18th Naples Workshop on Bioactive Peptides

PEPTIDES IN THE NATIVITY

ABSTRACTS

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Giancarlo Morelli, Michele Saviano, Menotti Ruvo, Paolo Grieco

NAPLES NOVEMBER 28TH - 30TH, 2024

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18th Naples Workshop on Bioactive Peptides

PEPTIDES IN THE NATIVITY

PROGRAM

PEPTIDES IN THE NATIVITY

Scientific day of the Research Centre
on Bioactive Peptides “Carlo Pedone” CIRPeB
&
18th Naples Workshop on Bioactive Peptides

THURSDAY 28TH NOVEMBER

10.30-19.00 **Registration**

SCIENTIFIC DAY OF THE RESEARCH CENTRE ON BIOACTIVE PEPTIDES "CARLO PEDONE"

10.55	**Welcome address**	

SESSION 1
Chair: C. Isernia – F. Merlino

11.00-11.12	**O1 Davide Esposito** IBB-CNR - Naples	*Insights into the interaction between the p60 subunit of chromatin assembly factor-1 and the histone chaperone anti silencing factor-1*
11.12-11.24	**O2 Angela Arciello** University "Federico II" - Naples	*Cryptic antimicrobial peptides hidden in human endopeptidases: the case of three novel peptides identified in metalloproteinase 19 by a computational-experimental platform*
11.24-11.36	**O3 Giulio Pota** University "Federico II" - Naples	*Bioactive light-responsive melanin-like nanostructures modulated by ascorbic acid reduction*
11.36-11.48	**O4 Roberta Della Marca** University "L. Vanvitelli" - Naples	*HR-derived antiviral peptides: a path to rapid response to emerging viral diseases*
11.48-12.00	**O5 Marina Sala** University of Salerno	*Moving towards a sustainable development of therapeutic peptides*
12.00-12.12	**O6 Jussara Amato** University "Federico II" - Naples	*Harnessing protein-derived peptides to target G-quadruplex DNA: Linking molecular recognition to anticancer activity*
12.12-12.24	**O7 Maria Carmina Scala** University of Salerno	*Rational design of the zonulin inhibitor AT1001 derivatives as potential anti SARS-CoV-2*
12.24-12.36	**O8 Rosanna Lucignano** University "Federico II" - Naples	*Development of new bionanomaterials based on bioactive peptides*
12.36-14.00	***Free Lunch***	

SESSION 2
Chair: P. Grieco – C. Diaferia

14.00-14.12	**O9 Ivana D'Angelo** University "L. Vanvitelli" - Caserta	*Boosting Antimicrobial Peptide Potential: Engineered Nanoparticles for Targeted P. aeruginosa Lung Infections*
14.12-14.24	**O10 Alessandra Monti** IBB-CNR - Naples	*Characterization of autofluorescent amyloid-like assemblies formed by PREP1-derived peptides: correlations between spectroscopic and structural properties*
14.24-14.36	**O11 Annalisa Chianese** University "L. Vanvitelli" – Naples	*Virucidal activity of frog-derived peptides against arboviral diseases*

14.36-14.48	**O12 Rosaria Schettini** University of Salerno	*Design of chiral DOTA complexes for MRI*
14.48-15.00	**O13 Rita Maria Di Lorenzo** University "Federico II" – Naples	*Biomimetic Peptides in Cosmetics Interfering with Neurotransmission*
15.00-15.12	**O14 Mariantonietta Pizzella** University "Federico II" – Naples	*Self-assembled biomaterials based on Transthyretin-derived peptide sequences*
15.12-15.24	**O15 Domenica Musumeci** University "Federico II" – Naples	*Exploring the nucleic acid-targeting and self-assembling properties of nucleoamino acids and nucleopeptides*
15.24-15.36	**O16 Vincenzo Maria D'Amore** University "Federico II" – Naples	*iRGD Peptide-Tumor Binding Dynamics: Toward Improved Activity and Selectivity Profiles*
15.36-15.48	**O17 Gaetano Malgieri** University "L. Vanvitelli" - Caserta	*Circular oligomers formed by Ros/MucR family members act as mediators of DNA condensation in a-proteobacteria*
15.48-16.00	**O18 Elisabetta Rosa** University "Federico II" – Naples	*Structural and mechanical insights of multi-LARKS assemblies: from biology to materials*

18TH NAPLES WORKSHOP ON BIOACTIVE PEPTIDES

16.50-17.00	**Welcome address**

SESSION 1

Chair: L. Russo – F. Formaggio

17.00-17.40	**Opening Lecture** **Horst Kessler** TUM Garching (Germany)	*Integrin-ligands for medical applications*
17.40-18.00	**O1 Mariano Venanzi** University of Rome Tor Vergata (Italy)	*Ultrasound-assisted formation of therapeutic peptide microcapsules*
18.00-18.20	**O2 Oliver Zerbe** University of Zurich (CH)	*Novel macrocyclic peptide antibiotics against Gram-negative bacteria based on the Thanatin scaffold*
18.20-18.40	**O3 Consiglia Tedesco** University of Salerno (Italy)	*From cyclic peptoids to beta turn in proteins*
19.00-20.30	***Welcome Party***	

FRIDAY 29TH NOVEMBER

SESSION 2

Chair: P. Gomes – A. Moretto

9.00-9.30	**PL1 Meritxell Teixido** Gate2Brain – Barcellona (Spain)	*Gate2Brain blood-brain barrier shuttle peptides, from discovery to applications and going beyond small molecules.*
9.30-9.50	**O4 Valentina Borghesani** University of Parma (Italy)	*Redesign of SpyTag/SpyCatcher complex into artificial Cu(II) metalloenzymes. The SpyTag, Our Playground!*
9.50-10.10	**O5 Kaliroi Peqini** University of Milan (Italy)	*Peptidomimetics Inhibiting Amyloid Aggregation: The Agel Case*
10.10-10.40	***Coffee Break***	

SESSION 3

Chair: A. Accardo – P. Grieco

10.40-11.10	**PL2 Rosalba Mansi** University Hospital Basel (CH)	*Radiopharmaceuticals Targeting GPCRs: Exploring the Features of Antagonists*
11.10-11.30	**O6 Marco Antonio Pometti** Cannizzaro Hospital Catania (Italy)	*Synthesis of a Radio-immuno-theranostic Agent for Breast Cancer*
11.30-11.50	**O7 Alberto Signore** Sapienza University – Rome (Italy)	*Biochemical characterization of 68Ga-interleukin-2 for imaging activated T-lymphocytes*
11.50-12.20	**O8 Frank Caruso** University of Melbourne - Australia	*Nanoengineering Biomaterials: From Assembly to In Vivo Delivery and Function*
12.20-12.40	**O9 Valeria Bentivoglio** Sapienza University – Rome (Italy)	*99mTc-FGF2 for nuclear medicine imaging of tumor associated fibroblasts: pre-clinical studies*
12.40-13.00	**O10 Nicola Salvarese** ICMATE-CNR – Padua (Italy)	*LC-MS-Based Metabolomics Analysis of [99m/99gTc][Tc(N) PNP]-labeled RGDechi-Cys Peptide Derivatives*
13.00-13.20	**O11 Luigi Aloj** Cambridge University (UK)	*Radiolabelled peptides targeting Gastrin Releasing Peptide Receptors for cancer theranostics*
13.20-14.30	***Lunch on site***	

SESSION 4 – COMPANIES

Chair: M. Teixido – M. Ruvo

14.30-15.00	**PLC Elisabetta Bianchi** IRBM – Pomezia (Italy)	*Macrocycles as inhibitors of protein/protein interactions: the PCSK9/LDL receptor complex case study*
15.00-15.10	**OC1 Raimund Maier** IRIS Biotech (Germany)	*Novel Orthogonal Protecting Groups for Amino Acid Side Chains*
15.10-15.20	**OC2 Giorgio Marini** CEM (Italy)	*Innovations in Sustainable Peptide Production*

15.20-15.30	**OC3 Antonio Colantuono** Arterra Biotech Naples (Italy)	*Bioactive peptide hydrolysates in beauty industry: Sources, chemical characterization and biological properties*
15.30-15.40	**OC4 Lars Irving Michael Meuser** Sibylla Biotech (Italy)	*A Quantum Algorithm for de novo Drug Design*
15.40-15.50	**OC5 Carmine Talarico** Dompé Farmaceutici S.p.a. (Italy)	*Computer-based peptides library design and optimization to address new therapeutic needs*
15.50-16.40	***Coffee Break & Poster Session*** *from P1 to P24*	

SESSION 5

Chair: M. De Zotti – D. Tesauro

16.40-17.10	**PL3 Paolo Rovero** University of Florence (Italy)	*AAT11RI: a retro-inverso collagen modulator peptide derived from Serpin A1 is a valuable cosmeceutical active ingredient*
17.10-17.30	**O12 Beatrix Miralles** Institute of Food Science Research – Madrid (Spain)	*Screening in vivo digestion resistant whey peptides capable to stimulate enteroendocrine sensing receptors*
17.30-17.50	**O13 Saan Voss** University of Cambridge (UK)	*Peptide-bismuth bicycles*
17.50-18.10	**O14 Mauro Adamo** RCSI Dublin (Ireland)	*Desulfurative Fluorination: an efficient methodology for late-stage introduction of 19F or 18F in amino-acids, peptides and biomolecules*
18.10-18.30	**O15 Sara Aquilia** University of Florence (Italy)	*Development of rapeseed meal-based biomaterials plasticized with his own protein-hydrolysates*
Free Evening		

SATURDAY 30TH NOVEMBER

SESSION 6

Chair: M. Saviano – N. Davey

9.00-9.30	**PL4 Marc Devocelle** RCSI Dublin (Ireland)	*Combining Medicinal Chemistry approaches to modify Antimicrobial Peptides*
9.30-9.50	**O16 Marta De Zotti** University of Padua (Italy)	*Aib-containing peptides as plant protection products*
9.50-10.10	**O17 Chiara Falciani** University of Siena (Italy)	*Antibacterial and anti-inflammatory activity of branched peptides derived from natural host-defence sequences*
10.10-10.30	**O18 Jaspreet Kaur Jandoo** Silesian University of Technology Gliwice (Poland)	*Comparison of antimicrobial assays to analyse bioactive low molecular peptide derived from Lucilia sericata maggot secretion*
10.30-10.50	**O19 Alessandro Pini** University of Siena (Italy)	*Design, synthesis and characterization of anti-TNFα peptides for pharmaceutical purposes*
10.50-11.20	***Coffee Break***	

SESSION 7

Chair: L. Zaccaro – G. Morelli

11.20-11.50	**PL5 Norman Davey** Institute of Cancer Research – London (UK)	*Comprehensive characterization of peptide-binding pockets using high-throughput screening*
11.50-12.10	**O20 Bruno Casciaro** University of Rome "La Sapienza" (Italy)	*Effect of acidic pH and of a single amino acid substitution on the antimicrobial/antibiofilm activity of the peptide Esc(1-21)*
12.10-12.30	**O21 Andrea Caporale** IC-CNR – Trieste (Italy)	*SAR STUDY OF THE HUMAN HOST-DEFENCE PEPTIDE LL-37*
12.30-12.50	**O22 Irina Naletova** IC-CNR – Catania (Italy)	*NGF mimic cyclic peptide demonstrates copper ionophore capability and Ctr1/CCS-driven signaling*
12.50-13.10	**O23 Alessandro Moretto** University of Padua (Italy)	*Conformationally interconnected amides: a remote transfer of stereochemical information over 40 bonds*
13.10-14.30	***Lunch on site***	

SESSION 8

Chair: P. Rovero – O. Zerbe

14.30-15.00	**PL6 Paula M.T. Ferreira** Universidade do Minho Braga (Portugal)	*Self-Assembly of Small Peptides Featuring Dehydroamino Acids for Advanced Nanomaterials*
15.00-15.30	**PL7 Paula Gomes** University of Porto (Portugal)	*From Merrifield to Meldal – Nobel-prized chemistries behind the development of new candidates for topical use on chronic wounds*

15.30-15.50	**O24 Giuseppe Pappalardo** IC-CNR – Catania (Italy)	*Rationally designed peptide conjugates in Alzheimer Disease: implications for diagnosis and therapy*
15.50-16.10	**O25 Adriana Di Stasi** University of Trieste (Italy)	*Proline-rich antimicrobial peptides as lead compound for the development of new anti-mycobacterial drugs*
16.10-16.30	**O26 Daniela Trisciuzzi** University of Bari (Italy)	*Understanding peptide-protein binding based on GRID-MIFs and machine learning*
16.30-16.50	**O27 Aparna Palakkurussi Rathessan** Freie Universität Berlin (Germany)	*Structure-Activity Relationship Study of β-Hairpin Peptides*
16.50-17.30	***Coffee Break & Poster Session*** *- from P25 to P47*	

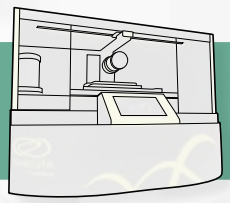
SESSION 9

Chair: P. Ferreira – M. Venanzi

17.30-17.50	**O28 Alberto Dal Corso** University of Milan (Italy)	*Salicylaldehyde-Tagged Peptides for the Reversible-Covalent Engagement of Protein Lysine Residues*
17.50-18.10	**O29 Luisa Calcinai** University of Parma (Italy)	*In silico mapping and proteomic analysis of digestion resistant allergenic peptides in Arginine Kinase from Hermetia illucens*
18.10-18.30	**O30 Luigi de Pascale** University of Trieste (Italy)	*An in vivo approach to study the inhibitory effect of Antimicrobial Peptides on bacterial protein synthesis*
18.30-18.50	**O31 Ren Lai** Chinese Academy of Sciences Kunming (China)	*Peptidic products from Chinese traditional 'worm' medicines*
18.50-19.00	**Concluding Remarks**	
21.00	***Gala Diner***	

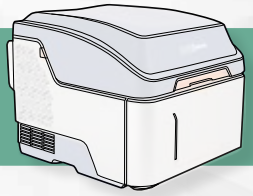
Soluzioni analitiche per la NANOMEDICINA

TEST IN VITRO ED IN VIVO



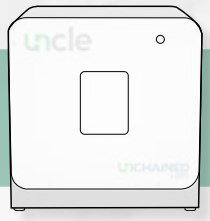
Live-cell imaging

saggi live di proliferazione
saggi di motilità e chemotassi
lineage colture cellulari
metriche su singola cellula



Microscopia confocale

imaging ad alta velocità
tecnologia spinning-disk



SLS, DLS e Fluorescenza

piattaforma di screening formulazione
stabilità colloidale, cinetica e termica
caratterizzazione di proteine e vettori virali
range di temperatura 15 - 95°
fino a 48 campioni

Spettrofotometria UV/VIS

quantifica proteica e di acidi nucleici
misure senza diluizione
fino a 96 campioni (2 µL)
misura in 10 minuti

DLS, SLS, Potenziale Zeta e MADLS

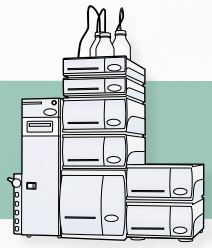
dimensione nanosistemi in dispersione
concentrazione di particelle in numero
polidispersità
stato di aggregazione
stabilità

Nanoparticle Tracking Analysis (NTA)

analisi di liposomi e nanoparticelle
dimensione e concentrazione in numero
discriminazione tramite fluorescenza
stato di aggregazione

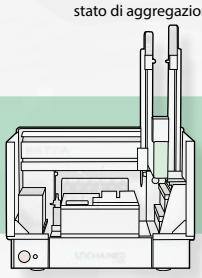
Single-Vescicle Multiparametric Analysis

EVs, esosomi e virus da fluidi biologici
analisi dimensionale e di concentrazione
fenotipizzazione completa
cattura attraverso anticorpi custom
analisi del cargo delle vescicole



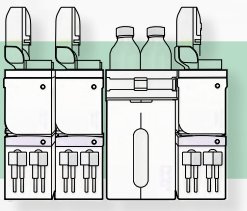
Field Flow Fractionation (FFF)

peso molecolare e dimensione
analisi ad alta risoluzione
frazionamento delle nanoparticelle
misure di efficienza di incapsulamento
fenotipizzazione delle nanoparticelle
stato di aggregazione



Robot

completamente automatizzato
buffer Exchange su piastra
pulizia campioni su piastra
concentrazione campioni su piastra
preparazionie in meno di 15 minuti



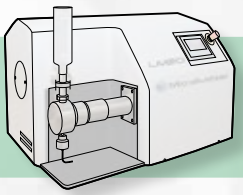
Microfluidica

produzione di NPs polimeriche
produzione di LNP's e liposomi
elevata efficienza di incapsulamento
scale-up GMP e non GMP
screening formulativo rapido

UV/Vis, DLS e RADLS combinati

concentrazione, quantifica,
distribuzione dimensionale e
payload vettori (LNP, Virus etc.)
soli 2 uL di campione
high throughput

PRODUZIONE E CARATTERIZZAZIONE



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produzione di nanoemulsioni
produzione di liposomi, LNPs
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18th Naples Workshop on Bioactive Peptides

PEPTIDES IN THE NATIVITY

SCIENTIFIC DAY OF CIRPEB

Insights into the interaction between the p60 subunit of chromatin assembly factor-1 and the histone chaperone anti silencing factor-1

Davide Esposito[1], Vincenzo Alterio[1], Valeria Menchise[2], Sergio Padovan[2], Giuseppina de Simone[1] and Simona Maria Monti[1]

1 Institute of Biostructures and Bioimaging, CNR, Naples, Italy
2 Department of Molecular Biotechnologies and Health Sciences, University of Turin, Turin, Italy

The proper assembly of newly synthesized and repaired DNA into chromatin is fundamental for maintaining genomic stability. Nucleosomes, which represent the repeating unit of chromatin, are made of four histones (H2A, H2B, H3 and H4) wrapped around with DNA.[1] The transport and deposition of H3/H4 histone dimers in human cells involve four different histone chaperones: Chromatin Assembly factor 1 (CAF-1), Histone cell cycle regulator (HIRA) and two paralogs Anti-Silencing Factors (ASF-1a and ASF-1b).[2] It has been demonstrated that ASF-1 works in synergism with CAF-1 and the interaction between the two chaperones is mediated by one of the three subunits of CAF-1, namely p60.[2] In details, the association with ASF-1 occurs via the C-terminal domain of p60, involving two specific sequences known as "HIRA B-domain like motifs".[2] Here we studied the interaction between different peptides, which encompass the B-domain like motifs of p60, and the N-terminal domain of ASF-1. We confirmed the in vitro binding of the molecules and determined the dissociation constant (Kd) of the interaction by means of Enzyme Linked Immunosorbent Assay and Microscale Thermoforesis. Our data showed a Kd in the micromolar range, thus confirming the hypothesis of a weak binding between ASF-1 and CAF-1, which likely favors a transient contact and a rapid histone transfer between the two chaperones. Moreover, the X-ray crystal structure of ASF-1 in complex with both the B-domain like motifs of p60 has been determined, thus elucidating the structural and molecular determinants responsible of the interaction. Together, our data provide new information on the biological and structural principles that guide the assembly and activity of these fundamental chaperone complexes, leading to innovative prospects for the understanding of their physiological function and their usage as diagnostic, prognostic and eventually therapeutic targets.

