarzyna Papaj, Silesian University, Gliwice, Poland**
Influence of microbial exposure on the peptide profile and biological activity of Lucilia sericata larval secretions

13:00-14:30 **Lunch on site**

SESSION 9
Chair: L. Cigliano – F. Merlino

14:30-15:00 **PL10 - Michal Lebl, Spyder Institute Prague, Czech Republic**
Reminiscences of 50 years in peptide chemistry

15:00-15:30 **PL11 - Govindaraju Thimmaiah, JNCASR, India**
Peptide Modulators of Multifactorial Alzheimer's Pathology

15:30-15:50 **O15 - Robert Vacha, Masaryk University Brno, Czech Republic**
Computational design of antimicrobial peptide nanopores

15:50-16:30 **Coffee Break & Poster Session**

SESSION 10
Chair: L. Ronga – C. Diaferia

16:30-16:50 **O16 - Salvatore Di Maro, University Vanvitelli, Italy**
Self-Assembling Amphiphilic Dendrimers (SAADS) for the detection of CXCR4 positive tumors by PET Imaging

16:50-17:10 **O17 - Alice Romagnoli, University of Marche, Italy**
A CYFIP1-Inspired Peptidomimetic Modulates eIF4E-Dependent Translational Control in Cancer and Neurodevelopmental Disorders

17:10-17:30 **O18 - Andrea Caporale, Italian CNR, Italy**
Novel Antimicrobial Peptides from Parasitic Flatworms with Activity against ESKAPE Pathogens

17:30-17:50 **O19 - Domenica Musumeci, University of Naples, Italy**
Nucleobase-functionalized aromatic amino acids and dipeptides: self-assembled nanostructures for potential drug encapsulation

17:50-18:10 **O20 - Valeria Scalcon, University of Padua, Italy**
Milk permeate-derived bioactive peptides: from identification to in vivo validation of their antioxidant properties

18:10-18:20 **Concluding Remarks**

20:30 **Gala Dinner**

PROGRAM

Opening Lecture

Redefining Peptide Synthesis: Emerging Strategies for Sustainable Future

Fernando Albericio[1,2], *Beatriz G. de la Torre*[1]

1 – School of Chemistry and Physics, University of KwaZulu-Natal, Durban, South Africa

2 – Department of Inorganic and Organic Chemistry, University of Barcelona, Barcelona, Spain

Despite decades of innovation, peptide synthesis in practice remains largely anchored in established methodologies, with Fmoc/tBu-based solid-phase peptide synthesis (SPPS) continuing to dominate both research and industrial production. This enduring reliance on gel-type resins, conventional coupling reagents (carbodiimides), and TFA-based deprotection strategies highlights the robustness, but also the limitations, of current approaches.

Today, the field is undergoing a significant transformation driven by evolving therapeutic demands. The rapid expansion of GLP-1 receptor agonists has shifted production requirements to unprecedented scales, while structurally complex, medium-sized peptides enriched in non-canonical amino acids are redefining the boundaries of synthetic feasibility. These developments expose critical inefficiencies in traditional workflows and underscore the urgent need for more sustainable and adaptable technologies. In this lecture, we will explore how these challenges are catalyzing a redefinition of peptide synthesis.

We will present our recent contributions toward greener and more efficient processes, including low-solvent-consumption solid supports, recyclable solvent-compatible materials, and cleaner coupling chemistries. We will further discuss strategies to eliminate polyfluorinated reagents and introduce novel liquid-phase peptide synthesis (LPPS) concept that expand the synthetic toolbox. Together, these advances point toward a new generation of peptide manufacturing strategies that integrate efficiency, scalability, and sustainability.

Plenary Lectures

Cancer-selective targeting of the NF-κB pathway via GADD45β/MKK7 inhibition: From target discovery to human trials

Aristeidis Chaidos, Dimitrios Potolidis, Jessica Cornice, Holger Auner, Gary Acton, Ceri Bygrave, Gordon Cook, Rakesh Popat, Andrew Davies, Christopher Parrish, Maria Atta, Kim Linton, Dean Smith, Christopher Fox, Iain McNeish, Elizabeth Hadley, Elena Ferrer Martinez Del Peral, Mario Pellegatti, Nigel Adams, Albert Chau, Precious Nnebedum, Daria Capece, Daniela Verzella, Daniel D'Andrea, Jan Rekowski, Christina Yap, Laura Tornatore, ***Guido Franzoso****

* Presenter and corresponding author

Background: NF-κB transcription factors are major drivers of multiple myeloma (MM) aetiopathogenesis, malignant cell survival, and therapy resistance. However, developing clinically useful IκB kinase (IKK)/NF-κB inhibitors has not proven possible, due to the preclusive on-target toxicities of systemic NF-κB blockade. To overcome this problem, we developed a targeted approach that disrupts the essential, cancer-specific survival module GADD45β/MKK7 – located downstream of NF-κB – rather than NF-κB itself [PMID: 25314077]. This strategy led to the development of the first-in-class GADD45β/MKK7 inhibitor, DTP3, a *D*-tripeptide which selectively kills human MM cells via MKK7/JNK-dependent apoptosis *ex vivo* and *in vivo* and is not toxic to normal cells [PMID: 25314077; PMID: 31080744].

Here, we report the results of a Phase I, multi-centre dose-escalation study evaluating the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary efficacy of DTP3 monotherapy in patients with relapsed or refractory MM and diffuse large B-cell lymphoma (DLBCL; EudraCT: 2021-004028-13).

Methods: Since May 2022, 16 patients provided informed consent, and 14 were enrolled (13 with MM and 1 with DLBCL). At study entry, median age was 64 years, and all patients had progressive disease, after receiving a minimum of 4 previous lines of therapy (median 5.5; range 4–11). Six MM patients (46.15%) had triple-refractory disease, and high-risk features, such as adverse cytogenetics, elevated serum LDH, or extramedullary disease, were present in 8 MM patients (57.1%).

Dose escalation followed a 1-patient per dose-level study design, unless a dose-limiting toxicity (DLT) occurred. DTP3 was administered intravenously on days 1, 3, and 5 of each week in 28-day cycles, over an overall range of escalating doses from 0.5 to 67 mg/kg of body weight. The minimum evaluable duration of treatment was 1 cycle, and median treatment duration was 58 days (range 29-142 days). Adverse events (AEs) were graded by CTCAE v5.0 criteria, and clinical responses were assessed by IMWG criteria. PD responses were evaluated by measuring JNK activation and apoptosis in $CD138_+$ plasma cells post-treatment compared to baseline, using flow cytometry. For PK analysis, plasma samples were collected on Cycle 1, Day 1 (C1D1) at baseline up to 24 hours post-dosing and, further, on Cycle 1, Day 6 (C1D5) at baseline up to 4 hours post-dosing.

Results: The best observed clinical response was a Very Good Partial Response (VGPR) in the single MM patient treated at 30 mg/kg; stable disease was observed in 6 patients (46.15%), and progressive disease was observed in a further 6 patients (46.15%). Duration of the VGPR was 109 days. The dose was escalated to 45 mg/kg for 4 weeks before treatment discontinuation, due to disease progression.

No haematological toxicity was observed in any of the study participants. Grade 3 non-haematological AEs included hypotension and pre-/syncope in the 2 patients treated at 67 mg/kg, fulfilling the DLT criteria. Grade 3 self-limiting QTc prolongation

post-treatment was observed in 2 patients. In total, 42 treatment-related AEs Grade 1-2 were recorded, most commonly dysgeusia and/or perioral dysesthesia (n=15), dizziness (n=6), and QTc interval prolongation (n=6). On the basis of the toxicity data, the Continual Reassessment Method (CRM) identified 45 mg/kg as the Maximum Tolerated Dose (MTD).

Mechanistically consistent PD responses of varying degree – i.e., selective activation of JNK signalling and apoptosis in MM cells, with no drug-related responses in normal cells – were observed in about 50% of patients. PK analyses demonstrated a favourable clinical profile across patients at doses ranging from 0.5 to 67 mg/kg, confirming linearity of plasma exposure (AUC) and maximum plasma concentration (C_{max}) with dose level and no accumulation upon repeated administration. The median plasma half-life was 8.1 hours (range 5.5-11.1 hours).

Conclusions: In summary, DTP3 exhibited a favourable PK, PD, safety, and initial efficacy profile upon intravenous administration at doses up to 45 mg/kg. A VGPR was observed in the patient treated at 30 mg/kg, and the cumulative PD and initial efficacy signals suggest broad clinical activity. A Phase IIa dose-expansion study is ongoing to further evaluate the safety and clinical activity of DTP3 in patients with relapsed or refractory MM and DLBCL, alongside development of predictive biomarkers for patient stratification.

The impact of posttranslational modifications on chaperone function and protein degradation

C.F.W. Becker[1]

1 – University of Vienna, Faculty of Chemistry, Institute of Biological Chemistry, Währinger Str. 38, 1090 Vienna, Austria

More than 80% of all human proteins experience one or more posttranslational modification (PTM) during their life cycle. These PTMs can result from enzymatic or non-enzymatic reactions. Enzymatic PTMs are well-known for being involved in regulating many cellular events such as gene expression, signal transduction, protein-protein as well as cell-cell interactions and protein degradation. Non-enzymatic posttranslational modifications (nPTMs) are increasingly recognized to affect such events as well, with a special emphasis on age-related, metabolic and neurodegenerative diseases.

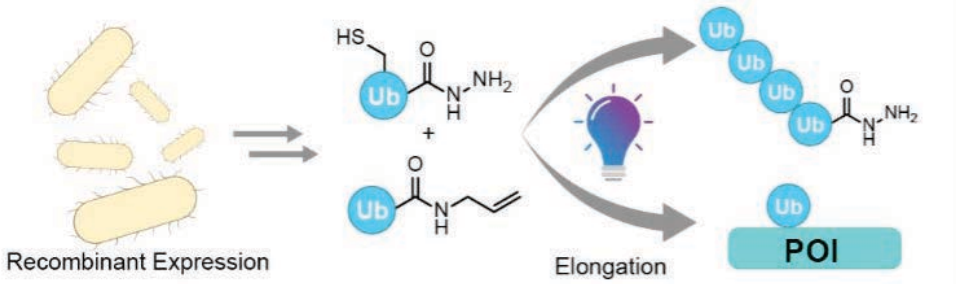

Figure 1. Generating functionalized ubiquitin building blocks via expression paves the way to different di-, tri- and tetra-ubiquitin chains. Light-induced thiol-ene click reactions, combined with a masking and protection strategy, are harnessed to establish controlled ubiquitin chain assembly and attachment to proteins of interest (POIs).

Here examples will be discussed that involve different semi-synthesis routes to access site-specific PTMs[1] within heat shock proteins such as Hsp90[2] and access to proteins carrying defined ubiquitin (Ub) chains.[3] (Figure 1). To obtain such modified proteins, our semi-synthetic strategy relies on expression of Ub-intein fusion constructs giving access to high quantities of defined ubiquitin building blocks with versatile C-terminal reactive handles. Linking Ub monomers or target peptides and proteins is achieved site-selectively via photoinitiated thiol-ene click chemistry.[4] We demonstrate the synthesis of ubiquitin chains up to tetramers as well as the site-selective installation of mono- or di-Ubiquitin on proteins such as Tau or α-Ssynuclein. The presented work combines minimal synthetic effort with high fidelity linkage chemistry paving the way towards homogeneously ubiquitinated proteins.

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1. Conibear, A.C. et al. Chem Soc Rev 2018, 47, 9046-9068.
2. Gajsek, O. et al. Chem. Eur. J. 2025, e202403676
3. Urschbach, M. et al. Angew. Chem. Int. Ed., 2025, e202502638.
4. Valkevich, E.M. et al. J. Am. Chem. Soc., 2012, 134, 6916–6919.

Diverse cyclic peptide architectures for targeting melanocortin receptors

P.E. Thompson[1], *K. W. Yue*[1,2], *N. Barlow*[1], *J. Zhu*[1], *P. Adriaan*[1], *T. Zhang*[1] *and R. Shandre-Mugan*[1]

1 – Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville 3057

2 – Department of Radiopharmaceutical Sciences, Cancer Imaging, Sir Peter MacCallum Department of Oncology, The University of Melbourne, 3010

Cyclic peptide development builds upon the separate but crucial notions that cyclic peptides can adopt stable bioactive conformations and resist proteolytic degradation, as compared to their linear counterparts. They may also be able offer improved permeability though biological membranes.

The design of cyclic peptides has largely been built around synthetically accessible methods, such as disulphide bond formation, lactam bridges, or peptide-orthogonal click reactions or alkene stapling. In our group we use and optimise these methods, but have also been trying to diversify the cyclic peptide architectures so that optimal ligands for our targets with improved pharmaceutical profiles can be achieved. We find that the investment in synthetic know-how allows us to explore deeper into peptide SAR.

In this presentation, selected examples will be presented that highlight the power of these approaches in the development of the melanocortin receptor GPCRs.[1]

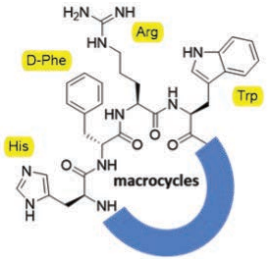

Figure 1. The melanocortin receptor pharmacophore

References

1. Yue, K. W., Barlow, N., Thompson, P. E., RSC Medicinal Chemistry, DOI: 10.1039/D6MD00115G

Update on applications of cyclotides in drug design

D.J. Craik[1]

1 – The University of Queensland, Institute for Molecular Bioscience and Australian Research Council Centre of Excellence for Innovations in peptide and Protein Science, 4072– Brisbane, Australia

Naturally occurring macrocyclic peptides offer great potential as leads for drug design and as next-generation crop protection products.[1-3] Our work focuses on a class of macrocyclic peptides known as cyclotides that are exceptionally stable and are resistant to enzymatic or thermal degradation by virtue of their cyclic cystine knot structural motif (Figure 1).

Figure 1. 3D structure of the prototypic cyclotide kalata B1

Cyclotides are excellent scaffolds for the incorporation of bioactive peptide epitopes to stabilise them. More than two dozen examples have now been reported where biologically active epitopes have been grafted onto cyclic peptide frameworks to produce drug lead molecules with potential in the treatment of cancer, cardiovascular disease, infectious disease, autoimmune disease (multiple sclerosis) and pain. This presentation will describe our work on the discovery, structural characterisation and applications of cyclotides in medicine, with recent examples using display technologies to combinatorially vary backbone loops in the cyclotide scaffold.[4] This will be illustrated by the development of a potent inhibitor of the human plasma protein, factor XII, to minimise clotting in extracorporeal membrane oxygenation (ECMO) procedures without bleeding complications.[5]

References

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Permeation-Enhancer Mediated Peptide Transport Across Biological Membranes: Mechanism, Simulation, and Emerging Single-Molecule Approaches

S. T. Schneebeli[1,2], *K. J. Colston*[1], *K. T. Faivre*[2], *D. Nie*[1], *J. E. Funk*[1] *and A. Soliman*[2]

1 – Purdue University, Dept. of Industrial & Molecular Pharmaceutics, West Lafayette, IN 47907, USA.

2 – Purdue University, Tarpo Dept. of Chemistry, West Lafayette, IN 47907, USA.

Peptide therapeutics are among the fastest-growing drug classes, yet nearly all require injection, limiting patient compliance. Oral delivery would be transformative, but the gastrointestinal epithelium is a formidable barrier to large, polar biomolecules. Permeation enhancers offer the most promising strategy for overcoming this challenge, yet oral bioavailability remains below 1% even for semaglutide co-formulated with salcaprozate sodium (SNAC),[1] because the underlying molecular mechanisms are poorly understood. Using all-atom constant pH molecular dynamics (CpHMD) with over 400 titratable groups, validated by multidimensional NMR and dynamic light scattering (DLS),[2] we reveal a two-stage mechanism: SNAC co-aggregates with the peptide in solution, monomerizing and delivering it to the bilayer, where environment-sensitive pK_a shifts drive SNAC into fluid, π-stacked defect domains through which the peptide sinks in a quicksand-like fashion without compromising membrane integrity. Building on these insights, we are developing single-molecule methods to resolve peptide–enhancer interactions at sequence-level resolution, establishing a general framework for rational optimization of enhancer formulations for oral peptide therapeutics.

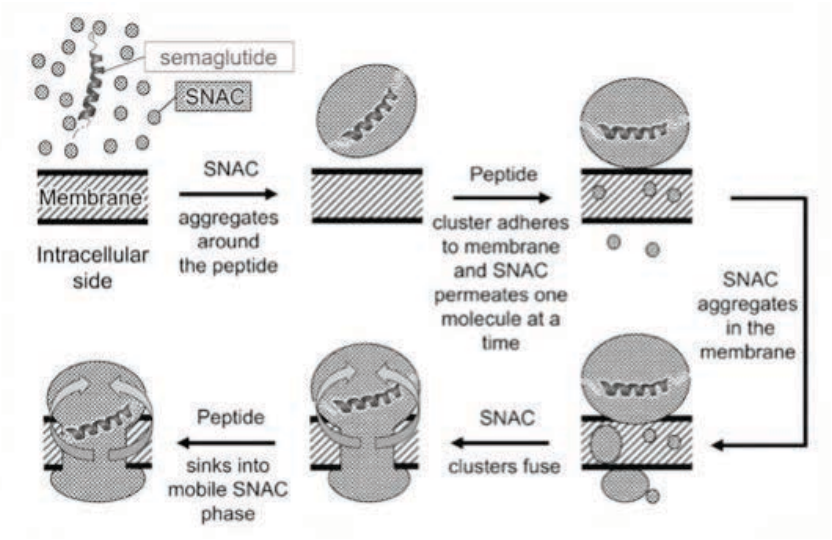

Figure 1. Quicksand-like mechanism for SNAC-assisted membrane permeation of semaglutide. SNAC molecules (dotted pattern) form dynamic, fluid defects within the lipid bilayer (diagonally striped region), enabling the peptide (solid dark gray helix) to progressively sink through the membrane. Curved arrows indicate the redistribution of mobile SNAC molecules that sustains permeation. Adapted from ref. 2.

References

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Molecular Details for activation and deactivation of the sarcoplasmic reticulum Ca2+-ATPase (SERCA) by membrane polypeptides

Gianluigi Veglia

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Calcium homeostasis is fundamental to heart muscle contraction and is primarily maintained by the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA), which is responsible for the bulk of intracellular calcium transport in humans. The activity of SERCA is finely regulated by a family of single-pass membrane peptides known as regulins, which ensure calcium levels remain within a precise physiological range. Disruption of this regulation has been implicated in the development of cardiomyopathies and heart failure. Among these regulins, phospholamban and DWORF play crucial but opposing roles in the beat-to-beat modulation of cardiac contractility. Unphosphorylated phospholamban inhibits SERCA by decreasing its affinity for calcium, whereas phosphorylation by protein kinase A lifts this inhibition and restores SERCA function. In contrast, DWORF enhances SERCA activity independently of β-adrenergic signaling. Notably, overexpression of DWORF can displace phospholamban and rescue impaired cardiac contractility, yet the molecular mechanism underlying SERCA activation by DWORF remains poorly understood. Here, we employ NMR spectroscopy and molecular dynamics simulations to dissect the molecular mechanisms governing both the activation and deactivation of cardiac SERCA. Our findings offer new mechanistic insights that could inform the development of targeted therapies for cardiomyopathies stemming from aberrant calcium homeostasis.

Design of cell-permeable DHHC peptides targeting S-palmitoylation dynamics

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Protein palmitoylation is a reversible post-translational modification in which cysteine residues of substrate proteins become modified with palmitate. This lipid tail enables proteins to anchor to the plasma membrane, and plays a role in protein trafficking, stability, interactions and activity.[1] Protein palmitoylation is catalysed by the 23 members of the family of palmitoyl-S-acyltransferases (ZDHHC enzymes), all containing the catalytic DHHC motif. Abnormalities in protein palmitoylation have been associated with many diseases including immunodeficiency conditions, neurological disorders, or cancer.[2]

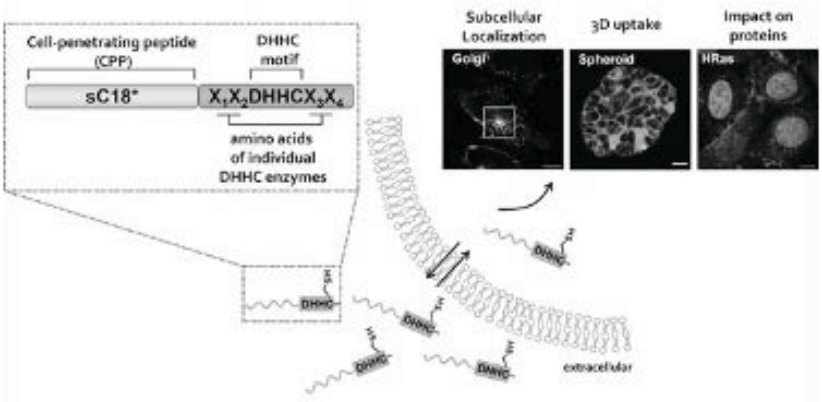

Figure 1. DC-peptides contain a DHHC palmitoylation motif and are linked to the CPP sC18.*

In this work, we investigated how we can influence the homeostasis of S-palmitoylation by cell-permeable peptides containing a DHHC palmitoylation motif.[3] A small library of peptides was generated and screened for cellular uptake and cell compatibility. The newly designed peptides internalized to high extent into different cell lines and human breast cell spheroids dependent on their palmitoylation motif. Out of this screen, we investigated in more detail the peptide DC-2 concerning its impact on palmitoylated proteins that are connected to cancer progression. Indeed, DC-2 affected the localization of the oncoprotein HRas and altered S-acetylation-related signaling cascades of EGFR. Our findings point to a peptide-driven impact on proteins having palmitoylation sites and highlight cell-permeable DHHC-peptides as an interesting tool to modulate S-palmitoylation dynamics and to be further evolved in the context of cancer.

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Gate2Brain, An Entrepreneurial Journey Towards Patients

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Gate2Brain is a deep-tech biotechnology venture addressing one of the most critical bottlenecks in central nervous system (CNS) therapeutics: the inability of most drugs to cross the blood–brain barrier (BBB). Founded in 2020 as a spin-off from IRB Barcelona, the University of Barcelona, and Sant Joan de Déu Hospital, the company has developed a proprietary platform of shuttle peptides capable of transporting a broad spectrum of therapeutic cargoes—including small molecules, biologics, and nucleic acid-based therapies—into the brain.

These peptide shuttles exploit endogenous BBB transport mechanisms without disrupting physiological function, preserving brain homeostasis while enabling efficient and targeted delivery. The technology has demonstrated strong preclinical validation, including the successful transport of antibody–drug conjugates that significantly reduce brain metastases, highlighting its transformative potential for next-generation CNS therapies.

By enhancing drug penetration into the CNS, Gate2Brain provides a dual value proposition: unlocking the potential of new therapeutics and rescuing previously abandoned drug candidates that failed due to poor brain accessibility. This approach not only accelerates clinical translation but also enables lower dosing, thereby reducing systemic toxicity and overall treatment costs. The platform's versatility, protease resistance, low immunogenicity, and scalable production further position it as a disruptive solution in drug delivery.

The company's lead program, G2B-002, focused on pediatric brain tumors such as diffuse intrinsic pontine glioma (DIPG) and pediatric glioblastoma, has received Orphan Drug Designation from both the EMA and the FDA, underscoring its clinical relevance and regulatory momentum toward human trials.

Beyond the science, Gate2Brain embodies a compelling entrepreneurial journey from over 15 years of academic research in peptide chemistry and biological barriers to becoming a patient-oriented biotech company. Gate2Brain is driven by a clear mission: to "bring medicines beyond barriers" and translate cutting-edge science into tangible benefits for patients, particularly in high unmet medical needs such as pediatric CNS diseases.

Ultimately, Gate2Brain represents not only a technological breakthrough but also a paradigm of translational entrepreneurship—where overcoming biological barriers goes hand in hand with overcoming the challenges of bringing innovation from the lab to the clinic.

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Novel covalent strategies for therapeutic peptides

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The design of covalent drugs in oncology research has proven beneficial in drug discovery in the past decade, resulting in the approval of several Cys-covalent anti-cancer therapeutics. The increased pharmacodynamics of covalent ligands makes this approach particularly attractive to increase the binding affinity of ligands targeting protein-protein-interactions (PPIs) for which it has been notoriously difficult to attain greater potency. In one application, I will report on an innovative ligand design strategy in which Cys-covalent peptide-mimetics targeting Pin1, act as *molecular crowbars*, destabilizing the target that is then degraded in cell and *in vivo*. [1,2] Moreover, recently, several new electrophiles have been proposed to target not only Cys but also other residues such as Lys, His, or Tyr. [3-7] Among those, we and others reported that certain aryl-sulfonyl fluorides and aryl-fluorosulfates can react to those residues when properly incorporated in carrying ligands to be juxtaposed to a targeted residue. We also recently found that targeting His residues is particularly attractive given their favourable nucleophilicity and their frequent occurrence in binding sites. Both aryl-fluorosulfates and chloroacetamides can be used to target His residues, making it a very promising residue, second only to Cys, for the design of covalent agents. I will report how those covalent design strategies seem particularly suited for optimization of peptide-ligands and therapeutic peptides with examples in targeting the anti-apoptotic protein Mcl-1 and the receptor tyrosine kinase EphA2.

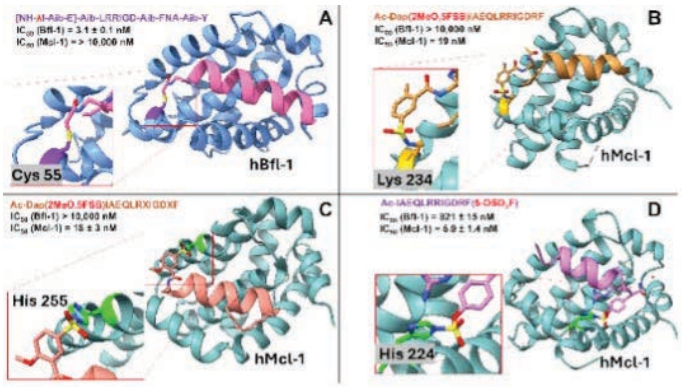

Figure 1. Examples of covalent peptides targeting Cys, Lys, or His residues.