References

1 Kornberg RD. Structure of chromatin. Annu Rev Biochem. 1977;46:931-54.
2 Tang Y, Poustovoitov MV, Zhao K, Garfinkel M, Canutescu A, Dunbrack R, Adams PD, Marmorstein R. Structure of a human ASF1a-HIRA complex and insights into specificity of histone chaperone complex assembly. Nat Struct Mol Biol. 2006 Oct;13(10):921-9.
3 Mello JA, Silljé HH, Roche DM, Kirschner DB, Nigg EA, Almouzni G. Human Asf1 and CAF-1 interact and synergize in a repair-coupled nucleosome assembly pathway. EMBO Rep. 2002 Apr;3(4):329-34.

Cryptic antimicrobial peptides hidden in human endopeptidases: the case of three novel peptides identified in metalloproteinase 19 by a computational-experimental platform

Rosa Gaglione [1], Martina Schibeci [1], Erika Piccolo [1], Eugenio Notomista [2], Angela Arciello [1]

1 *Department of Chemical Sciences, University of Naples Federico II, 80125, Naples*
2 *Department of Biology, University of Naples Federico II, 80125, Naples*

Several peptides produced during maturation processes of precursor proteins play central roles in innate defence systems in all complex life forms [1,2]. We recently identified three human antimicrobial cryptides in metalloproteinase 19 (MMP-19) by a computational-experimental platform [3]. The three putative antimicrobial peptides (AMPs) were named r(P)YLL19, r(P)YLL33, and r(P)PRT33, since they correspond to regions 1-19, 1-33, and 247-279 of MMP-19 precursor protein, respectively. P refers to the presence of a Pro residue at the N-terminus of recombinant peptides obtained upon acidic hydrolysis of an Asp-Pro labile peptide bond located between the sequences of the AMP and that of the carrier protein onconase. The three peptides were recombinantly produced [4] and found to be endowed with broad-spectrum anti-microbial properties, being active against both Gram-negative and Gram-positive bacterial strains comprising also clinically isolated and antibiotic-resistant bacteria. MMP-19-derived AMPs were also found to be endowed with anti-biofilm properties being able to affect all the three main stages of biofilm development, *i.e.*, attachment, formation, and detachment. Interestingly, recombinant peptides were also endowed with antiviral properties selective for enveloped virus particles. Importantly, neither toxic nor haemolytic effects were detected when MMP-19-derived peptides were tested on eukaryotic cell lines. MMP-19-derived AMPs were also found to display anti-inflammatory properties and to synergistically act in combination with conventional antibiotics when tested on bacterial planktonic cells. Furthermore, MMP-19-derived AMPs were found not to induce the development of resistance phenotype even after prolonged treatment rounds, what opens interesting perspectives to the future applicability of these peptides. Indeed, to perform a step forward the applicability of MMP-19-derived AMPs, they have been also selected as suitable bio-agents to be loaded into bacterial cellulose nanoparticles (BCNPs), with the aim to set-up a system to efficiently deliver AMPs for applications in biomedical, food or cosmeceutical fields.

References

1. Tooze, S. A., Martens, G. J. M., Huttner, W. B. Trends Cell Biol (2001) 11, 116–122;
2. Hancock, R.E., Haney, E.F., Gill, E.E. Nat Rev Immunol (2016) 16, 321-334;
3. Pane, K., Durante, L., Crescenzi, O., Cafaro, V., Pizzo, E., Varcamonti, M., Zanfardino, A., Izzo, V., Di Donato, A., Notomista, E. J Theor Biol (2017) 419, 254-265;
4. Gaglione, R., Pane, K., Dell'Olmo, E., Cafaro, V., Pizzo, E., Olivieri, G., Notomista, E., Arciello, A. New Biotechnol (2019) 51, 39-48.

Bioactive light-responsive melanin-like nanostructures modulated by ascorbic acid reduction

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Melanin polymers, widely available pigments composed of polyhydroxyindole planar structures, might behave as active phase of versatile bioinspired redox-active nanoplatforms. They can dynamically alter their redox activity in response to external stimuli, such as acidification or light exposure, thereby demonstrating both antioxidant and ROS-generating capabilities.[1] More specifically, reduction by ascorbate anions is reported to affect the oxidation state and thus the electron-transfer behavior of melanin chains. Herein, the design of versatile light-triggered redox-active melanin-based biointerfaces is reported. Melanin-like nanostructures were obtained following a green photocatalytic/solvothermal approach, whereas 2D biointerfaces were produced by depositing thin layers of the nanoparticles on glass slides. Obtained systems experienced an enhancement in the radical scavenging power after post-reduction by ascorbate, whereas ROS generation under IR and UV irradiation was completely turned off, as assessed by Electron Paramagnetic Resonance. The key findings herein presented supported the potential of melanin-based nanoplatforms as multifunctional tools able to selectively act as pro- or anti-oxidant tools, with potential and innovative biomedical applications.

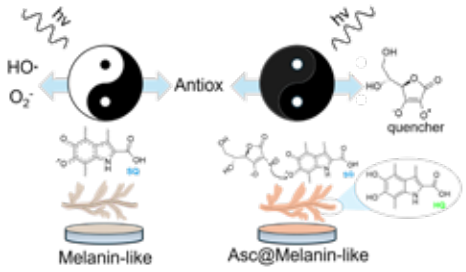

Figure 1. Graphical sketch describing the presented work

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HR-derived antiviral peptides: a path to rapid response to emerging viral diseases

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Zoonotic transmission of the paramyxoviruses Nipah (NiV) and Hendra (HeV) from their natural fruit bat reservoirs to humans has been widely reported and continues to threaten human health worldwide. Ghana virus (GhV) is phylogenetically related to NiV and HeV. Its zoonotic potential is unknown but serological studies have provided evidence that fruit bats, domestic animals, and humans harbor reactive neutralizing antibodies[1]. Menangle virus (MenV) is a member of genus Rubulavirus and it is the etiological agent of a single outbreak of reproductive disease in domestic pigs in Australia, in which two humans in close contact with infected pigs contracted a severe influenza-like illness, demonstrating a zoonotic potential that is not yet fully defined[2]. Therefore, detailed studies of GhV and MenV mechanism of infection are important to elucidate their pathogenicity. The viral entry stage is an appealing target for developing new antivirals against emerging viruses. GhV and MenV share common features of the entry mechanism with other paramyxoviruses, including the interaction between the complementary heptad repeat (HR) regions at the N- and C-terminal ends of the protein (HRN and HRC). It has been widely reported that peptides derived from HRC of viral fusion proteins inhibit viral infection, since they complement the respective HRN, thus interfering with the transition of F protein into its postfusion state and subsequent viral-host membrane fusion. Here, we describe the design and evaluation of several peptides derived from the consensus sequences of HRC and HRN of GhV and MenV F proteins. Since HR ectodomains are conserved among pathogenic viruses, we have also evaluated whether the HR-derived peptides shared broad antiviral properties among paramyxoviruses. Furthermore, we are currently improving our strategy by considering some standard peptide modifications, as dimerization, lipidation, or the use of a PEG-linker. The platform we use here for assessing the peptides antiviral potency, relies on functional assays that mimic the viral entry into a biosafety level (BSL)-2 containment for viruses that normally require BSL-3 facilities. We provide and consolidate evidences that HR-derivates peptides are robust and promising antiviral molecules against emerging and potential zoonotic viruses.

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Moving towards a sustainable development of therapeutic peptides

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The development of greener synthesis processes is a real necessity to transform the industrial landscape, especially the pharmaceutical sector, into a more sustainable reality. Due to the increasing demand from the chemical and pharmaceutical markets for synthetic peptides in the last years, the attention to the greening of their production represents a significant challenge droving researcher toward the introduction of sustainable processes to prepare highly pure, active pharmaceutical ingredients (APIs). [1]

Nowadays the preferred method to obtain peptides is the solid phase peptide synthesis (SPPS), which, unfortunately, does not respect the principles of green chemistry due to the large amount of toxic reagents and solvents used. Because the synthetic procedures do not admit a reduction in the amount of solvent, several attempts have been reported in recent years for replacing DMF with greener solvents, [2,3] according to well-known solvent-selection guides. In this contest, some of the efforts to make peptide synthesis more sustainable in our lab were made. We report a study focused on the replacement of DMF in the fluorenyl methoxycarbonyl (Fmoc) solid-phase peptide synthesis with Dipropylene glycol dimethyl ether (PROGLYDE™, DMM)[4], a well-known green solvent with low human toxicity following oral, inhalation and dermal exposure and easily biodegradable and a mixture anisole/NOP (75:25)[5]. The ability of selected solvents to swell different resins and their capability to solubilize all Fmoc protected amino acids was investigated and model peptides Aib-enkephalin and Aib-ACP were synthesized resulting in favorable outcomes in terms of peptide synthesis efficiency.

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Harnessing protein-derived peptides to target G-quadruplex DNA: Linking molecular recognition to anticancer activity

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Noncanonical nucleic acid structures, particularly G-quadruplexes (G4s), are promising therapeutic targets in cancer treatment.[1] Most G4-targeting bioactive small molecules suffer from poor selectivity, resulting in unfavorable pharmacokinetics and toxicity. In this study, we aimed to develop peptide-based G4 ligands with improved selectivity and anticancer activity. To achieve this, we started from the crystal structure of a G4 in complex with the DNA-binding domain of the yeast protein Rap1.[2] Biophysical techniques were employed to assess the interaction of a peptide derived from the G4-binding domain of the protein with various biologically relevant G4 structures (Figure 1). Through alanine scanning mutagenesis, key amino acids crucial for G4 recognition were identified, leading to the discovery of two peptides with improved G4-binding properties. However, despite their *in vitro* efficacy, these peptides showed limited cell penetration and anticancer activity. To overcome this challenge, cell-penetrating peptide (CPP)-conjugated derivatives were designed, some of which exhibited significant cytotoxic effects on cancer cells. Interestingly, selected CPP-conjugated peptides exerted potent anticancer activity across various tumor types via a G4-dependent mechanism. These findings underscore the potential of peptide-based G4 ligands in cancer therapy.[3]

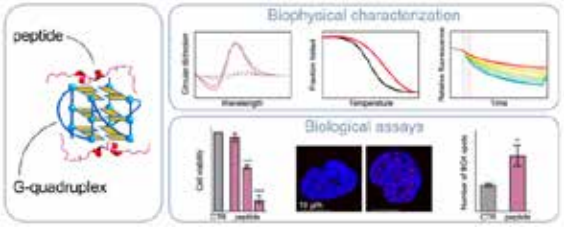

Figure 1

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Rational design of the zonulin inhibitor AT1001 derivatives as potential anti SARS-CoV-2

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The outbreak of novel coronavirus disease caused by the pathogen SARS-CoV-2 has resulted in over 61.8 million infections and over 1.4 million deaths worldwide.[1] These reported estimations highlight the need for drug discovery and development of antiviral treatments to combat this deadly virus. Most of anti-COVID therapeutics resulting from several repurposing campaigns are penalized by a specific administration intended exclusively for hospital practices coupled with most severe cases of infections. We experimentally confirmed the AT1001 binding towards of Mpro enzyme, providing us a structural rational to design and synthesize AT1001 derivatives.[2]
In the present work, we discuss the process leading to the development of a new series of Larazotide tripeptide derivatives.[3] Considering the previous results concerning AT1001 analogues, we collected pivotal clues to design a new series of more potent M^{pro} inhibitors.[4]

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Development of new bionanomaterials based on bioactive peptides

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The aim of the present study is to describe a modern and biocompatible strategy to develop new nanoparticles decorated with bioactive peptides for use in the medical and biomaterial fields. A suitable scaffold to this purpose is provided by ferritins, natural ubiquitarian proteins that spontaneously self-assemble forming ordered nanocages with a hollow core able to store small and medium-sized molecules.[1] Thanks to the capability to interact with the TfR1 human transferrin receptor, over-expressed on the surface of cancer cells [2], homopolymeric nanocages constituted by the heavy chain of the human ferritin (hHFt) can be used for the selective delivery of anticancer drugs. In addition, the ferritin shell avoids unspecific side-effects associated to the cargo molecules such as, for instance, the unspecific hemolytic activity of antimicrobial peptides [3]. Thanks to this peculiar behavior, there is a significant interest in ferritins in the scientific community, i.e. in using them as carriers in drug delivery or storage and optimizing the conditions to enhance encapsulation yield in different environments [4,5]. Moreover, their stability in solution allows ferritins to be used as a scaffold to functionalize their surfaces with peptide sequences. To this purpose, we functionalized by protein engineering a human-derived Ferritin composed of only the H-chain (hHFt) with two classes of peptides. The first class includes SARS-CoV-2 spike (S)-deriving sequences, which proved to be highly immunogenic, as many monoclonal antibodies target these moieties and give an individual susceptibility to the different variants. The decoration of the outer surface of the nanocage with the S-derived epitopes can provide a tool able to elicit an immune answer in view of the development of novel vaccine strategies that can also be applied to other critical pathologies, for which there is no therapy. The second class comprises the marine diatom's silaffin- and sylacidin deriving peptides, which have been shown to induce the formation of silica nanoparticles with an approximate size of 500 nm from phosphate containing silica solutions. The characterization of hHFt functionalized with the silaffin peptide R5 suggests that the nanocage scaffold can improve the bio-silicification and open the way for the development of hybrid nanomaterials with controlled architecture.

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Boosting Antimicrobial Peptide Potential: Engineered Nanoparticles for Targeted P. aeruginosa Lung Infections

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Antimicrobial peptides (AMPs) are one of the few alternatives to traditional antibiotics for treating multi-drug resistant *P. aeruginosa* lung infections. However, their activity is often hindered by interactions with airway barriers. Here we proposed the design and development of engineered nanoparticles (NPs) for the sustained pulmonary delivery of a model AMP, colistin (Col). Poly(lactide-co-glycolide) (PLGA)-based NPs with a core-shell structure were engineered using hydrophilic polymers, including poloxamer (PLX), to provide controlled drug release, improved diffusion across lung barriers (mucus and bacterial biofilm), and targeted delivery for multi-drug resistant lung infections. The PLGA/PLX blend addresses challenges like fast and complete peptide release. NPs were formulated using RG PLGA 502H and Pluronic® F127 or F68 at different ratios through an emulsion/diffusion method, with PVA as stabilizer. Optimized prototypes were loaded with Col and characterized for size, PDI, ζ-potential, encapsulation efficiency, production yield, and interaction with mucus. The effect on P. aeruginosa activity and the eradication of bacterial biofilm of Col-loaded NPs were also evaluated.

Blank PLGA/PLX NPs at various ratios, exhibiting favorable size (~ 200 nm) and ζ-potential (~ -25.0 mV), were achieved. The analysis of shell thickness (FALT) indicates that PLX F127 provides a 5 nm thickness shell on the particle surface while, using PLX F68, the shell thickness is 1.6 nm, suggesting greater adherence of PLX F127 to the PLGA matrix. Optimized formulations were selected for antibiotic encapsulation and NPs loaded with Col maintained an appropriate size, small PDI, negative ζ-potential and low interaction with mucin. Analysis of encapsulation efficiency revealed that the presence of PLX inversely correlates with drug encapsulation efficiency. Col-loaded NPs demonstrate comparable efficacy to free Col in reducing bacterial viability and effectively eradicating biofilms.

In conclusion, PLGA/PLX blend-based NPs represent a promising formulation strategy for the lung delivery of antimicrobial peptides.

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Characterization of autofluorescent amyloid-like assemblies formed by PREP1-derived peptides: correlations between spectroscopic and structural properties

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Proteins and peptides self-assembled into cross-β structures can generate complex supramolecular architectures with uncommon and poorly understood spectroscopic properties [1]. In this context, we have recently identified and characterized self-assembled peptides extracted from the PREP1 protein [2], endowed with interesting structural and spectroscopic properties including conformational versatility and the ability to emit blue-green-red fluorescence with an unusual sharp maximum at 520 nm in neutral buffers, in the solid state and also in cells [3-4]. In particular, the PREP1[117-132] peptide, devoid of aromatic residues, has the ability to emit an unusually strong green fluorescence (λem= 520 nm) after excitation not only with visible light (λex= 400 ÷ 480 nm) but, surprisingly, even with UV radiation (λex= 360 ÷ 390 nm). This is the first evidence for an internal (F)RET (resonance (Förster) energy transfer) in amyloid-like complexes, where the blue emission of some complexes can become the excitation radiation for others [5]. Furthermore, with the characterization of the PREP1[117-132] variants we provided evidence on the correlation between sequence/structure and structure/spectroscopic relationships. We discovered that although all the studied peptide variants present structures similar to beta-amyloid and preserve the blue fluorescence, most of them lose the green fluorescence, probably because, unlike the parent peptide that adopts antiparallel β-sheet states, the mutated ones form parallel β-sheets and exhibit different morphologies in the solid state, including compact aggregates and elongated fibers [5].

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Virucidal activity of frog-derived peptides against arboviral diseases

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Following the emergence of the coronavirus disease 2019 (COVID-19), zoonoses have gained increasing attention within the scientific community. Arboviral diseases are zoonoses caused by arthropod-borne viruses and affect both humans and animals. Viruses are spread through the bite of infected vectors, such as mosquitoes, ticks and sand flies. Over 100 viruses are currently classified as arboviruses, and are known to cause diseases in humans. Most of them belong to the Togaviridae (Alphavirus), Flaviridae (Flavivirus) and Bunyaviridae (Bunyavirus and Phlebovirus) families. Unfortunately, there are currently no vaccines or approved treatments available for human use. As a result, the development of new broad-spectrum antiviral molecules is essential to prevent, control, and eliminate future outbreaks. In this context, antimicrobial peptides (AMPs) with antiviral effect, also known as AVPs, have been already reported as potent inhibitors of the viral infection by affecting different stages of virus lifecycle. We recently reported the strong potential of AVPs as pan-inhibitors of several viruses, including the coronaviruses HCoV-229E and SARS-CoV-2, measles virus, human parainfluenza virus type 3 and influenza virus H1N1[1], herpes simplex viruses (HSV)[2], and animal viruses. In the present study, our aim was to investigate frog-derived AVPs potential against arboviral infections. Peptides were synthesized using the solid-phase Fmoc chemistry method, followed by purification by reversed-phase HPLC. The toxicity was determined via MTT assay and the hemolysis was investigated on human erythrocytes. Different time of addition assays were performed to evaluate in which stage of the viral lifecycle peptides could be involved and results were confirmed by Real-Time PCR and Western blot. AVPs were able to reduce the viral infection in a dose-dependent manner by interfering with the early phase of infection targeting the viral surface. Probably, the antiviral effect of AVPs was due to their interaction with the negative-charged viral membrane, resulting in a physical damage of the lipid envelope. Considering the huge therapeutic potential of these AVPs, further experiments are required to confirm the precise mechanism of action and to increase their stability by modifying the native sequence.

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Design of chiral DOTA complexes for MRI

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DOTA (1,4,7,10-tetraazacyclododecane) macrocycle is the "gold standard" chelator for gadolinium as magnetic resonance imaging (MRI) contrast agent. However, the development of new chelators that guarantee robust thermodynamic stability, kinetic inertness and high relaxivity is still a research challenge to enable diagnostic and therapeutic applications.

In recent years it has been demonstrated that chiral DOTAs, thanks to the formation of preorganized cavities, lead to faster complexation and slower metal decomplexation.[1] Taking advantage of the well-established solid-phase synthesis of cyclic peptoids, the rational design of various azamacrocycles, with different cavity size and various side chains, was already accomplished.[2]

In this work, the introduction of various stereocenters on the cyclic peptoid backbone led to the design and synthesis of novel chiral DOTA-like chelators. The strategic construction of these chiral DOTA complexes (Figure 1) can give rise to an improved library of compounds useful as contrast agents for MRI applications.

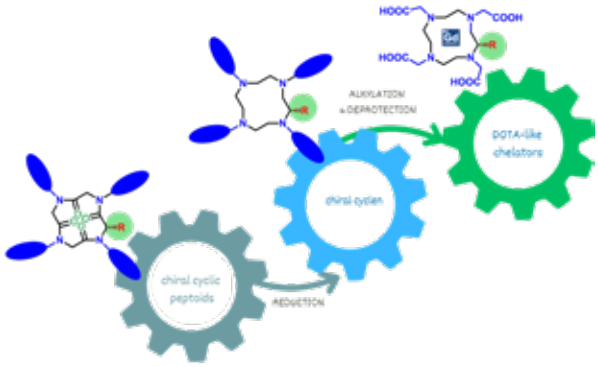

Figure 1. Novel DOTA chelators derived from cyclic peptoids.

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Biomimetic Peptides in Cosmetics Interfering with Neurotransmission

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The process of skin aging involves intrinsic and extrinsic mechanisms that lead to biochemical and structural modifications in the epidermis, dermis, fat tissue and underlying muscles. A variety of natural compounds is currently marketed as active ingredients of topical formulations aimed to contrast or minimize the signs of aging skin. Biomimetic peptides are synthetic analogues of natural peptides. They mimic the basic pharmacological activity of the natural ones but also provide advantages such as smaller molecular weight, easier synthesis or supply, better pharmacokinetic profiles, increased target affinity, and lower toxicity [1]. Biomimetic peptides used has shown valuable properties as topical skin anti-aging agents thanks to their ability to interfere with neurotransmission.[2]
SH-Pentapeptide-5 is an endorphin peptide associated with a delivery system based on conjugated linoleic acid linked to glutathione. The peptide was tested with a single-blind, placebo controlled clinical trial on 40 women for 30 days. SH-Pentapeptide-5 exhibited i) soothing action, ii) ability to restore damaged skin and iii) lifting effect on skin wrinkles. In conclusion, biomimetic peptides, such as SH-Pentapeptide-5, hold significant potential in anti-aging skincare formulations due to their ability to mimic natural peptides while offering enhanced pharmacokinetic profiles and lower toxicity. The promising results from the clinical trial underscore the therapeutic benefits of this peptide. As research in this area advances, biomimetic peptides are likely to play an increasingly important role in the development of cosmeceuticals aimed at addressing the signs of skin aging through targeted mechanisms like neurotransmitter inhibition.

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Self-assembled biomaterials based on Transthyretin-deriving peptide sequences

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Studies carried out in the last decades have demonstrated that, due to non- covalent interactions, proteins and peptides can organize into highly stable supramolecular assemblies, including amyloids and nanotubes. By exploiting the fast-growing amyloid-related structural information present in the Protein Data Bank, heterotypical regions can represent a database of bio-inspired self-assembling sequence. To verify the feasibility of this approach, a sequence of transthyretin (namely P3, residues 14-32) was selected as model. P3 consists of two strands (P3A and P3B) linked by a short loop of four amino acids (RGSP) P3 arranges in a β-harpin, stabilized by P3A and P3B interactions.
With the aim of mimicking the P3 supramolecular arrangements, P3, P3A and P3B sequences were synthesized and multiscale characterized their supramolecular behavior.
Stimulatingly, the systems formed by the co-aggregation of P3A and P3B is able to reproduce the native P3 sequence structuration. All the peptides were also found able to generate self-supporting hydrogels after a solvent switch procedure, thus demonstrating that heterotypical regions can inspire novel biomaterials.

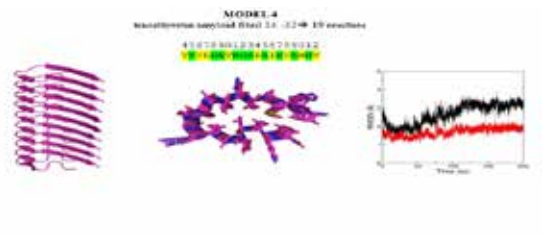

Figure 1. Transthyretin amyloid fibril 14-32

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Exploring the nucleic acid-targeting and self-assembling properties of nucleoamino acids and nucleopeptides

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Synthetic conjugates composed of nucleobases inserted into an amino acid or peptide backbone, referred to as nucleoamino acids or nucleopeptides (Figure 1), may show many interesting properties for various biomedical applications including nucleic acid-binding ability or formation of supramolecular networks.[1,2]
In the last two decades, we have explored several nucleopeptides made of different nucleoamino acid monomers, and found in various cases interesting properties in terms of binding to specific biomolecular targets, and of self-assembling ability affording novel nanostructures useful in the nanomaterials and drug delivery field.[1-4]

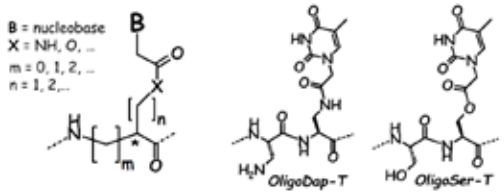

Figure 1. Generic nucleopeptide structure composed of a (α-, β-, γ-, δ-)peptide backbone on which nucleobases are anchored through a suitable linker, and two examples of nucleopeptides based on L-diaminopropanoic acid and L-serine backbones

Here, some examples of nucleoamino acids/nucleopeptides' synthesis and characterization, together with the investigation of their nucleic acid recognition ability, by exploiting CD and UV spectroscopy, as well as self-assembling properties, explored essentially by DLS and fluorescence techniques, will be presented.

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iRGD Peptide-Tumor Binding Dynamics: Toward Improved Activity and Selectivity Profiles

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Poor tumor penetration and low selectivity over healthy cells are major limitations to the development of effective anti-cancer agents.[1] Drug carriers of different nature have been thus largely explored for tumor-targeted delivery.[2] Most of them exploit typical molecular signatures of tumor microenvironments, like the overexpression of specific membrane receptors. A valid example is the cyclic nonapeptide iRGD[3] (CRGDKPGDC, 1) that shows striking tumor-homing properties based on a multi-step mechanism of action where the primary event is the recognition of RGD integrins. Despite the huge number of preclinical and clinical applications of iRGD, very few information is currently available about its structural and binding properties. In this work, we experimentally evaluate the capability of iRGD to interact with three different, clinically relevant, RGD integrin subtypes, namely avb3, avb5 and avb6. Furthermore, we provide an accurate description of the folding of iRGD and of its interaction with integrin receptors. In particular, we first use metadynamics simulations to characterize the bioactive conformation of the peptide and then extensive molecular dynamics calculations to investigate at atomic resolution its binding mechanism to the three receptors. These studies allow us to rationalize the molecular basis of the iRGD integrin potency and selectivity profile, also providing valuable pharmaceutical hints for the development of new peptides with fine-tuned integrin binding features.

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Circular oligomers formed by Ros/MucR family members act as mediators of DNA condensation in α-proteobacteria

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The transcriptional regulator MucR from Brucella species controls the expression of virulence and many others genes by binding to AT-rich DNA. MucR and its homologs constitute the Ros/MucR family, whose members occur in α-proteobacteria[1]. Recently, MucR has been classified as a new histone-like nucleoid structuring protein (H-NS)[2]. Despite the lack of sequence homology, MucR shares many functional similarities with H-NS and H-NS-like proteins, which play a role in structuring the bacterial genome and act as global transcriptional regulators. In this study, we combined cryo-electron microscopy (cryo-EM), nuclear magnetic resonance (NMR), and structural modeling approaches to define a structural model of the oligomers formed by MucR and its homolog Mll5 from Mesorhizobium loti. Our data reveal that MucR and Mll5 constitute a distinct type of H-NS-like proteins, as their circular quaternary structure differs from that found in other DNA structuring proteins. The ability of Ros/MucR family members to oligomerize and bind AT-rich DNA targets was also explored by molecular docking simulations. Our study sheds light on the mechanism by which Ros/MucR family members bridge and condense DNA, providing insights into the interplay between nucleoid structure and transcriptional regulation in α-proteobacteria.

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Structural and mechanical insights of multi-LARKS assemblies: from biology to materials

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In the last decades, the underling of inner workings of protein aggregation has occupied a central spot to inspire fabrication of supramolecular structures to be used in biomedical fields.[1] Low complexity domains, recognized, among the others, in hnRNPA1, hnRNPA2, and FUS proteins, and containing glycine, serine and tyrosine residues, are responsible for liquid-liquid phase separation phenomena *in vivo*, meaning the formation and disaggregation of membraneless organelles in mammalian cells as response to specific stimuli.[2] These fragments, known as LARKS (Low Complexity Aromatic Rich Kinked Segments) give rise to less tight association of the β-sheets inside the assemblies compared to steric zipper detected in amyloids, making the aggregates sensible to external stimuli. Taking inspiration from this evidence, we synthesized four different peptide sequences which were designed to comprise domains deriving from the FUS protein. These sequences were the three LARKS containing 26-residue one, that we named L1-L2-L3, and three shorter peptide sequences composed by only two of the three domains, differently combined to give L1-L2, L1-L3 and L2-L3 peptides (Figure 1). Supramolecular behavior of these peptides, both at the liquid and solid state, was investigated through a series of spectroscopic techniques. The gelation capability and rheological analyses were conducted to detect the mechanical responsivity of these materials to different temperatures.

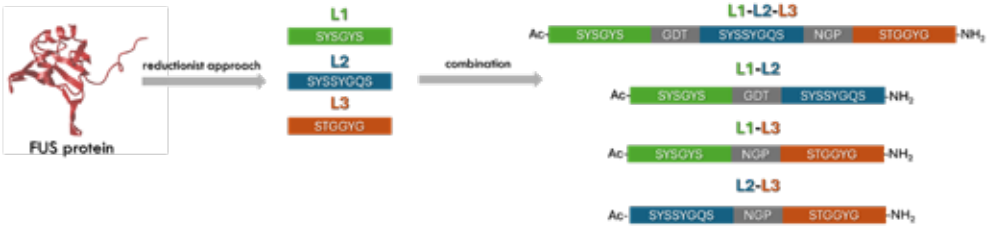

Figure 1. Schematic representation of LARKS peptides design.

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18th Naples Workshop on Bioactive Peptides

PEPTIDES IN THE NATIVITY

18TH NAPLES WORKSHOP

Integrin-ligands for medical applications

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Transmembrane heterodimeric integrins are well-characterized cell adhesion and signaling receptors, important for many (patho-)physiological functions[1]. Thus, peptidic and peptidomimetic ligands have been developed for medical applications in several fields[2]. However, it is critical to apply them without considering their concentration-dependent functional switch in their specific behavior on the respective integrin receptor: at high dose they behave as antagonists, at low dose as agonists [3] via triggering integrin-mediated signaling. We have shown that the interaction of the integrin transmembrane helices following ligand engagement is crucial for the observed effects. Ligand induced dissociation of the α- and the β-subunit is followed by (often ignored) homo-association (α-dimers and ß-trimers)[4] and further clustering to form highly complex focal adhesions[5]. As such, a simple use of highly affine and selective RGD-recognizing integrin ligands as putative inhibitors of integrin adhesive functions may result in lack of clinical efficiency, like noticed for Cilengitide[3]. However, meanwhile the use of integrin ligands as low-dose agonists was shown to open new interesting avenues for their medical use, e.g. to stabilize the vasculature for improved drug delivery in cancer and immune therapy[6], for addressing cytotoxic agents ("cargo") to distinct integrin expressing cancer cells ("logistic properties") [7] as well as "Molecular Imaging" agents[8], and as theranostics in personalized therapy. Moreover, coating of selective ligands onto implants is explored to improve their tissue integration and longevity.
These findings underscore the need for an in-depth evaluation of dose-dependent ligand actions in order to facilitate the decision-making for their most promising medical use.

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18th Naples Workshop on Bioactive Peptides

PEPTIDES IN THE NATIVITY

PLENARY LECTURES

Gate2Brain blood-brain barrier shuttle peptides, From discovery to applications and going beyond small molecules

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Gate2Brain shuttle peptides represent salvage for new or previously rejected CNS drug candidates by providing a way to cross the blood-brain barrier (BBB).

Gate2Brain technology consist on a toolbox of peptides able to cross the BBB and carry compounds covalently attached (including small molecules, peptides, proteins, antibodies, plasmids, siRNA or mRNA loaded nanoparticles, etc…) that cannot cross this barrier unaided. They have proofed to carry these cargoes in vitro and in vivo. These peptide shuttles use the existing transport mechanisms at the BBB without affecting the normal functioning of these mechanisms and preserving brain homeostasis.

By improving the delivery of therapeutic candidate to the CNS, we will ensure immediate impact in many CNS diseases patients. In addition, in a broader perspective, Gate2Brain technology may help to repurpose existing therapies previously rejected because of difficulty to reach the brain, accelerating the translation towards clinical development. Gate2Brain will also result in the application of lower concentrations of therapeutic agent, thereby significantly lowering systemic side effects and reducing the cost of the treatment.

Gate2Brain peptides combine protease resistance, capacity to carry a wide range of cargoes thanks to their versatility, low production costs, and low immunogenic risk. They provide a non-invasive, non-antigenic, permeable, stable, soluble and receptor-specific way to transport drugs across the BBB and into the CNS.

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Radiopharmaceuticals Targeting GPCRs: Exploring the Features of Antagonists

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The field of peptide radiopharmaceuticals for cancer theranostics has experienced significant growth over the past years. A key milestone in this area was the development of radiolabeled somatostatin ligands for targeting somatostatin receptors, particularly somatostatin receptor 2 (SST_2), which is overexpressed in most neuroendocrine tumors (NETs). Since the approval of OctreoScan®, the first peptide radiopharmaceutical for the diagnostic imaging of NETs, substantial progress has been made. A significant achievement is represented by the FDA approval of Lutathera® (^{177}Lu-DOTA-TATE), the first peptide radiopharmaceutical approved for treatment of gastroenteropancreatic (GEP)-NETs patients. The advancements in this field now extend beyond targeting SST_2 to include other cancers expressing G-protein coupled receptors (GPCRs), such as gastrin-releasing peptide receptor (GRPR) and cholecystokinin-2 receptor (CCK2R).
In this context, GPCR-antagonist-based radioligands are becoming increasingly important. Radiolabeled somatostatin receptor antagonists have demonstrated great clinical impact due to prolonged tumor retention and favourable pharmacokinetics. This shift towards antagonists is also expanding to other peptide families, especially for GPCR types where agonistic ligands may cause adverse effects following systemic administration, as seen with GRPR and CCK2R. The development of radiolabeled GRPR-antagonists has led to clinical studies, raising hopes for effective and safe peptide receptor radionuclide therapy (PRRT) across a broader spectrum of cancers. Similarly, the advancement of gastrin antagonist-based radioligands promises exciting new developments.
This lecture will focus on the structural characteristics of GPCR-antagonists used as radioligands and on their influence on *in vivo* properties.

AAT11RI: a retro-inverso collagen modulator peptide derived from Serpin A1 is a valuable cosmeceutical active ingredient

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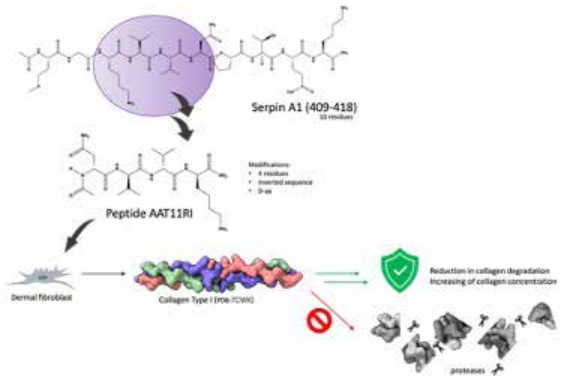
The rising demand for novel cosmeceutical active ingredients has recently driven a surge of interest toward peptides. Building on the collagen turnover modulation properties of SA1-III, a previously described decapeptide derived from the serine protease inhibitor serpin A1,[1,2] we developed AAT11RI, a shorter, second-generation peptide endowed with improved properties. We used the retro-inverso approach, based on the use of D-amino acids, to significantly enhance peptide stability against human dermal proteases. Extensive in vitro data underscore the ability of this peptide to enhance collagen concentration in cultured human dermal fibroblasts and suggest a mode of action based on inhibition of collagen degradation.



Altogether, AAT11RI is a promising candidate as a cosmeceutical dermal anti-aging active ingredient, whose claim is substantiated by a robust research approach.[3]

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Combining Medicinal Chemistry approaches to modify Antimicrobial Peptides

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Antimicrobial Peptides (AMPs) are membranotropic biopolymers with a broad spectrum of antimicrobial activities associated with high fitness cost of resistance. They are currently developed in multiple antimicrobial products ranging from human medicine to biomaterials.

However, AMPs have a number of shortcomings which hinder the exploitation of these unique features, such as proteolytic liability and high cost of production, for clinical and biotechnological applications, respectively.

Techniques of pharmacokinetic improvement developed for therapeutic peptides, such as conjugation, including low molecular weight agents and polymers, peptidomimetic conversion and prodrug modification can be applied to membranotropic peptides. Examples of candidates obtained through these approaches (Figure 1), developed individually or concurrently, will be presented. They are mainly aimed at therapeutic applications, but could also be extended to other products such as antimicrobial coatings.

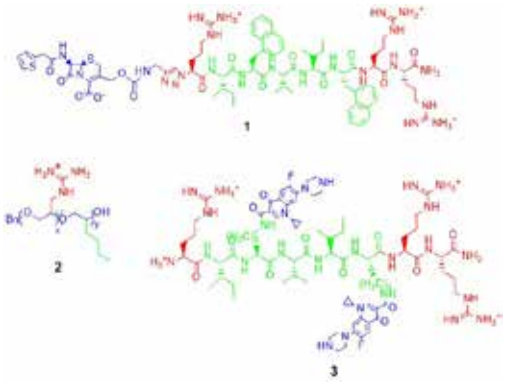

Figure 1. Examples of AMP prodrug (1), polymer-based peptidomimetic (2) and hybrid mimetic (3).