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Reminiscences of 50 years in peptide chemistry

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Artificial intelligence can be extremely useful in designing peptides with properties required for planned application – if there is enough information about peptides already tested for this use. However, if the use is novel, the only solution is the old-time trial and error approach. It means synthesis and evaluation of as many as reasonably possible structures. And for that task the classical solid phase synthesis either in the individual or library approach is used. This contribution will suggest several approaches and tricks which are the results of lifetime experience in peptide and oligonucleotide syntheses which are difficult to find by searching the literature since they were not the main topic of the particular article, or they were not even published. As an example of synthetic approach evolution will be the development of super economical solid phase centrifugal synthesizer.[1]

Another focus will be given to the synthetic aspects of solid carrier handling and distribution for library construction with possibility of artificial "nose"[2] for quick detecting contaminants in chemical processes and in food and drink production, or continuous production of peptides in the industrial settings.[3]

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Peptide Modulators of Multifactorial Alzheimer's Pathology

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder and a leading cause of dementia. Despite extensive research, effective diagnostic and therapeutic approaches for AD remain limited, primarily due to the lack of a comprehensive understanding of its underlying disease mechanisms. The hallmark features of AD include the production, accumulation, misfolding, and aggregation of amyloid-β (Aβ) and tau proteins in the brain. This pathological condition is exacerbated by elevated levels of reactive oxygen, nitrogen and sulfur species, leading to biomolecular and mitochondrial damage, and resulting in oxidative stress within neuronal cells. Furthermore, oxidative stress, mitochondrial dysfunction, ferroptosis, cuproptosis, pyroptosis, neuroinflammation, and the role of microglia significantly contribute to the pathogenesis of AD. In this talk, I will discuss the latest and comprehensive insights into the complex mechanisms underlying AD. I will present peptide-based strategies developed in our laboratory aimed at understanding and mitigating multifaceted Alzheimer's pathology.

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Keynotes

Peptide Nucleic Acids (PNAs) as scaffolds for multi-functional compounds with multi-purpose applications

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Peptide Nucleic Acids (PNAs) have long been known as a crossover between nucleic acid function and peptide chemistry. They have been used in many biomedical applications, from very sensitive and selective advanced probes for diagnostic genoassays and sensory systems to potential new therapeutic agents targeting RNA/DNA (antisense, anti-miR, anti-gene) and as supramolecular components of 'smart' nanomaterials.[1,2] Some of the DNA and RNA-binding properties of PNA can be modulated by using chemical modification at the backbone and nucleobase level. Recently, the use of PNA as aptamers interacting with high affinity with proteins has also been described.[3] PNA can thus be viewed as a versatile scaffold for multi-functional molecules able to recognize not only nucleic acids, but a variety of biomolecules, including proteins.

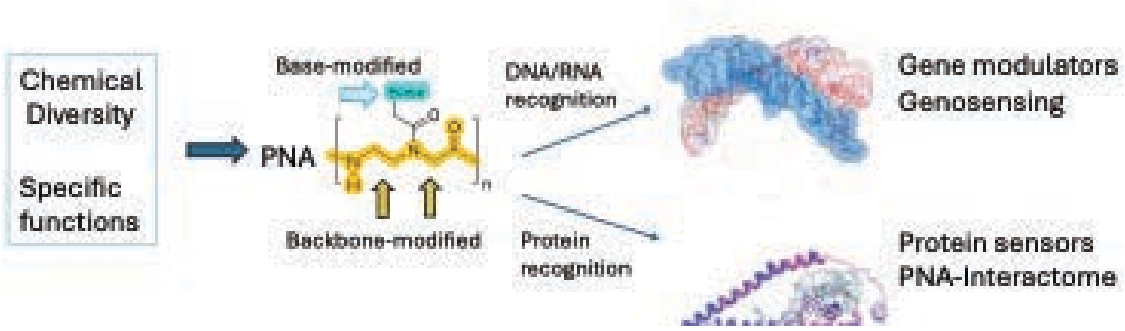

Figure 1. PNA as scaffold for multifunctional molecules

In this talk, examples taken from our group experience on the advantages and drawbacks of PNA in these fields will be discussed, with a special focus on structure-activity of base and backbone in the interactions with nucleic acids and proteins. Challenges posed by the synthesis of these very versatile class of molecules will be discussed.

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Disrupt Peptide Aggregation for Difficult Peptide/Protein Synthesis

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Despite advances in peptide synthesis, chemically assembling proteins that contain aggregating or colloidal peptide segments remains a major challenge for both solid-phase peptide synthesis and peptide ligation. New methods and practical strategies are still needed to overcome these obstacles. We have focused on developing simple yet effective approaches for peptide and protein synthesis and modification. Building on Ser/Thr ligation (STL) and Cys/Pen ligation (CPL), we have devised several strategies—STL/CPL$_{RST}$ (reducible solubilizing tags), STL/CPL$_{LEAD}$ (ligation-embedding aggregation disruptor), CPL$_{TBM}$ (tunable backbone modification), STL$_{NBD}$ (N,O-benzylidene acetal dipeptides), and CPL$_{NBT}$ (N,S-benzylidene thioacetals)—to address difficult syntheses. Using these approaches, we have successfully synthesized challenging targets including PD-1, PD-L1, and Interleukin-2. In this presentation I will describe our recent progress and ongoing developments.

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From endogenous and natural peptides to functionalized pharmacological tools: synthesis, development and applications in neurodegenerative diseases and cancer

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Peptides derived from endogenous sequences and natural sources represent a unique and versatile class of biomolecules with high specificity and biological activity, making them attractive scaffolds for the development of innovative pharmacological tools. In this work, we explore the transformation of natural and bioinspired peptides into functionalized systems for applications in neurodegenerative diseases and cancer.

Our approach integrates advanced solid-phase peptide synthesis strategies with chemical biology to access complex architectures, including cysteine-rich toxins and selenocysteine-containing analogues. Particular attention is devoted to controlling peptide folding, stability, and resistance to proteolytic degradation through rational modifications. These strategies enable fine-tuning of peptide structure–function relationships while preserving biological activity.

In the context of neurodegenerative diseases, we investigate amyloid-β (Aβ1–42) aggregation using synthetic peptide models and fluorescent probes, aiming to better understand early oligomer formation and to develop tools for the detection of pathological species. Complementary efforts focus on the design of peptide-based imaging agents and modulators targeting protein–protein interactions involved in amyloidogenesis.

In parallel, natural peptide ligands and toxins are exploited as high-affinity targeting vectors in oncology. By engineering peptide conjugates with improved pharmacokinetic properties—such as enhanced plasma stability, reduced immunogenicity, and prolonged circulation time—we develop novel systems for receptor targeting and radiotherapeutic applications.

Altogether, this multidisciplinary work highlights how peptide synthesis, structural engineering, and functionalization can be combined to bridge fundamental studies of peptide self-assembly with the development of next-generation diagnostic and therapeutic tools in complex diseases.

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Novel antibiotic- and lipid-peptide conjugations applied to antimicrobial peptides

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Antimicrobial Peptides (AMPs) are archetypal bioactive peptides, fulfilling multiple functions in innate immunity and beyond.[1] Their exogenous counterparts intended for anti-infective applications benefit from modifications such as conjugation to enhance their activity, stability and/or selectivity.[2]

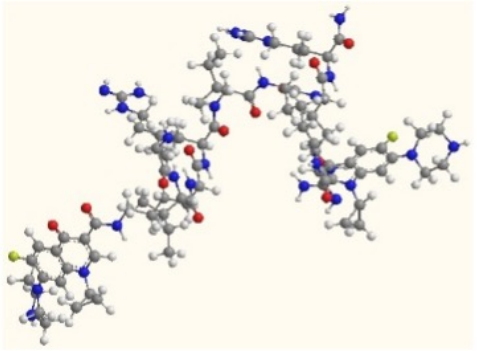

Figure 1. AMP (Bac8c)-ciprofloxacin hybrid conjugate

of peptides with a lipid and a classical antibiotic, respectively. The former allows the versatile, conditionally stable, cholesterylation of sequences with antiviral properties, reminiscent of lipopeptides as fusion inhibitors. The latter affords the hybridisation of AMPs and fluoroquinolones with concomitant replacement of naturally occurring (tryptophan) residues, combining therefore conjugation and partial peptidomimetic conversion to generate peptides with emergent properties, in particular targeted, and in some cases improved, activities.[3]

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Miniproteins: design, synthesis, and applications

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Miniproteins, by definition, are small proteins adopting tertiary structure in solution with a molecular weight not exceeding 10 kDa.[1] They are often considered a bridge between peptides and proteins due to the combination of features from both aforementioned classes. Miniproteins can be synthesized and evaluated spectroscopically, but they also have relatively complex, stable three-dimensional structures. Due to these properties, miniproteins are excellent candidates for developing molecules with advanced functions.[2] In particular, several applications of miniproteins for the construction of biologically active compounds have already been published, mainly including inhibitors of protein-protein interactions, which are of particular interest due to the potentially large interaction surfaces of miniproteins.

In this contribution, the miniproteins workflow, comprising computer-aided design, flow synthesis, and various applications, will be presented. First, miniproteins of various geometries and secondary structure composition are designed *de novo* using the FastDesign algorithm (Rosetta software).[3] In order to increase conformational stability, cyclic β-amino acids that induce specific local conformation are incorporated into the miniprotein structure. Second, designed miniproteins are obtained by applying flow solid-phase synthesis. Custom equipment combined with an AI-supported synthesis protocol enables the production of miniproteins with sequences up to 120 amino acid residues. Third, miniproteins are evaluated for their conformational stability, three-dimensional structures, and functions, including inhibition of the PD-1/PD-L1 interaction[4] or enzyme mimicry.[5]

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Designing peptides with generative AI

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In an era increasingly shaped by artificial intelligence (AI), peptide discovery is shifting from a resource-intensive, trial-and-error process guided largely by human intuition toward a data-driven paradigm characterized by high predictive accuracy and scalability. However, the large size of the peptide sequence space, together with our still-limited understanding of sequence-to-function relationships, makes the identification of new functional or bioactive peptides challenging.[1] To address this challenge, we integrate machine learning with a genetic algorithm-based exploration strategy to identify sequences with strong self-assembly propensity.[2] A neural network trained on experimentally validated peptides and aggregation propensity scores obtained through coarse-grained molecular dynamics achieves an accuracy of 81.9%, enabling the discovery of self-assembling peptides in previously unexplored regions of sequence space, with low similarity to the training set. This framework can be readily extended to therapeutic peptide discovery through multi-objective optimization, balancing desirable bioactivity, such as antimicrobial potency, while minimizing toxicity. Beyond improving the exploration of unknown peptide spaces, we leverage generative AI to accelerate peptide discovery by supporting experts in selecting promising candidates for experimental validation, accelerating innovation in the field.

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Microscale Thermophoresis as a Tool for Quantifying Peptide-Membrane Interactions in Bacteria

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Antimicrobial peptides (AMPs) are key components of innate immunity and represent promising candidates for the development of new anti-infective agents.[1] Although they are commonly classified as membrane-permeabilizing or non-permeabilizing, their mechanisms of action are highly context-dependent and often involve multiple, overlapping pathways influenced by peptide concentration, membrane composition, and environmental conditions.[1,2] Notably, membrane interaction represents a critical early step in AMP activity, even for peptides that primarily exert intracellular effects. Current biophysical approaches used to study peptide-membrane interactions, including surface plasmon resonance, isothermal titration calorimetry, and nuclear magnetic resonance, rely predominantly on simplified model systems such as synthetic lipid bilayers.[2,3] While highly informative, these systems do not fully capture the complexity of bacterial cell envelopes.

In this work, we present a strategy to directly probe peptide interactions with Gram-positive and Gram-negative bacteria using microscale thermophoresis (MST). MST is an immobilization-free technique that quantifies molecular interactions by detecting changes in the movement of molecules along microscopic temperature gradients, reflected in fluorescence signal variations arising from ligand-induced microenvironmental perturbations.[4,5] Although MST has been previously applied to investigate AMP binding to model membranes,[6] here we extend its application to intact bacterial cells. By employing fluorescently labelled bacteria as targets, we demonstrate that MST can be used to monitor interactions of AMPs with live bacterial membranes, providing a direct and versatile platform for studying peptide-bacteria interactions in a biologically relevant context.

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Conjugation with Nucleobases Amplifies Peptide-Mediated Liposome Fusion

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The discovery of novel fusion peptides with enhanced properties is crucial for advancing our understanding of membrane fusion processes and developing more efficient tools for biotechnology and medicine. Currently, fusion peptides are mostly identified as portions of viral fusion proteins, and this limits their versatility. In this presentation, we demonstrate that end-capping a cationic helical peptide with a nucleobase substantially enhances its ability to induce liposome fusion. First, we show that a helical trichogin (Tric) analog, Oct-K2569Tric-Lol, containing four lysine residues, induces reversible aggregation of negatively charged liposomes in a concentration-dependent manner. Then, we demonstrate through several complementary techniques that the addition of a single thymine (T) nucleobase at the C-terminus of this peptide (Oct-K2569Tric-T) transforms its activity from reversible aggregation to irreversible membrane fusion. The presence of complementary nucleobases at both peptide termini (T-K2569Tric-A; A, Adenine) further promotes membrane fusion. Our findings provide a new strategy for designing more potent fusion peptides, opening possibilities for improved applications in drug delivery, cell biology research, and nanotechnology.

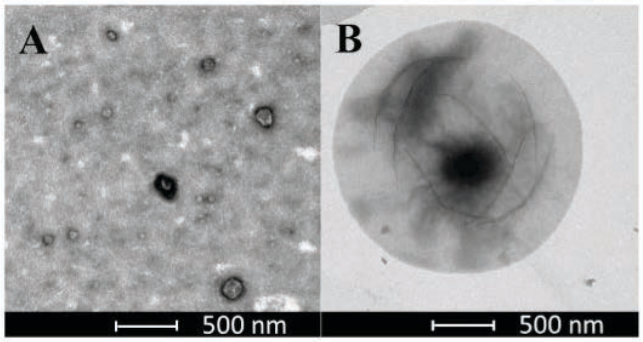

Figure 1. TEM images of: (A) Freshly made small unilamellar vesicles composed of phosphatidylethanolamine and phosphatidylglycerol 7/3. (B) The same liposomes after addition of T-K2569Tric-A (measured diameter: 1938nm)

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Searching for Nanostructured or pOre fOrming Peptides for therapY – SNOOPY COST Action

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The COST Action *Searching for Nanostructured or pOre fOrming Peptides for therapY* (SNOOPY) establishes an interdisciplinary network of experts to address key challenges in understanding and harnessing peptides and their activities or functions in a broad range of applications. Bringing together experts from chemistry, biology, biochemistry, materials science, nanotechnology, physics, computer science, and medicine, SNOOPY focuses on investigating the ability of bioactive peptides to form functional nanostructures, including membrane-active assemblies capable of pore formation. This rapidly expanding field offers strong potential for targeted cancer therapy, drug delivery, amyloidosis inhibition, regenerative medicine and antimicrobial development. Beyond therapeutics, peptide nanostructures offer opportunities in diagnostics, catalysis, optics, and bioelectronics.

The Action is structured into four synergistic Working Groups: computational methods (WG1), peptide production and characterization (WG2), biological studies and applications (WG3), and dissemination (WG4). Key deliverables include (i) creation of structured datasets from molecular dynamics and data mining, (ii) machine learning-driven identification of bioactive or functional peptide candidates, (iii) best-practice guidelines for synthesis and characterization, (iv) advanced synthesis and analytical protocols and (v) a roadmap for biological applications. SNOOPY also prioritizes training and career development of young researchers, fostering geographical inclusivity and collaboration to deliver socio-economic impact by addressing antimicrobial resistance, amyloid diseases, and enabling AI-accelerated discovery.

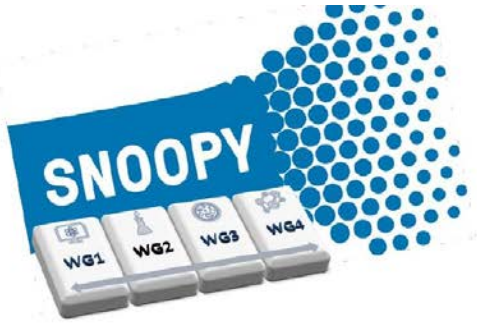

Figure 1. CA23111 logo and WG-based structure.

The role of selenoenzymes in the toxic and therapeutic activity of metallic compounds

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Mammalian selenoproteins containing selenocysteine (Sec), the sulfur-to-selenium substituted variant of cysteine (Cys), at their active site, play critical roles in redox homeostasis, antioxidant defense, and cellular signaling. Due to the high nucleophilicity and reactivity of selenocysteine, these proteins represent potential targets of toxic trace metals (Hg) and metallodrugs (Au, Pt).[1]

This presentation will elucidate the key role of Thioredoxin Reductases (TrxR) and Glutathione Peroxidases (Gpx), two main families of selenoenzymes, both in the toxicity of $MeHg_+$, a potent neurotoxicant, and in the therapeutic activity of gold-based drugs.

The reactivity of TrxR and Gpx with $MeHg_+$ or gold(III) cyclometallated compounds was evaluated by an integrated experimental, theoretical and cellular approach.

Our data offer a clear molecular explanation for the inhibition of the selenoenzymes TrxR1 and GPx1 by $MeHg_+$, as well as for the associated toxic effects. This process involves $MeHg_+$ preferentially reacting with SeCys, followed by the involvement of neighboring Cys residues.[2] We also provide mechanistic details and evidence supporting the use of selenopeptides for $MeHg_+$ detoxification strategies.[3]

Moreover, the intrinsic preferences of cyclometallated gold(III) compounds for Sec- over Cys-arylation was demonstrated in peptide models[4] and translated to functional inhibition of selenoenzymes.[5] This activity can mediate the cytotoxicity of these metallodrugs, highlighting their potential in anticancer therapy.

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Communications from Companies

TAPS – Where to put which Tag in Tag Assisted Peptide Synthesis

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Discovery and production of synthetic peptides has significantly progressed in the last years resulting in the fact that rather complex peptides like GLP-1 analogues, e.g. semaglutide and similar sequences, are being produced in large quantities for commercial consumption. Length, special sequences and modifications challenge to develop a robust process.

Figure 1. Peptide synthesis with C-terminal TAG attachment

We are demonstrating how different tags placed on different positions on a sequence can help to manufacture difficult peptides such as beta-amyloid sequences in high yield and how such tags can be removed in a traceless manner. This approach includes replacing conventional solid support (resin) on the C-terminus by a small molecule tag leading to tag-assisted LPPS technology.

Each technology has benefits and drawbacks which will be highlighted, in order to help the process chemist to develop an efficient process. We also will comment on the “greenness” of this technology.

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Industrial Strategies for Peptide Manufacturing: Combining Sustainability and Cost

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We are living in the golden era of peptides, dominated by the Incretin agonist for the treatment of diabetes and weight control but also sustained by the application of other peptide derivatives in several other therapeutic areas [1]. Driven by this wave, Academic and Industrial realities are jointly developing innovative strategies in both upstream and Downstream processes to minimize the manufacturing cost, ensure proprietary process and reduce the environmental impact. During the lecture, selected case studies focused on these aspects and under development in our laboratory will be presented and discussed [2,3].

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The New Golden Age of Peptides: Manufacturing Evolution and Emerging Therapies

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The peptide field is entering a new golden age, driven by both therapeutic breakthroughs and industrial scale-up. From a market intelligence perspective, this talk highlights how innovation in peptide manufacturing is adapting to the rapid rise of GLP-1–based anti-obesity and metabolic therapies, which are redefining demand in terms of volume, complexity, and reliability. Beyond GLP-1s, emerging modalities such as cyclic peptides, exemplified by ICOTYDE™, underline the expanding therapeutic relevance of peptides. Together, these advances are transforming peptides into large-volume, life-changing medicines requiring multi-ton API production at industrial scale.

Microwave Peptide Synthesis

Giorgio Marini
CEM Corporation

Method development for further advancing the efficiency of SPPS is of the utmost importance. Microwave irradiation provides simplified optimization, higher peptide purity, and an overall “greener” process. Compared to conventional heating methods, microwave irradiation provides rapid and direct energy exchange with the reagents.

Over the last two decades, we improved coupling efficiency and speed. The result, difficult and long sequences are effectively synthesized in a fraction of the time using much less solvent. Advances have been made which further reduce the cycle time to under 4 min, offer an overall solvent reduction greater than 90 % compared to other SPPS processes, and are readily scalable to generate up to 200 mg purified peptide.

Rapid scale-up for clinical trials and peptide production has been accomplished using similar technology.

In addition, recent improvements in microwave peptide synthesis related to the total elimination of all washing steps after each cycle (resulting in a massive waste reduction) will be highlighted, with application to both R&D; and production scale synthesis. A single wash is sufficient at production scales.

Microwave Peptide Synthesis provides the lowest PMI and most efficient process for peptide production.

Finally, a new HPLC process for peptide purification that completely eliminates the use of acetonitrile in place of ethanol has been developed. This new process is based on a novel integrated heating system that not only improved peptide recoveries by 50% on average, but also increased the final isolated purity. Significant cost savings are granted from using ethanol vs acetonitrile.

Oral Communications

Engineering Therapeutic-Relevant Snake Peptide Toxins with Selenocysteine to Investigate Folding and Stability

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Many bioactive peptide toxins rely on disulfide networks to adopt and maintain active conformations. However, these bonds are sensitive after *in vivo* administration, leading to structural destabilization and loss of activity, thus limiting their therapeutic potential. To overcome this, replacing cysteine residues with selenocysteine (Sec) is a promising strategy. Indeed, diselenide bonds are nearly isostructural to disulfides but exhibit a lower pK_a and a more negative redox potential, enabling faster formation and greater resistance to reduction.[1] Pioneering studies on diselenide-substituted peptides (insulin,[2] apamin,[3] conotoxins)[4] have already demonstrated that Sec incorporation can guide oxidative folding, preserve structure and function, and improve stability in reducing environments.

Here, we investigated Sec substitution in two snake venom peptide toxins: mambalgin-1 and mambaquaretin-1. Using optimized solid-phase peptide synthesis, Sec residues were introduced at key cysteine positions to generate selenium-containing analogues. These Se-mimetics were characterized through structural, biochemical, and preliminary functional assay to evaluate the impact of sulfur-to-selenium substitution on folding, redox stability, and activity. Altogether, this work provides new molecular insights into how selenocysteine can strengthen the structural robustness of disulfide-rich scaffolds and highlights Sec incorporation as a promising and generalizable approach for designing redox-stable bioactive venom peptides.

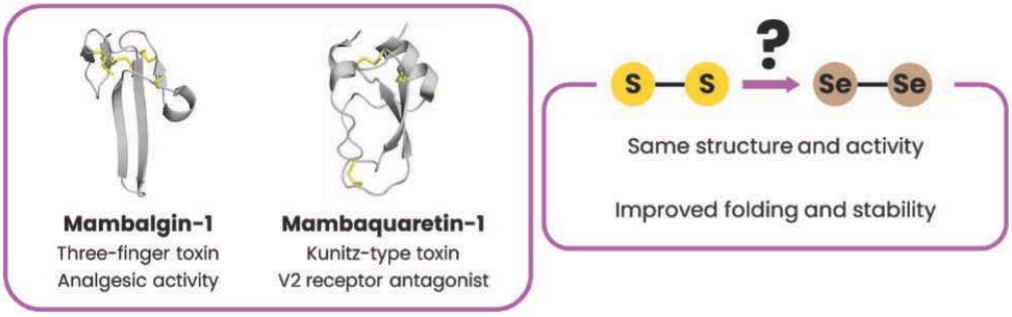

Figure 1. Cys-to-Sec substitution in mambalgin-1 and mambaquaretin-1: probing the effects of S–S to Se–Se bond substitution on folding, stability, and function.

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Redirecting the Peptide Cleavage Causes Protease Inactivation

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The typical feature of peptidic inhibitors of cysteine proteases includes an N-capped peptide structure bearing an electrophilic warhead (e.g. an aza-nitrile, as established in our group[1,2]) in place of the scissile peptide bond. A linker can direct a carboxylic group to the S′ region to allow for an advantageous salt bridging with the histidine residues of a special structural feature of cathepsin B, the occluding loop, thus enhancing cathepsin B selectivity. Human cathepsin B is a cysteine protease of significant therapeutic importance.

We have designed cathepsin B inhibitors with dipeptide portions directed towards the occluding loop and equipped with fine-tuned, inversely oriented warhead structures which are cleaved upon the action of the active site cysteine leading to irreversible inhibition. The peptidic inhibitors undergo a redirected cleavage and the primed part of the inhibitor remained covalently attached to the protease, an opposite situation when compared to the doctrinal cysteine protease-catalyzed peptide cleavage. Such a non-canonical scenario has not been achieved so far in an experimental realization. The fate of the resulting, covalently modified protease was unknown. We deciphered that such carbamoyl enzymes are inhibited in a virtually irreversible manner. Hydrolysis of the resulting covalently modified protease complexes is catalytically unsupported rendering inhibition irreversible. Crystallographic analysis of the enzyme-inhibitor complexes was pursued to corroborate the transcarbamoylation reaction and fine-tune the binding mode of our inhibitors.[3]

Convergent syntheses have been devised for an extended series of around 200 final compounds. The representatives of this chemotype were biochemically characterized and kinetic data at four human cathepsins demonstrated an impressive selectivity for cathepsin B over the closely related human cathepsins L, S and K and allowed to draw detailed structure-activity relationships. Representatives of this chemotype exhibit second order rate constants of inactivation of human cathepsin B greater than 10,000 $M^{-1}s^{-1}$. The exciting mode of action involved the interaction with the occluding loop of cathepsin B and the attack of the active site cysteine at the warhead of the inactivator by a crucial enzyme-catalyzed deprotonation step.

The novel mechanism of action comprises a significant extension of the peptide drug space.

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Peptide-based fluorescent conjugates for targeted imaging, fluorescence-guided surgery, and phototherapy in solid tumors

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Background. Compounds able to selectively accumulating in neoplastic lesions may have a major impact on cancer diagnosis and therapy. We designed a chromogranin-A derived peptide (peptide 5a) containing an RGDL motif that selectively binds αvβ6 and αvβ8 integrins, which are overexpressed in several solid tumors.[1] We conjugated 5a to the near-infrared (NIR) fluorescent dye IRDye800 (5a-IRDye) and investigated its tumor-targeting properties.[2] Subsequently, we coupled peptide 5a to the dye indocyanine green (ICG) (5a-ICG) for combined imaging and photo-therapy applications.

Methods. 5a-IRDye and 5a-ICG conjugates were coupled to dyes via maleimide chemistry and characterized by RP-HPLC and mass spectrometry. Binding to purified αvβ6 and αvβ8 integrins and to αvβ6+/αvβ8+ cancer cell lines of pancreatic ductal adenocarcinoma (PDAC) and glioblastoma was assessed using direct and competitive binding assays. *In vivo* biodistribution and tumor accumulation of 5a-IRDye and 5a-ICG were evaluated by NIR fluorescence imaging in αvβ6+/αvβ8+ PDAC tumor models. In parallel, phototherapeutic potential of 5a-ICG was assessed *in vitro* on cancer cells irradiated with an 808 nm diode laser.

Results. Both conjugates exhibited high affinity for αvβ6 and αvβ8 integrins with nanomolar affinity, and showed specific binding to cancer cells *in vitro*. *In vivo* imaging in PDAC-bearing mice showed a strong tumor accumulation of 5a-IRDye and 5a-ICG, with favorable tumor-to-background contrast and limited uptake in most non-target tissues. Preliminary *in vitro* phototherapy experiments showed that 5a-ICG displays efficient photothermal conversion and cytotoxic effects in αvβ6+/αvβ8+ cancer cells.

Conclusions. These findings indicate that 5a-based fluorescent conjugates possess strong tumor-targeting properties and promising theranostic potential for imaging, fluorescence-guided surgery, and phototherapy of $\alpha v\beta 6^{+}/\alpha v\beta 8^{+}$ solid tumors.

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Engineered Supramolecular Nanostructures for Tissue Regeneration

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Advances in regenerative medicine require materials that provide precise control over nanoscale organization, mechanical behaviour, and biological function. We develop bioinspired supramolecular scaffolds based on short aromatic peptides that undergo solvent-regulated self-assembly and polymorphic phase transitions, enabling fine-tuning of crystalline structure, rigidity, and bioactivity. Co-assembly of complementary dipeptides yields exceptionally rigid hydrogels that support cell mechanotransduction and osteogenic differentiation. The incorporation of biopolymers, such as hyaluronic acid and gelatin, yields injectable, 3D-printable composite hydrogels that exhibit thixotropic behavior and enhanced calcium mineralization. These materials result in substantial bone regeneration *in vivo*.

Expanding their functionality, we fabricate patient-specific, bioactive titanium implants via additive manufacturing and design conductive peptide-MXene hybrid hydrogels that function as piezoresistive sensors for real-time tissue monitoring and electrical stimulation. Furthermore, we developed an autologous electrospun polymer-peptide skin equivalent for treating severe burn injuries, demonstrating accelerated wound closure and a reduction of T_{50} from 12 to 4 days.

Together, these studies establish a modular platform that integrates mechanical tunability, printability, and sensing capabilities to fabricate multifunctional biomaterials and hybrid bioelectronic interfaces. This supramolecular approach enables the design of personalized regenerative systems with translational potential across soft and hard tissue repair.

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AI-driven cyclic peptides inhibiting undruggable intracellular PPIs: application to the Calcineurin-NFAT interface for improved immunosuppressants

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Targeting intracellular Protein-Protein Interactions (PPIs) remains an attractive therapeutic path in drug discovery. This requires modulators with high affinity, cellular permeability, and metabolic stability. While peptides offer an ideal modality for inhibiting large PPI interfaces, filtering the exponential sequence space to identify high-affinity binders and translating linear hits into metabolically stable, cell-permeable therapeutics remains a challenge. Herein, we targeted the Calcineurin-NFAT intracellular PPI axis. Upon activation, Calcineurin dephosphorylates the transcription factor NFAT, triggering its nuclear translocation and downstream release of inflammatory cytokines. The clinical drug Cyclosporine, which blocks this pathway, relies on Calcineurin catalytic site inhibition, leading to severe nephrotoxicity and broad off-target effects. Targeting the specific Calcineurin-NFAT PPI interface offers a safer, more precise therapeutic strategy but remains elusive due to the flat, featureless nature of the binding interface.

Herein, we applied an AI-driven approach that overcomes these hurdles by discovering novel linear binders and transforming them into cell-permeable cyclic peptides. By synergizing machine learning algorithms with real-time biophysical feedback, we screened vast sequence libraries to identify “out-of-the-box” linear peptides and structurally constrain them into cyclic peptides that inhibit the Calcineurin-NFAT PPI interface. We further show that these AI-designed cyclic peptides retain high binding affinity but also exhibit enhanced stability and efficient cellular activity, successfully inhibiting NFAT translocation and downstream immune signalling. These findings establish a foundation for the development of new immunosuppressants based on cyclic peptide therapeutics.

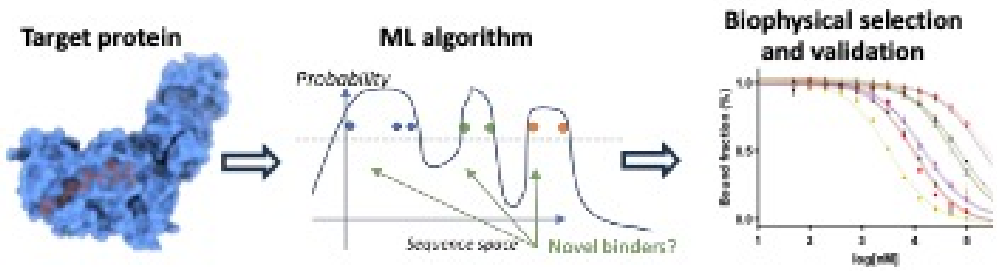

Figure 1. Discovery and characterization of peptides targeting PPI interfaces.

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Towards green solid phase peptide synthesis: DBN for sustainable Fmoc removal

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Developing greener synthetic methodologies requires replacing hazardous reagents with more sustainable alternatives.[1-4] In solid-phase peptide synthesis (SPPS), piperidine is the standard base for Fmoc group removal, but its toxicity, environmental concerns, and regulatory restrictions have prompted the search for safer substitutes. This study evaluates various green bases based on their GSK greenness score and chemical–physical properties similar to piperidine. Their performance was assessed through deprotection kinetics tests in different green solvents, followed by efficiency evaluations in solid-phase synthesis.

Among the tested bases, DBN demonstrated the highest efficiency, achieving rapid Fmoc removal at the lowest concentration across all green solvents. Additionally, DBN effectively minimized side reactions such as racemization, particularly in Anisole/NOP (75:25). Finally, DBN was tested in the synthesis of a model peptide, yielding comparable results to piperidine. These findings highlight DBN as a promising green alternative for SPPS, contributing to the advancement of more sustainable peptide synthesis strategies.[5]

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Antimicrobial peptides: Mechanism of action, synergy and mimetics

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Biophysical and structural studies of peptide-lipid interactions, peptide topology and dynamics have changed our view how antimicrobial peptides insert and interact with membranes. Clearly, both the peptides and the lipids are highly dynamic, change and mutually adapt their conformation, membrane penetration and detailed morphology on a local and a global level. As a consequence, the peptides and lipids can form a wide variety of supramolecular assemblies in which the more hydrophobic sequences preferentially, but not exclusively, adopt transmembrane alignments and have the potential to form oligomeric structures similar to those suggested by the transmembrane helical bundle model. In contrast, charged amphipathic sequences tend to stay intercalated at the membrane interface, where they have been found to adopt mesophase structures in a lipid dependent manner. Although the membranes are soft and can adapt, at increasing peptide density they cause pronounced disruptions of the phospholipid fatty acyl packing. At increasing local or global concentrations the peptides result in transient membrane openings, rupture and ultimately bacterial lysis.

We have extended such studies to combinations of antimicrobial peptides that act in synergy. Notably, the much higher antimicrobial activity of the mixtures is strongly lipid dependent in a manner that potentially increases their therapeutic index. We also investigated a series of peptide mimetics such as peptoids and small molecules which show different interaction characteristics with membranes.

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Antiviral peptides against SARS-CoV-2

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The devastating pandemic of severe acute respiratory syndrome known as COVID-19 is caused by the coronavirus SARS-CoV-2. The trimeric spike (S) protein expressed on the virus outer bilayer leaflet plays a crucial role in the viral infection molecular mechanism, through multiple interactions with host cell substrates. In particular, the spike S1 subunit interacts with the cell surface angiotensin-converting enzyme-2 (ACE2) receptor and subsequently the S2 subunit mediates the final event of membrane fusion.

Since both these processes represent promising targets for the development of antiviral drugs, we implemented strategies based on synthetic peptides to develop new compounds able to interfere either with the ACE2/Spike protein interaction, or with the membrane fusion process, to prevent entry of the virus in the host cell.

In a first approach, we focused our attention on the minimal ACE2 fragment involved in binding to the spike S1 subunit, i.e., ACE2(24–42), and, in order to increase its conformational propensity to form an alpha-helix, we designed different triazole-stapled analogs, changing the position and the number of bridges. The peptide featuring a triazole-containing bridge linking positions 36–40 showed increased helical conformation, as determined by circular dichroism, and promising antiviral activity at micromolar concentration, assessed by plaque reduction assay.[1]

In another approach, we studied the spike S2 subunit internal fusion peptide (IFP) region, which is supposed to interact with the membrane proximal external region (MPER) of the same S2 subunit, in the fusion-competent S2 coiled-coil structure. We prepared several peptides and lipo-peptides, some of which appear to bind to the target, exerting interesting anti-viral activity.[2]

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The next generation peptide sequencing with AI

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The identification of clinically relevant peptide biomarkers remains a central challenge in translational medicine due to the extreme complexity, dynamic range, and post-translational heterogeneity of biological peptidomes. Recent advances in next-generation peptide sequencing (NGPS) have enabled direct, single-molecule peptide readouts, offering unprecedented opportunities to interrogate native peptide landscapes beyond the limits of conventional mass spectrometry.

In this study, we present an integrated NGPS–artificial intelligence (AI) framework leveraging the Quantum-Si Platinum peptide sequencing platform combined with a proprietary, in-house AI-engaged reference library for high-confidence biomarker identification. Biological samples derived from cell lysates, secretomes, and extracellular vesicle fractions were sequenced to generate high-resolution peptide fingerprints, including low-abundance and post-translationally modified peptides.

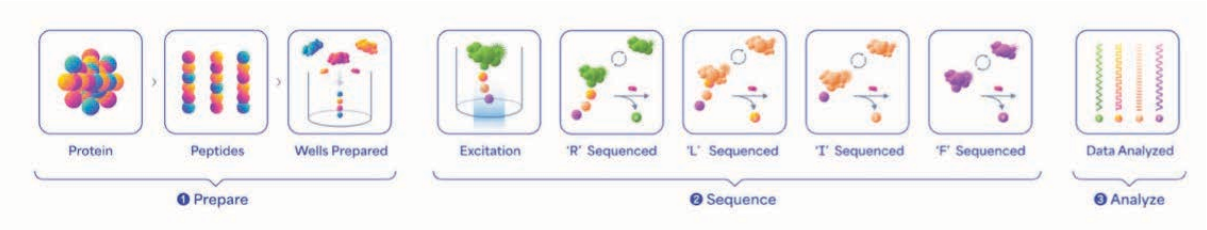

Figure 1. NGPS Workflow.

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Artificial intelligence-guided design of Kisspeptin-10 analogs reveals bioactive peptides with cytotoxicity activity in breast cancer cells

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Kisspeptin-10 (KP10), an endogenous peptide derived from the KISS1 metastasis suppressor, plays a critical role in regulating tumour progression and metastasis through activation of the G protein-coupled receptor KISS1R. Increasing evidence suggests that structural modifications of KP10 may enhance its biological activity and reveal new therapeutic opportunities in cancer treatment. In this study, artificial intelligence-based computational approaches were used to design novel analogs of KP10 with potentially improved bioactivity.

A library of peptide analogs was generated through *in silico* design strategies guided by machine learning-assisted predictions of structural and functional properties. Selected candidates were synthesized by solid-phase peptide synthesis and purified prior to biological evaluation. The antiproliferative activity of the peptides was assessed in breast cancer cell lines using cell viability assays to determine their potential anticancer effects. Two AI-designed KP10 analogs showed enhanced biological activity compared with the native peptide, leading to significant reductions in breast cancer cell viability. These findings suggest that targeted sequence modifications can modulate the biological properties of kisspeptin-derived peptides and generate bioactive molecules with potential therapeutic relevance. The integration of artificial intelligence-driven peptide design with experimental validation represents a promising strategy for the discovery of novel peptide-based anticancer agents.

Overall, this work highlights the potential of AI-assisted peptide engineering to accelerate the development of optimized kisspeptin analogs and expands the landscape of bioactive peptides targeting cancer-related signalling pathways.

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A comprehensive multi-language database and conversion toolkit for macrocyclic peptides

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Nowadays, peptides represent versatile therapeutics bridging small molecules and biologics due to their high specificity, low immunogenicity, and ability to bias "undruggable" targets, such as protein-protein interaction systems.[1] This field has evolved from mimicking natural hormones to designing novel sequences with tailored pharmacological properties, opening new frontiers in *de novo* peptide discovery.[2] In this respect, cyclic peptides represent a promising class of therapeutic agents, as their constrained structure can enhance stability, binding affinity, and selectivity, ultimately leading to improved pharmacokinetic and pharmacodynamic properties compared to linear peptides.[3]

In this scenario, we developed new computational approaches for better dealing with the complexity of macrocyclic peptides. In particular, we crafted a new toolkit for translating the chemical structures of macrocyclic peptides into diverse easy-readable string-based notations, such as SMILES, HELM,[4] BILN,[5] or graph representation. Our toolkit demonstrated high accuracy in converting between different macrocyclic peptide representations, enabling seamless integration and aggregation of robust data from multiple existing sources.

On this basis, we built HERMES (an acronym standing for HELM and smilEs Repository for Macrocyclic pEptideS), a curated dataset of ~21,000 macrocyclic peptides obtained by harmonically integrating information from multiple existing databases to ensure the retrieval and collection of high-quality, standardized, and comprehensive data. Based on fragmentation, a dataset of approximately ~4,000 monomers was also derived from HERMES enabling a consistent and unambiguous molecular-level understanding of macrocyclic peptide building blocks. In perspective, HERMES could be exploited for developing advanced machine learning approaches, including large language models (LLMs) based on string representations and graph neural networks (GNNs) built on graph-based structures. Taken together, these resources will provide a robust framework for advancing computational methodologies and are expected to facilitate the broader adoption of AI-driven strategies in peptide-based drug discovery.

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Branched Oncolytic Peptides (BOPs) against cancer

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Cold tumors, defined by low immune cell infiltration and limited immune activation, represent approximately 60–70% of all solid cancers. These tumors are largely unresponsive to current immunotherapies, with clinical response rates often below 10–20%, compared to significantly higher responses in *hot* tumors. This creates a major therapeutic gap, particularly in aggressive cancers such as pancreatic cancer, where over 80% of tumors are immunologically cold and the 5-year survival rate remains below 10%.

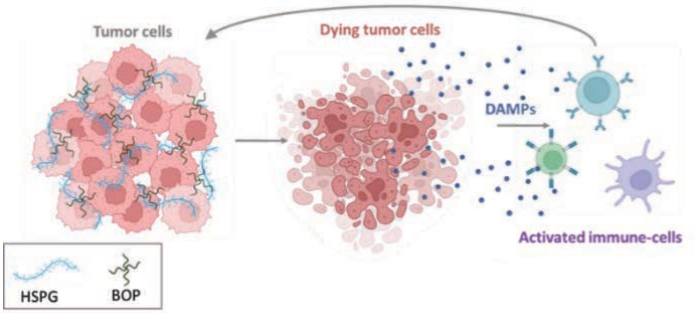

Figure 1. BOPs are cytotoxic to tumor cells and promote immune reactivation.

BOPs have strong cancer selectivity and are proteolysis-resistant peptides, unlike their linear analogues. Their repeated cationic sequences enable multivalent binding to negatively charged tumor-cell membranes, particularly heparan sulfate proteoglycans (HSPGs), which are often overexpressed in pancreatic cancer cells. BOPs reduce cancer cell adhesion and migration and show selective cytotoxicity with low hemolysis. They also induce immunogenic cell death, promoting sustained release of DAMPs (e.g., HMGB1, IFN-β, ATP). *In vivo*, BOP9 inhibits tumor growth and metastasis and demonstrates effective systemic distribution.

These properties highlight BOPs as promising candidates for combination therapies, including immune checkpoint inhibitors, particularly for immunologically *cold* tumors.

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Cashing in on Fragments: A Peptide-Based Discovery of KCASH2 Binding to MAD2A

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MAD2A and KCASH2 play complementary roles in cell cycle control, acting respectively in the spindle assembly checkpoint and in ubiquitin-mediated regulation of oncogenic signaling.

Here, we report for the first time their interaction and adopt a peptide-centered strategy to define its structural basis. A fragmentation-based *in silico* approach[1] was used to screen short KCASH2-derived peptides against MAD2A using AlphaFold Multimer, enabling identification of binding regions.

These results guided the reconstruction and molecular dynamics (MD) characterization of the full-length KCASH2–MAD2A complex, revealing a conserved binding mode driven by a previously unrecognized linear motif (MIM) in KCASH2. Building on these insights, a minimal 8-residue peptide derived from the identified interface and designed based on MD-extracted data was synthesized. Remarkably, this short peptide is sufficient to recapitulate the key interactions of the full-length protein, as confirmed by *in silico* and *in vitro* evidence.

Overall, this work establishes a closed-loop, peptide-driven workflow, starting from fragment screening and culminating in a minimal functional motif, demonstrating the utility of peptides as both discovery tools and functional mimetics of protein–protein interactions.

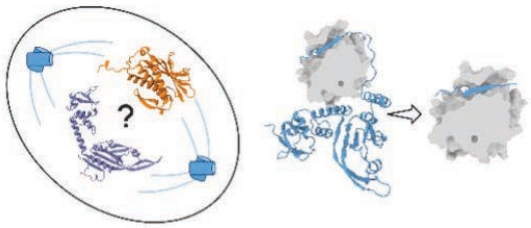

Figure 1. Structural characterization of the KCASH2-MAD2A interaction through in silico modelling and peptide design.

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Influence of microbial exposure on the peptide profile and biological activity of *Lucilia sericata* larval secretions

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Insects thriving across diverse environmental conditions constantly encounter numerous pathogens, including viruses, bacteria, and fungi. In order to defend themselves, they have evolved three primary immune defence mechanisms: anatomical and physiological barriers, cellular defence mechanisms, and humoral defence mechanisms.[1] The humoral response is crucial for recognizing specific pathogens *via* pattern recognition receptors, which detect pathogen-associated molecular patterns characteristic of distinct microbial groups. This molecular interaction triggers the targeted production of specific antimicrobial peptides to effectively combat the infection.[1]

The current research examines how exposure to Gram-positive and Gram-negative bacteria and fungi influences the composition and biological activity of secretions produced by fly larvae. Specifically, it investigates necrophagous *Lucilia sericata* larvae, whose secretions contain numerous biologically active compounds possessing broad applications, particularly in regenerative medicine and the treatment of hard-to-heal wounds.[2] The larvae were immunized with specific bacteria and fungi, and their resulting secretions were collected and separated on 3 kDa MWCO filters. The fraction below 3 kDa was further fractionated using size-exclusion chromatography. These fractions were subsequently evaluated for their antimicrobial activity and their direct effects on endothelial cell proliferation.

Our findings demonstrate that exposing larvae to different types of microorganisms significantly alters the overall peptide composition, inducing both qualitative and quantitative shifts depending on the specific inducing agent. Furthermore, we observed distinct variations in the biological activity of the secretions, including altered selectivity toward reference pathogen strains and dermal endothelial cells. These results unveil novel research avenues, particularly concerning the targeted modulation of secreted material composition. In the future, this approach may facilitate tailoring these biological properties for highly specific applications in medicine and biotechnology.

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Computational design of antimicrobial peptide nanopores

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Antibiotic resistance is a global health threat, driving the need for new molecules that kill bacteria via non-traditional mechanisms. Here, we present a computational *de novo* design strategy for α-helical peptides that self-assemble into large, stable, membrane-spanning nanopores with antimicrobial activity — including *in vivo* efficacy against drug-resistant pathogens. Molecular dynamics simulations guided the selection of sequences predicted to form barrel-stave pores, validated by microscopy, electrophysiology, and fluorescence assays.

Based on computational and experimental analyses, including negative design controls, we derived design guidelines and proposed 52 modular sequence templates that can be tuned for antimicrobial and functional pore-forming properties. Mechanistic studies confirmed that antimicrobial activity is driven by bacterial cytoplasmic membrane disruption via designed nanopore formation. Tuned peptides selectively killed drug-resistant ESKAPEE bacteria at nanomolar concentrations without harming human cells and demonstrated anti-infective efficacy in skin abscess and thigh infection preclinical mouse models of *Acinetobacter baumannii* infection. This work provides a general strategy for designing synthetic peptide nanopores, enabling future applications in precision antimicrobials, anticancer agents, molecular sensors and stimuli-responsive delivery systems.

Self-Assembling Amphiphilic Dendrimers (SAADs) for the Detection of CXCR4 Positive Tumors by PET Imaging

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Self-assembling amphiphilic dendrimers (SAADs) were developed as the first PET probe targeting CXCR4-overexpressing tumors, combining dendrimer-mediated EPR with active CXCR4 targeting. The amphiphilic peptidomimetic dendrimer–NOTA conjugate (1,4,7-triazacyclononane-1,4,7-triacetic acid), C18-NOTA, was coupled with the potent and selective CXCR4 antagonist R54.[1,2] This strategy enabled the preparation of four formulations with increasing amounts of R54 (F1–F4), along with corresponding non-targeted control molecules (F5–F6). F1–F6 and their Ga(III) derivatives (Ga-F1 to Ga-F4) exhibited nanomolar affinity for the CXCR4 receptor, with IC_{50} values decreasing from Ga-F1 to Ga-F4 in correlation with increasing R54 content.

PET imaging and biodistribution analyses showed that only [^{68}Ga]Ga-F3 accumulated specifically in CHO-hCXCR4 tumor-bearing mice. As Ga-F1 through Ga-F4 shared similar particle sizes and positive surface charges, the lack of active targeting by [^{68}Ga]Ga-F4, despite its high R54 density and strong receptor affinity, suggests that both ligand density and spatial arrangement are crucial for effective receptor binding and tumor targeting. Overall, these findings identify CXCR4-targeted SAADs as a promising PET imaging platform and highlight the broader potential of multivalent dendrimer-based tracers for future theranostic applications, particularly for tumors with complex microenvironments, such as pancreatic ductal adenocarcinoma (PDAC), where CXCR4 is highly expressed across multiple cell types.[3]

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A CYFIP1-Inspired Peptidomimetic Modulates eIF4E-Dependent Translational Control in Cancer and Neurodevelopmental Disorders

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The eukaryotic translation initiation factor 4E (eIF4E) is a central regulator of cap-dependent translation and a compelling pharmacological target in disorders marked by protein synthesis dysregulation, including cancer and neurodevelopmental disorders, such as Fragile X Syndrome (FXS).[1] When upregulated, eIF4E drives the selective translation of oncogenic mRNAs involved in proliferation, angiogenesis, EMT, and metastasis in cancer.[2] Conversely, FXS, the most common inherited cognitive developmental disability, exhibits disrupted synaptic plasticity attributed to abnormal protein synthesis within neurons.[3,4] eIF4E activity is controlled by dynamic interactions with eIF4G, which promotes translation, or with 4E-binding proteins (4E-BPs), which act as translation repressors. Cytoplasmic FMRP Interacting Protein 1 (CYFIP1) is an atypical 4E-BP with emerging roles in both tumorigenesis and synaptic dysfunction.[5,6] It binds eIF4E via a non-canonical α-helix with low sequence conservation, suggesting a unique inhibitory mechanism.[7,8] The CYFIP1-eIF4E interaction is uniquely selective, offering a framework for designing targeted translation modulators.

Here, we report Cy-9B, a rationally engineered stapled peptidomimetic derived from CYFIP1. Enhanced-sampling free-energy simulations indicate that Cy-9B engages eIF4E through a non-canonical binding mode. Stapling stabilizes its α-helical conformation and confers high resistance to proteolysis (>30 h). Cy-9B binds eIF4E with high affinity (K_d = 57 nM) and disrupts the eIF4E–eIF4G interaction, thereby inhibiting cap-dependent translation. In lung cancer cells, Cy-9B penetrates membranes, downregulates global protein synthesis, and reduces cell proliferation, migration and invasion. In neurodevelopmental disease models, Cy-9B partially normalizes excessive translation in FXS hippocampal neurons, where it modulates translation without detectable toxicity, and rescues social behavior deficits in a haploinsufficient *Drosophila melanogaster* model, restoring wild-type–like performance. Cy-9B emerges as a first-in-class therapeutic candidate for disorders sharing translational dysregulation, highlighting targeted modulation of eIF4E as a broadly applicable and physiologically compatible therapeutic strategy.

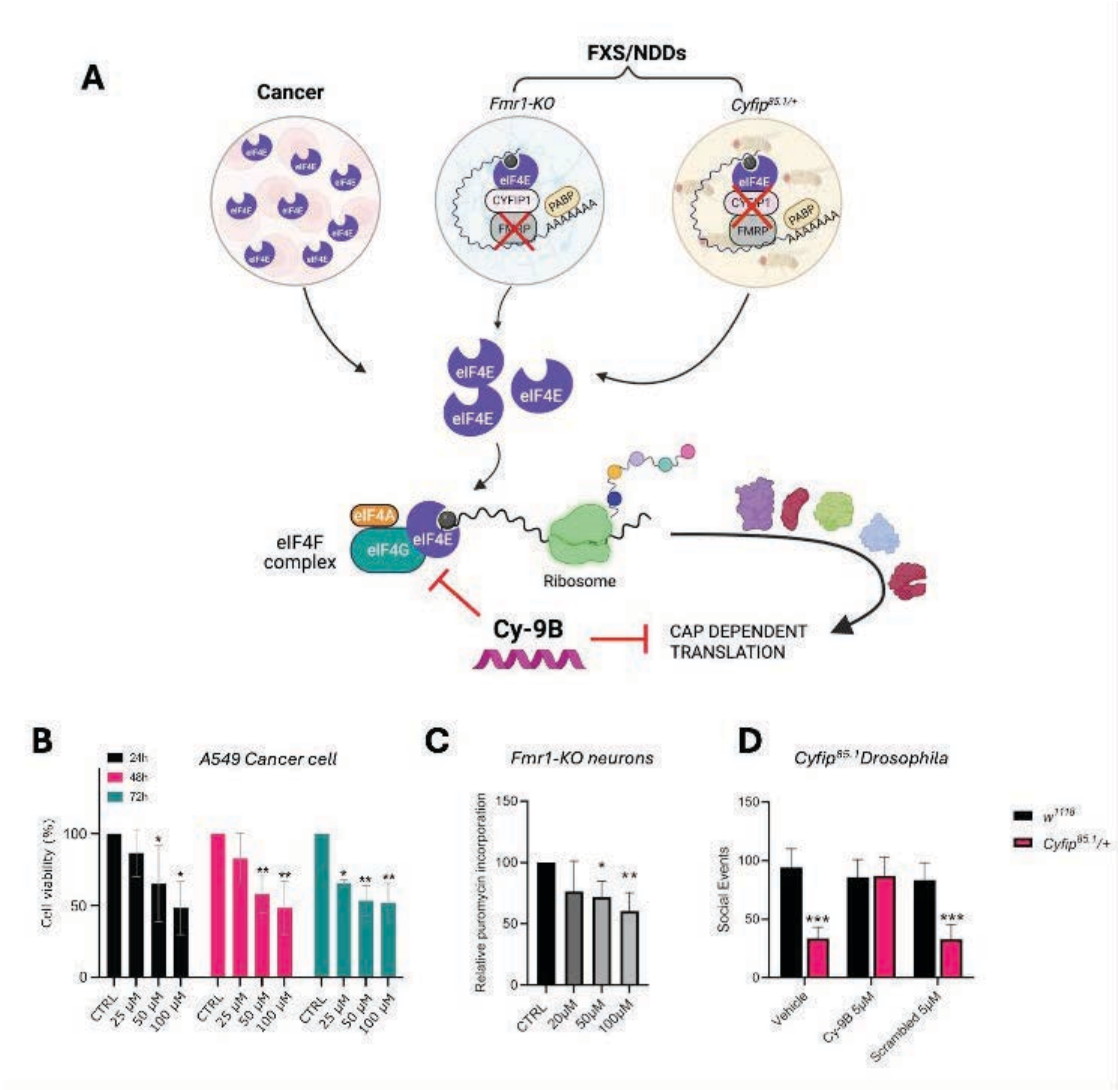

Figure 1. (A) Dysregulation of cap-dependent translation in cancer and neurodevelopmental disorders (NDDs). Conceptual schematic illustrating disease-specific perturbations converging on enhanced eIF4E-driven translation. The proposed mechanism of action of the Cy-9B peptidomimetic, namely disruption of the eIF4E–eIF4G interaction, is indicated. (B) MTT assay of A549 lung cancer cells treated with increasing concentrations of Cy-9B. (C) Global protein synthesis in Fmr1-KO neurons (FXS model), measured by the SUnSET assay following treatment with increasing concentrations of the peptide. (D) Social behavior (competition for food) of Cyfip85.1/+ flies upon treatment with 5 µM of Cy-9B or scrambled peptides.