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Comprehensive characterisation of peptide-binding pockets using high-throughput screening

Norman Davey

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Many proteins harbour extensive regions that lack stable secondary or tertiary structure in their native unbound states. These protein segments, known as intrinsically disordered regions (IDRs), are predicted to constitute up to 40% of the residues in higher eukaryotic proteomes. IDRs engage in diverse sets of activities that include providing enzyme docking sites regulating protein modification states, controlling protein stability (by recruiting ubiquitin ligases), acting as signals to target proteins to specific subcellular locations, directing dynamic complex formation or driving concentration-dependent phase transitions. Various estimates have suggested that these regions contain upwards of one hundred thousand interaction interfaces. However, to date, only a small portion of the functional peptides predicted to reside within these regions have been characterised. In this talk, I will introduce our recent work functionally characterising regulatory short, linear motifs (SLiMs), the most common class of interaction interfaces found in the unstructured regions of the human proteome. I will describe the development and application of scalable methods for SLiM interaction discovery that allow thousands to millions of human peptides to be screened in a single experiment. Finally, I will describe examples where in-depth follow-up has resulted in exciting discoveries.

Self-Assembly of Small Peptides Featuring Dehydroamino Acids for Advanced Nanomaterials

P.M.T. Ferreira[1], J.A. Martins[1], A. Carvalho[1] and C.B. Oliveira[1]

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Supramolecular dehydropeptide hydrogels represent an innovative class of biomaterials that integrate the unique structural and functional properties of dehydroamino acids with the self-assembling nature of small peptides. These materials are gaining significant attention as versatile platforms for theranostics combining therapeutic and diagnostic capabilities in a single system. The incorporation of dehydropeptides into hydrogels enhances their biostability and biocompatibility. Their ability to form nanofibrous networks facilitates the encapsulation and sustained release of therapeutic agents. Moreover, the incorporation of functional moieties for imaging and sensing allows for real-time monitoring of therapeutic efficacy and disease progression. This dual functionality positions supramolecular dehydropeptide hydrogels as promising candidates for applications in targeted drug delivery, cancer therapy, and regenerative medicine, offering new avenues for personalized and precision medicine. In this work a small library of dehydrotripeptides (Figure 1) N-capped with aromatic moieties were prepared and tested as hydrogelators.

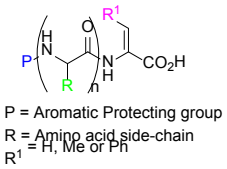

Figure 1. General structure of supramolecular hydrogels

These compounds gave hydrogels with low critical gelation concentration and with self-healing capabilities. The hydrogelators were tested as drug delivery platforms using model compounds. Magnetic hydrogels, with incorporated SPIONs, displayed concentration-dependent T2-MRI contrast enhancement. Moreover, upon magnetic excitation (alternating magnetic field –AMF–) the SPIONs were able to generate a significant amount of heat. Hence, magnetic hyperthermia can be used as a remote trigger for release of drug cargos and SPIONs incorporated into the self-assembled dehydrodipeptide hydrogels.

From Merrifield to Meldal – Nobel-prized chemistries behind the development of new candidates for topical use on chronic wounds

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Chronic wounds are spiralling worldwide due to a combination of factors that include unhealthy lifestyles (sedentarism, inadequate diets), increased life-expectancy, and growing antibiotic resistance. One critical case is that of diabetic foot ulcers (DFU), whose healing is hampered by several diabetes-related complications, e.g., chronic inflammation, hyperglycaemia, peripheral neuropathy, peripheral vascular disease, hypoxia, poor angiogenesis, and altered skin microbiome. Moreover, microbial pathogens easily thrive in the DFU environment, which further aggravates the condition and increases the risk of ischemia, necrosis, and sepsis, ultimately leading to severe amputation or even death.

We combined Merrifield's solid-phase peptide synthesis with Meldal's copper(I)-catalyzed azide-alkyne cycloaddition to produce novel peptide-ionic liquid conjugates (PILC) as candidates for topical treatment of DFU. PILC showed excellent properties in vitro, namely, wide-spectrum antimicrobial activity and collagen-boosting effects on human dermal fibroblasts.[1,2] Biophysical studies revealed a membranolytic mode of bactericidal action, which is less likely to favour bacterial resistance.[3] One of the best PILC was tested in vivo as a topical treatment for wounds in diabetic mice. A multifactorial healing action was observed, encompassing: (i) anti-inflammatory; (ii) antioxidant; (iii) pro-collagenic; (vi) pro-angiogenic; (v) antimicrobial; and (vi) improved wound maturation effects.[4] In other words, a valuable candidate for topical treatment of DFU and other chronic wounds is advanced.

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18th Naples Workshop on Bioactive Peptides

PEPTIDES IN THE NATIVITY

ORAL COMMUNICATIONS

Ultrasound-assisted formation of therapeutic peptide microcapsules

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Core-shell microcapsules made by proteins and polysaccharides have found wide applications in food science, nanomedicine and cosmetics. In this contribution we describe the ultrasound-assisted preparation of microcapsules stabilized by a peptide coating at the oil/water interface. The peptide shell is formed by the growth hormone releasing hexapeptide (GHRP-6), a bioactive peptide used in the treatment of cardiovascular disease. The Centre of Genetic Engineering and Biotechnology (CIGB) of Havana (Cuba) developed a therapeutic formulation, denoted as CIGB-500, and based on GHRP-6, at the moment in clinical phase 2. The aggregation properties of GHRP-6 have been investigated by spectroscopic techniques, determining its critical aggregation concentration. We show that by ultrasound-induced oil/water emulsification, GHRP-6 formed microcapsules, filled by soybean oil, and stabilized by a peptide shell at the air/water interface (Figure 1). The morphology of peptide microcapsules was characterized by fluorescence spectroscopy, differential light scattering and microscopy (optical, confocal fluorescence, scanning electrons) techniques. It will be also shown that the synthesized GHRP-6.coated microcapsules can successfully encapsulate a commercialized oily drug named “One Primary Wound Dressing” (OPWD), the controlled release of which was achieved by temperature and pH stimuli. These findings make GHRP-6 microcapsules a suitable candidate for the development of active ingredient carriers.

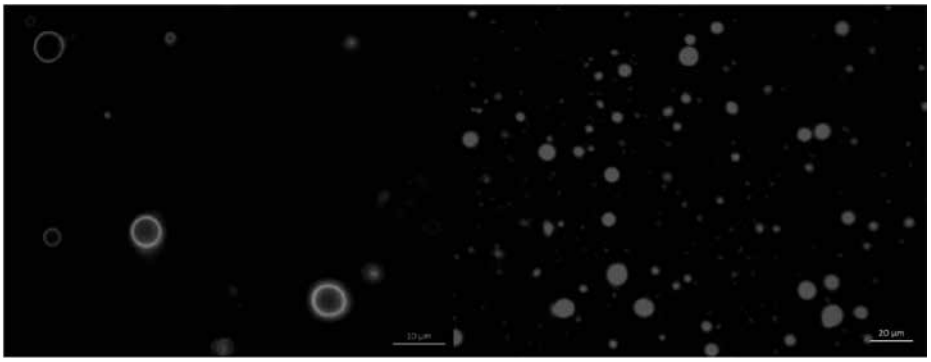

Fig1. GHRP-6/soybean oil microcapsules imaged by fluorescence confocal microscopy. Left: FITC-GHRP-6/soybean oil microcapsules; right: GHRP-6/Nile red/soybean oil microcapsules. The FITC dye was covalently linked to GHRP-6, while Nile red was dissolved in the soybean oil inner phase.

Novel macrocyclic peptide antibiotics against Gram-negative bacteria based on the Thanatin scaffold

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Resistance to antibiotics represent an enormous thread to mankind and the death toll due to antimicrobial resistances was recently estimated as 4.7 millions (1). As a consequence alternative antibiotics with novel mode of actions are desperately desired.

Recently, novel cyclic peptide antibiotics based on the protegrin scaffold were developed (2) that target the lipopolysaccharide (LPS) transport pathway Lpt (3,4,5). They represent the first new class of antibiotics developed in the last 50 years.

We recently discovered that the naturally occurring peptide thanatin binds to LptA, that is part of the LPS transport bridge across the periplasm (5). In my presentation I will show examples of derivatives of thanatin, developed in collaboration with Spexis, that bind to components of the periplasmic protein bridge involved in the LPS transport pathway(6). Peptide protein complexes are determined by solution NMR techniques for two major pathogenic bacteria, E. coli and K. pneumoniae. For all protein-protein and protein-peptide interactions binding affinities were determined by fluorescence depolarization. By NMR and size-exclusion chromatography using suitably modified Lpt proteins LptA and LptC we could demonstrate that the protein bridge is completely dissembled upon addition of the peptides in vitro.

The peptides were optimized for PK and ADME properties, and are demonstrated to be highly active in lung and thigh infection models. In particular, they display low MICs even against many multi-resistant strains from the WHO ESKAPE priority list.

I will also describe the development of a mimic of LptD, suitable for screening, that binds to LptA and Thanatin but does not contain the beta-barrel and is much easier to produce.

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From cyclic peptoids to beta turn in proteins

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Peptoids are N-substituted polyglycines with useful biological activities and interesting chemical properties both in solution and in the solid state. The lack of the amide proton prevents the formation of NH···CO hydrogen bonds and makes peptoids the ideal platform for evidencing the influence of CH···OC and CO···OC interactions in stabilizing molecular conformations and the solid state assembly [1]. Recently, we highlighted the role of intramolecular backbone-to-backbone CO•••CO interactions and CH•••OC hydrogen bonds in the stabilization of enantiomorphic right- and left-handed polyproline type I helices in cyclic dodecapeptoids [2].

The analysis of CO•••CO interactions in cyclic peptides, depsipeptides and peptoids evidenced that ϕ values in the range between [-40°, -90°] and [40°, 90°] correspond to CO···CO distances below 3.22 Å (Figure 1). By extending the analysis of CO•••CO interactions to different types of beta turns in proteins, we highlighted the role of direct or reciprocal carbonyl-carbonyl in stabilizing the beta turn conformation for each specific type, showing that the occurrence of such interactions could be advocated to explain the diversity of turn types from a chemical point of view [3].

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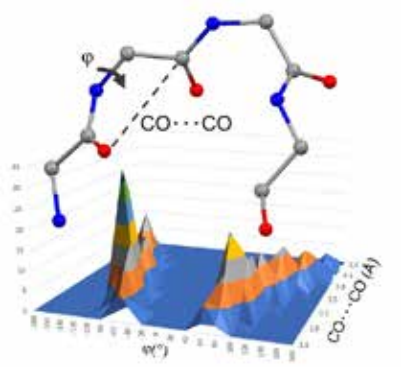

Figure 1. CO···CO distance (Å) vs ϕ angle (°) in cyclic peptides, depsipeptides and peptoids.

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Redesign of SpyTag/SpyCatcher complex into artificial Cu(II) metalloenzymes. The SpyTag, Our Playground!

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Metalloproteins promote several of the most complex biomolecular processes in Nature. The design of new metalloproteins is therefore of interest in the field of the development of new efficient biocatalysts. We present here results related to the redesign of the Spy protein into an artificial metalloenzyme. The Spy complex is an artificial protein system in which a peptide (SpyTag, ST) binds to a protein (SpyCatcher, SC) through an isopeptide bond, to give rise to a recombined Spy protein.[1] Design a metal site on the peptide component allow a straightforward introduction of metal binding sites on the final protein without redesigning the entire protein construct. Moreover, the peptides can be synthesized by solid-phase synthesis, making the SpyTag a playground where we can easily introduce different binding site for different metal ions, expanding the space of redesign of the Spy protein toward applications in industry, biotechnology and nanotechnology.

Here we present a new copper protein designed using the SpyCatcher/SpyTag construct bearing a catalytic bis-histidine site, capable to bind copper in both +1 and +2 oxidation states and to promote reactions of oxidation of catechols. These results have prompted us to try to achieve stereoselectivity in L/D-DOPA oxidation, which will be discussed.

Learning from our first (failing) attempts to obtain the SpyTag/SpyCatcher adduct, we learn the rules we have to respect to have fun in our SpyTag playground. Despite the presence in the peptide of a consensus sequence, which is a recognized “rule” to be respect (its presence is mandatory) for Spy Complex formation, initially we were not able to obtain the Spy complex. We herein proving that it is essential to also have charge complementary in the terminal region.

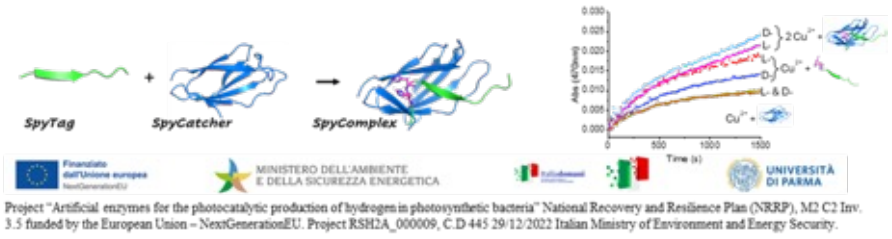

Figure 1. Left: Representation of the SpyTag and SpyCatcher and their complex. Right: Representation of the cathecol oxidation.

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Peptidomimetics Inhibiting Amyloid Aggregation: The Agel Case

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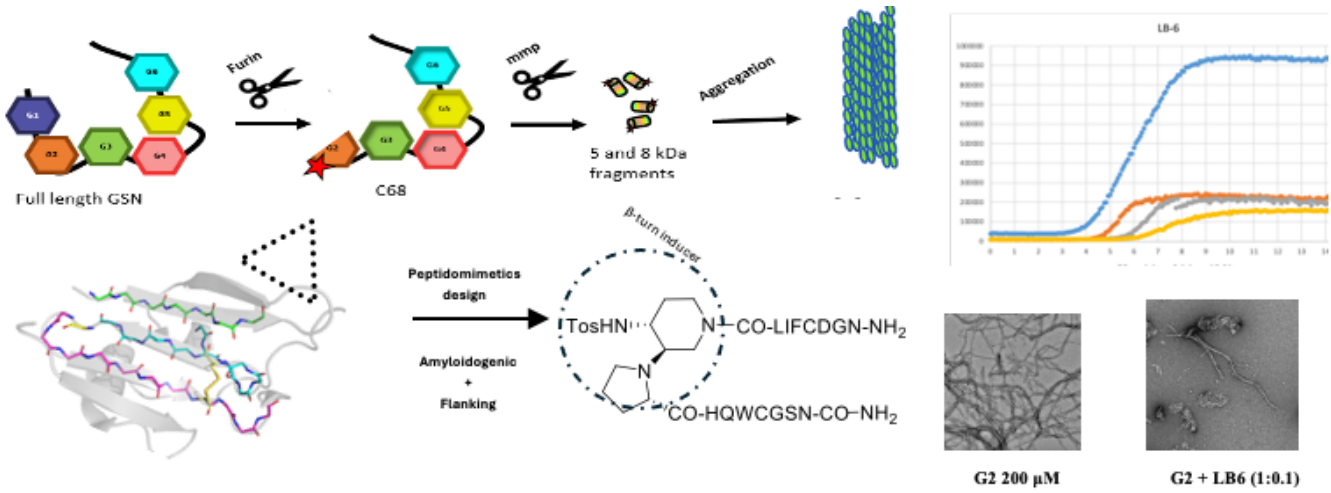
Gelsolin amyloidosis (AGel) is a rare familial disorder characterized by multiple systemic and renal features resulting from pathological tissue deposition of the gelsolin (GSN) protein. This protein is organized into six homologous domains (named G1–G6) that share the same folding. The most common forms of AGel are caused by mutations in the G2 domain, including D187N/Y, N184K, and G167R that lead to a local destabilization and proteolytical susceptibility of the protein. The latter causes the production of two aggregation-prone fragments of 5 and 8 kDa that readily aggregate and eventually form the mature fibrils which are deposited in various organs of the body.[1] To date, no therapeutical treatment is available for any form of AGel. Following a structure-based approach, we designed and synthesized a library of sequence-specific peptidomimetics built on a piperidine-pyrrolidine unnatural scaffold.[2] ThT experiments, and TEM analysis allowed identifying a lead compound LB-6 able to inhibit G2 aggregation at sub-stoichiometric concentration.[3] This peptidomimetic resulted also effective in vivo, in a C. elegans-based assay, in counteracting the proteotoxicity of aggregated G2 domain. These data pave the way to a novel pharmacological strategy against AGel and also validate a toolbox exploitable in other amyloidogenic diseases.



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Synthesis of a Radioimmunotheranostic Agent for Breast Cancer

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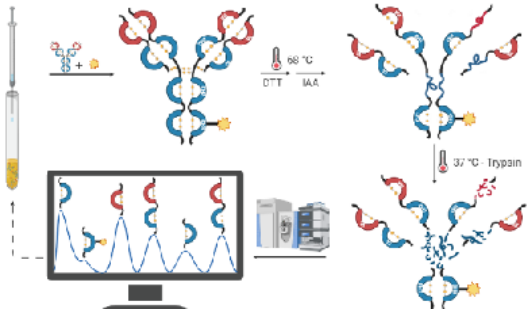
Introduction

Human epidermal growth factor receptor 2 (HER2) is overexpressed in 25-30% of breast cancers and it is associated with poor prognosis. Although the use of Trastuzumab, a monoclonal antibody targeting the receptor, is associated with a survival benefit, tumors will often develop resistance. The creation of a Trastuzumab-based theragnostic agent, enabling receptor nuclear imaging and boosting the treatment through radio ligand therapy (RLT), might increase overall survival and progression-free survival for HER2+ patients. Here, we report a kinetically controlled bioconjugation between Trastuzumab and the N- hydroxysuccinimide ester of DOTA, [1] a chelator suitable for radiolabeling with several radionuclides. A new proteolysis protocol named domain mapping, based on a selective domain unfolding, allowed for quantification of chemical modification at a domain level (figure 1). Finally, data analysis based on LC–MS quantification of different analytical levels (intact, reduced chains, and domains) provided a molecular formulation of the mixture of immunoconjugates.

Materials and methods

The immunoconjugate was synthesized by adding 0.01 eq per min. of DOTA-NHS to Trastuzumab at room temperature and pH 7.2. The total time of synthesis was 500 min. Therefore, the reaction mixture was purified through size exclusion gel filtration. The resulting immunoconjugate was digested by a trypsin enzyme developing a domain mapping mass spectrometry workflow.