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Novel Antimicrobial Peptides from Parasitic Flatworms with Activity against ESKAPE Pathogens

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The increasing problem of multidrug antibiotic resistance has prompted research into alternative antimicrobial agents from diverse sources, including plants and animals. One promising approach is genome-wide mining. In this context, parasitic flatworms are of particular interest because they inhabit microbe-rich environments and have evolved by adapting to their hosts and the surrounding microbial communities throughout their life cycle. Antimicrobial peptides (AMPs) derived from helminths may display activity against human pathogens, making them promising candidates for clinical applications.[1,2]

Here, we present several examples of synthetic AMPs identified from helminths that exhibit potent broad-spectrum antibacterial activity against *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *S. aureus*.[2] These peptides possess specific sequence features that highlight a direct relationship with their mechanism of action against pathogens. Notably, they share a conserved C-terminal CXXXC motif, which is common in many flatworm-derived AMPs and typically occurs within unstructured regions.[3] Molecular modelling revealed that these regions represent key modular elements responsible for conformational flexibility and antimicrobial activity. Mechanistic studies further indicated a membrane-targeting mode of action, as demonstrated by circular dichroism spectroscopy, which revealed distinct structural conformations in different environments. In neutral membranes, no structural changes are observed, suggesting selective antimicrobial activity. Finally, flow cytometry and fluorescence imaging confirmed a membrane-related mechanism of action.[3]

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Nucleobase-functionalized aromatic amino acids and dipeptides: self-assembled nanostructures for potential drug encapsulation

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In the last decades, the study of molecular self-assembly has emerged as a field of great importance for obtaining increasingly sophisticated and finely-tunable functional nanomaterials.[1] Interestingly, even small molecules such as dipeptides, amino acids, nucleobases or metabolites in general, can, under appropriate conditions (physiological, pathological or artificial), give self-assembly by providing nanostructures with unique mechanical, optical and electronic properties.[2] Currently, self-assembling nanomaterials find a wide variety of applications in the field of nanotechnology, from imaging to biosensors and drug-delivery.[3,4] Nucleoamino acids and nucleopeptides, whose study represents one of our research lines for many years,[5,6] constitute an interesting class of hybrid biomolecular scaffolds with unique potential in medicinal chemistry, combining peptide-like structural versatility with nucleobase-mediated recognition and supramolecular organization.

Herein, a comprehensive biophysical characterization of a set of compounds mainly based on L-tyrosine and L-tryptophan functionalized with nucleobases, particularly thymine, is reported. The selected molecules, obtained by solid phase synthesis as amidates, were purified by HPLC and characterized by NMR and LC-ESI-MS. Their self-assembling ability in pseudo-physiological buffer was investigated using a combination of spectroscopic, mainly fluorescence and DLS, and microscopy-based (SEM) techniques.[7] Preliminary drug- and metal-binding experiments, showing that the formed nanostructures could interact with the hydrophobic drug curcumin and Cu^{2+} ions, together with the non-toxic profile of the molecules on healthy cells, indicate them as promising building blocks for drug delivery or metal-responsive nanomaterials.

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Milk permeate-derived bioactive peptides: from identification to *in vivo* validation of their antioxidant properties

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The antioxidant potential of bioactive peptides derived from milk permeate, a dairy industry by-product, was investigated contributing to both sustainable food systems and nutraceutical development. In the first approach, peptide-enriched fractions were isolated from milk permeate using solid chromatography and tandem mass spectrometry, and their antioxidant properties were evaluated as a pool. In the second approach, individual synthetic peptides were selected through an *in silico* molecular docking strategy targeting the Keap1/Nrf2 pathway, with eight candidates synthesized and tested to identify which specific peptides are responsible for the observed antioxidant activity.

Both the peptide pool and the individual peptides demonstrated free radical scavenging activity and effectively protected cultured cells — including Caco-2 and HCT116 lines — from oxidative stress, reducing reactive oxygen species production and partially mitigating lipid peroxidation. At the mechanistic level, both approaches consistently showed activation of the Keap1/Nrf2 pathway, with stimulation of Nrf2 nuclear translocation, upregulation of downstream antioxidant gene transcription, and increased expression of related proteins. Permeability studies using Caco-2 cells in Transwell inserts confirmed that the peptides can cross the intestinal monolayer, suggesting varying degrees of bioavailability and potential for systemic effects. The antioxidant efficacy of the peptide pool was further validated *in vivo* in a zebrafish model, where it provided significant protection against acute cold stress-induced oxidative damage.[1]

Collectively, these findings highlight the multifunctional antioxidant properties of milk permeate-derived peptides, both as a complex mixture and as individual molecules, supporting their potential application as functional food ingredients, nutraceuticals, or therapeutic agents for oxidative stress-related conditions.

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Flash Communications

Expanding the Role of RNase 7: A Novel Player in Neuroimmune Regulation

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Human ribonuclease 7 (RNase 7), originally isolated from skin, is a member of the RNase A Superfamily and primarily known to be among the endogenous proteins with the most pronounced antimicrobial properties[1]. Nevertheless, to date, studies on this enzyme are mostly limited to its antimicrobial effects on epithelial tissue, which is surprising considering the numerous districts of the human body potentially susceptible to infections[2]. This inference inspired the present work, which is mainly dedicated to uncovering new roles of the RNase 7 in human cells that have not yet been explored in relation to this antimicrobial agent: neuronal cells. In this context, we decided to address possible host defense properties of RNase 7 in the nervous system, and to do this, we have selected both neuroblastoma SH-SY5Y and glioblastoma U-87 MG cells as experimental models.

As a result, we found that, in addition to endogenously expressing RNase 7 under altered growth conditions, both cell lines are also responsive to its administration. More specifically, we highlighted for the first time that recombinant RNase 7 reduces the expression levels of pro-inflammatory cytokines, the release of nitric oxide, and the production of reactive oxygen species in LPS-stimulated SH-SY5Y and U-87 MG cells, contributing to their innate immune response. Moreover, here we highlighted that internalized recombinant RNase 7 can contribute to bacterial clearance in both SH-SY5Y and U-87 MG. These findings extend the functional role of RNase 7 beyond epithelia, indicating its potential involvement in neuroimmune regulation and suggesting novel therapeutic implications.

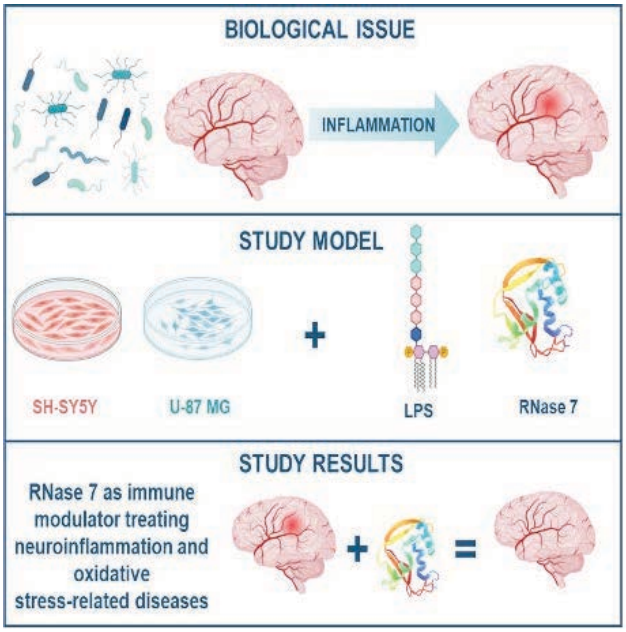

Figure 1. RNase 7 functions in neuronal cells to limit inflammation and support antimicrobial defense.

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Coronavirus M^{pro} Peptidomimetic Inhibitors: from a tetrapeptide to *in vivo* efficacy proof-of-concept study

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Over the past two decades, coronaviruses (CoVs) demonstrated pandemic potential proved by SARS outbreak in 2003, MERS in 2012–2013, and the most devastating COVID-19 pandemic caused by SARS-CoV-2 in 2020[1]. In this context, a drug discovery program targeting the essential viral enzyme SARS-CoV-2 main protease (M^{pro}) was initiated. Through a structure-based drug design (SBDD) approach, novel series of peptidomimetic covalent reversible M^{pro} inhibitors were designed and synthesized. Our initial structural analysis of the M^{pro}–N3 complex revealed a β-turn-like motif spanning the P1–P3 residues, leading to the introduction of a P2 proline residue to stabilize the ligand's bioactive conformation, thus leading to the identification of F2F-2020094, behaving as a nanomolar SARS-CoV-2/MERS M^{pro} inhibitor in enzymatic assays, and displaying nM-to-low µM antiviral activity across different coronaviruses with high selectivity index[2,3]. Our design was confirmed by X-ray co-crystal structure of F2F-2020094 in complex with SARS-CoV-2 M^{pro}.

Further SAR exploration led to F2F-2020339 as a second generation low-nanomolar M^{pro} inhibitor bearing a C-terminal nitrile warhead. While this work was ongoing, Nirmatrelvir, the first in class M^{pro} inhibitor, was disclosed, sharing similar structure and mechanism of action with F2F-2020339[4]. To further assess the value of our findings, an *in vivo* proof-of-concept (POC) study was performed to compare F2F-2020339 and Nirmatrelvir in a mouse model, evaluating the efficacy and the effects on adaptive immune response[5].

F2F-2020339 resulted a potent broad spectrum CoVs M^{pro} inhibitor closely related to Nirmatrelvir and for the first time, the *in vivo* efficacy POC study revealed a negative impact on adaptive immune responses *in vivo* in the second infection.

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NMR-Based Structural Characterization of Full-Length and Truncated HS-1, an Antimicrobial Peptide of *Hypsiboas semilineatus*

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Multidrug-resistant bacteria have become a significant public health risk. In this context, antimicrobial peptides (AMPs) derived from animals and plants have been developed as promising new drugs that can substitute conventional antimicrobial treatments. In this context, a precursor sequence, isolated from the skin of the Brazilian treefrog *Hypsiboas semilineatus*, encodes a novel antimicrobial peptide (AMP) called HS-1. The peptide, constituted by 20 amino acids and with a molecular weight of 2144.6 Da[1], shows an antimicrobial activity against Gram-positive, but does not demonstrate any effects against Gram-negative bacteria. By several *in silico* methods, the peptide was reported to assume a predominant amphipathic-helix conformation required for its antimicrobial activity against Gram-positive bacteria. This conformation allows the insertion of a well-defined hydrophobic region into the lipid bilayer[2].

Here, we investigate the tendency of HS-1, full length (1–20) and truncated fragment (7–20), to assume the α-helical structure in aqueous solution and after TFE addition, by low and high resolution techniques such as Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR). Our results expand our understanding of the antimicrobial activity of these peptides and stimulate further investigations in the direction of novel peptidic drugs for therapeutic applications.

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Fmoc-FF matrices induce a multicolour BODIPY emission

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Fmoc-FF (fluorenylmethoxycarbonyl-diphenylalanine) is a versatile low-molecular-weight hydrogelator that self-assembles in aqueous environments into nanofibrous matrices, forming self-supporting hydrogels through non-covalent interactions. The solvent-switch preparation method further enables the incorporation of additional chemical entities within the Fmoc-FF hydrogel network, providing a flexible strategy to tune its structural, functional, and physicochemical properties for biomedical, sensing, and materials applications.[1-3] In this work, the inclusion of BODIPY dyes (BP18, BP15 and BP11) in Fmoc-FF hydrogels was investigated, revealing an unexpected multicolour emission under UV irradiation at 365 nm, which depended on the concentration of the fluorescent probe (**Figure 1**). By incorporating chemically distinct BODIPY derivatives, the peptide hydrogel matrix was shown to enhance dye dispersibility in water and provide a confined supramolecular environment that promotes local dye organization, crystal formation, J-aggregate assembly, and protection of the fluorophores against irreversible, photon-induced chemical destruction of a fluorophore (photobleaching).

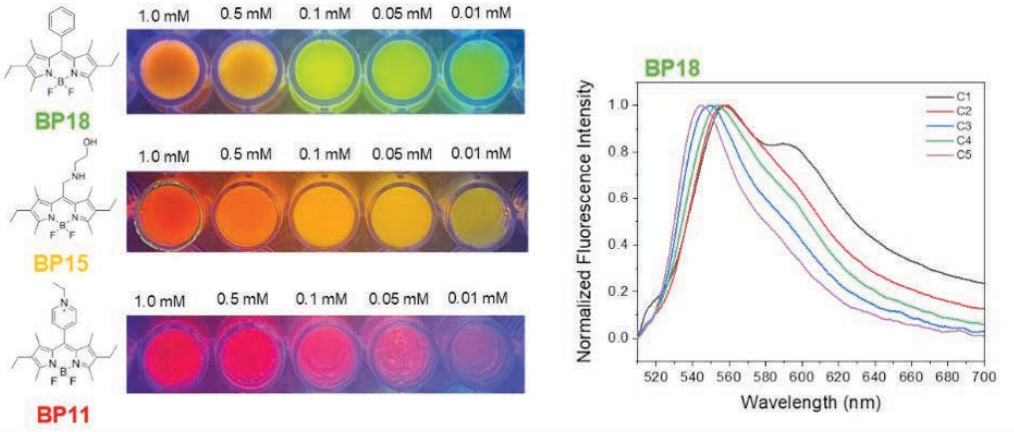

Figure 1. On the right: molecular structure and multicolour emission at different concentrations for BP18, BP15 and BP11; *on the left*: Emission characterization at solid state for BP18 at the analysed concentrations (from C1 1.0 mM to C5 0.01 mM).

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Biomimetic peptides for h20S proteasome activation in protein misfolding disorders

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Protein conformational diseases (PCDs), including Alzheimer's, Parkinson's, and prion-related disorders, are characterized by the progressive accumulation of misfolded and toxic proteins.[1] The main issue contributing to the pathophysiology of PCDs is the damage of the proteostasis network (PN), the complicated system responsible for maintaining protein homeostasis through regulation of synthesis, folding, and degradation processes. In this context, the proteasome, an essential multicatalytic complex involved in the selective protein turnover, has gained considerable attention as a potential therapeutic target. Proteasomal activity is tightly controlled by structural rearrangements of its core particle (CP), which are induced by interactions with specific regulatory particles (RPs).[2] Recently, increasing interest has targeted the design of biomimetic compounds able to mimic the natural regulators of the human 20S proteasome (h20S). This approach opens new opportunities for modulating protein systems that have been traditionally considered difficult to target pharmacologically.[3,4]

In the present work, we adopted a multidisciplinary strategy, combining advanced computational techniques with high-resolution Nuclear Magnetic Resonance (NMR) spectroscopy, to characterize the interactions between newly synthesized peptides and the human 20S proteasome investigating their impact on proteasome activation and function.

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Cytotoxicity and selectivity of Temporin L Analogues towards target cells and their molecular mechanism

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The main limitation of conventional cancer treatments is their lack of tumour selectivity, which leads to harmful side effects[1]. Additionally, cancer cells can develop resistance to these drugs due to the overexpression of multidrug resistance proteins, which pump them out of the cells, rendering them ineffective[2]. Antimicrobial peptides (AMPs, also known as host defence peptides) have been shown to possess potent anticancer effects both *in vitro* and *in vivo*, attracting attention as a new class of anticancer agents. These peptides offer several advantages over current cancer drugs, including selective cytotoxicity towards tumour cells, the ability to bypass multidrug resistance mechanisms, and synergistic effects when used in combination therapies[3]. Temporin L (TL) (FVQWFSKFLGRIL) is a potent antimicrobial peptide active against gram-positive and gram-negative bacteria[4]. Moreover, TL has demonstrated synergistic antitumour, anti-endotoxin, and immunomodulatory activities when tested alongside other Temporin members. However, it also exhibited significant haemolytic activity at bactericidal concentrations and showed cytotoxicity against various human tumour cell lines[5].

Using solid-phase synthesis, TL and its analogues (TL-34, TL-42, TL-46, TL-47, TL-48, and TL-51) were prepared by substituting natural or unnatural amino acids. The ability of TL to inhibit cell viability was assessed on five tumour cell lines of different tissue origins: MCF-7, MDA-MB-231, LoVo, HCT 116, and PC-3, over 24, 48, and 72 hours using the MTT assay. All peptides showed dose-dependent, significant cytotoxicity against the tested cell lines, although sensitivity varied considerably across cell lines. Notably, the analogue TL-47 exhibited a more pronounced growth inhibitory effect than the others. Consequently, TL-47 appears to be a promising candidate for anti-cancer applications. TL-47-induced growth inhibition occurs through the activation of apoptosis, as shown by Annexin V-fluorescein isothiocyanate/propidium iodide (Annexin V-FITC/PI) double staining in MCF-7, LoVo, HCT 116, and PC-3 cancer cell lines. Furthermore, TL-47 induces apoptosis via the mitochondrial pathway, as evidenced by western blot analysis of proteins. Additionally, a comprehensive PCR array-based screening was performed to evaluate inflammation-related genes in the MCF7 and MDA-MB-231 cell lines after TL-47 treatment and to identify differentially expressed genes in TL-47-treated cells compared with non-treated cells. In conclusion, TL-47 could serve as a new anticancer agent owing to its selectivity for tumour cells.

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Honeybee venom peptide characterization and encapsulation for triple-negative breast cancer treatment

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Bee venom (BV) is a natural toxin produced by honeybees, known for its dual toxic and therapeutic properties[1], with increasing interest in its application in the treatment of various cancers, including renal, hepatic, and prostate malignancies[2]. Its biological activity is attributed to a complex mixture of bioactive components; among these, melittin is the main peptide responsible for BV cytotoxicity, inducing cell-cycle alterations, inhibiting proliferation, and triggering apoptotic and necrotic death through mechanisms involving caspase and matrix-metalloproteinase activation[3]. In this study, we performed a comprehensive physicochemical characterization of BV, focusing not only on its chemical composition but also on its surface properties, to enable successful encapsulation. High-performance liquid chromatography (HPLC) coupled with liquid chromatography–mass spectrometry (LC–MS) analyses was employed to define the compositional profile of the BV extract, confirming melittin as the predominant peptide[1,4]. Furthermore, circular dichroism (CD) spectroscopy was used to investigate the secondary structure of BV peptides, highlighting the presence of melittin and its characteristic α-helical conformation[1,3]. Structural stabilization was further evaluated through trifluoroethanol (TFE) titration, revealing that the helical structure is maximally achieved at 30% TFE. In addition, surface tension measurements of BV aqueous solutions were conducted to assess their interfacial behavior, providing further insight into their physicochemical properties.

As a translational step, BV was encapsulated into poly(lactic-co-glycolic acid) (PLGA) nanoparticles to allow a controlled delivery. The developed nanoparticles exhibited an average size of approximately 167 nm with a low polydispersity index (PDI ≈ 0.1), indicating a homogeneous population suitable for biomedical applications. We also evaluated the cytotoxic activity of BV-loaded nanoparticles on triple-negative breast cancer (TNBC) cell lines, aiming to assess their potential as an innovative anticancer strategy. Future steps involve the *in vitro* evaluation in a more complex system to assess the efficacy in the tumour microenvironment.

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New azamacrocycle-based siderophores as potential MRI agents

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Over the past forty years, contrast-enhanced MRI has transformed radiology and diagnosis, especially for imaging vascular disorders and tumor tissues.[1]

Low-molecular-weight Fe(III) complexes have recently gained significant attention for MRI applications due to their enhanced relaxivity at higher magnetic fields, low long-term toxicity, and strong thermodynamic stability.[2] Despite these advances, significant challenges remain in both their chemistry and clinical application, making careful molecular design essential to overcome these limitations. Furthermore, for high-spin Fe(III) complexes — unlike their gadolinium-based contrast agent (GBCA) counterparts — it remains challenging to form complexes with rapidly exchanging inner-sphere water molecules, a key factor for achieving higher relaxivity.

We previously reported the synthesis of new cyclopeptoid-based siderophores featuring three catecholamide chelating units, which unexpectedly formed dinuclear rather than mononuclear iron species.[3] In this communication, we propose a series of different trimeric hosts. Specifically, we describe the design and synthesis of novel siderophore mimics based on the 1,4,7-triaminocyclononane (TACN) scaffold.

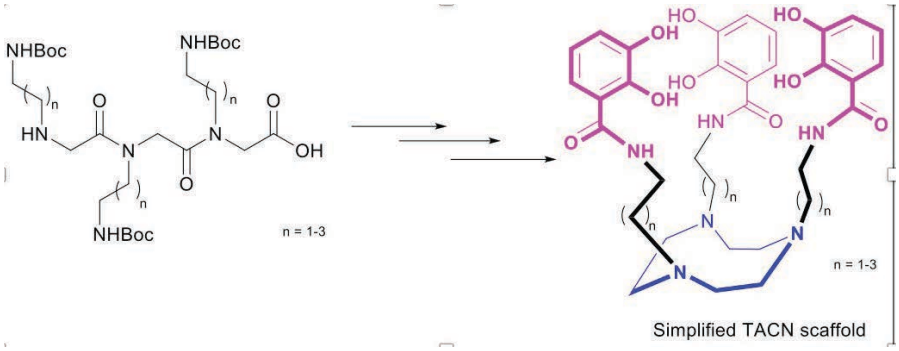

Figure 1. Synthetic scheme for new siderophores as potential MRI agents.

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Peptide-Integrated Polymeric Microneedles for TNF-α Monitoring in Interstitial Fluid

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Peptide-based recognition elements offer a promising alternative to antibodies for biosensing applications, combining high specificity with improved chemical stability, lower production costs and easy surface functionalization. In this work, we explore a synthetically engineered high-affinity P52 peptide (NH_2-K(FITC)-HAIYPRH-$CONH_2$) as a capture probe for the specific detection of the pro-inflammatory cytokine TNF-α in simulated subcutaneous interstitial fluid (ISF). To demonstrate their potential in biosensing platforms, the P52 peptide was covalently attached to polymeric PLGA microneedles (MNs) and integrated in a 3D fluorescence sandwich assay for TNF-α detection. The developed platform enabled sensitive detection of TNF-α with a limit of detection of approximately 0.2 pM (~3 pg/mL). Notably, peptide immobilization on the MN surface significantly enhanced binding affinity, shifting it from the nanomolar to the picomolar range. These findings highlight the potential of peptide-based capture elements as robust and scalable alternatives to antibodies for the development of minimally invasive biosensing devices.[1,2]

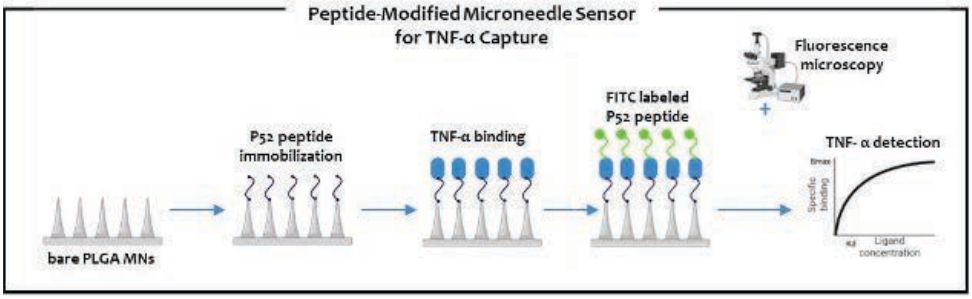

Figure 1. Schematic illustration of the sensor integrating microneedles and peptide for TNF-α detection.

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Macrocyclic Peptide Inducers of PD-L1 Internalization and Degradation Enhance Antitumor Immunity in NSCLC

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Immune checkpoint blockade targeting the PD-1/PD-L1 axis has revolutionized cancer therapy, yet antibody-based agents still present limitations in terms of pharmacokinetics, tissue penetration, and diagnostic applicability.[1,2] In this context, low-molecular-weight modulators and macrocyclic peptides are emerging as promising alternatives to antibodies for the regulation of immune checkpoints.[3,4] Here[5], we report the design and biological characterization of a focused library of anti-PD-L1 macrocyclic peptides. Among them, FM2 and FM213 emerged as potent inhibitors of PD-1/PD-L1 interaction. Using a compound-centric proteomic approach, we demonstrated that FM213 selectively binds PD-L1 on NSCLC cells and tumor-derived exosomes. At non-cytotoxic concentrations (2 µM), FM213 significantly enhances PBMC-mediated killing of NSCLC cells and promotes the internalization of membrane PD-L1, leading to its degradation through a lysosome-dependent pathway.

Importantly, combined inhibition of PD-L1 and TIGIT, achieved using FM213 and the TIGIT inhibitor DTBP-3, resulted in a further increase of antitumor immune response, supporting a cooperative immune-checkpoint modulation strategy. Overall, these results identify macrocyclic peptides as a versatile platform for the development of next-generation PD-L1 modulators capable of inducing target degradation and potentiating antitumor immunity, highlighting their potential as innovative tools for cancer immunotherapy.

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Posters

Novel biomolecules modulating viral RNA capping machinery in CoV infection

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The progression of the COVID-19 pandemic in 2020 highlighted the urgent demand for the development of selective antiviral therapies,[1] alongside vaccination strategies aimed at strengthening immune protection. Current treatments against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, are focused on different stages of the viral replication cycle. Although remarkable advances have been achieved, the identification of innovative therapeutic approaches remains highly desirable. Future research should therefore address viral mechanisms that are still insufficiently explored.[2]

In this framework the multiprotein assemblies formed by the non-structural proteins nsp10/nsp14 and nsp10/nsp16 are involved in the viral RNA capping machinery,[3] a crucial process that allows the viral genome to evade host immune surveillance.[4] Mutations occurring at the nsp10/nsp14 and nsp10/nsp16 interfaces have been reported to impair the protein–protein interactions required for complex formation, ultimately resulting in reduced viral replication.

The primary objective of this project is to expose and unveil the viral RNA to the host recognition through the development of novel peptides and peptidomimetics able to target nsp10 and prevent its association with the partner proteins nsp14 and nsp16. Structural and bioinformatic analyses of the interacting regions and key residues of nsp14 or nsp16 involved in nsp10 binding were performed to guide the design of peptide-based inhibitors of nsp10 function.

The chemical synthesis of the newly designed peptides is currently underway; their interaction with the target protein will be evaluated by NMR spectroscopy and biophysical techniques.

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Copper calling: myxinidin and the ATCUN motif in metal binding

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Metal-binding motifs offer an interesting way to develop peptides with improved biological activity. In addition to their use in developing specific drugs, metal-binding motifs can improve the efficacy of antimicrobial peptides, providing a new weapon against antimicrobial resistance. Amino-terminal copper (ATCUN) binding motifs, involved in the formation of reactive oxygen species (ROS) upon metal binding, are part of the mechanism of action of many naturally occurring antimicrobial peptides (AMPs).[1] The interaction of ATCUN sequences with copper ions produces stable metal-peptide complexes with nuclease and protein cleaving activities, causing lysis through oxidative damage generated during copper redox cycling.[2] Beyond metal transport, this motif shows promise for incorporation into rationally designed peptide therapeutics with increased antimicrobial potency.[3]

Among naturally occurring AMPs, Myxinidin (GIHDILKYGKPS) is a membrane-active peptide isolated from hagfish mucous secretions and previously studied by our research team.[4,5] It contains the ATCUN motif GIH, indicating a dual mechanism of action: membrane disruption and metal-mediated oxidative damage. In this study, we researched the Cu(II) binding ability of Myxinidin using UV/Vis spectrophotometry and Circular Dichroism (CD). To understand structure–activity relationships, we created a family of Myxinidin analogues ranging from the minimal “GIH” motif to the full-length peptide. Their ability to bind Cu(II) and the geometric arrangement of copper were determined by UV/Vis spectroscopy, with CD providing supporting structural data. We also evaluated how physicochemical factors affect metal coordination under different pH conditions relevant to therapeutic environments. The functional consequence of copper binding was assessed using the ascorbate oxidation assay, providing insight into ROS generation and redox cycling efficiency of Cu–ATCUN complexes. These findings highlight the potential of integrating metal-binding motifs into peptide scaffolds to enhance antimicrobial activity and guide the development of next-generation therapeutics.

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Rational Design, Synthesis, and Immunogenicity of HER2-Derived Peptide Vaccines against Breast Cancer

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Breast cancer is one of the most prevalent malignancies worldwide, with 15–20% of cases characterized by HER2 overexpression, a key driver of tumour progression.[1] Although HER2-targeted monoclonal antibodies have achieved clinical success, therapeutic resistance and disease recurrence remain significant challenges, highlighting the need for alternative immunotherapeutic strategies. Peptide-based vaccines represent a promising approach due to their specificity, safety, and synthetic accessibility.[2] In this study, a rational design strategy was used to develop HER2-derived peptide candidates for cancer vaccines. Sequences were selected through literature analysis, structural mapping, and computational antigenicity predictions to enhance immunogenicity and antigen presentation. Three peptides (H2, H5, H6) were synthesized via Fmoc-based solid-phase peptide synthesis (SPPS), purified by RP-HPLC, and characterized by mass spectrometry.

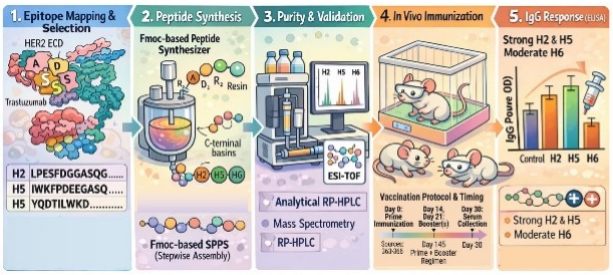

Figure 1. Workflow of the rational design, synthesis, and immunological evaluation of HER2-derived peptide vaccines.

Their immunogenicity was evaluated *in vivo* using a murine protocol. ELISA results showed that all peptides induced specific IgG responses, with H2 and H5 eliciting the strongest responses and H6 a moderate one. Based on these findings, extended peptide versions were designed to improve antigen processing and immune response. Further optimization includes incorporation of non-natural and D-amino acids, as well as cyclization strategies to enhance stability and efficacy. Overall, this work provides a rational framework for HER2-derived peptide vaccine design and highlights structure-guided peptide engineering as a promising strategy for next-generation anticancer immunotherapy.

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Preparation and Testing of Drug-loaded Lipid Nanoparticles decorated with anti-lysyl oxidase antibodies

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Antibody-Drug Conjugates (ADCs) merge the high potency of cytotoxic payloads with the exquisite precision of monoclonal antibodies. Representing a new frontier in oncology, dozens of these biotherapeutics have reached clinical use in recent years. Nanotechnology is now playing a pivotal role in the evolution of ADCs, offering innovative strategies for the loading and delivery of therapeutics to tumors. By immobilizing targeting agents on the nanoparticle surface, these systems preferentially accumulate within malignant tissues.[1] Following this approach, researchers previously described liposomes (LIPO) randomly functionalized with anti-lysyl-oxidase (LOX) antibodies and loaded with epirubicin (EPI).[2] LOX catalyzes the cross-linking of collagen and elastin to maintain extracellular matrix (ECM) stability and its overexpression alters the tumor microenvironment (TME), leading to ECM remodeling, tumorigenesis, and metastasis in various solid tumors. The initial formulation, LIPO-EPI-LOX, was shown to inhibit triple-negative breast cancer (TNBC) cell proliferation both *in vitro* and *in vivo*, significantly improving survival rates in orthotopic TNBC mouse models.[2]

We have now optimized the conditions to produce an advanced version of LIPO-EPI-LOX. This iteration features the anti-LOX antibody anchored in an oriented manner, resulting in more homogeneous preparations and potentially superior target recognition. Immobilization was achieved via hinge cysteines liberated under controlled conditions, with the anchoring process monitored in real-time through fluorescence. The advanced preparations were evaluated using SW1353 chondrosarcoma cells and HUVEC endothelial cells to assess their impact on proliferation and invasion. On HUVECs, the antiangiogenic potential was also investigated both *in vitro* and *in vivo*. The advanced LIPO-EPI-LOX preparation resulted highly effective in blocking cell invasion, while it did not exhibit cytotoxic effects on cell proliferation. It also very efficiently suppresses angiogenesis both *in vitro* and *in vivo*. These results support its continued investigation and further development in additional preclinical models.

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Generation and Characterization of Macrocyclic Peptide Ligands for Therapeutically Relevant Protein Targets

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Macrocyclic peptides combine the high affinity and specificity of proteins with the favourable stability and synthetic accessibility typical of small molecules. This unique combination makes them a versatile class of bioactive compounds for applications in therapeutics and chemical biology.

In this presentation, I will describe a series of macrocyclic peptide ligands developed in our research group that target proteins of biomedical relevance. Particular emphasis will be placed on the design and synthetic strategies used to access structurally diverse macrocyclic scaffolds and to explore structure–activity relationships.

We characterized the interactions between these peptides and their targets using complementary biophysical techniques to evaluate binding affinity and selectivity. Furthermore, I will discuss structural insights obtained through X-ray crystallography of selected peptide–protein complexes, highlighting key features, such as conformational preorganization, shape complementarity, and specific intermolecular interactions, that underpin molecular recognition.

Overall, these results underscore the potential of macrocyclic peptides as adaptable bioactive molecules capable of modulating challenging protein interfaces.

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The proline-rich antimicrobial peptide Lip1 as scaffold for the development of novel antimycobacterial agents: a Structure–Activity Relationship Study

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Tuberculosis remains a significant and escalating global health concern, largely driven by the spread of mycobacterial strains that exhibit resistance to standard therapeutic regimens.[1] The development of new antimicrobial agents is inherently challenging, and the evaluation of novel compounds against *Mycobacterium tuberculosis* is further complicated by biosafety constraints associated with laboratory handling, as well as by the organism's slow replication rate. In this framework, *Mycobacterium smegmatis* has emerged as a widely adopted model system, offering advantages such as enhanced biosafety, faster growth kinetics, and substantial genomic similarity to *M. tuberculosis*.[2]

Proline-rich antimicrobial peptides (PrAMPs) have attracted considerable interest as potential anti-infective agents, owing to their low toxicity toward host cells and their versatile mechanisms of action, which can vary from membrane-disruptive to non-lytic effects depending on the bacterial target.[3,4]

In this study, we describe the synthesis and purification of multiple fragments derived from the cetacean-derived peptide Lip1, followed by the assessment of their secondary structure and of their antimicrobial activity against *M. smegmatis*.

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Peptide-Like Benzenesulfonamides as Selective Inhibitors of human Carbonic Anhydrase IX and XII

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Carbonic anhydrases (CAs) are ubiquitous enzymes that catalyse the reversible hydration of carbon dioxide into bicarbonate and protons. In humans (h), fifteen CA isoforms have been identified, differing in their subcellular localization and tissue distribution.[1] These isoforms perform distinct physiological functions in different tissues, and their absence or malfunction can cause several pathological conditions.[1]

Isoforms IX and XII represent two of the most interesting members of the CA family, as they are overexpressed in several tumours while being largely absent in most normal tissues.[2] For these reasons, in recent years there has been a growing interest in hCA IX and hCA XII as promising targets for the development of molecules capable of modulating their catalytic activity, thereby opening new opportunities for the development of cancer therapies.

However, most of the pharmacological agents developed to date still require further investigation, as they often exhibit undesirable side effects due to limited isoform selectivity. Within a bilateral cooperation between Italy and Georgia,[3] we have designed and synthesized peptide-like benzenesulfonamide inhibitors bearing, at para position of the benzene ring, a variety of functional groups. These include lipophilic or hydrophilic moieties, as well as flexible or rigid tails, aimed at establishing favourable interactions with specific residues in both hydrophilic and hydrophobic halves of the CA active site, thereby enhancing the selectivity towards hCA IX and hCA XII.

Furthermore, inhibition studies combined with structural analyses by means of X-ray crystallography will provide a robust foundation for the rational design of new drugs targeting these enzymes.

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Cathepsin-B activable prodrugs of histone deacetylase inhibitors

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Histone deacetylases (HDACs) regulate gene expression by removing acetyl groups from histones, promoting chromatin compaction and silencing tumor-suppressor genes. HDACs are often overexpressed in cancer, making HDAC inhibitors (HDACi) such as vorinostat effective therapeutic agents.[1] However, most HDACi contain a hydroxamic acid group that contributes to systemic toxicity and poor pharmacokinetics.[2] Cathepsin B (CTSB), a cysteine protease overexpressed in tumors and upregulated after HDAC inhibition, offers an opportunity for tumor-specific drug activation.

This project aims to design a CTSB-activated prodrug to selectively release HDAC inhibitors in tumor tissue, reducing toxicity and improving stability and specificity.[3]

The prodrug masks the hydroxamic acid using a dual self-immolative linker with a CTSB-cleavable sequence. Upon cleavage, a CO_2-driven rearrangement triggers drug release. Analogues were synthesized by modifying residues adjacent to proline. SPPS was accelerated using ultrasound-assisted and SUS-SPPS protocols. *In vitro* assays included CTSB cleavage, stability, cytotoxicity, and Western blot analysis. Selected candidates were evaluated in PDX models.

Prodrugs showed high stability and rapid CTSB-mediated cleavage, achieving up to 100% conversion to vorinostat within 2 hours. Bulky side chains improved stability while maintaining enzymatic responsiveness.

This CTSB-activated prodrug platform enhances tumor specificity and reduces HDACi toxicity, supporting its potential as a next-generation targeted cancer therapy.[4]

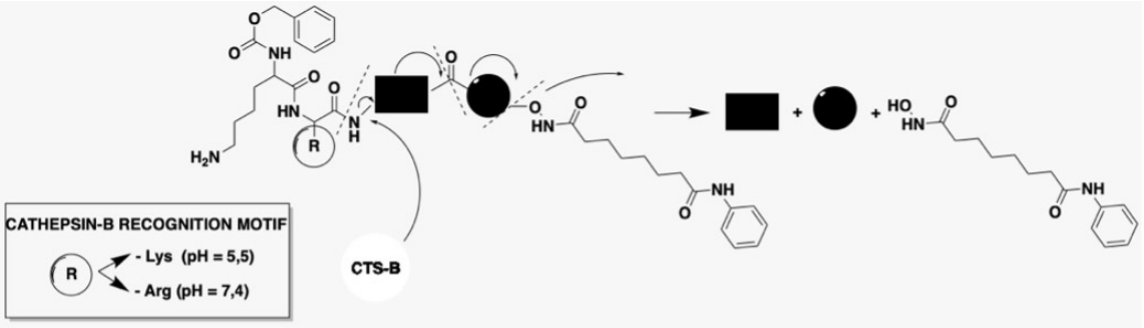

Figure 1. General mechanism of CTSB-activated HDACi prodrug.

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Structure–Activity Relationships of the proline-rich antimicrobial peptide Bac7(1–35) for the Development of Novel Antimycobacterial Agents

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Tuberculosis remains a major challenge and a growing global public health emergency, due to the emergence of mycobacterial strains resistant to the therapeutic drugs currently in use.[1] In addition to the difficulty of discovering new antimicrobial agents, the testing of novel compounds against *Mycobacterium tuberculosis* is hampered by safety concerns related to laboratory facilities and by the slow growth rate of the pathogen. In this context, the use of non-pathogenic *Mycobacterium smegmatis* as a model organism has become well established, combining improved biosafety, rapid growth, and high genomic homology with *M. tuberculosis*.[2]

Proline-rich antimicrobial peptides (PrAMPs) are promising candidates for the development of new anti-infective agents, mainly due to their low host toxicity and their multifaceted mechanism of action, which may range from membranolytic to non-lytic, depending on the targeted bacterial species.[3,4] Here, we report the preparation of several fragments of Bac7(1–35), the active domain of Bac7, followed by the evaluation of their antibacterial activity against *M. smegmatis*. In parallel, preliminary studies were conducted to investigate the mechanism of action of the most promising candidates, aiming to determine whether their primary targets are the bacterial membrane or intracellular components.

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Exploring the Synergistic AMP-combination Temporin A and the proline-rich B7-005 Against *Pseudomonas aeruginosa*

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The growing spread of antimicrobial resistance in respiratory pathogens such as *Pseudomonas aeruginosa* underscores the need for alternative therapeutic strategies. Antimicrobial peptides (AMPs) are attractive candidates because of their varied modes of action and their capacity for synergistic interactions.[1] In this work, we assessed the efficacy of a peptide combination involving the membrane-disrupting peptide Temporin A (TA) and the proline-rich peptide B7-005[2] in the context of pulmonary infections.

Synergy between TA and B7-005 was confirmed by checkerboard assays across multiple *P. aeruginosa* strains (FICI ≤ 0.5); cytotoxicity testing on bronchial epithelial cells highlighted a favourable selectivity profile for B7-005, whereas the combined treatment produced moderate effects on cellular metabolism. Repeated exposure experiments showed stable MIC values and no evidence of cross-tolerance, indicating a limited tendency for resistance development.

Importantly, the peptide pair preserved strong antibacterial activity in artificial sputum medium and markedly reduced the viability of established biofilms, supporting its effectiveness under conditions that mimic the lung environment. In addition to its antimicrobial action, TA promoted wound closure in bronchial epithelial cells, and this regenerative effect was retained in the presence of B7-005. Preliminary *in vivo* analyses and advanced airway epithelial co-culture systems further demonstrated a decrease in bacterial load following combined treatment.

Taken together, these findings point to the TA/B7-005 combination as a promising approach for managing difficult-to-treat pulmonary infections caused by *P. aeruginosa*.

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Peptide-based dual receptor targeting strategies for cancer diagnosis and therapy

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Cancer remains one of the leading causes of mortality worldwide, making of critical importance the early diagnosis for therapeutic efficacy and patient survival. Nevertheless, conventional diagnostic approaches frequently lack the sensitivity and specificity required to reliably detect malignancies at early stages. In this context, the accurate identification of tumour-associated receptors is essential for early cancer detection. Among emerging targeting strategies, receptor-specific peptides have attracted considerable attention due to their high binding specificity, structural stability, and facile chemical functionalization. These peptides can selectively recognize and bind receptors that are overexpressed on the surface of cancer cells, enabling sensitive and precise detection, characterization, and quantification of malignant cell populations.

The peptides presented in this study were designed to exhibit dual-targeting properties toward two cancer-associated receptors, integrin αvβ3 and neuropilin-1 (NRP-1). These constructs incorporate the RGD (arginine–glycine–aspartate) motif, a sequence commonly found in extracellular matrix (ECM) proteins that displays high affinity for integrin receptors, together with a CendR motif responsible for specific binding to NRP-1.

Through single-molecule and confocal microscopy, and lipid model membranes, we evaluated the receptor-binding efficiency and ability to penetrate lipid bilayers of these peptide structures. Incorporation of these molecules into detection platforms offers a promising, non-invasive, and reliable approach for the early diagnosis and therapy of cancer.

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Bicyclic peptide mimicry of the cripto-1 EGF-Like domain

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Human Cripto-1 protein is a member of the EGF-CFC growth factor-like superfamily. While its physiological role is the regulation of embryogenesis, Cripto-1 often assumes an oncogenic role.[1,2] Structurally, Cripto-1 consists of two well folded domains, the CFC domain and the EGF-Like domain, which appear to modulate distinct transduction.[3] The most characterized molecular complex involving Cripto-1 is the one who regulates the SMAD-2/3 signalling pathway in the TGFβ-dependent signalling. In this context, Cripto-1 serves as a coreceptor for Nodal facilitating its binding to ALK4 receptor. Recently, the Nodal-Cripto-ALK4 complex has been extensively investigated to determine whether Cripto-1 presents a functional division between its two domains. In particular, these studies indicate that Cripto-1 acts as a "bridge": while the EGF-Like domain binds to Nodal, the CFC domain binds to ALK4 receptor, facilitating Nodal dependent signalling activation.[4]

Aiming to analyse this highly specific compartmentalization of the Cripto-1 domains, we propose an integrated computational and experimental approach, based on the design of a synthetic bicyclic peptide mimicking the structure of the EGF-Like domain. Our finding not only highlight the specificity of Cripto-1 domain in partner's recognition and binding affinity but furthermore, reveal important implications for development of selective Cripto-1 modulators.

Figure 1. A Bicyclic peptide to inhibit TGFβ-dependent signaling.

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Peptide mapping of Zika virus EDIII reveals an immunodominant epitope for monoclonal antibody development

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Zika virus (ZIKV) infection can lead to severe neurological complications and congenital abnormalities, including microcephaly, following maternal infection during pregnancy. The absence of approved vaccines or specific antiviral therapies highlights the need for alternative strategies, such as monoclonal antibody-based approaches, particularly for vulnerable populations. Here, we investigated the immunogenicity of the envelope protein domain III (EDIII) of ZIKV already identified as a target for generating new neutralizing antibodies.[1]

For this aim, recombinant EDIII (Uniprot A0A0X8GJ44) was produced in *Escherichia coli* and used for mice immunization. To identify the potential protein epitopes, the protein was proteolytically digested with trypsin to generate smaller fragments which were next isolated by reversed-phase chromatography into various fractions and tested by iterative ELISA assay to detect those most immunoreactive with polyclonal antibodies raised against the protein following mouse immunization.[2] Sequences contained in the positive fractions were identified by mass spectrometry, chemically synthesized as individual peptides and retested. The region of EDIII spanning residues 76 to 96 displayed the strongest antigenic reactivity, suggesting this to be the main immunodominant portion of the protein.

The data obtained suggest that the C-terminal region of EDIII protein contains promising epitopes for the development of potential new monoclonal antibodies for diagnostic and therapeutic applications and with potential neutralising activity.[3]

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Fermentation improves bioactive peptides, polyphenol bioaccessibility and anti-inflammatory activity of chickpea flours

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Introduction. Legumes are an excellent source of beneficial compounds, and fermentation represents an effective way to enhance the concentration of bioactive peptides (BAPs) in the resulting product. This study aimed to develop innovative postbiotics from fermented chickpea-based flours with improved functional properties compared to the unfermented counterparts. Fourteen lactic acid bacteria strains were used to ferment chickpea purees and both the fermented (FP) and control flours (CP) were analyzed for their polyphenol content, BAPs, and total antioxidant capacity (TAC). The most promising FP and another FP obtained with *Lactobacillus plantarum 95* (C95) were produced at a larger scale and subjected to simulated gastrointestinal digestion to assess polyphenol and peptide bioaccessibility, TAC, and potential anti-inflammatory activity on lipopolysaccharide (LPS)-activated Caco-2 human colon cells. In addition, molecular modelling investigations were conducted on TLR4 receptor, to clarify the binding mode of the bioactive peptides from the fermented products.

Results. Fermentation significantly increased both soluble and direct TAC. *Leuconostoc mesenteroides* OM94 (C19) and *Lactiplantibacillus plantarum 299v* CN2 (C95) were the most effective strains. Interestingly, postbiotics treatment significantly reduced, in LPS-activated Caco-2 cells, both the activation of inflammatory pathway, namely TLR4 expression and NF-κB phosphorylation, as well as oxidative stress, with C95 FP being the most effective postbiotic. Subsequently, the identified BAPs were subjected to molecular docking calculations on the LPS binding site of TLR4 X-Ray structure and the predicted binding modes of the two best-ranked BAPs were further validated through 200 ns long molecular dynamics simulations (MDs), revealing a great stability of their predicted binding conformation with a detailed picture of the intermolecular interaction patterns.

Conclusions. Fermentation enhanced polyphenol bioaccessibility and bioactive peptide release in chickpea flours, leading to increased antioxidant and anti-inflammatory activity through modulation of the TLR4/NF-κB pathway, supporting their potential as sustainable functional ingredients.

Human Histone Fragments Display Antibacterial Properties against *Pseudomonas aeruginosa*

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Antimicrobial resistance (AMR) represents a major global health threat, responsible for approximately 1.27 million deaths in 2019 and projected to reach up to 10 million deaths annually by 2050.[1] In this context, antimicrobial peptides (AMPs) are emerging as promising alternatives to conventional antibiotics due to their broad-spectrum of activity and diverse mechanisms of action. Beyond classical AMP families, histones have recently been recognized as endogenous antimicrobial effectors with functions extending beyond chromatin organization. Here, we report the identification and characterization of novel antimicrobial peptides derived from human histone H1 variants.[2] Using a human hemofiltrate peptide library combined with *in silico* prediction, thirteen candidate peptides were identified and synthesized.

Functional screening against ESKAPE pathogens revealed that 12 out of 13 peptides exhibited strong and selective antibacterial activity against *Pseudomonas aeruginosa*, with no detectable effects on other tested species. Among these, a H1.2-derived peptide showed the highest potency and consistent activity across multiple clinical isolates, with maximal efficacy under acidic conditions. Kinetic analyses demonstrated rapid bactericidal activity, without membrane disruption or direct DNA binding, suggesting a non-lytic intracellular mode of action. Differential proteomic analysis revealed a significant downregulation of proteins involved in protein translocation and outer membrane biogenesis, including key components of the Sec pathway and β-barrel assembly machinery.[3] PCR investigation gave negative results suggesting a post-transcriptional regulation. Functional proteomics approach using a biotinylated peptide was used to investigate the H1.2 mechanism of action, leading to the identification of a network of peptide interactors involved in translation and RNA metabolism. Notably, components of the RNA degradosome[4] and RNA chaperone systems were enriched, supporting a role in RNA processing and stability. Collectively, these findings identify histone H1-derived peptides as selective antimicrobial effectors with a novel intracellular mechanism targeting RNA metabolism and protein biogenesis, highlighting their potential for the development of innovative therapies against multidrug-resistant pathogens.

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Insight into the molecular recognition of ZO1-PDZ2 by viral Envelope derived peptides

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The Envelope (E) protein, one of the structural proteins encoded by Coronavirus (CoV) genome, is involved in multiple processes of the virus life cycle and it directly related to the pathogenesis process.[1] The C-terminus of E contains an important virulence factor, a PDZ domain binding motif (PBM), whose deletion or modification is sufficient to attenuate the virus.[2] Notably, the PBM of E interacts with the second PDZ domain (PDZ2) of host Zonula Occludens-1 protein (ZO1), leading to delayed formation of tight junctions (TJs) and disruption of the respiratory epithelium.[3,4]

Although the binding reaction between ZO1-PDZ2 and E has been extensively studied since COVID-19 pandemic breakout, the structural determinants governing the binding process of ZO1-PDZ2 by E protein are still poorly understood. In this scenario, we performed, through a combination of experimental NMR and computational methods, a comparative study to structurally characterize the interaction occurring between ZO1-PDZ2 and three peptides mimicking the C-terminal PBM of E protein from SARS-CoV (now SARS-CoV-1), SARS-CoV-2 and MERS-CoV. Our results could provide the basis for the development of therapeutic strategies that may reduce cell damage and the morbidity and mortality associated with coronavirus infection.

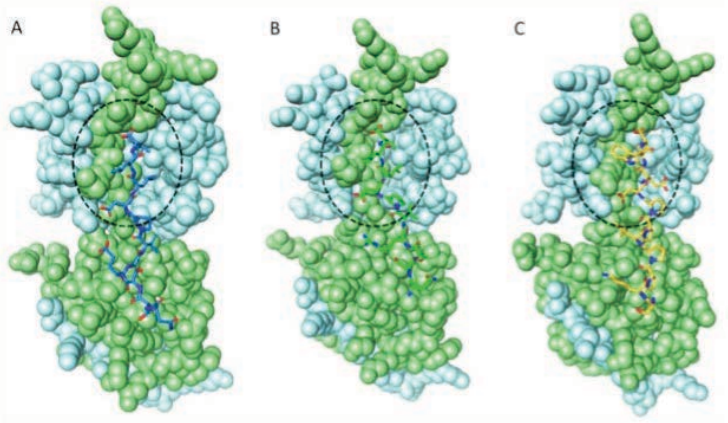

Figure 1. Structural models of A) SARS-CoV-1_E:ZO1-PDZ2, B) SARS-CoV-2_E:ZO1-PDZ2 and C) MERS-CoV_E:ZO1-PDZ2 complexes. ZO1-PDZ2 dimer is shown as a cartoon while the three E peptides are represented as sticks. The dotted circles indicate the α2-β2 groove onto the protein surface.