S	%
T	35
S_{CH3}	13,46
S_{CH2}	12,94
S_{VH}	11,39
S_{CL}	6,21
$S_{CH2\text{-}CH3}$	3,10
$S_{CH2\text{-}CH3}$	2,98
$S_{CH3\text{-}CH2}$	2,87
$S_{VH\text{-}CH3}$	2,62
$S_{VH\text{-}CH2}$	2,52
$S_{VH\text{-}VH}$	2,22
$S_{CH3\text{-}CL}$	1,43
$S_{CH2\text{-}CL}$	1,38
$S_{VH\text{-}CL}$	1,21
$S_{CL\text{-}CL}$	0,66

Figure 1. Schematic representation of kinetically controlled bioconjugation coupled to the domain mapping MS-workflow.
Table 1. Percentage composition of the immunoconjugate mixture.
T=naked Trastuzumab; S=generic species; subscripts=conjugated domain.

Results and Discussion

Although several site-selective bioconjugation methods have been implemented in recent years, most of them work on non-native forms of mAbs. Thus, non-specific bioconjugations using electrophilic reactants are still very relevant, *but have the disadvantage of generating heterogeneous mixtures. The immunoconjugate, here synthesized using nonspecific reactants but under kinetic control, showed unitary chelator to antibody ratio (CAR). Moreover, proteolysis experiments displayed that an intradomain regioselectivity was achieved, with the conjugated lysine residues not involved in the binding with the antigen. The immunoconjugate mixture was composed of 15 species (table 1), whereas up to 10^6 species are statistically possible employing one-step nonspecific bioconjugations.*

Conclusion

A DOTA-Trastuzumab derivative with features close to immunoconjugates obtained from site-selective procedures has been synthesized, which can be alternatively radiolabeled with imaging or therapy radionuclides enabling for theragnostic nuclear medicine applications. Notably, domain mapping might be useful for broader omics studies where proteolysis plays a key role, as well as a preparation method for single-domain antibodies.

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Biochemical characterization of ^{68}Ga-interleukin-2 for imaging activated T-lymphocytes

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Imaging activated T-lymphocytes in chronic inflammatory diseases and in tumor micro-environment, is a task that cannot be successfully accomplished ex-vivo with histological techniques or in-vivo with conventional radiological techniques. Interleukin-2 (IL2), a cytokine produced by T-cells, binds to receptors expressed on activated T-lymphocytes (T-cytotoxic and T-regs). Published studies on ^{123}I-IL2[1], ^{99m}Tc-IL2[2] and ^{18}F-IL2[3], highlighted the important clinical role of this imaging modality. Nevertheless, none of these radiopharmaceuticals is easily and cheaply synthetized, thus limiting its clinical use. Our goal was to develop a ready to use kit to label IL2 with ^{68}Ga for non-invasively imaging of activated T-cell trafficking by PET/CT.

Aldesleukin (desIL2) was chemically modified with a bifunctional chelating agent, the THP, to allow the radiolabelling with ^{68}Ga at room temperature. The THP-desIL2 was purified by 24h dialysis, freeze dried in aliquots and stored at -80°C. HPLC and mass-spectroscopy were performed to identify how many molecules of THP were bound to desIL2 and in which aminoacid residue. Radiolabelling was performed with GAIA synthesis module at room temperature for 30'. Radiochemical yield (RCY), radiochemical purity (RCP), specific activity (SA) and molar activity (MA) after 1, 3 and 6-months storage at -80°C, were calculated by HPLC and ITLC. For in vitro binding assay, lymphocytes were collected from donors and stimulated with PHA or with IL2 and negatively sorted by FACS to obtain different cell subsets (CD4+, CD8+, CD25+, B-cells, NK and Treg). Pre-clinical studies, in 12 healthy BALB/c mice, were performed to evaluate the ^{68}Ga-THP-desIL2 biodistribution at 15', 1h and 2h post injection (p.i.) of 3.7 MBq (100μCi, 1μg ^{68}Ga-THP-desIL2). Blood and major organs were collected and the % of injected dose per organ (%ID) and per gram (%ID/g) were calculated.

Mass spectrometry showed that more than 70% of the IL2 was conjugated with only 1 molecule of THP. QCs of ^{68}Ga-THP-desIL2 showed: RCY=53.6±10.4%, RCP=97.9±0.45%, SA=5.9±0.4 MBq/μg and MA=90.9±6.4 GBq/μmol, stable up to 6 months. In vitro binding on PHA-activated PBMCs showed a Kd=10^{-9}-10^{-10} M with similar results on stimulated Treg cells, B-cells and NKs. Slightly lower Kd (10^{-8} M) was measured for unstimulated CD8+ cells and CD4+ cells. ^{68}Ga-THP-desIL2 showed excellent biodistribution in mice, with a rapid kidney metabolism. At 1h and 2h p.i., the spleen had the highest %ID/g, given the physiological presence of activated T-cells. In conclusion, we set-up an efficient and reproducible kit to label desIL2 with ^{68}Ga at room temperature.

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Nanoengineering Biomaterials: From Assembly to In Vivo Delivery and Function

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Advances in nanoparticle-mediated therapeutic delivery are poised to revolutionize disease treatment and prevention. In particular, the formulation of mRNA into lipid nanoparticles to combat COVID-19 has highlighted the transformative potential of nanoparticle platforms in the pharmaceutical industry and clinical practice. However, distinct types of therapeutics are required to meet specific therapeutic purposes and their encapsulation is typically tailored on a case-by-case basis. This presentation will present a versatile and biomaterial-based nanoparticle platform, whereby diverse therapeutics, including functional small molecules, siRNA, mRNA, and proteins, can be readily assembled into nanoparticles. The encapsulated therapeutics maintain their intrinsic activity and can be released upon exposure to the biological milieu. This nanoparticle platform has potential for usage in a range of applications.

^{99m}Tc-FGF2 for nuclear medicine imaging of tumor associated fibroblasts: pre-clinical studies

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Fibroblast Growth Factor (FGF) exerts mitogenic activity by stimulating the growth of endothelial cells, fibroblasts, and cancer cells [1]. Several studies have shown its involvement in tumor associated fibroblasts (TAF) and cancer progression. Amongst the large family of FGF proteins, FGF-2 is an angiogenic factor that can activate neovascularization during tumorigenesis. The overexpression in tumors of FGF-2 receptor (FGFR-2c) has been associated with advanced clinical stages and increased metastatization [2]. We focused our research on the development of a specific radiopharmaceutical targeting FGFR2c, for early detection of cancer metastases. To this aim, we have conducted in-vitro and in-vivo experiments to find the best protocol to radiolabel the recombinant human FGF2 (hrFGF2) and evaluate its specificity to target tumors [3].

HrFGF2 was firstly modified with a heterobifunctional chelator, the 6-hydrazinonicotinamide (HyNic). Different HYNIC:protein molar ratios were tested. Once conjugated and purified, hrFGF2-HyNic was radiolabelled with technetium-99m. To find the best radiolabelling protocol, different amounts of tricine and stannous chloride ($SnCl_2$) were tested. Labelling efficiency (LE%) was evaluated with ITLC and HPLC analysis.

Accurate estimate of the binding kinetics and affinity of radiolabelled FGF2 to FGFR2c positive cells (human keratinocytes) were obtained from in vitro binding assay studies. The formulation with the highest LE% was selected to perform dynamic and targeting studies in BALB/c mice bearing murine reticulum cell sarcoma in the right thigh.

A high-resolution camera was used to acquire dynamic images (1 frame/min for 90 min) in 3 mice to evaluate the in vivo kinetic binding of ^{99m}Tc-FGF2 to tumor cells.

For targeting studies, after three time point (1, 3, 24h), three mice per group were sacrificed and major organs collected and counted in a single-well γ-counter. The percentage of injected dose per organ (%ID) and percentage of injected dose per gram (%ID/g) were calculated.

The results showed the highest LE% of 86.33 with 500 μg $SnCl_2$, 20 mg of tricine, and 5 mCi of ^{99m}Tc. 99mTc-HYNIC-FGF2 was purified with size exclusion chromatography to achieve 100% of LE. In vitro binding assays on human keratinocytes expressing FGFR2c, showed that our radiopharmaceutical can bind to the specific receptor within minutes and demonstrating slow dissociation from the cells (Kd 3.36 x 10^{-9} M).

In vivo dynamic imaging showed rapid accumulation in lungs, liver and spleen in the first 10 min, followed by uptake in implanted tumors. Ex-vivo evaluation of radioactivity in tumors showed a tumor/blood ratio of %ID/g at 3h = 4.1 and at 24h = 26.1.

In conclusion, the high LE% and the high affinity of radiolabelled 99mTc-FGF2 to its receptor, confirmed the validity of these new radiopharmaceutical for imaging TAF.

Currently, there is no option to evaluate in vivo the abundance and expression of FGFR2c and this could be achieved by using the radiopharmaceutical developed in the present project.

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LC-MS-Based Metabolomics Analysis of [$^{99m/99g}$Tc][Tc(N)PNP]-labeled RGDechi-Cys Peptide Derivatives

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RGDechi peptide is a potent and selective antagonist of $\alpha_v\beta_3$ receptors.[1] It was recently investigated as potential vector for the preparation of new SPECT imaging agents.[2] Animal studies of [^{99m}Tc][Tc(N)PNP]-labeled RGDechi peptide (PNP= bisphosphinoamine) confirmed the ability of the peptide to visualize integrins in vivo, showing promising biodistributions, but low absolute uptake in $\alpha_v\beta_3$ positive tumors. This could be a consequence of its proven degradation by enzymatic cleavage between the Pro^{17}–Ala^{18} residues of the peptide.[3]

To improve the biological stability of this compound and enhance its bioavailability and accumulation at the tumor site, two derivatives named RGDechi1-17 (1, which lacks the last two amino acid residues at the C-terminus) and RGDechiψ (2, in which a reduced amide bond -ψ[CH2–NH]- is introduced at the Pro^{17}–Ala^{18} proteolytic degradation site), were synthesized and the biodegradation products of the corresponding [$^{99m/99g}$Tc][Tc(N)PNP]-tagged agents ($^{99m/99g}$Tc1-2) were characterized.

$^{99m/99g}$Tc1-2 were synthesized under carrier-added conditions and characterized by RP-HPLC (UV/Radio) combined with LC-MS. To define the hydrolysis products, $^{99m/99g}$Tc1-2 were incubated, at 37 °C, in mouse and human sera and fresh mouse kidney homogenate. The mixtures were analyzed by RP-HPLC-UV/radio, LC-MS and MALDI-TOF-MS.

The chemical identity of $^{99m/99g}$Tc1-2 was established to agree with the proposed formulation. A different metabolic pathway was detected for $^{99m/99g}$Tc1-2 with respect to native peptide. Compounds were stable in sera, but not in mouse kidney homogenate, as the collected chromatograms showed a significant shift of the peaks. Similar outcomes were detected in HPLC analysis of urine collected from healthy mice injected with ^{99m}Tc1-2. Chromatograms revealed a good coincidence between stability in vivo and in tissue homogenates. For both radiolabeled peptides, a common cleavage site has been identified between the Asp^{7}–Asp^{8} at the echistatin portion.

The data obtained give important suggestions about possible approaches to improve the enzymatic stability of the peptide that might result in the overall pharmacokinetic enhancement of the corresponding radiolabeled compounds.

The project was funded by AIRC IG2020 (ID24528).

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Radiolabelled peptides targeting Gastrin Releasing Peptide Receptors for cancer theranostics

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There is increasing interest in the clinical application of receptor targeting radiolabelled peptides for cancer diagnosis and therapy. Imaging the distribution of these agents to visualise disease, quantify target expression and characterise biology of tumours is a main objective in early clinical development. The most sensitive and high-resolution imaging method in this field is positron emission tomography (PET). Well established chelator chemistry is available to couple peptides to radiometals such as the short-lived positron emitter ^{68}Ga commonly utilised for PET applications. When the imaging approach proves successful and high target to background delivery of these radiopharmaceuticals is achieved, labelling with different radiometals can be considered to use the same binding agents to deliver targeted radiation to cancer cells. The ß-emitter ^{177}Lu is the radionuclide most commonly utilised in this scenario. These so called "theranostic" pairs are being actively investigated by industry and academia [1]. To date the only clinically approved theranostic pair is a somatostatin receptors (SSTR) targeting agent known as DOTATATE. This conjugate is approved for clinical use to image and treat patients with SSTR overexpressing neuroendocrine tumours. The success of this approach has prompted attention to other peptide receptor systems of interest. Their potential as novel theranostic targets is being explored in other cancer types [2].

Gastrin Releasing Peptide Receptors (GRPRs), also known as Bombesin receptors, are overexpressed in a number of endocrine related cancers and in gastrointestinal stromal tumours (GISTs). Rare forms of GIST, driven by gene mutations that result in succinate dehydrogenase (SDH) deficiency, are tumours of particular interest as no effective treatments are available. A novel GRPR antagonist known as NeoB [3] is being developed as a theranostic agent. We conducted a pilot [^{68}Ga]NeoB PET imaging study in 12 patients with metastatic SDH deficient GISTs. 8 of 12 (66.7%) patients exhibited intense but heterogeneous [^{68}Ga]-NeoB uptake in tumour lesions, with variable tracer uptake both within and between lesions. Four patients (33.3%) displayed minimal or no uptake in tumour lesions. GRPR targeting with NeoB may have a theranostic role in patients with this rare disease.

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Screening in vivo digestion resistant whey peptides capable to stimulate enteroendocrine sensing receptors

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A novel promising strategy for type 2 diabetes and obesity management is the mobilization of the gastrointestinal hormone stores to control postprandial glucose levels and intake modulation. We have previously shown that milk protein digests and particular peptides thereof can stimulate GLP-1and CCK secretion from enteroendocrine cells by activating sensing receptors, and this effect highly depends on the amino acid composition [1]. Virtual screening using the intestinal calcium sensing receptor CaSR, one of the likely involved receptors, against short peptide sequences identified in human jejunal whey protein digests [2] has been performed.

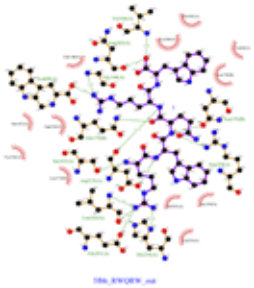

Figure 1. Molecular docking of peptide RWQWR

From 106 short peptides identified from β-lactoglobulin, α-lactalbumin, lactoferrin and bovine serum albumin, 64 sequences showed AutoDockVina scores over -7.5 and 19 over -8.5. A combination of the obtained scores and a range of diverse charge and hydrophobicity features has been used to select candidate sequences to experimentally evaluate the GLP-1 and CCK secretagogue effect. Four sequences with lengths between 5 and 10 amino acids, were capable to strongly induce GLP-1, a basic isoelectric point and high hydrophobicity being common but not necessary features of the most active forms. The proposed strategy linked to the study of the interaction sites is expected to uncover the characteristics needed to induce hormonal secretion that could be used in future screening studies.

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Peptide-bismuth bicycles

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Bicyclic peptides arise as next-generation pharmaceuticals.[1] We introduce peptide-bismuth bicycles as a new class of constrained peptides.[2] Like currently used alkylating agents,[3] bismuth(III) can link three thiols in linear peptide chains. Unlike conventional methods though, peptide-bismuth bicycles form instantaneously and selectively at physiological pH, enabling in-situ access to highly constrained peptides, directly in biological assays. Two screening campaigns, against viral proteases,[4,5] revealed a lead compound which was up to 130 times more active and up to 19 times more proteolytically stable than its linear congener. Compounds are stable at physiological pH over several weeks and tolerate glutathione at 100-fold access over two days at 37°C. Subsequent studies with rhodamine-labelled analogues revealed a potent series of bicyclic peptides that penetrate human cells at concentrations as low as 0.01 µM without significant cytotoxicity at 10 µM. In comparison to their linear precursors, peptide-bismuth bicycles enter mammalian cells 10 times more effectively and outcompete known cell-penetrating peptides such as Tat49-57 by more than an order of magnitude.[6]

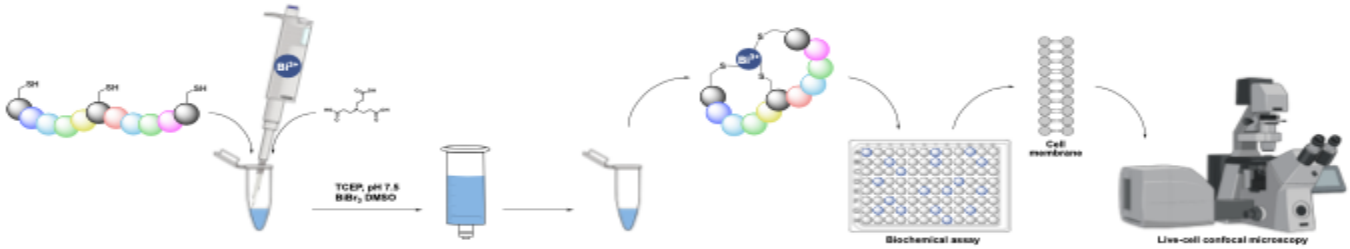

Figure 1. Facile approach for the generation of cell-penetrating peptide-bismuth bicycles.

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Desulfurative Fluorination: an efficient methodology for late-stage introduction of ^{19}F or ^{18}F in amino-acids, peptides and biomolecules

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Herein we report a new fluorination methodology that is fast (completed in minutes), compatible with a vast array of functional group present on peptides and oligonucleotides and is high yielding. Therefore, the technology is ready to be applied in the "late stage" introduction of ^{19}F or ^{18}F on amino-acids, peptides, proteins and oligonucleotides without the use of protection deprotection steps.[1] Introduction of fluorine on biomolecules is an important chemical transformation which can be exploited to (i) impart specific conformational properties;[2] (ii) alter the binding of small molecules and peptides to receptors via electrostatic (non H-bonding) interactions;[3] (iii) decrease the metabolic fate of bio-actives.[4] Furthermore, ^{19}F is an ideal nucleus for the determination of binding via Nuclear Magnetic Resonance (NMR)[2] meanwhile ^{18}F is considered the most "user-friendly" isotope for Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT).[2]

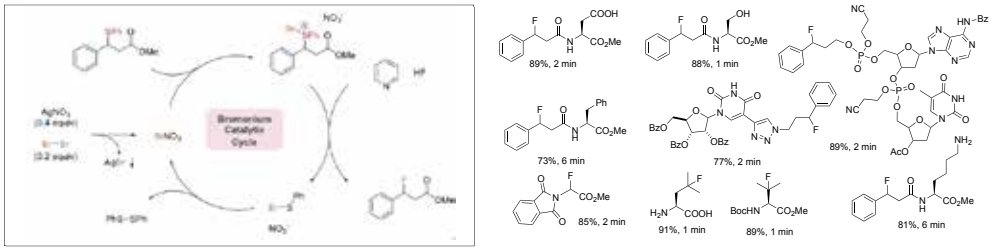

Figure 1. Reaction Mechanism and selected products available via desulfurative fluorination

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Development of rapeseed meal-based biomaterials plasticized with his own protein-hydrolysates

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Plastic materials have provided significant benefits to society due to wide-ranging applications and low cost. However, uncontrolled production and inadequate waste management have led to severe environmental problems, including the accumulation of plastic waste and microplastics in landfills and marine environment.[1] Therefore, developing sustainable alternatives to traditional fossil-based plastic has become a pressing challenge. Bioplastics derived from coproducts or waste materials offer the advantage of exploiting pre-existing, underutilized resources. Among these, natural biopolymers, specifically proteins, emerge as an attractive alternative to petroleum-based materials, both in terms of potential applications and environmental impact.[2] The objective of this study is to explore the use of rapeseed meal as a matrix for the development of cost-effective, biodegradable composites with potential industrial applications. Particularly, we introduced a novel method for the preparation of a flexible biomaterial derived from raw, protein-rich rapeseed meal, stabilized using appropriate interactive additives via compression molding. Mechanical properties have been significantly improved by addition of denaturing and disulfide bonds reducing agents which induce protein denaturation during thermal processing, thereby enhancing the strength and toughness of the final materials.[3] Furthermore, this research extends its focus to the proteolysis of rapeseed proteins using specific proteases directly on rapeseed meal, generating peptide mixtures. A preliminary screening of the hydrolysates was conducted via RP-HPLC and MALDI-TOF-MS, revealing the formation of hydrophilic peptides with masses ranging between 1000 and 1500 Da. These peptides are strategically used as additives in protein-rich materials, with the aim of achieving a full eco-sustainability. Tests are underway to evaluate their impact on material properties. The achieved results will contribute to the green transition achieving the goals of the European Green Deal.

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Aib-containing peptides as plant protection products

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Plant diseases are essentially controlled in the field with the use of copper-based products and antibiotics, raising environmental and safety concerns. Trichogin GA IV, a non-ribosomal, 11-residue long peptide naturally produced by the fungus Trichoderma longibrachiatum has the ability to perturb membrane integrity and permeability, thanks to its well-defined helical conformation stabilized by the presence of three Aib (α-aminoisobutyric acid) residues in its sequence. Recently, trichogin analogs modified at the level of specific residues were designed to be water-soluble and active against plant pathogens[1]. Here, we report on the role of glycine-to-lysine substitutions and of the presence of a C-terminal leucine amide on bioactivity against a variety of fungal and bacterial phytopathogens, affecting a wide range of crops worldwide, including tomato and kiwifruit. Our results show that trichogin GA IV analogs containing two or three Gly-to-Lys substitutions are highly effective in vitro, displaying minimal inhibitory and minimal bactericidal concentrations in the low micromolar range. Some lysine-containing analogs were able to significantly reduce bacterial titers and symptom development in infected plants. Our results point to a positive correlation between the number of lysine substitutions and the antimicrobial activity. This correlation was supported by microscopy analyses performed with mono-, di- and tri-Lys containing analogs that showed a different degree of interaction with microbial cells and ultrastructural changes that culminated in cell lysis (Figure 1).[2]

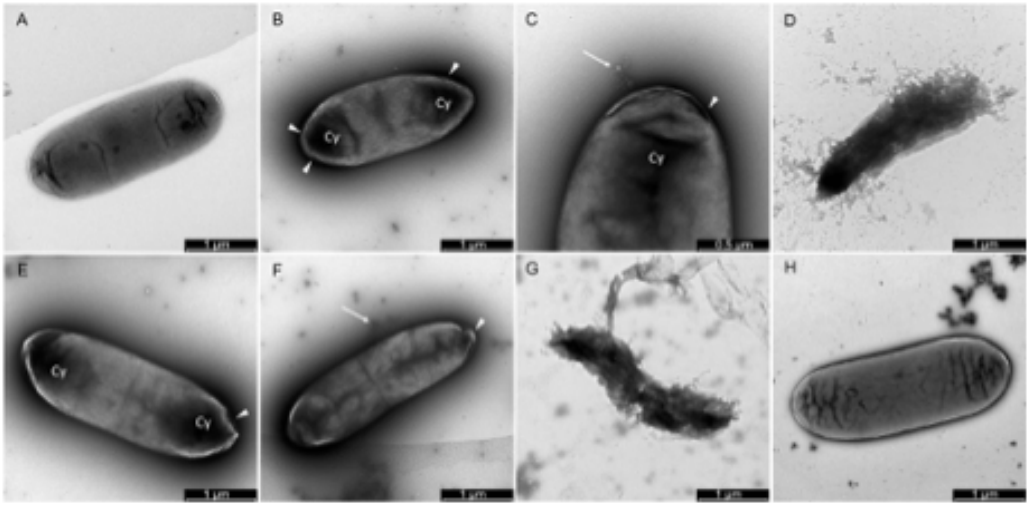

Figure 1. Representative transmission electron microscopy micrographs of Pseudomonas syringae pv. tomato (Pst) co-incubated with trichogin GA IV analogs. Untreated Pst cell (A), Pst cell co-incubated 2 h with the analogs K2,5,9 (B, C and D), K5,6 (E, F and G) or K6 (H) at the concentration of 10 μM.

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Antibacterial and anti-inflammatory activity of branched peptides derived from natural host-defence sequences

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Antibiotic resistance is a major global health threat, necessitating the development of new treatments and diverse molecules to combat severe infections and preserve the efficacy of existing drugs. Antimicrobial peptides (AMPs) offer a versatile arsenal against bacteria, and peptide structure branching can enhance their resistance to proteases and improve their overall efficacy. A small library of peptides derived from natural host defence peptides and synthetized in a tetrabranched form were selected against E coli. Six selected branched peptides were further studied for antibacterial activity against a panel of strains, biofilm inhibition, protease resistance and cytotoxicity. Their structure was predicted computationally and their mechanism of action was investigated using electron microscopy and fluorescent dyes.

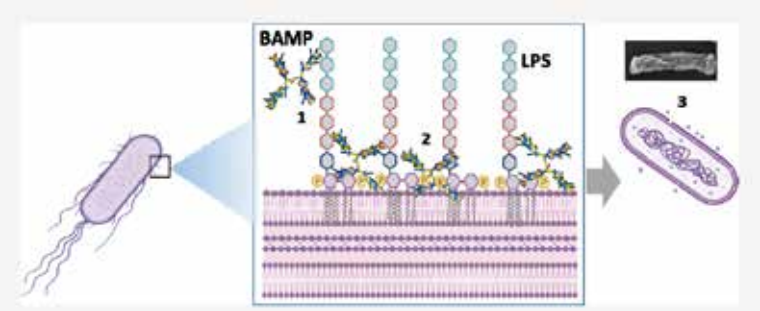

Figure 1. Model of the BAMPs 3-step mechanism of action: 1-electrostatic interaction of positive charges on the peptide and LPS, 2 -embedding into the membrane, thanks to amphipathic helix structure, and 3-loss of membrane functionality.

The peptide BAMP2 showed promise in a mouse skin infection model, indicating potential for local infection treatment.

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Comparison of antimicrobial assays to analyse bioactive low molecular peptide derived from Lucilia sericata maggot secretion

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Introduction. Medical maggots are being used in wound debridement therapy as they can accelerate the multidimensional wound healing by feeding on the necrotic tissue, promoting tissue regeneration and disinfecting the wound. [2] Studies have shown that maggot secret antimicrobial peptides and compounds that facilitates wound healing and eliminate infecting bacteria. Many researchers are interested in isolating and characterizing these compounds and peptides from maggot whole bodies or salivary gland secretions. Little is known about small molecular weight peptides composition and their antibacterial activity. [2,3]

Method. In this study small molecular peptides <3 kDa were extracted from Lucilia sericata maggot that were induced with S.aureus. The extract was fractionated via size exclusion chromatography from FPLC. Obtained fractions were tested against Gram positive and Gram negative reference bacterial strains using agar-based radial diffusion assay and broth-based microtiter cell viability assay.[4]

Result. Nine fractions were obtained through size exclusion chromatography namely, WF0-8. Obtained fractions have shown potential bioactivity against reference bacterial strains. Fraction WF2, in particular, exhibited a reduction in cell viability by 56% (±0.4), 54.8% (±0.1), 52.43% (±2.2) against Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus epidermidis respectively as determined by the microtiter broth-based assay. Furthermore, only fractions WF1 and WF4 showed a zone of inhibition against P. aeruginosa, in the agar-based radial diffusion assay.

Conclusion. The results suggest that both agar-based and broth-based assays should be used to examine the antibacterial effectiveness of maggot secretion, as they provided different outcomes. This may indicate a change in chemical interaction due to the peptide composition of the fractions in different environments.

Keywords Lucilia sericata; maggot therapy; antimicrobial peptides; angiogenesis

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Design, synthesis and characterization of anti-TNFα peptides for pharmaceutical purposes

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Tumor necrosis factor-alpha (TNF-α) is a potent pro-inflammatory cytokine exerting pleiotropic effects on various cell types. TNF-α is translated as a 26 kDa type II transmembrane protein that assembles into homo-trimers displayed on the cell surface of macrophages, lymphocytes and other cell types. Each subunit of the membrane trimer is cleaved at the cell surface by a specific enzyme, producing a soluble form of TNF-α trimer with a subunit mass of 17 kDa. It plays a critical role in the pathogenesis of chronic inflammation and chronic inflammatory diseases, such as rheumatoid arthritis (RA) and Crohn's disease, psoriasis, psoriatic arthritis and ankylosing spondylitis. Given its pivotal role in many inflammatory diseases, TNF-α has been proposed as a therapeutic target for a number of diseases [1].
Here we describe a rational design of peptides based on the structure of TNF-α aimed at inhibiting protein trimerization and binding to its receptors. This strategy allowed the select a panel of peptides the best of them were characterized for cytotoxicity in human cells, for its efficacy in maintaining cell survival under toxic stimulation of inflammatory triggers, and for the ability to reduce cytokines gene expression generally present in inflammatory processes.
The peptides here presented are part of a preclinical development process for the selections of novel lead compounds for the set-up of new therapeutic options for diseases where TNF-α is strongly involved.

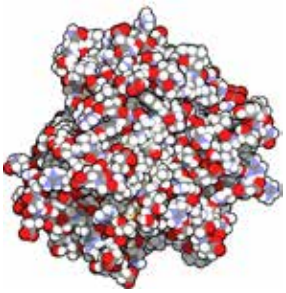

Figure 1. Trimeric structure of soluble TNF-α

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Effect of acidic pH and of a single amino acid substitution on the antimicrobial/antibiofilm activity of the peptide Esc(1-21)

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The antimicrobial peptide Esc(1-21) was previously characterized for its potent anti-Gram negative activity and it has been shown to have excellent biological activity and low cytotoxicity both in vivo and in vitro. However, its activity against Gram-positive strains such as Staphylococcus aureus is poor. In order to increase the content in alpha-helix of the peptide and its biostability, the Gly^8 was replaced with a residue of α-aminoisobutyric acid (Aib).[1] The obtained peptide, named [Aib^8]-Esc(1-21), resulted to be active against both planktonic and biofilm form of S. aureus, with a rapid bacterial killing, suggesting a membrane-perturbing mechanism of action. Structural analysis of [Aib^8]-Esc(1-21) evidenced that the improved biological activity is the consequence of a combination of (i) higher biostability, (ii) higher α helical content, and (iii) the distorted helix, bent in correspondence of Aib^8. In parallel, considering that the microenvironment of the infection is usually characterized by an acidic pH and this could influence the activity of antimicrobials, Esc(1-21) was tested at different pHs against a panel of Gram-negative strains, including Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae. We found that Esc(1-21) retained its activity against the sessile form of these bacteria grown in media with a neutral pH, and showed similar or higher effectiveness against the biofilm form of bacteria grown in acidic media, simulating a cystic fibrosis-like acidic microenvironment, compared to physiological conditions.[2]

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Acknowledgments

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Sar study of the human host-defence Peptide ll-37

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Antimicrobial peptides an important component of innate immunity that act as a first line of defence against the occurrence of infections. Among these natural antibiotics, those of the cathelicidin family [1], which includes LL-37[2] in humans and RL-37[3] in the primate Macaca mulatta, are intensively studied because of their diverse properties. For example, LL -37 can adopt an amphipathic helical structure in physiological solutions and then oligomerizing; RL-37, on the other hand, remains monomeric and disordered under the same conditions. The occurrence of intramolecular salt bridges could play an important role in determining the structure which is adopted[3]. These important structural aspects are highlighted by predictive applications, allowing us to identify a new stabilized analogue of RL-37 or destabilized analogues of LL -37 to probe positional effects in their sequences underlying their structural behavior. We were able to evaluate the effect on structural stability by monitoring thermal denaturation in plasma mimetic buffer, using circular dichroism assays. These data were compared with functional assays of efficacy against bacterial cells or cytotoxicity to host immune cells. Selected analogues were also evaluated for the mechanism of antimicrobial action monitoring permeabilization of bacterial membranes to fluorescent probes[3] and other roles in immunity, such as cytotoxicity towards, or triggering of proinflammatory M1 macrophages. These studies have shown a direct correlation between the structure and behavior of the peptides in terms of their direct antimicrobial and host-mediated activity.

Keywords: LL-37; RL-37; AMPs; Structural Requirements.
Funding: This study was funded by PRIN2022 - "PrAMP4KleBI"
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NGF mimic cyclic peptide demonstrates copper ionophore capability and Ctr1/CCS-driven signaling

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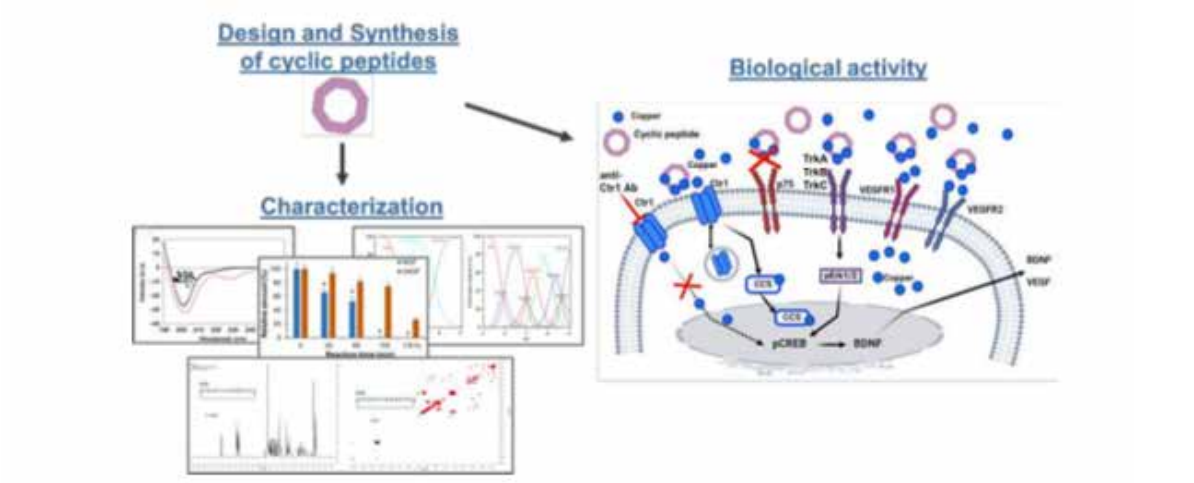
The neurotrophins (NTs) such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), are growth factors that regulate cell survival, differentiation, synaptic plasticity, neurite outgrowth and regeneration, in nervous system. Recent findings on NTs structure and their receptors, encourage now the design and development of small molecules that mimic NT activities and induce the synthesis of endogenous NTs. Considering that N-terminus domain is crucial for the binding selectivity/activation of their receptors, and that cyclization slows down degradation and facilitates cell membrane permeability, we design, synthetized and characterized three NTs cyclic peptides (cNTs): the first 13 residues of NT-3, 12 residues of BDNF and 14 residues of NGF.



The biological effects of cNTs were tested on neuronal model cell lines. Our results demonstrated that cNT peptides activated phosphorylation of Trk receptors, VEGFR and CREB, neurite sprouting, BDNF and VEGF expression. Since copper is a key factor in the neuropathologies development, we investigated the role of cNTs on cell metal homeostasis. Our findings might open new horizons proposing cNTs as promising therapeutic molecular entities which conjugate the advantages of small molecules with the ability to activate the trophic factors pathways also in response to a copper stimulus.

Conformationally interconnected amides: a remote transfer of stereochemical information over 40 bonds

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Stereochemistry can be viewed as a chemical means of transferring specific information during a synthetic process. Conventional methods of asymmetric synthesis of organic compounds employ the use of chiral catalysts or stoichiometric amounts of chiral substrates. In both cases, the reacting components must be in proximity to the entity that introduces chirality into the system. Thus a close spatial contact between the control center and the reaction site is required, and it can be expected that 1,2- or 1,3-asymmetric induction (where the two sites are separated by one or two bonds) normally gives high levels of stereoselectivity (Figure 1).[1]

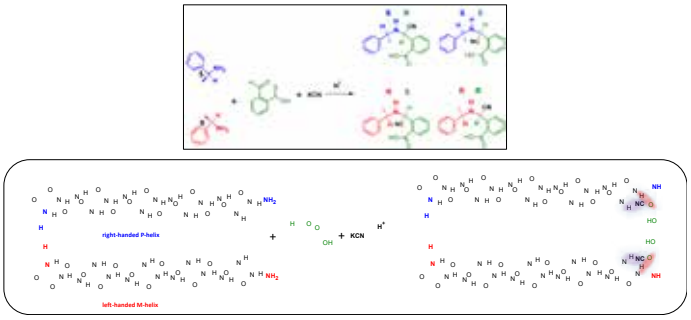

Figure 1. Upper part: example of strereochemical proximity effect in Strecker reaction. Lower part: our work.

In addition to our previous works,[2] we confirmed that stereochemical information can be transmitted efficiently across distances much greater than those commonly involved in asymmetric syntheses. In these new studies we establish that a chiral information can be delivered from a remote controller through numerous connections of achiral atoms,up to 40 bonds distances, with remarkable selectivity effects, to give efficient enatiomeric and diasteromeric excesses in chemical condensations. To study the diastereo- and enatio-selectivity, poly-Aib (Figure 1) or an appropriately modified version of them were used as catalysts, and three model of chemical reactions, all able to generate important building block for pharmaceutical-active molecules, were taken into consideration: (i) an aldol condensation, (ii) an epoxidation and finally, (iii) the Strecker reaction.

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Rationally designed peptide conjugates in Alzheimer Disease: implications for diagnosis and therapy

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Alzheimer's Disease (AD) is a central neurodegenerative disorder characterized by behavioural disturbance and progressive cognitive impairment. It is the major cause of dementia affecting prevalently elderly individuals worldwide. Unfortunately, there are challenges in diagnosing the early signs of the disease especially at the molecular level.

Studies on the KLVFF peptide, a short Aβ fragment consisting of the amino acid residues of Aβ16–20, have demonstrated its ability to block the conformational transition from the native monomeric state of Aβ to the toxic β-sheet conformation. We synthesized a variety of bio-conjugates with this sequence.
In this communication an overview of the design principles of the peptide conjugates, their neuroprotective activity, their capability in detecting Aß peptide in solution alongside their pharmacological activities are described in terms of potential use of these compounds as theragnostic agents.