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Exploiting conformational plasticity to target the CypA surface

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Peptides that mimic protein–protein interaction surfaces have emerged as powerful scaffolds for molecular recognition. Traditional design approaches usually focus on rigidifying a sequence into its native fold, with the implicit assumption that fidelity to the original structure guarantees optimal binding. However, the functional outcome often depends more on how the peptide's shape complements the target surface than on strict structural mimicry.[1,2]

Here, we explore the interaction of a cyclic peptide that mimics the interaction region of a protein that binds to cyclophilin A (CypA) on its relatively flat surface, which poses challenges for conventional ligand design.[3–5] Rather than locking the peptide in its native fold, we sought to modulate its conformational landscape to enhance adaptability to the CypA surface. This strategy led to the identification of a variant that adopts an alternative secondary structure, distinct from the original motif, which significantly improves surface engagement and binding affinity.

Our biochemical and structural results indicate that prioritizing conformational plasticity and surface complementarity over faithful preservation of the native fold can unlock novel, high-affinity binding modes at flat or shallow interfaces. This work provides a conceptual framework for designing dynamic peptide ligands that target challenging protein surfaces, including those historically considered "undruggable".

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Shielding siRNA by peptide-based nanofibers: An efficient approach for turning off EGFR gene in breast cancer

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Gene therapy has emerged as an innovative approach in cancer treatment, offering the potential to selectively modulate gene expression. This approach is crucial for the treatment of Triple-Negative Breast Cancer (TNBC), an aggressive form of breast cancer with no effective targeted therapies. TNBC is characterized by the overexpression of the EGFR (Epidermal Growth Factor Receptor) that drives tumour growth. Therefore, RNA interference (RNAi), using small interfering RNA (siRNA), represents a promising strategy to induce selective gene silencing. However, siRNA therapy faces significant challenges, including serum nuclease degradation, poor cellular uptake, and inefficient endosomal escape.[1]

To address these limitations, we designed a biodegradable and modular peptide-based nanofiber (NF) delivery system engineered for targeted siRNA delivery in TNBC cells. The platform is based on peptide amphiphiles (PAs) that self-assemble into elongated nanofibers through non-covalent interactions, such as van der Waals forces, hydrogen bonding, and π-stacking. The modular design integrates three functional domains: the membrane-penetrating peptide gH625 to enhance endosomal escape, the EGFR-targeting peptide P22 for selective delivery, and cationic arginine-rich sequences for electrostatic siRNA complexation.

Physicochemical characterization confirmed the formation of stable nanofibers with favourable morphology and efficient siRNA loading. *In vitro* evaluation on MDA-MB-231 TNBC cells demonstrated high cytocompatibility and efficient cellular uptake of siRNA:NF complexes. Functional assays showed significant downregulation of EGFR expression at both mRNA and protein levels, achieving efficacy comparable to Lipofectamine-siRNA complexes but with superior safety and minimal inflammatory responses. In conclusion, the peptide nanofiber system provides a safe and effective alternative to lipid-based transfection agents for siRNA delivery, with advantages in terms of biodegradability, modularity, and reduced immunogenicity.[2]

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Ferrocene-peptide conjugates with antibacterial properties

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Over the last five years, the research group has been producing various ferrocene (Fc)-peptide hybrid molecules to investigate how the peptide structure influences electron transfer processes, since Fc works as a convenient electrochemical probe.[1] Moreover, Fc can trigger the production of reactive oxygen species (ROS) under biological conditions, which can be toxic to cells. Therefore, these compounds are potentially useful as anticancer agents.[2]

In this study, we applied the same strategy to fight bacterial infections. We chose a group of very short antimicrobial peptides, characterized by the presence of a non-natural histidine analogue, which we recently published.[3] We covalently linked an Fc unit to them, using linkers of different lengths, to test whether the Fc could increase their antibacterial efficacy.

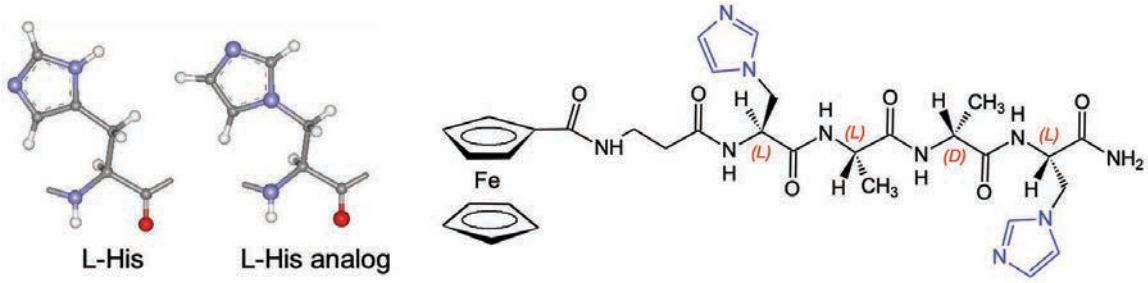

Figure 1. Molecular structures of His and its analog His* (left) and of Fc-βAla-His*-Ala-D-Ala-His*-NH_2.

The conformation of the new compounds was investigated through 2D NMR and FT-IR techniques. Stability tests in human serum showed that both Fc and the histidine analogue protect the peptide from enzymatic breakdown. Testing against both Gram-positive and Gram-negative bacteria is ongoing.

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Aggregation and neurotoxicity: synergistic effects of NPs and NTD-HuPrP

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Many neurodegenerative diseases are correlated with the aggregation and deposition of misfolded proteins into amyloid fibrils, which accumulate in the brain of the affected patients, leading to progressive cognitive and physical decline.[1] Among these, prion diseases are caused by the pathologic conformational conversion of native cellular prion protein (PrP^{C}) into misfolded oligomeric or fibrillary forms (PrP^{Sc}) which accumulates in the brain.[2] Recent researches indicate that, rather than the mature protein fibrils, oligomers are the most toxic species responsible of neuronal damage.[3] Currently, it is not clear what causes this phenomenon and no effective therapies are available to treat these pathologies.

In this scenario, we focused our attention on the intrinsically disordered N-terminal domain (23–89) (NTD) of the human prion protein (HuPrP), which is released in the extracellular space from the full-length protein after a proteolytic cleavage and, unlike the C-terminal domain (CTD) of HuPrP(90–231), it does not show any aggregation propensity under physiologic conditions.[4] In particular, we have investigated the effect of anionic polystyrene Nanoplastics (NPs) on NTD aggregation characterizing the structural features of the formed oligomers (NTD(o)) and evaluating their cell toxicity through a multidisciplinary approach. In recent years, NPs have attracted increasing interest as aggregation modulators and were found to either accelerate or inhibit protein fibrillation, depending on NPs properties, solution conditions, and protein aggregation propensities.[5]

We have demonstrated, by Dynamic Light Scattering (DLS) measurements, Nuclear Magnetic Resonance methodologies and fluorescence spectroscopy studies, that the NTD is absorbed on NPs surface and that this interaction induces the formation of NTD(o), whose formation is driven by an interplay between hydrophobic and hydrophilic interactions. Moreover, we have performed biological assays to evaluate the cytotoxicity *in vitro* of NTD(o) on neuronal cells. We have also developed a synthetic strategy to site-specifically label the NTD with a fluorescent probe to explore the cellular localization of NTD in the presence of NPs by fluorescence microscopy. Our findings highlight the significant impact of NPs on NTD amylogenic proprieties and suggest a potential synergy with prion diseases insurgence.

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Development of novel metal-RGDechi bioconjugates for integrin targeted cancer diagnosis and therapy

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$\alpha_v\beta_3$ is a member of the integrin family and plays a prominent role in many pathological conditions. This integrin exhibits a relatively limited distribution in normal tissues, while being highly expressed in many solid tumours. RGDechi is a bifunctional chimeric peptide that selectively interacts with $\alpha_v\beta_3$ integrin and displays antiangiogenic activity, antiadhesive, antiproliferative, and pro-apoptotic effects on human malignant melanoma cells.[1–4] Therefore, RGDechi represents a promising candidate both as a non-invasive diagnostic tracer for tumor imaging and as a drug delivery system for therapeutic applications.

Technetium-99m is the most widely used radionuclide in Nuclear Medicine for SPECT imaging. To prepare the [^{99m}Tc]-labeled RGDechi derivative peptides, a cysteine residue was selected as a bifunctional chelating agent and conjugated to the side chain of Lys1 to enable coordination with the [^{99m}Tc][Tc(N)(PNPn)]-synthon (PNP= bis-phosphino-amine). Cellular uptake and internalization studies of the resulting radiolabeled peptides were then performed.[5]

In cancer therapy, Au-based compounds have emerged as a promising alternative to Pt-based drugs as they exhibit lower toxicity and selective activity against specific cancers. In this context, new drug delivery systems that combine the targeting capability of RGDechi peptide with the therapeutic efficacy of gold-based drugs were developed for the treatment of highly aggressive integrin-expressing tumours.[6] Several Au-based compounds were synthesized, and novel selective gold-peptide bioconjugates were designed, synthesized and characterized to evaluate their selectivity for $\alpha_v\beta_3$-overexpressing tumors. The *in vitro* activity of the Au-RGDechi bioconjugates is being assessed in selected tumor cell lines.

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Discovery of G-quadruplex-binding peptides via structure-based and sequence alignment approaches

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Noncanonical nucleic acid structures, particularly G-quadruplexes (G4s), have emerged as attractive targets in anticancer therapy due to their structural uniqueness and involvement in key regulatory processes.[1] Their formation is modulated by G4-binding proteins (G4BPs), whose dysregulation has been associated with several diseases.[2] Despite the increasing number of G4-targeting ligands, achieving high selectivity and specificity remains challenging. In this context, peptides represent a promising class of ligands, offering structural adaptability and specificity.

Here, we describe two complementary strategies for the discovery of G4-binding peptides. The first is a structure-based approach involving a G4-binding domain of the Rap1 protein, whose crystallographic complex with G4 DNA enabled the design of a selective peptide ligand.[3] Subsequent alanine scanning and conjugation with cell-penetrating peptides (CPPs) led to derivatives exhibiting potent G4-mediated cytotoxicity across multiple cancer cell lines. In parallel, to address the limited structural data available for most G4-protein complexes, we adopted a strategy based on the sequence alignment of known G4BPs. This approach identified a 20-mer RGG-rich peptide motif, which we selected for experimental validation.[4]

Biophysical characterization of the resulting peptide ligands by circular dichroism (CD), microscale thermophoresis (MST), isothermal titration calorimetry (ITC), and nuclear magnetic resonance (NMR) confirmed their ability to bind multiple G4 topologies with high affinity. Alanine scanning further highlighted key residues involved in G4 recognition, providing a rational basis for ligand optimization. Collectively, these findings demonstrate the effectiveness of integrating structural and sequence-based approaches for the rational design of G4-binding peptides, paving the way for the development of next-generation G4-targeting therapeutics in oncology and beyond.

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Evolutive-based optimization of membrane-selective peptides

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Antimicrobial peptides (AMPs) are a viable alternative to conventional antibiotics due to their broad-spectrum activity and reduced resistance propensity.[1] One of the primary targets of AMPs is the microbial cell membrane, which AMPs usually disrupt by forming pores on it.[2] However, the clinical application of membrane-binding AMPs is often limited by poor selectivity, leading to cytotoxic effects on human cells.

To address this limitation, as well as identify more selective peptides, we implemented an evolutionary algorithm based on the calculation of binding free energy differences (ΔΔG) obtained from coarse-grained (CG) molecular dynamics simulations. An *E. coli* membrane model (POPG:POPE, 1:3) and a POPC membrane (simplified human-like model) were used to screen for peptides with preferential binding to the *E. coli* membrane and reduced interaction with POPC. After 25 iterations, the optimized peptides were further validated by all-atom (AA) simulations using the CHARMM36m force field, revealing a good correlation between the CG and AA models. The best-performing peptides are currently under experimental evaluation.

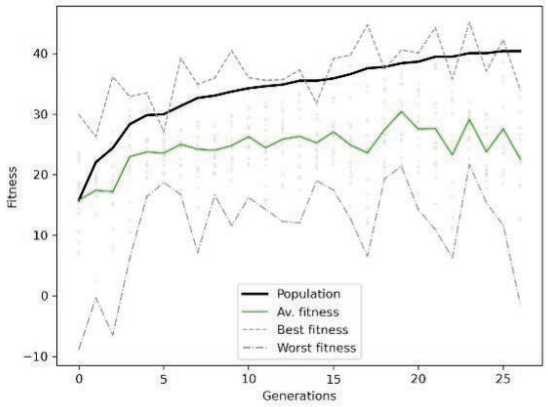

Fig 1. Evolutionary optimization. Peptide fitness was defined as the binding free energy difference (ΔΔG) between *E. coli* membrane and POPC membrane, where higher selectivity for the bacterial membrane corresponds to improved fitness.

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Synthesis of bioactive peptides with non-proteinogenic amino acids via solid-phase and activated ester approaches

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In this work, we report two complementary synthetic strategies for the preparation of bioactive peptides containing non-proteinogenic amino acids. Solid-phase peptide synthesis (SPPS) was employed for the efficient assembly of longer peptide sequences, whereas short di- and tripeptides were synthesized in solution via an activated ester approach. Using these methodologies, more than 60 peptides were successfully synthesized and characterized.

The obtained compounds exhibited notable antimicrobial and enzyme inhibitory activities. Selected derivatives demonstrated significant collagenase inhibition, with 9-fluorenylmethoxycarbonyl-(S)-β-[4-allyl-3-(pyridin-4-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine showing an IC_{50} value of 1.45 µM. In addition, antifungal activity against *Aspergillus versicolor* was observed for 9-fluorenylmethoxycarbonyl-(S)-β-[4-allyl-3-(2-methoxyphenyl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanylglycine (IC_{50} = 169.94 µM). Furthermore, Fmoc-protected peptides Fmoc-KLF(F)HK and KLF(Br)HK exhibited antibacterial activity against *Salmonella typhimurium* G-38 (MIC = 5 mg/mL), while Fmoc-KLF(F)HK also showed activity against *Escherichia coli* (MIC = 3 mg/mL). Additionally, (S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)-3-(5-methyl-2-thioxo-1,3,4-thiadiazol-3(2H)-yl)propanoic acid demonstrated strong antibacterial activity against *Salmonella typhimurium* G-38 (MIC = 1 µg/mL). A schematic representation of the synthetic approaches is provided in Figure 1.

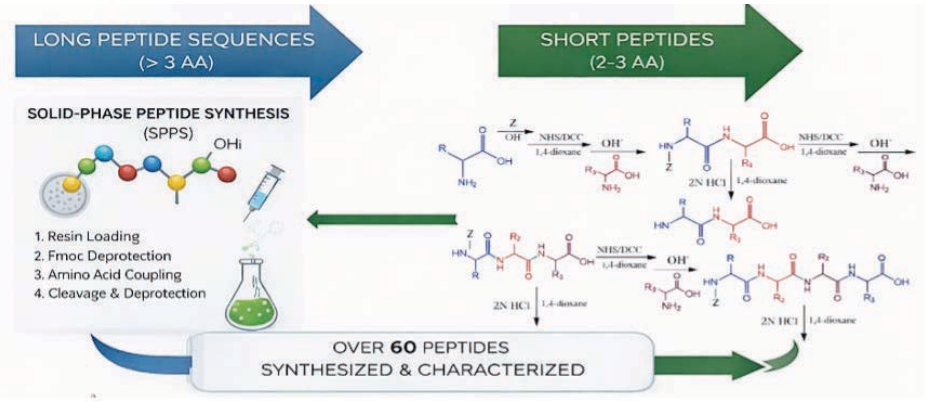

Figure 1. Synthetic routes to peptides.

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Synthesis and structural investigation of the GRP78 domain involved in Cripto-1 signalling

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The GRP78 protein, a member of the Heat Shock Protein family HSP70, is an endoplasmic reticulum chaperone that plays a central role in protein folding and cellular stress responses. Under stress conditions, including cancer, GRP78 translocates to the cell surface, where it interacts with Cripto-1 forming a complex that further favours growth and survival pathways by inhibiting tumour-suppressive TGF-β signaling and activating MAPK and PI3K/Akt pathways.[1–5] Because of these properties, the complex represents an excellent target for precision medicine and for tumour-specific therapeutic agents whose development would strongly benefit from structural details underlying the interaction regions.

In silico studies have shown that the GRP78 region involved in the interaction with Cripto-1 is the N-terminal portion spanning residues 19–68.[6] Structural data indicate that this region is partly unorganized but features a canonical antiparallel β-sheet–loop–β-sheet motif across residues 31–51.[7] With a view to developing inhibitors, we aim to verify whether the isolated region retains the conformation adopted in the full-length protein, investigate whether the domain is structurally stabilized in the presence of the interactor and identify the minimal regions of interaction with Cripto-1 which, as isolated synthetic molecules, can potentially act as soluble antagonists of the interaction. We have therefore prepared by chemical synthesis the 19–68 domain of GRP78 and other shorter variants that can possibly interact with Cripto-1, have performed circular dichroism studies to assess their structure in solution and tested their interaction with Cripto-1. The results confirm that the N-terminal region of GRP78 recognizes the target protein and lays the groundwork for the development of inhibitors interfering with Cripto-1 signaling pathways associated with cancer cell survival and of specific carriers for the selective delivery of therapeutic agents.

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The effect of pathogenic yeast stimulation on the composition and biological activity of *Lucilia sericata* larval secretions

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One of the biggest challenges in managing hard-to-heal wounds is multispecies biofilms containing both bacteria and fungi. Pathogenic yeasts frequently colonize the wound bed, particularly in patients undergoing intensive antibiotic therapy or those with diabetes. *Candida albicans* is among the most prevalent yeast species found within wound biofilms.[1] Fungal presence significantly complicates wound management and can diminish the efficacy of conventional therapies. Maggot debridement therapy is a highly successful treatment modality for hard-to-heal wounds. This approach utilizes *Lucilia sericata* (green bottle fly) larvae to debride the wound bed and stimulate healthy cell proliferation.

The study aimed to determine the composition and biological activity of *L. sericata* secretions following environmental exposure to *C. albicans*. Using size-exclusion chromatography, we isolated peptide fractions with a molecular weight below 3 kDa. These fractions were evaluated for antimicrobial activity against reference strains using agarose radial diffusion assay and microtiter cell viability assay. Furthermore, sample capacity to promote healthy endothelial cell line proliferation was tested using an MTS assay.

Results demonstrate that the secretion from *L. sericata* larvae exposed to inactivated *C. albicans* exhibits dual biological activity. Specific fractions were active against pathogenic reference strains while simultaneously enhancing cell growth. This investigation reveals the broad antimicrobial potential of induced larval secretions against diverse microorganisms, alongside their cellular-level support of wound healing processes.

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KLVFF Functionalized Graphene Oxide For Selective Alzheimer's Disease Biomarkers Detection

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Alzheimer's disease (AD) is the most common cause of dementia, contributing to morbidity and mortality among elderly patients.[1] Currently AD diagnosis mainly relies on medical imaging techniques, cognitive tests, and cerebrospinal fluid analysis; but these tests are generally performed when brain structures are already compromised. In this context, biosensors could provide a less expensive and noninvasive way to provide early diagnosis of AD.[2]

Previous studies report that the development of a biosensor made of the KLVFF peptide, as the bioreceptor, and the graphene oxide (GO) sheets as the transducer, has allowed to obtain a sensing platform capable of recognizing and detecting the well-known biomarkers of AD, $A\beta_{40}$ and $A\beta_{42}$, with a linear electrical response as a function of the biomarker concentration in a range between 3–9 µM.[2] In order to increase the sensitivity range of this system, we implemented the biosensor by acting on three different levels: increase of the size of the device and thus the sensitive area, increase of the number of active sites of the functionalized layer and thus the density of KLVFF unit by conjugation of two copies of KLVFF, and increase of the number of detectable biomarkers by functionalizing the GO with the monoclonal antibody (mAb) 12A12, a cleavage specific antibody that *in vivo* selectively neutralizes the harmful tau fragment(s).[3]

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Benchmarking Evo2 for enzyme generation

G. Lobinska

Enzymes are proteins that catalyze nearly all reactions necessary for life. They often operate in pathways, in which a molecule is sequentially modified by a series of enzymes. This organization reaches its most sophisticated form in biosynthetic gene clusters (BCG), particularly present in bacteria, where such clusters produce valuable secondary metabolites. Engineering these clusters is therefore of significant interest, especially for the creation of new antibiotics.

The current state-of-the-art methods for BCG engineering recombine related clusters to generate alternative compounds. However, identifying suitable fusion points remains challenging; hence, many hybrid biosynthetic gene clusters ultimately prove to be nonfunctional. Large language models (LLMs) present a new possibility for BCG design: instead of fusing segments from clusters of different species, it may become possible to generate functional alternatives by conditioning on a part of a given cluster.

How well do current DNA generation models perform at designing enzymes? Can they effectively exploit useful information contained in DNA context to generate functional sequences? To address these questions, we propose several benchmarks to evaluate Evo2, a state-of-the-art DNA generation model. Our results show that Evo2 can generate multidomain enzymes with plausible architecture. However, it sometimes relies on non-informative contextual cues rather than functionally relevant signals.

These results reveal the strengths and limitations of current models and chart a path toward generative models capable of designing functional enzymatic pathways for the production of useful metabolites such as antibiotics.

Targeting CFTR Dysfunction and Airway Infection in Cystic Fibrosis with Multifunctional Frog skin-derived Peptides

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Cystic fibrosis (CF) is caused by mutations in the CFTR ion channel, leading to impaired chloride transport, chronic lung infections, and progressive respiratory damage.[1] Although CFTR modulators have improved patient outcomes, a subset of individuals remains unresponsive or continues to suffer from persistent infections,[2] highlighting the need for alternative therapeutic strategies. We previously identified frog skin–derived antimicrobial peptides (Esc peptides) that reduce bacterial burden in murine models of *Pseudomonas aeruginosa* lung infection and promote epithelial repair. Notably, Esc(1-21) also acts as a potentiator of F508del-CFTR, enhancing chloride transport in both cell lines and primary bronchial epithelial cells.[3]

Here, combining molecular dynamics, mutagenesis, and disulfide cross-linking assays, we show that Esc(1-21) directly interacts with the CFTR nucleotide-binding domain interface. Confocal imaging confirms peptide internalization and co-localization with CFTR. These findings identify Esc peptides as multifunctional molecules with antimicrobial, pro-repair, and CFTR-modulating activities, supporting their development as novel therapeutic candidates for CF.

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D. melanogaster and *C. capitata* transformer-2 proteins: identification of the basis of target RNA recognition

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Sex determination in insects is an intricate and highly diversified process that involves many key players. Its elucidation at the molecular level represents an important step for both basic and applied sciences. Indeed, the possibility of modulating this process represents an effective way to limit the populations of agricultural pests and disease vectors. In the last few years, we have focused on the study of the molecular and structural basis of sex determination in Tephritidae,[1,2] a fly family that includes highly invasive species such as *Ceratitis capitata* (medfly), *Bactrocera dorsalis* (oriental fly), and *Bactrocera oleae* (olive fly).

We are currently working on the molecular characterization of the splicing factor transformer TRA2 from *C. capitata* and for the model system *D. melanogaster*. By combining a repertoire of computational and experimental techniques, we have achieved a detailed characterization of the RNA recognition process of these proteins. Moreover, we have elucidated the structural mechanism underlying the (dys)function of two DmTRA2 temperature-sensitive mutants. These observations, which have been replicated on Medfly, provide a solid basis for developing effective strategies to determine the sex fate of these pests, which could be used in programs such as the sterile insect technique.

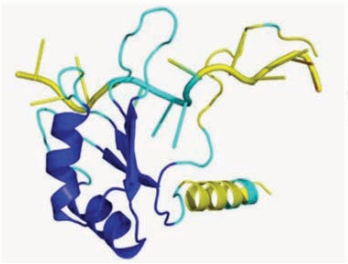

Figure 1. AlphaFold model of the complex formed by CcTRA2 with its RNA target.

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Tuning formulation parameters of Fmoc-FF Nanogels

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Peptide-based nanogels (NGs) are core-shell nanoplatforms with a promising role as drug delivery systems. Fmoc-FF dipeptide, a well-known small molecular weight gelator, can be used to generate hydrogel-like NGs core. In 2020, we proposed three different formulative routes mixing both bottom-up and top-down approaches. These three strategies were, namely, inverse emulsion technique (W/O), "nanogelling in water" and top-down methodology. Among them, top-down method was selected as the best route, avoiding the use of non-green chemical solvents and assuring a faster process. In this method the macroscopic Fmoc-FF hydrogel undergoes homogenization and tip-sonication steps, generating stable NGs with 174 nm diameter. The external covering shell is composed by TWEEN60/SPAN60 52/48 w/w (HLB=10).[1]

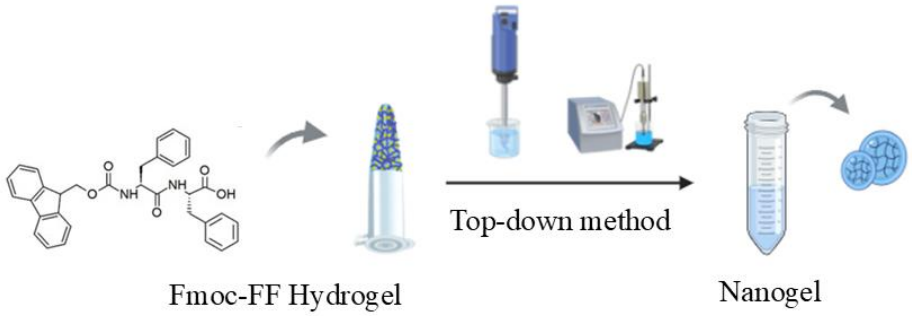

Figure 1. Schematic representation of formulation procedure by using top-down methodology.

In order to further optimize the proposed formulation, the role of four different parameters was investigated. Specifically, it was analysed the impact of the starting Fmoc-FF core concentration (0.25 wt%, 0.5 wt%, 0.75 wt%, 1.0 wt%, 1.5 wt% and 2.0 wt%), the peptide/surfactant ratios (1/2, 1/1 and 2/1 mol/mol), the mechanical homogenization speed (11,000 rpm, 16,000 rpm, 25,000 rpm and 35,000 rpm) and tip-sonication amplitude (20%, 35%, 50% and 65%). The resulting formulations, in terms of dimensions, stability and ζ-potential, were studied using Dynamic Light Scattering analysis.

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Unlocking miRNA Detection with PNA-Based Sensing Technologies

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Recent advances in carbon nanomaterials have highlighted a novel class of fluorescent nanoparticles known as carbon nanodots (CNDs) ranging in dimension from 1 to 10 nm. These materials possess outstanding properties, including high water solubility, low toxicity, and excellent chemical stability, making them promising for a wide range of applications.[1] In this work, we employed a synergistic strategy that integrates the distinctive features of CNDs with peptide nucleic acid (PNA), a synthetic mimic of natural nucleic acids.[2] This combination enabled the development of an effective sensing platform for detecting nucleic acid biomarkers such as microRNA (miRNA) and messenger RNA (mRNA).[3] Water-soluble carbon nanodots were prepared via modified thermal decomposition of citric acid[4] and exhibited excitation-dependent fluorescence (360–540 nm), with a strong emission peak at 504 nm under 400 nm excitation.