Proline-rich antimicrobial peptides as lead compound for the development of new anti-mycobacterial drugs

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The proline-rich antimicrobial peptides (PrAMPs), are gaining attention as candidates for the development of new therapies against antibiotic-resistant bacterial infections. To kill bacteria, PrAMPs do not disrupt their membranes but instead enter the bacteria cytosol, primarily via the inner membrane transporter SbmA. Once inside, they bind to bacterial ribosomes and inhibit protein synthesis, leading to bacterial cell death. Given these desirable attributes, we investigated whether the PrAMPs Bac7(1-35), Bac7(1-16), Bac5(1-31), Bac5(1-25), and Tur1A [1]. These peptides could be active and maintain the same advantageous mechanism of action in more difficult pathogens with SbmA transporter orthologues. This study focuses on mycobacteria, microorganisms causing infections represent a serious threat to global health, particularly with the increasing incidence of drug-resistant strains, such as for Mycobacterium tuberculosis [2]. The discovery of new anti-mycobacterial drugs is hindered by several factors, including the slow growth rates or by the dangerousness of most of these microorganisms [2]. Consequently, the use of non-pathogenic and fast-growing mycobacteria, such as Mycobacterium smegmatis as a model, is helpful for the study of new dedicated drugs [3]. Here, we optimised the growth conditions of M. smegmatis in GYM mediums to ensure unexpensive but reliable and reproducible results in antimicrobial assays. Subsequently, PrAMPs were tested for their anti-mycobacterial activity. We demonstrate that peptides Bac7(1-31) and Bac5(1-25) can inhibit the growth, not only of the model organism M. smegmatis, but also of M. tuberculosis. The potential of PrAMPs to fight drug-resistant strains of mycobacteria could significantly improve tuberculosis treatment outcomes, and PrAMPs could emerge as a new class of antimycobacterial agents, gaining the fight against one of the world's most stubborn and deadly infectious diseases.

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Understanding peptide-protein binding based on GRID-MIFs and machine learning

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Peptide-protein interaction systems play a key role to tackle the onset of several diseases such as cancer and neurodegenerative pathologies. The spread of artificial intelligence is supporting the understanding of peptide-protein molecular recognition although the identification of peptide interacting cavities is still an open challenge. Based on an in house non-redundant collection of high- quality 3D crystallographic structures, we explored the property space of peptide-protein complexes by employing interpretable 3D GRID-MIFs descriptors. The GRID-MIF energetic distribution of the most frequent peptide-protein residue pairs at the peptide-protein interface has been carried out in order to study the peptide affinity-enhancing interactions compared to the protein-protein systems.[1] Subsequentially, an innovative machine learning based model has been developed demonstrating to be highly predictive in detecting the putative protein binding regions of small peptides (Figure 1).

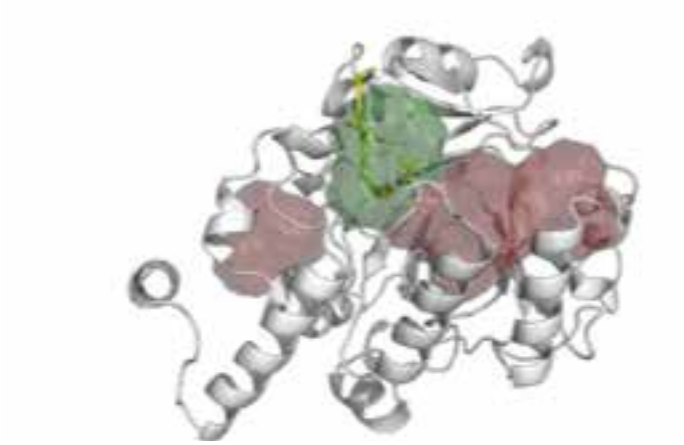

Figure 1. Example of pockets detecting in a peptide-protein complex by using machine learning based model.

Based on 3D GRID-MIF molecular descriptors, a clustering algorithm integrated with a Linear Discriminant Analysis-based protocol implemented in BioGPS software[2] has been employed to detect actual interacting druggable peptide regions at peptide-protein interface with respect to the rest of target surface. Our model has been successfully tested on two high-quality external benchmarks of peptide-protein pockets to be effective to further run peptide-protein virtual screening campaigns [3].

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Structure-Activity Relationship Study of β-Hairpin Peptides

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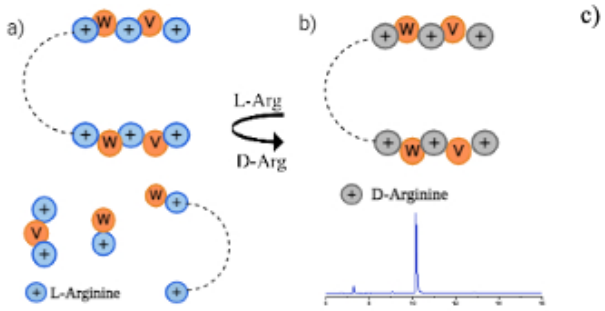
The increasing resistance of pathogenic microbes to antibiotics poses a major threat and calls for methods to improve the pace of antibiotic discovery. Antimicrobial peptides (AMPs) are effective against antibiotic-resistant bacteria in many cases. The peptide SAJO-2 developed by Sarojini and co-workers represent a tryptophan zipper-like motif involving a central D-Phe-Abz unit, which is a peptidomimetic beta turn including the conformationally inflexible ortho-aminobenzoic acid.[1] By fine-tuning the intrinsic hydrophobicity of SAJO-2 by distinctive degrees of fluorination, Chowdhary and co-workers enhanced antimicrobial activity; however, undesired proteolytic degradation by β-trypsin was also enhanced. [2]



Peptide	S. Typhimurium ATCC 14028	E. Coli ATCC 25922	E. Faecalis ATCC 29212	S. Aureus ATCC 29213	C. Albicans IMT 9655
SAJO - 2	32	16	128	64	32
SAJO - D	32	16	1024	256	32
SAJO -2DR	16	32	1024	256	32
SAJO -1DR	32	32	1024	128	32
SAJO -PfpGlyDR	32	32	128	64	32

Figure: a) Proteolytic degradation of SAJO-2 by β-trypsin, b) Proteolytic degradtion of D-amino acid substituted SAJO analogues by β-trypsin followed by HPLC, c) MIC analysis of different library members

This work involves an effort to improve the proteolytic stability and antimicrobial activity of SAJO-2 by the introduction of D amino acids. The incorporation of D enantiomers into the peptide backbone introduces structural changes due to an altered stereochemistry that prohibits sufficient enzyme-substrate binding, beneficial for enhancing peptide stability. In the present study, we synthesized D-arginine substituted analogues of SAJO-2 including a pentafluorinated amino acid to determine the change in stability with fluorination. Satisfyingly, all peptides were found to be stable against enzymatic degradation and the antimicrobial activity of D-analogues was comparable to their L-counterparts against E. coli, S. Typhimurium and fungal pathogen C. Albicans. Therefore, the incorporation of D-amino acids into the design of SAJO-2 analogues offers great potential for improvement of the therapeutic profile of the peptidomimetic to defend against the infection of microbes by substantially increasing the biological half-life.

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Salicylaldehyde-Tagged Peptides for the Reversible-Covalent Engagement of Protein Lysine Residues

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The design of synthetic small drugs capable of forming a covalent bond with the target protein of interest typically aims at stabilizing the drug-protein complex and to improve the pharmacological effects. The amino acid Lysine (Lys) is highly abundant in the proteome and one of the most frequent residues on the outer structural layers of proteins. Due to this high frequency, the derivatization of synthetic ligands with aldehyde tags capable of imine bond formation with Lys ε-amino groups may represent a general strategy for the discovery of potent small-molecule inhibitors.
Ortho-hydroxy aldehydes such as pyridoxal or salicylaldehyde (SA) derivatives have been used to form imines in aqueous media, stabilized by an intramolecular H-bond between the imine N atom and the ortho-phenolic proton. By virtue of this reactivity, SA derivatives are being installed into various classes of protein ligands, aimed at the reversible-covalent engagement of protein Lys residues.[1,2]
This talk will describe our recent contribution to this field, with focus on the installation of the Lys-engaging SA module into peptide ligands.[3,4]

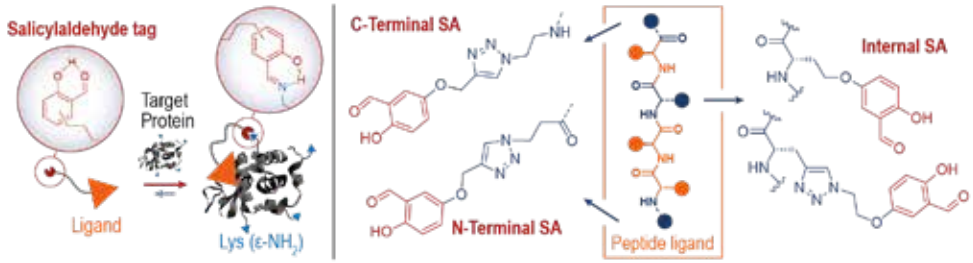

Figure 1. Left: Binding mechanism of a reversible-covalent ligand equipped with a salicylaldehyde (SA) tag. Ideally, SA forms a remarkably stable imine bond with a Lys(ε-NH_2) residue proximal to the ligand binding site. This covalent ligand-protein connection is stabilized by a H bond between the OH phenolic proton and the imine N atom. As a result, the final ligand-protein complex is stabilized by a combination of non-covalent ligand-protein interactions and the covalent imine bond. Right: Current options for the SA tag installation at different peptide positions, recently developed by our group.

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In silico mapping and proteomic analysis of digestion resistant allergenic peptides in Arginine Kinase from Hermetia illucens

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Arginine kinase (AK) plays a key role in invertebrate energy metabolism by maintaining constant ATP levels. However, it is also a major allergen in insects and crustaceans and can cause IgE-cross-reactivity in individuals sensitized to similar proteins. Bioinformatics analyses were performed to predict possible linear and conformational epitopes, and in vitro simulated gastrointestinal digestion (SGD) was performed to investigate a significant reduction of linear epitopes resulting in lower allergenicity of AK from Black soldier fly (BSF). The sequence encoding for H. illucens AK was identified by tBLASTn algorithm using the allergenic AK sequence from white shrimp (L. vannamei, GenBank ABI98020) as a query by NCBI database, which led to the identification of LOC119647699 gene. Then, linear epitope mining with non-redundant sequences from insects, crustaceans, Arachnida and molluscs was retrieved from the Immune Epitope Database (IEDB) and analyzed for their inclusion in the BSF AK sequence, considered both as a whole protein and as hydrolysate after SGD, by an ad hoc python script. The conformational epitope mining had been performed by IEDB, searching for “discontinuous epitope” for the AK of insects, crustaceans and arachnids. The amino acid substitution had been scored according to Grantham substitution matrix (dissimilarity above a score of 50). The isoform AK X4 (XP_037904712) from H. illucens was identified as the sequence most similar to AK enzymes recognized as allergens in crustacean and mites, with sequence identities of 77–88%. The sequence regions experimentally identified as IgE epitopes in the AK of mud crab S. paramamosain turn out to be highly conserved in all aligned sequences, suggesting that AK X4 might trigger cross-reactivity. Afterwards, to study the effect of the digestion process on the integrity of putative linear IgE epitopes, AK X4 was subjected to SGD in duplicate[1], and then studied by LC-HRMS. 68 and 56 peptides were identified by HRMS in the two digestion replicates, for a total protein coverage of 69 and 63%, respectively, and were analyzed by bioinformatic tools. Only 1 out of 18 epitopes (WPTGRGIYHNDNKTF, IEDB ID 418907) was still detected after the SGD. Furthermore, conformational epitopes are susceptible to breakdown in acidic and proteolytic environments, such as those occurring during SGD. In conclusion, the possible allergenicity of AK should be taken in consideration when dealing with Novel foods containing H. illucens or its derivatives.

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An in vivo approach to study the inhibitory effect of Antimicrobial Peptides on bacterial protein synthesis

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The spread of antibiotic-resistant pathogens has driven the search for new antimicrobial agents, such as antimicrobial peptides (AMPs). However, for any molecule considered for antibiotic development, its antimicrobial mode of action must be well understood. Unlike most AMPs, which permeabilize bacterial cell membranes, proline-rich antimicrobial peptides (PrAMPs) kill bacteria by impairing protein synthesis. To investigate disruptions in key bacterial biosynthetic pathways, such as translation and transcription, in vitro approaches or in vivo studies using Escherichia coli cells with radioactive amino acids or nucleosides have traditionally been employed.
In this study, we evaluated the suitability of Bioorthogonal Non-Canonical Amino Acid Tagging (BONCAT) as a versatile alternative to radioactive analogues for studying the inhibition of newly synthesized proteins by non-lytic AMPs. BONCAT enables the in vivo incorporation of alkyne-carrying amino acid analogues into newly synthesized proteins, which can be detected via click-chemistry conjugation with an azide-labeled fluorophore.
Using BONCAT, we confirmed the inhibitory effects of PrAMPs Bac7(1-35), Bac7(1-16), and B7005 on protein synthesis in living E. coli and K. pneumoniae cells. Additionally, we were able to distinguish between reversible and irreversible inhibition of protein synthesis. This method can be easily extended to study the effects of antimicrobial peptides on both Gram-negative and Gram-positive bacterial pathogens, as well as eukaryotic cells.

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Peptidic products from Chinese traditional 'worm' medicines

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Worms such as wasps, horseflies, cockroaches, ground beetles, scorpions, centipedes, spiders, leeches, frogs, toads and snakes, which are from different taxonomic animal groups, have a long history of traditional use as medicines in China. However, their pharmacological constituents and mechanisms are largely unknown. The peptidic products of Chinese traditional 'worm' medicines are mainly from the secretions of exocrine glands such as the skin, salivary and venomous glands. We recently identified and characterized pharmacological components from Chinese traditional 'worm' medicines and investigated their pharmacological mechanisms, revealing that most of them are peptides targeting receptors, enzymes, ion channels and other membrane components. More than 2000 bioactive peptides have been identified from the 'worms', especially from venomous animals. Highly specific and extremely diversified peptides from animal venoms can rapidly target key physiological elements, such as the nervous, cardiovascular and immune systems. These molecules have proven excellent amenability to drug discovery and development. We have successfully developed antimicrobial peptides from Krait snakes and proteinase inhibitors from forest leeches into novel drugs, demonstrating the transformative potential of this research. This exploration into the peptidic world of worm medicines presents a compelling opportunity to revolutionize therapeutic approaches.

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18th Naples Workshop on Bioactive Peptides

PEPTIDES IN THE NATIVITY

COMPANIES

Macrocycles as inhibitors of protein/protein interactions: the PCSK9/LDL receptor complex case study

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In drug development targets have been historically limited to receptors which function by interacting with small substrates that can be engaged by 300-500 Da synthetic drugs in a highly specific manner. With deeper understanding of complex biological mechanisms, which function through interactions mediated by a relatively large surface area, it has become apparent that traditional drug discovery approaches leave a larger number of disease mediators unavailable to therapeutic intervention.

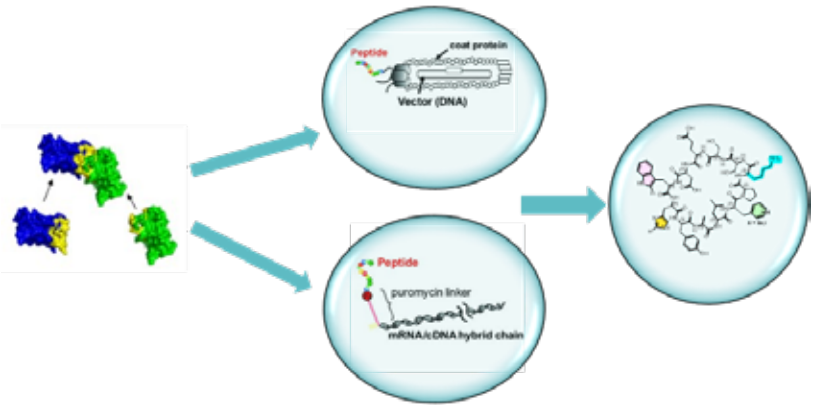

Figure1. Phage and mRNA peptide libraries as sources for macrocycle ligands to target protein/protein interactions

Peptides and in particular macrocycles offer a unique opportunity and represent a powerful chemical modality for large surface area receptor interactions. Phage and mRNA display peptide libraries are unique sources for macrocycles as protein ligands (Figure 1). One protein target is PCSK9 a key regulator of plasma LDL-cholesterol levels and a validated target for hypercholesterolemia. I will be sharing our experience on the development of a macrocycle inhibitor of PCSK9 that is now in phase 3. This study validates the approach that a particularly wide and shallow protein surface, can be targeted by a macrocycle optimized from an hit originally selected from a mRNA display library.

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Novel Orthogonal Protecting Groups for Amino Acid Side Chains

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We are presenting a novel selectively cleavable side chain protecting group for lysine which can play an important role during peptide synthesis, e.g., for modification of the lysine side chain on the synthesis resin. As Trityl-based protecting groups such as Mtt or Mmt are incompatible with strongly acid-labile resin linkers like Trt, amino acid derivatives carrying the hydrazinolysis-susceptible protecting group Dde or ivDde[1] are frequently used for the synthesis of branched, cyclic, or side chain-modified peptides. However, Dde might migrate to free lysine epsilon-amino groups (“scramble”) [2], and for the more robust ivDde, total removal is hardly possible. A newer group for the orthogonal protection of amino groups is MeDmb (methyl dimethylbarbituric acid)[3]. In our study we present the possibilities and limitations of novel lysine Dmb derivatives (MeDmb, EtDmb, ivDmb) and compare their properties with the existing protecting groups Dde and ivDde.

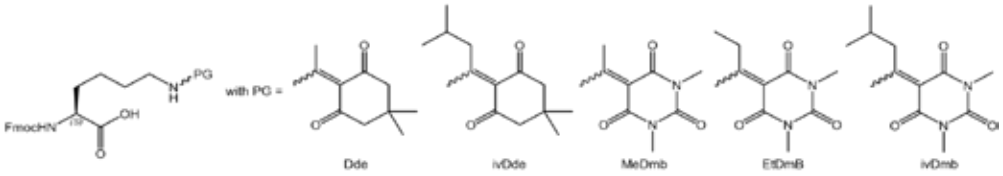

Figure 1. Dde- and Dmb- based protecting groups for amino functions

Furthermore, we are presenting new safety-catch protecting groups. Among these, the arylalkyl-sulfoxides Msz [4-(methylsulfinyl)benzyl-], MsbH [bis(4-(methylsulfinyl)benzhydryl)-], Mmsb [2-methoxy-4-(methylsulfinyl)benzyl-] and Msib [4-(methylsulfinyl)benzyl-] have proven to be useful for masking functional groups with free electron pairs, making them suitable to protect the side chains of Lys, Tyr, Asp, Glu, Cys, Ser, and Thr, and of other building blocks.[4] Especially in the case of cysteines, these protecting groups are a valuable addition to the peptide chemist’s toolbox.