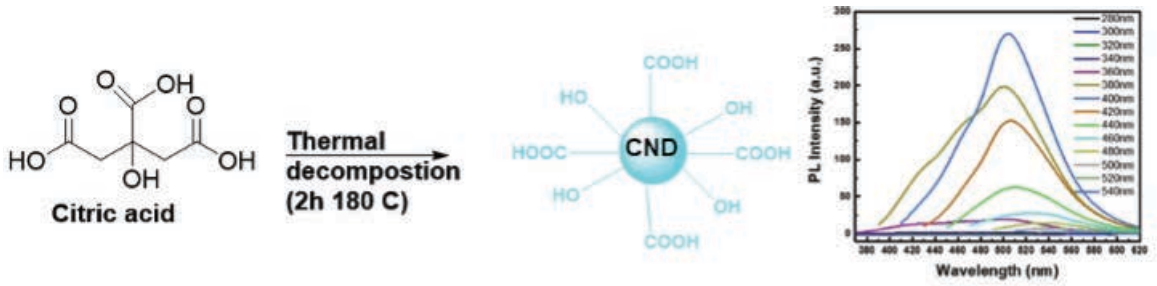

Figure 1. Synthesis of carbon nanodots and fluorescence properties.

The sensing platform was built using a PNA probe tailored to recognize a specific miRNA target. This probe adsorbs onto the surface of carbon nanodots, generating distinct signals that differentiate between the single-stranded PNA, and the double-stranded hybrid formed upon target binding. The sensing platform will be evaluated using sequences that differ from the target even for a single base. As a result, the method enables highly sensitive, rapid, direct, and simple detection of trace biomarkers.

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Design of *tert*-Leu–containing Lonidamine scaffolds bearing arginine side-chain mimetic substitutions on the indazole ring as novel β-arrestin-avoiding CB1 ligands

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Several synthetic cannabinoid receptor agonists targeting the CB1 receptor have been developed to treat chronic pain; however, they are often associated with CNS side effects and tolerance. A cryptic pocket inside the CB1 receptor, that opens rarely and leads to the conserved residue $D^{2.50}$, has been recently identified. In order to target this site, a positively charged group can be introduced through an appropriate linker.[1] Starting from a Lonidamine scaffold,[2,3] we designed novel analogues with structural modifications performed on the indazole moiety. An arginine side chain mimetic was introduced at the 6-position of the indazole ring, through an ether linkage, bearing the guanidine group, to confer a positive charge. This modification promotes an activity on peripheral CB1 receptors, with a reduced brain penetrance and a lower β-arrestin signalling, which could reduce tolerance.

The Lonidamine-analogues were synthesized via tandem solution- and SPPS approach, starting from 6-methoxy-1*H*-indazole-3-carboxylic acid as a scaffold, following a well-established protocol. In solution phase, the scaffold was coupled with *tert*-leucine and further derivatized as methyl ester, acid, and *N*-methyl amide. The amide analogue was obtained via SPPS, using Rink-amide resin with a coupling-cleavage protocol, yielding four Lonidamine-analogues.

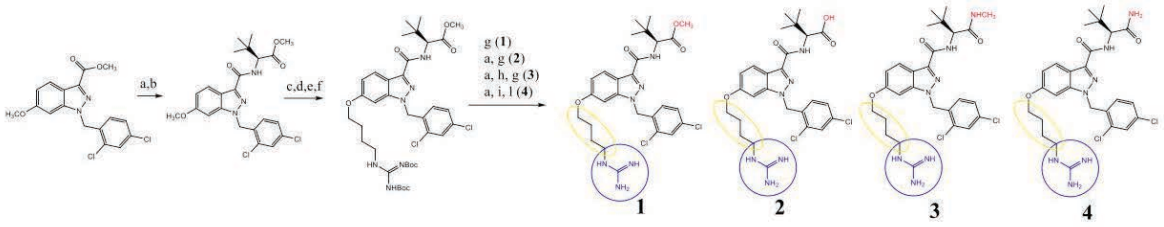

Figure 1. Synthesis scheme of the novel analogues 1–4.

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A combinatorial platform for the discovery and characterisation of yeast-encoded macrocyclic peptide ligands

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Macrocyclic peptides (MPs) are an emerging class of therapeutic agents that effectively bridge the gap between small molecules and biologics. By combining the high target specificity and binding affinity of proteins with the stability, synthetic accessibility, and favourable diffusion properties of small molecules, MPs represent a versatile modality for drug discovery.

Herein, we present a platform for the rapid generation and quantitative screening of genetically encoded, disulfide-cyclized peptide libraries using yeast display technology. Through flow cytometry-based selection, billions of structurally diverse peptide variants can be finely screened in real time, enabling the efficient identification of binders with optimized properties. Promising candidates are chemically synthesized using orthogonal cysteine protecting group strategies, followed by cyclization, purification, and detailed characterization.

To demonstrate the platform's utility, we report the discovery of high-affinity and selective macrocyclic peptide ligands against six protein targets, including low-nanomolar inhibitors of human angiotensin-converting enzyme 2 (hACE2). These results highlight the capability of our platform to generate potent, selective macrocyclic peptides, supporting its application in the development of next-generation therapeutics.

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Peptide inhibitors of CHCHD4: leveraging substrate-binding motifs to tune mitochondrial redox homeostasis

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CHCHD4 (coiled-coil-helix-coiled-coil-helix domain-containing protein 4, also known as MIA40) is a central component of the mitochondrial disulfide relay system in the intermembrane space (IMS), where it acts as a redox chaperone for oxidative protein folding. Through its conserved CPC motif, CHCHD4 captures unfolded, cysteine-rich nuclear-encoded proteins and catalyzes intramolecular disulfide bond formation, stabilizing their active conformation and ensuring correct localization within the IMS. This pathway is essential for mitochondrial biogenesis, respiratory complex assembly, and mitochondrial proteome integrity, all of which are tightly linked to neuronal health and energy homeostasis.[1]

In the context of neurodegeneration, studies in mouse models and cellular systems indicate that moderate reduction of CHCHD4 levels or activity can exert neuroprotective effects, promoting a preconditioning-like adaptation of mitochondrial metabolism.[2] This suggests that fine-tuning CHCHD4 activity may represent a promising strategy to modulate neuronal vulnerability in neurodegenerative and injury-related contexts.

In this work, we aim to develop peptide-based inhibitors that selectively target the redox activity of CHCHD4, in order to probe the impact of its downregulation on mitochondrial reprogramming in damage-resistant cells and to better understand its neuroprotective effects. We designed and synthesized peptide mimetics of known CHCHD4 substrate binding sites using Fmoc-based solid-phase peptide synthesis.[3] All peptides were purified and characterized and recombinant CHCHD4 was expressed and purified to homogeneity. Peptide–CHCHD4 interactions were analyzed by ELISA and circular dichroism (CD) spectroscopy. Comparative analysis identified one peptide sequence as the best CHCHD4 ligand. These findings provide a promising foundation for the development of peptide-based regulators of CHCHD4, which may help elucidate its role in cellular processes and inform novel therapeutic strategies for neurodegenerative and injury-related conditions.

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Peptide-based site-specific conjugation of ageritin to antibody fragments to generate cell-permeable ribo-immunotoxins

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Antibody-drug conjugates (ADCs) and other similar biotherapeutics are a new class of highly effective therapeutic agents, many of which are clinically approved. Efficacy is inherently linked to the high selectivity of the targeting agent component and the notable potency of the cytotoxic payload anchored on it. A subset of ADCs are immunotoxins (ITs) which contain a protein toxin instead of small cytotoxic agents. Several toxins have been investigated in this field, including small toxic proteins from fungi that inhibit protein synthesis by cleaving a single phosphodiester bond within the conserved alpha-sarcin loop (SRL) of 23–28S rRNAs.

We have designed and prepared a prototypical immunotoxin linking the ribotoxin-like protein ageritin (14.8 kDa) to the C-terminal free cysteines of Fabs.[1,2] Immunotoxins contain one or two copies of ageritin linked to 1 copy of Fab and have been prepared using peptide-based linkers that site-specifically connect the toxin free cysteine to Fab hinge cysteines (Cys228 and/or Cys231) liberated by treatment with pepsin. Immunotoxins have been successfully obtained, purified and fully characterized at analytical level. Purified products bind the target antigen and show cytotoxic activity on cancer cell lines far superior to the isolated Fab and ageritin in consequence of the Fab-driven toxin internalization. Data suggest that the site-specific conjugation of ageritin to Fab fragments leads to new ribo-immunotoxins that preserve toxin and Fab' structure and show enhanced toxicity compared to the isolated components.

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Development of humanized anti-Nodal monoclonal antibody

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Nodal is a powerful signaling protein belonging to the TGF-beta superfamily that acts as master morphogen during embryonic development. In healthy adults, Nodal is almost entirely silent but "re-appears" as a biomarker in many aggressive tumors, including melanoma.[1] When Nodal is reactivated in a tumor, it forces cancer cells to maintain their stemness through several mechanisms supporting drug resistance, relapse and metastasis. By virtue of these onco-fetal features, Nodal is considered a potential target for anti-cancer strategies.

Starting from a murine monoclonal antibody (3D1) raised against the Nodal region (43–69) directly involved in the interaction with Cripto-1,[2,3] we have moved forward to obtain humanized antibodies. Here, we reported the biochemical and biophysical characterization of humanized recombinant 3D1 full-length monoclonal antibody prepared in a CHO expression system and preliminary data on its functional effects on Nodal-driven stemness and epithelial–mesenchymal transition in a panel of melanoma cells.

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Modular Surface Functionalization of Liposomes Nanoparticles via Post-Insertion of Lipopeptides

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Due to their high biocompatibility and loading capacity, liposome nanoparticles are currently regarded as one of the most versatile carrier platforms for drug delivery. However, conventional liposomes lack targeting cues to promote their accumulation in specific tissues or cellular compartments, suffering low therapeutic efficacy. Beyond drug loading, surface decoration with targeting ligands is therefore a major field of investigation to generate next-generation therapeutic agents for precision medicine. The use of small portions of lipids modified with specific functional groups (functional lipids), added during initial lipid film hydration, is the main route typically adopted for surface functionalization. This method, however, often leads to partial sequestration of functional lipids within the aqueous core or to their random insertion in the opposite and unfruitful orientation. This leads to non-homogeneous particles whose preparation can be very expensive due to the high costs of functional lipids.

Small lipopeptide linkers (SLLs), short peptides bearing a fatty acid tail, may represent a less expensive and more versatile alternative, especially when coupled with post-insertion approaches for incorporation into pre-formed liposomes. Following their oriented insertion into the lipid bilayer, SLLs can guarantee homogeneous and density controlled external decoration through specific chemical groups that enable further covalent modifications, consensus peptide sequences suitable for enzymatic bioconjugations or small ligands acting as baits for larger functional molecules. Additionally, they can bear fluorescent dyes to monitor liposome localization or their anchoring on the particle surface.

In this study, we designed and studied several SLLs for decorating liposomes with different ligands, carefully optimized the conditions for their proper post-insertion, creating an array of reactive chemical groups, dyes, and ligands. In one specific study, we generated and tested a fluorescent SLL bearing an external biotin, which is used to first capture multivalent streptavidin with further functionalization capability. A full characterization of these systems has been achieved using approaches such as DLS and fluorescence measurements, together with interaction studies to confirm their functional features.

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Making Solid-Phase Peptide Synthesis Greener: Exploring Ultrasound-Driven Sustainable Strategies

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Peptides represent a major class of bioactive molecules, with growing interest reflected by the increasing number of drugs on the market and in clinical pipelines.[1] Solid-phase peptide synthesis (SPPS) remains the most widely adopted strategy for their production.[2] Although considered a greener alternative to solution-phase synthesis, SPPS is still waste-intensive and relies on large reagent excesses and hazardous solvents, particularly N,N-dimethylformamide (DMF).[3]

Our previous studies demonstrated that ultrasonication (US) enhances peptide bond formation and Fmoc-deprotection improving both reaction times and reagent equivalents.[4] In parallel, several reports have shown that SPPS can be performed using greener solvents or solvent mixtures as alternatives to DMF. Here, we combine these approaches and investigate whether US can further enhance the performance of greener solvent systems, enabling protocols with efficiencies and yields comparable to those obtained using DMF. Despite being recognized as a green enabling technology,[5] the potential of US to redesign SPPS from a sustainability perspective remains underexplored.

We therefore investigated ultrasonication as a driver for solvent substitution in SPPS. Using a model pentapeptide, multiple green solvents and mixtures were evaluated for coupling and Fmoc deprotection. Among the tested systems, selected solvents and/or mixtures such as **NBP/EtOAc** showed enhanced coupling efficiency. Also, alternative basic systems such as NaOH in greener solvents enabled both piperidine- and DMF-free Fmoc deprotection. Ongoing investigations will focus on optimizing the process to reduce the number of reaction steps, as well as improving the synthesis of longer or aggregation-prone sequences. These findings indicate that ultrasound-assisted SPPS can improve solvent substitution strategies while preserving synthetic efficiency, supporting the transition toward DMF-free and more sustainable peptide synthesis.

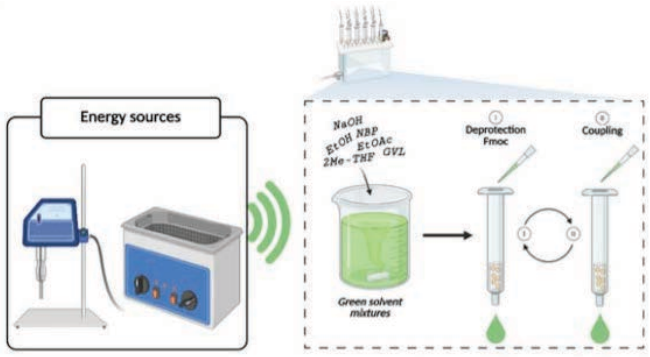

Figure 1. Ultrasonic energy enables the use of green solvent mixtures for both Fmoc deprotection and peptide coupling.

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Mitigating Pyroglutamate in Microwave-assisted SPPS: side-reaction control and Sequence–Structure Influence

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Lactam bridge construction represents a central strategy in peptide design, enabling intramolecular amide bond formation between suitably selected residues. This approach aims to increase conformational rigidity, thereby promoting secondary structure stabilization and enabling macrocyclization strategies.

Cyclization strategies frequently rely on orthogonally protected residues such as Glu(OAll), which can, however, undergo undesired pyroglutamate (pGlu) formation.[1,2] This side reaction is particularly pronounced under microwave-assisted solid-phase peptide synthesis (SPPS), compromising synthetic efficiency and product accessibility.

In this work, we demonstrate that both synthetic conditions and sequence features play a critical role in modulating pGlu formation, systematically investigating this side-reaction using a 10-mer model peptide with intrinsic helical propensity. An optimized synthetic protocol was established using an automated CEM Liberty Blue synthesizer by fine-tuning deprotection and coupling steps at the glutamic acid residue.

Our results provide a practical protocol and valuable insights to mitigate pGlu formation, highlighting the combined impact of local structure and synthetic conditions in microwave-assisted SPPS.

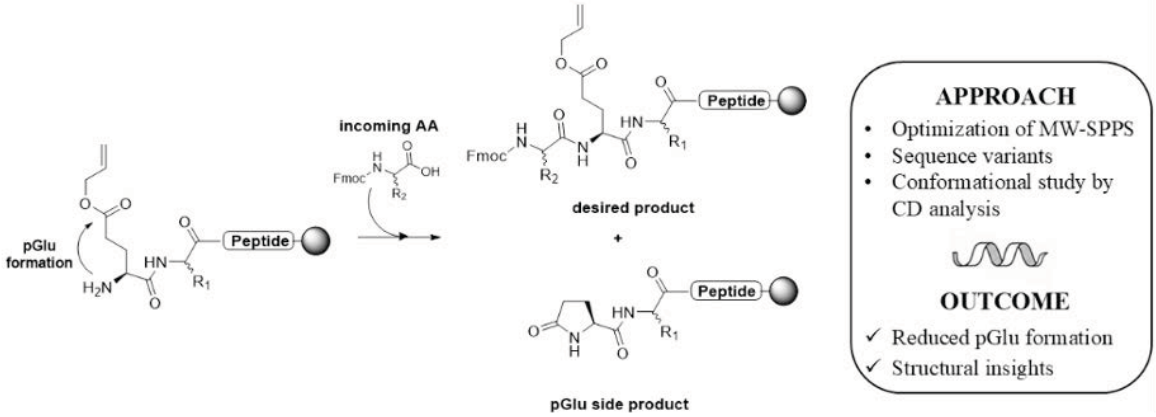

Figure 1. Representative synthetic scheme.

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Poly-L-Lysine doped FmocFF Nanogels as delivery platforms for siRNA

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Due to their intrinsic advantages (*e.g.* biocompatibility, low toxicity and reduced immunogenicity), peptides can serve as promising building blocks for the construction of macro- and nanostructured materials intended for the delivery of active ingredients. Among them, cationic nanogels (NGs) have been investigated as potential carriers for the intracellular transport of siRNA (short interfering RNA). In this context, we formulated novel NGs containing FmocFF hydrogelator in combination with polylysine (PLL) at three different concentrations (1.0, 3.5 and 7.0 mg/mL). Empty NGs and NGs loaded with cyanine 3-labelled siRNA (siRNA-Cy3) were formulated using a top-down methodology through submicronization of the macroscopic hydrogel (HG) in the presence of two surfactants: polysorbate 80 (TWEEN®80) and sorbitan monooleate 80 (SPAN®80).[1]

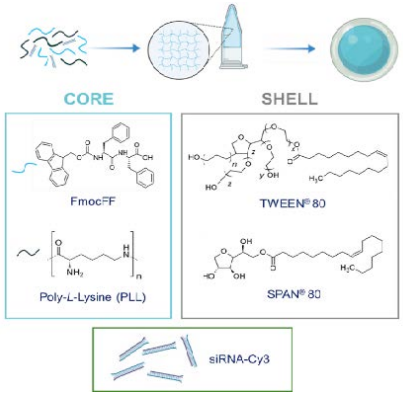

Figure 1. A schematic representation of formulative route for siRNA-Cy3 loaded hydrogels and nanogels.

HGs and NGs were fully characterized from the structural point of view using various experimental techniques (DLS, fluorescence spectroscopy, rheology, SEM). siRNA-Cy3 loading and release were evaluated by fluorescence spectroscopy, with and without Ribogreen marker, and electrophoresis. NGs, with a hydrodynamic diameter of 284 nm and a zeta potential of +31 mV, were found able to efficiently encapsulate siRNA-Cy3 (98%) and release it (70% after 72 h) in simulated biological fluids. Finally, preliminary *in vitro* assays carried out on Human Embryonic HEK293 Kidney cells pointed out that NGs are non-toxic and able to be internalized by active endocytic processes.

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Peptide-based nanogels for potential siRNA delivery

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Supramolecular nanostructures such as micelles, liposomes, and polymeric nanoparticles have been widely studied as innovative carriers for various active pharmaceutical ingredients (APIs). The success of these nanosystems is related to their capability to improve APIs bioavailability and biodisponibility, by changing its pharmacokinetic and pharmacodynamic profiles, while also offering physical protection against *in vivo* degradation.[1] These aspects are connected to both small drugs and biotechnological APIs, such as small interfering RNAs (siRNA), which are being developed as an efficient treatment method against various diseases, such as cancer.[2] Peptides are highly attractive for nanostructure design thanks to their tunable nature, as sequence and terminal modifications can significantly influence material properties.[3] Among all the possible formulations, cationic peptide-based hydrogels (HGs) and nanogels (NGs) were formulated, characterized and evaluated. These systems were obtained using three tripeptides 2NapKFF, 2NapFKF and 2NapFFK. All the nanostructures, empty and filled with a siRNA model, were fully structurally characterized and, the more promising ones also underwent *in vitro* biological assays.

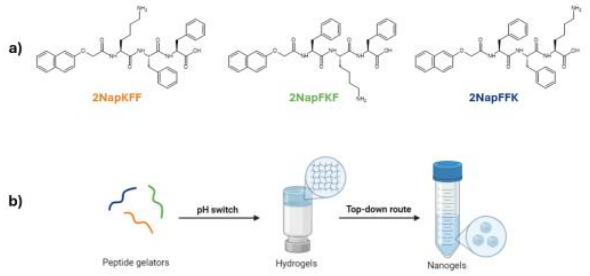

Figure 1. a) Chemical structures of peptides. b) Schematic representation of hydrogels and nanogels formulation.

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Structural Determinants of G-Quadruplex in a Vimentin-derived Peptide

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Vimentin is a key intermediate filament protein involved in epithelial-mesenchymal transition (EMT) and cancer progression.[1] Recent studies have shown that its soluble form can selectively bind G-Quadruplex (G4) DNA structures, suggesting a potential regulatory role in gene expression.[2] In this study, we investigated the molecular determinants underlying G4 recognition by applying an alanine-scanning approach to the N-terminal Vimentin-derived peptide 11–30.

The reference peptide and a panel of alanine-substituted analogues were synthesised via Fmoc-based solid-phase peptide synthesis combined with fragment-based convergent strategies. To enhance oxidative stability, methionine was substituted with norleucine without significantly altering the physico-chemical properties.

Peptide-G4 interactions were characterized by using fluorescence melting assays to assess G4 stabilisation, and surface plasmon resonance to determine binding affinity and kinetic parameters.

Systematic alanine substitutions targeting aromatic and positively charged residues highlighted their key contribution to G4 recognition. In particular, arginine residues were found to play a central role via electrostatic interactions, while aromatic residues contributed through π-stacking interactions. Notably, multiple substitutions led to a marked decrease in binding affinity, highlighting the cooperative nature of the interaction.

The study provides the first structure-activity relationship analysis of a Vimentin-derived G-quadruplex binding peptide. It identifies critical residues for molecular recognition and offers a foundation for the rational design of peptide-based modulators of Vimentin-DNA interactions.

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A selective JNK3-targeting miniprotein confers neuroprotection in a mouse model of ischemic stroke

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Ischemic stroke remains a leading cause of death and long-term disability. Current reperfusion therapies do not prevent progressive neuronal loss and neurological decline. The neuron-restricted kinase c-Jun N-terminal kinase 3 (JNK3) is a key mediator of ischemia-induced neuronal death. Efforts to therapeutically inhibit this pathway are still limited by poor isoform selectivity (JNK3 over JNK1 and JNK2) and toxicity.

We have tested whether specific disruption of pathological JNK3 signaling could provide sustained neuroprotection after stroke. Using structural information underlying the interaction between JNK3 and its selective scaffold protein β-arrestin2 (BA2), we have designed a BA2-derived miniprotein to attenuate ischemia-induced JNK3 signaling. This strategy has led to SIMBA2, a brain-penetrant miniprotein that selectively inhibits JNK3 signaling and prevents phosphorylation of the pro-apoptotic effector c-Jun.

In a mouse model of transient middle cerebral artery occlusion, administration of SIMBA2 3 h after ischemia significantly reduces infarct volume, brain edema and cytotoxic damage, improved functional recovery, and maintained neuronal as well as synaptic integrity. These effects persist for at least 7 days, indicating sustained neuroprotection. Circulating JNK3 blood levels increase after ischemia but remain unchanged in SIMBA2-treated mice, indicating effective target engagement. Significantly, the reduction of plasma JNK3 correlates with neuroprotection, supporting JNK3 as both a pathogenic effector and a peripheral biomarker.

Together, these results identify SIMBA2 as a selective JNK3 inhibitor that confers long-lasting neuroprotection following ischemic stroke, highlighting its potential as both an independent neuroprotective therapy and an additive strategy alongside approved thrombolytic reperfusion treatments. Moreover, plasma JNK3 levels emerge as a potential peripheral biomarker for monitoring neuronal dysfunction and therapeutic efficacy.

Biochemical and Biophysical characterization of anti-CD7 scFv fragment targeting human T-cell acute lymphoblastic leukemia

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CD7 is a transmembrane glycoprotein that is highly expressed in the majority of T-cell Acute Lymphoblastic Leukemia (T-ALL) cases, including early T-cell precursor ALL, making it a promising target antigen for the development of novel CAR T-cell therapies.[1]

On the way to developing second-generation CAR-T systems expressing scFv directed against human CD7 cell marker, we have produced and biochemically and biophysically characterized a soluble single-chain variable fragment (scFv)[2,3] targeting the ectodomain of the human CD7 protein. The humanized recombinant anti-CD7 scFv TH69 has been obtained in high yield in a eukaryotic expression system and has been characterized for purity and identity by mass spectrometry, by SEC to assess monomericity and by circular dichroism to confirm correct fold. The scFv has been also characterized for its ability to bind the soluble recombinant CD7 ectodomain using CGI-based label free technique. The scFv TH69 is monomeric, well folded and binds the antigen with a sub-nM affinity. FACS analysis, performed on T-cells CD7 positive, confirmed the specificity and selective binding of scFv TH69. The functional effects of this anti-CD7 scFv into CAR-T cells are currently in progress. In addition, given the ability of CD7 to internalize, it will be used to generate an immunoribotoxin as novel therapeutic approach for leukemia related disease.

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A semi-synthetic approach for the generation of BisAb using recombinant Fab fragments

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Bispecific antibodies (BisAb) are engineered antibodies that concurrently bind two different epitopes either on the same or different antigens. During the past decades, the innovation in chemical and genetic engineering methods has contributed enormously to the generation of BisAbs, leading to an extensive collection of several different formats that cover a broad spectrum of applications, including diagnosis, imaging, and therapy.[1]

Here, we have explored a two-step hybrid strategy aiming to get a bispecific Fab_2 through the heterodimerization of engineered recombinant Fab fragments. Fabs anti-HER2 and anti-Cripto-1,[2,3] two breast cancer biomarkers, have been recombinantly produced and have been site-specifically functionalized at the C-terminus of the heavy chains using microbial transglutaminase (MTGase), introducing two "complementary" dipeptide linkers capable of undergoing a chemoselective thioalkylation reaction. Following purification, the two modified Fabs have been heterodimerized and the resulting bispecific HER2/Cripto-1 Fab_2 has been purified. The final molecule has been identified by mass spectrometry, analytically characterized and tested for its ability to recognize both targets using SPR direct binding assays. *In vitro* studies to evaluate the efficacy are currently underway.

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Exploring the KCASH2–MAD2A axis: from experimental identification to computational structural insights and rational peptide design

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MAD2A is a core component of the spindle assembly checkpoint (SAC), ensuring proper chromosome segregation, while KCASH2 acts as a tumor suppressor by promoting Cullin3-dependent degradation of Hedgehog pathway components. Prof. De Smaele identified KCASH2 as a novel interactor of MAD2A that induces its degradation, linking SAC dysregulation to tumorigenesis and revealing crosstalk between Cullin3 ubiquitin ligase activity and SAC control.

To structurally characterize this interaction, we combined *in vitro* data with computational modeling. Using an optimized AlphaFold Multimer fragment-based approach,[1] we identified minimal KCASH2 regions required for MAD2A binding and generated a full-length complex. Molecular dynamics simulations (15 μs total) under physiological and stress conditions showed that KCASH2 stabilizes the MAD2A interface and undergoes conformational rearrangements in its BTB/POZ domain, likely facilitating Cullin3 recruitment. Finally, *in vitro* validation by structure-guided peptide design and testing, coupled with mutagenesis profiling, fully confirmed a minimal linear motif able to recapitulate interfacial interactions. Overall, these findings provide a starting point for elucidating the KCASH2–MAD2A axis in cell cycle control and oncogenesis.

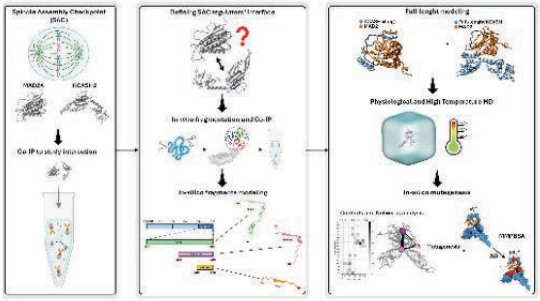

Figure 1. Schematic overview of the *in vitro* and *in silico* discovery and characterization of the KCASH2-MAD2A complex.

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Study of Human Antimicrobial Peptides Active Against Some Bacteroidota Species of the Oral Cavity

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The increasing problem of antibiotic resistance is a critical global health issue, necessitating the development of alternative therapeutic strategies to manage infections effectively. Among the promising solutions are human antimicrobial peptides (AMPs), naturally occurring molecules known for their broad spectrum of antimicrobial activity.

Background/Objectives: This study investigates the potential of some AMPs, selected through a bioinformatic approach, as alternatives to conventional antibiotics, particularly focusing on their efficacy against species within the Bacteroidota phylum. These species, including pathogens such as *Porphyromonas gingivalis*, *Capnocytophaga ochracea*, and *Capnocytophaga canimorsus*, are well known for their roles in various human infections and related diseases. Non-pathogenic environmental species, such as *Flavobacterium johnsoniae*, are also included in this group, frequently used as a model organism.

Methods: By analyzing the antimicrobial efficacy, mechanisms of action, and potential therapeutic applications of human AMPs, this research underscores their significance in addressing the challenge of antibiotic resistance.

Results: This study identified three peptides, KTL24, LIR23, and MFP22, as particularly interesting. These peptides are derived from specific human proteins, namely SPI1, NAPSA and SCUB1.

Conclusions: Their notable antimicrobial potential suggests that AMPs could serve either as a complementary treatment alongside traditional antibiotics or as a standalone therapy, mitigating the ongoing spread of antibiotic resistance and offering an alternative in global health strategies.

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Rational design and synthesis of bioactive miPEP205-derived fragments as modulators of Wnt/β-catenin signaling in triple-negative breast cancer

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Background: Triple-Negative Breast Cancer (TNBC) is the most aggressive subtype of breast cancer, the most commonly diagnosed cancer and the second highest cancer-related death rate.[1] Around 15–20% of breast cancer is accounted as TNBC type and the median survival with the actual therapies is 10.2 months.[2] Therapeutical approaches for TNBC include surgery, radiotherapy and chemotherapy, with the latter being most effective due to the frequent late diagnoses. Despite the effectiveness of chemotherapeutics, tumor relapse rates are high due to the immunosuppressive tumor microenvironment. This challenge has led to the investigation of new approaches and molecular targets. In this framework, **micropeptides** may be an appealing avenue. Recently an increasing number of transcripts from different **lncRNAs** have shown their different pathways toward tumor progression and therapeutic benefits, like **miPEP205**, a 49aa micropeptide coded by lncRNA MIR205HG that acts as a negative regulator of the canonical **Wnt/β-catenin pathway**, which is a central contributor to TNBC pathogenesis. miPEP205 interacts with **GSK-3β**, a protein that increases phosphorylation of β-catenin inducing its degradation and consequently inhibiting cell proliferation.[3]

Design & Strategy: Our research focused on the micropeptide miPEP205 and its functional properties by creating five segments, each containing 8–12 amino acids (FT1–5). The aim was to employ overlapping peptide mapping to generate these segments, thus pinpointing the smallest peptide portion that demonstrated functional activity on tumor cells. The peptides were produced using microwave-assisted solid-phase peptide synthesis (SPPS) with the Liberty Blue 2.0 system. We adopted an eco-friendly synthetic method to enhance the coupling-deprotection cycles, reducing solvent consumption. All segments were subjected to *in vitro* testing using MDA-MB-231 TNBC cell lines. In this presentation, we present the initial *in vitro* findings of the synthesized segments and outline the future directions of this project.

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Temporin-A Targets the PilSR Regulatory System to Inhibit Motility and Virulence in *Pseudomonas aeruginosa*

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Antimicrobial peptides (AMPs) have emerged as promising therapeutic agents to combat multidrug-resistant (MDR) pathogens such as *Pseudomonas aeruginosa*, an opportunistic bacterium endowed with intrinsic resistance mechanisms and a remarkable ability to form robust biofilms. These biofilms limit antibiotic penetration and induce metabolic dormancy, thus severely complicating the treatment of *P. aeruginosa*-associated chronic infections.[1]

Due to the urgent need for innovative anti-biofilm strategies, the present work focuses on Temporin-A (TA), a short, naturally occurring AMP derived from the skin of *Rana temporaria*. Temporins are typically characterized by a short sequence (10–14 amino acids), a C-terminal amidation, and a weak cationic charge. While TA exhibits strong direct activity against Gram-positive bacteria, it lacks bactericidal efficacy against *P. aeruginosa*.[2] However, Sytox Green assays revealed that TA perturbs the bacterial membrane without causing cell death, suggesting an indirect anti-biofilm action driven by its ability to translocate and reach intracellular targets.

To elucidate its molecular mechanism of action (MoA), a functional proteomics approach was employed,[3] revealing PilR as its specific interaction partner. As the primary response regulator of the PilS-PilR two-component system (TCS),[4] PilR plays a pivotal role in regulating pili and flagella formation, which are crucial for bacterial motility, surface attachment, and biofilm maturation. A multidisciplinary strategy was applied to validate this interaction. *In silico* molecular docking simulations predicted a specific interaction between TA and PilR. The production and purification of the recombinant PilR protein allowed subsequent *in vitro* fluorescence-based binding assays to successfully confirm the target engagement. Furthermore, *in vivo* motility assays demonstrated that TA significantly impairs both swimming and swarming motility in *P. aeruginosa*, altering flagellar and pili functions and thereby preventing biofilm maturation. In conclusion, these findings unveil a novel mechanism of action for TA, highlighting its ability to interfere with a key regulatory system.

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pH-Sensitive Dipeptide Hydrogel for Injectable and Stimuli-Responsive Delivery

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Ultrashort peptide-based hydrogels represent an attractive class of supramolecular soft materials due to their minimalistic design, chemical versatility, and strong potential for translational applications.[1] Here, we report a pH-responsive injectable hydrogel based on a dipeptide incorporating the unnatural amino acid Fmoc-β-(3-pyridyl)-L-alanine (Fmoc-3-Pal-OH). The strategically positioned pyridine ring ($pK_a \approx 6.0$) enables finely tuneable protonation–deprotonation equilibria, allowing the dipeptide to self-assemble into supramolecular hydrogels under mild, physiologically relevant conditions. The hydrogel system was comprehensively characterized as a function of concentration and buffer conditions using fluorescence spectroscopy, circular dichroism, FT-IR spectroscopy, SEM, TEM, and rheological analysis. The pH-dependent assembly behaviour was further exploited to regulate drug release. Encapsulation of curcumin as a hydrophobic model compound demonstrated high loading capacity and sustained release under neutral conditions, while acidic pH triggered accelerated release due to protonation-induced network collapse.

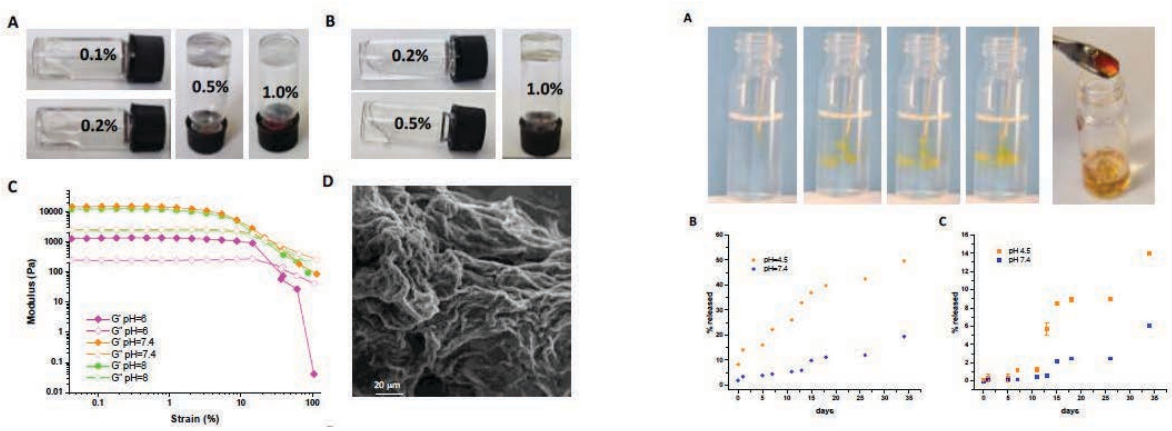

Figure. Three-dimensional hydrogel characterization and pH-dependent drug release profiles of the Fmoc-$(3\text{-Pal})_2$-NH_2 system.

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Target Mapping in Cancer: Ligandable Protein Pockets on 3D OncoPPI Networks

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The investigation of protein–protein interaction (PPI) networks plays a fundamental role in cancer research, both in characterizing different phenotypes and in achieving a deeper molecular understanding of the disease. In this context, identifying specific oncogenic proteins involved in particular cancer types is essential for the rational design of targeted therapies.[1,2]

In this work, we propose a flexible and systematic framework focusing on PPIs involved in 12 different cancer types (oncoPPIs), aiming to unveil protein pockets that serve as key sites for modulating protein function.[3] First, we constructed a comprehensive pocketome based on 314 crystallographically solved oncoPPIs and identified all potentially ligandable protein pockets by employing geometric and energetic 3D GRID-MIF descriptors.

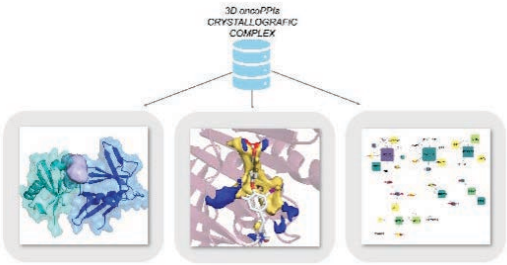

Figure 1. 3D oncoPPI pocketome.

The pockets were then classified according to their suitability for hosting oncoPPI modulators or PROTACs, and ligand-bound pockets in crystallographic structures were compared to assess differences across cancer types. Finally, 3D oncoPPI networks were generated for each cancer type to identify the most connected and central proteins (i.e., hubs), allowing not only the identification of key cancer proteins but also the recognition of pockets and residues involved in critical interactions.

All data are freely available at https://github.com/moldiscovery/OncoPPI-pocketome, paving the way for future research in the cancer field.

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Liquid-liquid separation and gel formation of CIGB-814, a therapeutic peptide with anticancer activity

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Peptide gels are intensively investigated for applications as drug delivery carriers, topical therapy, and biocompatible matrices for tissue engineering. In this contribution, we report on the gelation kinetics and the morphology at the gel state of CIGB-814, a therapeutic peptide with anticancer activity.[1]

Gelation of CIGB-814 has been studied by fluorescence spectroscopy, dynamic light scattering and optical microscopy measurements at a peptide concentration of 10 mg/ml. Under these experimental conditions, CIGB-814 forms aggregated structures that in 8 hours undergoes gelation.

CD spectra showed that the formation of the hydrogel takes place through the initial formation of peptide chiral aggregates structured as helical fibers. The amyloid-like nature of the peptide fibril network in the hydrogel phase was also confirmed by confocal fluorescence microscopy experiments carried out on CIGB-814 conjugated with Congo red. FTIR spectra also showed that in the hydrogel the peptide predominantly populates β-sheet structures.

The kinetics of the gel phase formation was also studied by fluorescence spectroscopy and dynamic light scattering experiments at a 20 mg/ml peptide concentration, revealing a multistep pathway that initiates with the formation of small peptide aggregates of nanometric dimensions, proceeds to the formation of peptide droplets via liquid-liquid separation and ends with the formation of a network of peptide fibers characteristic of the gel phase.

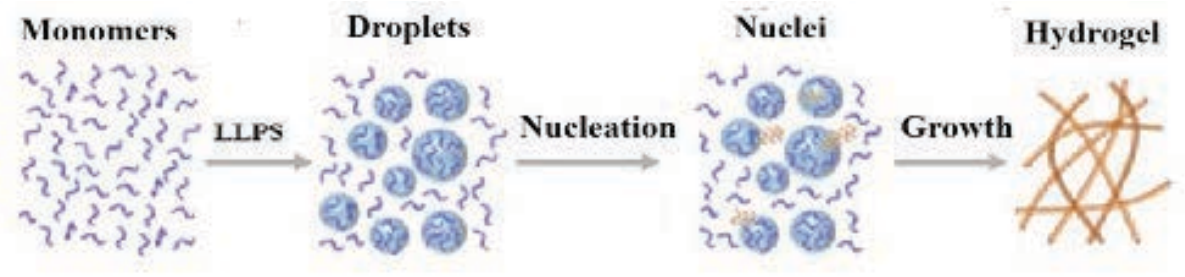

Figure 1. Mechanism of gel phase formation of CIGB-814.

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Disarming *Pseudomonas aeruginosa*: a biological study of Temporin A as an Antivirulence Agent

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Pseudomonas aeruginosa is a major opportunistic pathogen whose ability to form robust biofilms underlies its persistence in chronic infections and contributes to increased antibiotic resistance. In this context, antimicrobial peptides (AMPs) are emerging as promising agents capable of targeting bacterial virulence and biofilm-associated tolerance.[1]

In the present study, we focused on the AMP Temporin A (TA). Despite its limited direct bactericidal activity against Gram-negative bacteria, we investigated its biological effects at sub-inhibitory concentrations against *P. aeruginosa*,[2] with the aim of assessing its potential as an antivirulence agent. To this end, a range of biological assays were performed, including biofilm inhibition assays, pyoverdine quantification, and motility analyses. TA significantly inhibited biofilm formation in a dose-dependent manner, reduced pyoverdine production (thereby indicating attenuation of virulence) and markedly impaired both swimming and swarming motility, suggesting interference with the early stages of surface colonization. In addition, SYTOX Green assays revealed membrane perturbation at concentrations corresponding to maximal antibiofilm activity, supporting a mechanism of action involving both membrane-level effects and potential intracellular targets.

In parallel, functional proteomics approaches were employed to elucidate the peptide's molecular mechanism of action (MoA),[3] identifying PilR, the response regulator of the PilS–PilR two-component system, which regulates pili and flagella biosynthesis through activation of *pilA*, as a putative interaction partner. This intracellular target was further validated by fluorescence-based binding assays and RT-qPCR analysis, which demonstrated downregulation of *pilA* expression following TA treatment.

Overall, these findings demonstrate that TA exerts a multitarget antivirulence effect by interfering with regulatory pathways that control the early stages of biofilm development.

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On Demand Activation of Cryptophycin Drug Conjugates by Click Chemistry

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Cryptophycins have emerged as highly promising payloads for drug conjugates in targeted cancer therapy, owing to their cytotoxicity in the single-digit picomolar range and their strong activity against multidrug-resistant (MDR) cell lines. This exceptional potency necessitates precise target selectivity, making the click-to-release approach an ideal complementary strategy. Payloads attached to the benzylic position to a 1,2,4,5-tetrazine (Tz) can be rapidly and efficiently liberated upon initiation of a Diels–Alder reaction with a strained alkene such as trans-cyclooctene (TCO).[1]

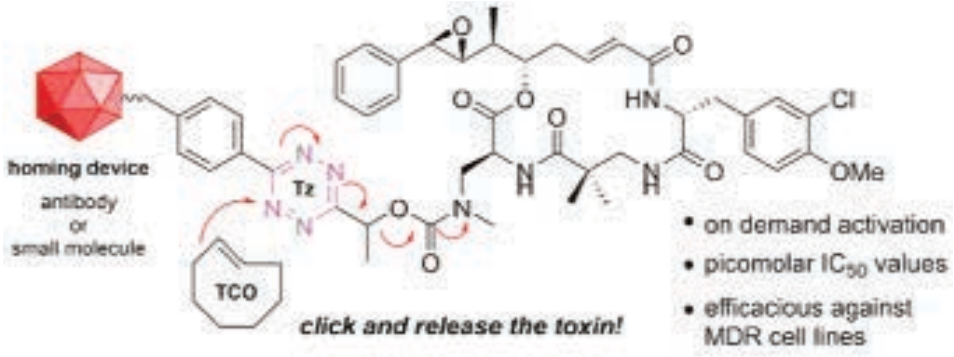

Figure 1. Click-to-release platform for cryptophycin drug conjugates.

By combining one of the most potent cryptophycins developed by our group[2] with a click-cleavable tetrazine linker, we established a platform of drug conjugates featuring on-demand activation via click chemistry. Antibody–drug conjugates (ADCs) based on both internalizing and non-internalizing antibodies were designed, and *in vitro* studies demonstrated cytotoxicity in the single-digit nanomolar range, which could be further enhanced to picomolar levels upon activation with TCO. This activation mode proved superior to that of analogous systems relying on enzymatic cleavage.[3] The use of highly target-specific ligands enabled the development of click-cleavable small molecule–drug conjugates (SMDCs) combined with a fluorophore allowing for monitoring of potential internalization pathways.

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Lysine rich d,l peptide based amphiphiles with antimicrobial activity

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One of the major modern medicine's challenges is laid by the increasing drug-resistance developed by bacteria. Among the many classes of compounds exploited as potential antimicrobials, Antimicrobial Peptides (AMPs)[1] are naturally occurring peptides whose cationic and amphipathic nature leads to self-assembly and bioactivity properties. Some of the natural AMPs have a regularly alternated enantiomeric sequence (D,L), which increases their proteolytic resistance, lends special conformation accessibility and may also be responsible for their biological activity.

In this context, we have designed a series of peptide amphiphiles to evaluate this stereochemistry effect on both the self-assembling properties and the bioactivity. The hydrophilic region was formed by cationic peptide sequences of increasing length, built by repeating one, two or three times a unit of valine (Val) and lysine (Lys). The palmitic acid (Palm), chosen as hydrophobic tail, was conjugated to the N-terminus of the di-, tetra- and hexapeptides; obtaining molecules with a different hydrophilicity/hydrophobicity ratio, since this feature seems to be important for antimicrobial activity. For all Palm(D-Val-L-Lys)$_n$ (n=1,2,3) conjugates, the corresponding homoconfigurational (all-D or all-L) sequences were synthesized.

The structural characterization carried out with dynamic light scattering, small angle X-ray scattering, scanning electron microscopy and circular dichroism spectroscopy showed that the lipopeptides with homoconfigurational residues self-assembled into fibrous structures stabilized by β-sheet conformation, while the peptides with regularly alternating sequences adopt a random coil conformation self-organizing into fibrous structures at higher pH values and concentrations. Biological tests on Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus* showed that only the tetrapeptide and hexapeptide conjugates with alternated enantiomeric sequence had antimicrobial activity. These findings could be the result of the optimal hydrophilicity/hydrophobicity balance and the higher flexibility of the peptide moiety that allow a better interaction with the bacteria membranes.[2]

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Evolving short FPR1 peptide antagonists into a platform for theranostic molecular imaging of FPR1-expressing tumours: design and screening of labelled derivatives

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Formyl Peptide Receptor type 1 (FPR1) is overexpressed in various solid tumors, including high-grade gliomas and sarcomas, where it serves as a negative prognostic factor by promoting cell motility, invasion, and angiogenesis. This study aimed at transforming the *retro-inverso* peptide RI-3 (Ac-(D)-Tyr-(D)-Arg-Aib-(D)-Arg-NH_2), a stable and potent FPR1 antagonist that blocks its binding to uPAR,[1,2] into a theranostic asset for molecular imaging and targeted therapy. For this purpose, we designed and synthesized a set of new peptide derivatives by conjugating RI-3 to chelating agents (DOTA) for radiolabeling or fluorophores for optical imaging. To minimize structural interference, various flexible linkers were inserted at the N- or C-terminus of the tetrapeptide.

Structural integrity and thermal stability were assessed by Circular Dichroism (CD) and other spectroscopic investigations as well as NMR analyses. Functional screening included competitive binding assays on FPR1-transfected RBL-2H3 cells. Synthesis yielded high-purity products (>95%) with stable, folded conformations similar to the parent RI-3 peptide. CD analysis confirmed that DOTA-conjugates complexed or not with Ga^{3+} ions, retain their structure after thermal stress, a critical requirement for radiolabeling. The screening identified the RI-3 analogues RI3-1 and RI3-2 as lead candidates. Both peptides exhibited nanomolar affinity (IC_{50}) for FPR1, establishing a promising theranostic toolset for FPR1-positive tumors. The binding affinity coupled with inhibitory capacity, and successful radiolabeling, warrants further validation in *in vitro* and *in vivo* models to confirm their utility in molecular imaging and targeted radionuclide therapy.

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Funding: *European Union – Next Generation EU – NRRP M6C2, Investment 2.1 (project: POC-2023-12377318; Cup Master H63C24000320001).*

Evolving short FPR1 peptide antagonists into a platform for theranostic molecular imaging of FPR1-expressing tumour: stability, brain permeability and anti-tumor activity

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The Urokinase Plasminogen Activator Receptor (uPAR) is a master regulator of cell migration through the formation of composite regulatory units with the Formyl Peptide Receptor Type 1 (FPR1). High levels of uPAR are associated with poor prognosis and increased risk of metastasis, while accumulating evidence demonstrates that FPR1 is involved in tumor progression of a variety of tumors, including sarcomas and gliomas. In the past years, we have designed a number of modified uPAR-derived peptides that inhibit cell migration and angiogenesis by preventing uPAR-FPR1 interaction. This study aimed at transforming the *retro-inverso* variant of one of these peptides, named RI-3 (Ac-(D)-Tyr-(D)-Arg-Aib-(D)-Arg-NH_2), a stable and potent FPR1 antagonist that prevents uPAR signalling, into a theranostic platform for molecular imaging and targeted therapy.

New RI-3 analogues named RI3-1 and RI3-2, selected as lead candidates by a cell-binding assay, were investigated to assess stability, anti-tumour efficacy, brain permeability and brain targeting, as well as to validate their theranostic properties *in vivo*. The study confirmed the stability of the compounds in human and murine sera while demonstrating their potent ability to inhibit sarcoma and glioma cell migration, Matrigel invasion, and endothelial tubule formation (angiogenesis). Additional experiments verified that the new derivatives successfully cross the blood-brain barrier (BBB) with an efficiency comparable to the reference compound RI-3. *In vivo* studies are currently ongoing to evaluate their functional activity in nude mice xenografted with sarcoma cells and glioblastoma cell line. Altogether, the data suggest RI3-1 and RI3-2 as new candidates for theranostic use in sarcomas and gliomas.

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Evolving short FPR1 peptide antagonists into a platform for theranostic molecular imaging of FPR1-expressing tumours: preclinical imaging studies

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Formyl Peptide Receptor 1 (FPR1) is overexpressed in several solid tumors, including high-grade gliomas and sarcomas, and is associated with poor prognosis. To exploit FPR1 as a target for tumor imaging and theranostic applications, RI-3, a highly stable retro-inverso tetrapeptide antagonist of FPR1, was used to generate novel derivatives for optical imaging, fluorescence-guided surgery, and radiolabeling. Novel RI-3-derivatives were coupled to either IRDye800, a near-infrared fluorophore, or DOTA, a radionuclide chelating agent, generating four compounds: RI3-1-IRDye, RI3-2-IRDye, RI3-1-DOTA, and RI3-2-DOTA. The binding properties of the fluorescent derivatives were then assessed in FPR1-positive RBL-2H3 cells and receptor-negative RBL-2H3/ETFR cells. Both peptides bound FPR1-positive cells, but not receptor-negative cells ($K_{d,app}$: 100–200 nM).

In an orthotopic glioblastoma model based on U87 cells, RI3-1-IRDye selectively accumulated in the tumor and showed significantly higher tumor uptake than unconjugated IRDye800, as assessed by *ex vivo* quantification of tumor-to-normal brain fluorescence ratios (T/B ratios: RI3-1-IRDye *vs* IRDye800, 10 *vs* 5). RI3-1- and RI3-2-DOTA were radiolabeled with the positron emitters ^{68}Ga, yielding both compounds with radiochemical purity greater than 95%. The *in vitro* uptake of [^{68}Ga]DOTA-RI3-1 evaluated in U87 and LN229 glioma cell lines peaked at 60 min and correlated with FPR1 expression. *In vivo* PET in mice bearing orthotopic U87 tumors with [^{68}Ga]DOTA-RI3-1 and [^{68}Ga]DOTA-RI3-2 revealed a rapid tumor uptake with a plateau between 25 and 45 min, confirming preferential uptake in the tumor compared with normal brain (T/B ratios: 2.29 ± 0.58 and 2.18 ± 0.58, respectively). Overall, these preliminary results identify RI3-1 as a promising platform for optical and nuclear imaging of gliomas, with potential for targeted radionuclide therapy using lutetium-177.

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Conformational peculiarities of Pantinin-derived peptides probed by natural-abundance NMR spectroscopy

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Pantinin peptides are bioactive molecules isolated from the venom of the scorpion *Pandinus imperator*, known for their broad-spectrum antimicrobial properties [1]. Their ability to act on cellular membranes with low cytotoxicity makes them suitable for the development of therapeutic applications. In this study, we investigated the structural properties and the antiviral activity against caprine herpesvirus 1 (CpHV-1) and bovine herpesvirus 1 (BoHV-1) [2] of two pantinin-derived antimicrobial peptides (AMPs), called pantinin-1 and pantinin-2 [3]. Specifically, we analysed their conformational behaviour in aqueous solution and in membrane-mimicking conditions (TFE/H_2O) using natural abundance NMR methodologies. Our NMR-based structural data clearly indicated that both peptides, in aqueous solution, sample random coil conformations with pantinin-2 showing greater α-helical propensity. In the presence of TFE (30 % v/v), both peptides transitioned to stable α-helical structures, which are often associated with membrane interaction and antiviral activity. Furthermore, both peptides exhibited antiviral activity against the veterinary alphaherpesviruses bovine herpesvirus 1 (BoHV-1) and caprine herpesvirus 1 (CpHV-1). Overall, our study offers valuable insights for the rational design of pantinin analogues with enhanced antiviral efficacy.

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