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Innovations in Sustainable Peptide Production

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Increased global demand for peptide therapeutics has placed a renewed emphasis on improving the efficiency and sustainability of peptide production. This presentation will highlight workflow and methodological improvements for high-throughput peptide synthesis (96-well plates), enabling more efficient access to longer sequences at higher purities. 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 synthesis1. Finally, a new HPLC process for peptide purification that completely eliminates the use of acetonitrile in place of ethanol will be demonstrated. 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.

Bioactive peptide hydrolysates in beauty industry: Sources, chemical characterization and biological properties

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Bioactive peptides (BPs) are specific protein fragments that possess a wide range of positive effects in living organisms. BPs are largely used in the skin and hair care, as they are capable of triggering signal transduction mechanisms in skin cells, leading to the activation of important gene regulators. Beside the biological activity, BPs desires characteristics by formulators is their ability to penetrate the skin easier and to reach the deeper skin layers. This presentation will explore compositions and applications of bioactive peptide hydrolysates used as cosmetic active ingredients. Specifically, three different products were obtained by the application of three sequential enzyme-assisted extraction (EAE) processes to different vegetal sources mainly including plant cell cultures of Nicotiana sylvestris (NS), the microalga Arthrospira platensis (AP) and rice bran (RB), a by-product derived from rice industry. After EAE extraction, peptide profiles of mixtures were characterized by LC-ESI MS/MS and the extracts were tested in skin cells and hair follicle cells and hair shaft in order to investigate their specific biological properties. Our results showed that in NS extract were identified 57 peptide sequences and about 80% of identified peptides showed a sequence with 8-15 amino acids with a molecular weight comprises between 800 and 1500 Da. Peptides from NS showed a high amount in proline and hydroxyproline in line with their collagen-like biological activities. The peptide mixture derived from NS cell cultures, is active at 360° in counteracting aging signs on skin. In AP extract were identified 26 peptide sequences derived from the hydrolysis of phycocyanin. The molecular weight distribution of AP peptides mainly ranged from 1000 to 2000 Da (more than 55% of all AP peptides was in this range) thus indicating that a large fraction of identified peptides was composed by 10-20 amino acids. Our data demonstrated that AP extract is effective in protecting skin cells form osmotic stress and improving hydration state of skin. Finally, 318 different peptides, mainly including anionic and cationic peptides, were identified in the extract from rice bran. About 90% of identified peptides showed a molecular weight comprises between 1000 and 2000 Da thus indicating that a large fraction of identified peptides was composed by 10-20 amino acids. Rice bran peptides are instead able to stimulates hair density and to protect hair from damages.
In conclusion, our data effectively demonstrated the correlation between the chemical profile and the resulting biological activities of three different hydrolysate obtained from natural vegetable sources.

A Quantum Algorithm for de novo Drug Design

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The identification of ligands for a given binding site typically involves screening extremely large databases, containing billions of compounds. An alternative approach is de novo design, in which a suitable ligand is constructed using a set of molecular building blocks. The search space grows exponentially with each building block added to the compound, making an exhaustive search using classical computing increasingly difficult and expensive.

Quantum computing offers a potential solution for that problem, as the number of states represented by the quantum computer grows exponentially with every added qubit, the quantum analog of the classical bit. Current quantum hardware is not yet strong enough to outperform classical computers but grows at an increasingly fast pace.

Here, a quantum encoding for de novo design is presented which builds upon an algorithm developed by Ghamari et al.[1] Low-energy configurations of the quantum encoding correspond to potential ligands. The model generates a coarse-grained polypeptide docking to a pocket in a protein. Low-energy configurations are obtained through a quantum annealer as well as using a classical optimizer. The algorithm was applied to a set of protein-peptide complexes obtained from the LeadsPep dataset.[2] Among the dataset, the eukaryotic protein LC8 was contained for which a dataset set of 110 experimentally known polypeptide binders are available.[3] The de novo generated polypeptide binders were compared to the experimentally known ones. The code successfully replicated key characteristics of experimentally identified binders without prior exposure to the dataset.

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Computer-based peptides library design and optimization to address new therapeutic needs

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Therapeutic peptides are highly specific, low in toxicity, and can target various diseases. [1] Computer-aided drug discovery (CADD) accelerates peptide identification, optimization, and validation by integrating computational models with biological data. Key methodologies include molecular docking, molecular dynamics simulations, and AI approaches. [2,3] CADD also addresses challenges like peptide degradation and poor bioavailability by designing stable peptide analogues. This synergy transforms drug discovery, making it faster and more cost-effective for treating specific pathological conditions. Leveraging experimental structural data within the Exscalate platform, [4] it was possible to optimize peptide structures to meet structural and physicochemical requirements suitable for specific therapeutic needs. X-ray crystallography methods were then employed to validate the computational hypotheses.

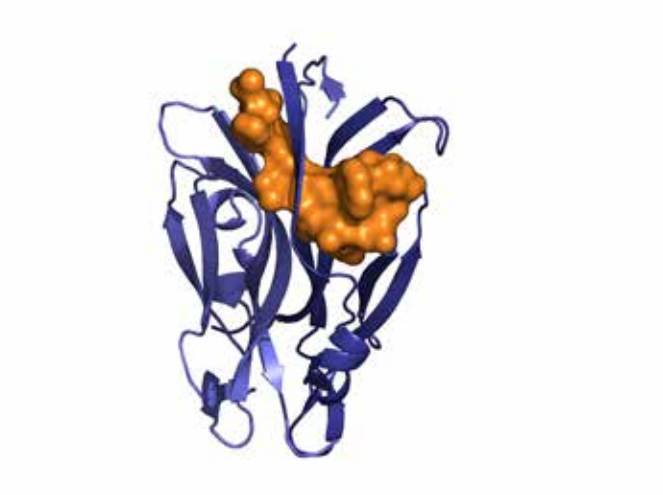

Figure 1. Crystal structure of an identified peptide (orange surface) binding the Interleukin homo-dimer 17A

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18th Naples Workshop on Bioactive Peptides

PEPTIDES IN THE NATIVITY

POSTER COMMUNICATIONS

Novel potent macrocyclic peptidomimetic inhibitors from HRV to SARS-CoV-2 Main Protease Inhibitors

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Viral proteases play an essential role in the viruses replication, and without a human counterpart making them primary targets for the development of effective and safe antiviral drugs. In the context of the COVID-19 pandemic, caused by SARS-CoV-2,[1] significant efforts in drug discovery have led to Nirmatrelvir,[2] the first covalent reversible peptidomimetic inhibitor of SARS-CoV-2 Mpro. Due to the high sequence conservation of the substrate binding site among the Orthocoronavirinae subfamily this protease offers an opportunity to develop pan-coronavirus inhibitors.[3]
Our approach to designing new Mpro inhibitors was inspired by macrocyclic peptidomimetics previously reported as inhibitors of the human rhinovirus (HRV) 3C protease.[4] Since both HRV and SARS-CoV-2 proteases recognize glutamine at the P1 position of the substrate, we decide to take advantage of macrocyclic structure to improve binding entropy and potency. We replaced the γ-lactam ring commonly found in Mpro inhibitors with proline, preserving the essential hydrogen bond interaction with His163 in the S1 pocket of Mpro active site, analogous to the interaction with His161 in the HRV 3C protease active site. Additionally, leucine was introduce at P2 to better fit the S2 pocket of SARS-CoV-2 Mpro active site.
Using solid-phase peptide synthesis (SPPS), we prepared three sets of novel macrocycles varying P3 capping groups and ring macrocycle sizes, exploiting a nitrile warhead for covalent reversible inhibition with the catalytic Cys145 of SARS-CoV-2 Mpro. The compounds exhibited nanomolar IC50 values in biochemical assays against SARS-CoV-2 Mpro, providing a promising starting point for further structural refinement aimed at developing a new class of macrocycles targeting SARS-CoV-2 Mpro active site.

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In-depth understanding of hCA IX/XII multitarget inhibitors targeting cancer cells

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Carbonic anhydrases (CAs) are ubiquitous enzymes, which catalyze one of the simplest physiological reaction, the inter-conversion of the carbon dioxide into bicarbonate ion and protons. In humans, fifteen CA isoforms have been described with very different sub-cellular localizations and tissue distributions. These isoforms have different physiological functions in different tissues and their absence or their malfunctioning can cause several pathologies (1). As a consequence, in the last years CAs have become interesting targets for pharmaceutical research. Most of the available pharmacological agents directed towards these enzymes and developed until now still need investigations since they possess many undesired side effects due to lack of selectivity for the different isoforms. By a bilateral cooperation between Italy and Poland we have identified and developed novel molecules targeting tumour-associated CA IX and CA XII enzymes likely able to inhibit the growth of primary tumours and metastasis (2). The hit compounds showing the highest inhibition activity against CA IX/XII and the highest selectivity over the other hCAs, have been subjected to X-ray studies.
Here we report the crystal structures for 5-(dimethylamino)-2-naphthalenesulfonamide (2-NSA) in adduct with the physiologically dominant isoform II, as well as with the tumor-associated isoform XII. The structures give an interesting explanation for the experimentally observed different affinities of this molecule toward hCAs. Indeed, its binding to the CA active site follows the Lock-and-Key mechanism, since the protein did not substantially change its structure upon ligand binding.

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Semax, a peptide analog of the melanocortin ACTH fragment (4- 10), modulates the inflammatory response in LPS-activated murine macrophages affecting the wound healing process

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Cutaneous wound healing is a complex process involving inflammation, new tissue formation, blood clotting and tissue remodelling. Many experimental and clinical studies have demonstrated varied effects of wound-healing peptides to promote skin regeneration and affect inflammation processes. ACTH(4-7)PGP (Semax) is a synthetic peptide based on the ACTH(4-10) melanocortin derivative regulatory peptide that preserves the entire behavioural effects of his precursor with an increased stability to the proteolysis, exerts marked neuroprotective activities on brain cognitive functions and affects inflammation processes after the brain stroke.[1,2]
In previous works, we demonstrated the Semax ability to chelate copper and zinc ions and the protective ability against metal-induced cell toxicity, the Cu(II)-mediated ascorbic acid oxidation and copper-induced aβ aggregation and amyloid formation in artificial membrane models.[3-5]
The aim of this work was to evaluate the anti-inflammatory and wound healing activity of Semax in vitro models of lipopolysaccharide (LPS)-induced inflammation in murine macrophages and of epithelial damage on keratinocytes, respectively. We demonstrate that Semax can reduce the inflammation level process influencing the expression and release of pro-inflammatory mediators, such as NOS2, ROS, pro-interleukin-1beta (pro-IL-1β), formed during the inflammatory response, and delay of the macrophage's activation of towards the phenotypic state of M1-inflammation by morphological analysis using the Incucyte live cell imaging system. Moreover, the accelerating the wound healing has been demonstrated by scratch test. Therefore, the Semax bioactive peptide with high anti-inflammatory efficiency may be considered as potential therapeutic candidates for wound healing.

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"The Long Road to Radiochemical Process Development: Optimizing ^{68}Ga-Labeled Peptide Tracers for CXCR4 Imaging"

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Overexpression of CXCR4 is present in the majority of cancers. This phenomenon is often correlated with an aggressive tumor phenotype, elevated risks of metastasis and recurrence of the primary tumor, and a poor prognosis. Several radiolabeled peptides targeting CXCR4, have been developed and evaluated for clinical applications. Our team developed various cyclic radioligands based on eptapeptide structure derived from CXCL12 and among these the R54 peptide showed interesting characteristics as potential candidate for clinical use.

The R54 has been conjugated with both DOTA and NOTA chelators and radiolabeled with ^{68}Ga. To examine their properties and potential PET radiopharmaceuticals. These derivatives were tested in vitro in binding assays on different cell lines and in vivo in nude mice bearing xenograft tumors. The ^{68}Ga labeling process was initially developed using micro-scale experiments evaluating concentrations of the peptide, different buffers, range of temperatures and duration of the reaction. The final step was to adapt the pharmaceutical formulation for humans use used an automated synthesis module. TLC and HPLC analyses were conducted to thoroughly evaluate and determine quality parameters such as radiochemical purity (RCP) and radiochemical yield (RY).

Under similar/equivalent labeling conditions, the R54-DOTA showed lower RY (max 53%) than R54-NOTA derivative (> 90%). Among the evaluated buffers (citrate, acetate, HEPES), the best results in term of RCP were obtained using a HEPES 1 M. The limitations in using HEPES for human studies influenced the choice of an alternative buffer. Acetate buffer was then selected and the labeling of R54-NOTA were carried out in an automated synthesis module (at 100 °C for 20 minutes whit a 10 mM peptide molar concentration). After the purification step, RY was 75% and the RCP was 100%, indicating successful and efficient labeling for a diagnostic tool.

The ^{68}Ga labeling initially has been optimized in micro-scale experiments (defining the key parameters of reference, the affinity binding by cell-binding assays in vitro and in vivo in nude mice). Such parameters have been then adopted for automated labeling procedures with a commercially available model. This step is pivotal to meet at once regulatory guidelines and to obtain highly repeatable results.

Proline-Rich Antimicrobial Peptides: A Novel Approach to fight Klebsiella pneumoniae bloodstream

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Bloodstream infections require rapid and effective intervention due to their high mortality risk[1]. However, the number of antibiotics available to treat these conditions has been significantly reduced due to the spread of antibiotic-resistant pathogens. Bloodstream infections caused by antibiotic-resistant Klebsiella pneumoniae, which have a concerning mortality rate of 40% to 50%, are representative of the current antibiotic crisis[2]. The need for new antimicrobial drugs is evident. In this context, antimicrobial peptides (AMPs) have gained interest as potential compounds for developing new anti-infectives. AMPs possess potent antimicrobial activity with a low rate of bacterial resistance development. Unfortunately, most AMPs are also quite cytotoxic. The proline-rich antimicrobial peptides (PrAMPs) are an exception. They exhibit a specific and non-membranolytic antimicrobial mechanism of action, killing bacteria by binding to bacterial ribosomes and blocking protein synthesis. This makes PrAMPs selective for bacteria and generally well tolerated by eukaryotic cells[3]. This work aimed to identify a lead compound from a group of selected PrAMPs (Bac7(1-16), Bac7(1-35), Tur1A, and B7-005) suitable for combating antibiotic-resistant K. pneumoniae bloodstream infections. the study, supported by MUR PRIN 2022 (Project n. DCM.PN008.002 / 2022EKWRHB_LS6_PRIN2022_), aimed to identify the PrAMP with: (i) the best in vitro antimicrobial activity against multidrug-resistant clinical isolates of K. pneumoniae; (ii) the best tolerability to human cells; and (iii) potentially anti-inflammatory and LPS-inactivating properties. Peptides of different lengths and positive charge numbers were synthesized to determine how these factors influence AMP efficacy. Preliminary MIC assays against clinical K. pneumoniae were carried out to identify peptides with the most effective antimicrobial activities. Additionally, non-natural peptides differing in size and rigidity were synthesized to verify whether such modifications can influence the conformational stability of the peptides and their interaction with biological molecules. Circular dichroism experiments were performed to assess the impact of these substitutions on peptide structure and properties. This work could pave the way for the development of further optimized antimicrobial drugs based on PrAMPs..

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Fight-TB: Design of novel vaccine antigens to fight Tuberculosis

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In 2022, TB was the world's second leading cause of death from a single infectious agent, after coronavirus disease (COVID-19), and caused almost twice as many deaths as HIV/AIDS. More than 10 million people continue to fall ill with TB every year. Inadequate treatment, multidrug-resistance and delays in diagnosis contribute to the severity and mortality of the disease. Therefore, preventing TB disease rather than curing the infection has been the primary target for vaccine development. BCG is still the only vaccine approved to prevent TB, despite its known inefficacy. Currently, the global TB vaccine portfolio includes over 16 distinct vaccine candidates at different stages of clinical development [1]. Among these, subunit vaccines are extremely promising candidates, as they overcome safety concerns and optimise antigen targeting.
In collaboration with the University of Chungnam (South Korea), we are investigating a number of potentially effective mycobacterial antigens, as they effectively induce macrophages or DC maturation and possess anti-mycobacterial action [1,2]. All of these antigens possess promising vaccine potential and constitute valid starting points for peptide-based vaccine development. Among these identified antigens, we are characterizing the chaperone-like protein HtpG (Rv2299c) with the aim to improve its functional role and immunogenicity [2]. Moreover, another ongoing study is aimed at the biophysical characterization of Rv3463, a novel macrophage-activating factor, conserved in mycobacteria. Recombinant Rv3463 activates mouse bone marrow-derived macrophages to induce the expression of surface molecules and secrete pro-inflammatory cytokines via the TLR2 and TLR4 pathways [3]. Interestingly, Rv3463-activated macrophages, but not those activated by LPS, Ag85 or ESAT6, significantly suppress Mtb growth [3]. In this framework, we also adopted computational approaches to identify the most immunogenic region of Rv3463 to design peptide-based vaccine antigens. In particular, we have produced overlapping peptide libraries (mimotopic approach) which will be tested in vitro (TLR2 and TLR4 binding) and in immunization assays [4]. Different conjugation methodologies will be adopted to enhance the immunogenic properties of the identified antigens.

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Synthesis and characterization of membrane-active peptides promoting fusion between model liposomes

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Extensive studies on viral fusion have led to the identification of small segments of the fusion proteins, consisting of 20-30 residues, with a predominant role in the membrane fusion process.[1] With this in mind, the term fusion peptide refers not only to the corresponding region of the fusion proteins, but also to peptide sequences with fusogenic capabilities that mimic these segments.[2] It becomes clear that investigating how these sequences mediate membrane interactions is a key aspect for the development of new preventive and pharmacological strategies.
In this work, how structural modifications of a peptide can modulate its membrane activity was investigated. The starting point was the structural modification of the following peptide:
- Ade-Aib^{1}Lys^{2}Leu^{3}Aib^{4}Lys^{5}Lys^{6}Leu^{7}Aib^{8}Lys^{9}Ile^{10}Leu^{11}-NH-$(CH_2)_2$-NH_2-Thym.
Previous studies have shown the ability of the sequence to promote fusion between negatively charged model membranes of the PE/PG type; whereas with zwitterionic, similarly sized membranes of the PC/Ch type, only the phenomenon of leakage has been shown.
The modified sequences synthesized on solid phase are:
- Ade-Leu^{1}Lys^{2}Leu^{3}Leu^{4}Lys^{5}Lys^{6}Leu^{7}Leu^{8}Lys^{9}Ile^{10}Leu^{11}-NH-$(CH_2)_2$-NH_2-Thym
- Cbz- Leu^{1}Lys^{2}Leu^{3}Leu^{4}Lys^{5}Lys^{6}Leu^{7}Leu^{8}Lys^{9}Ile^{10}Leu^{11}-NH-$(CH_2)_2$-NH_2-Cbz.
Optimized the synthesis process, conformational analyses were performed. Studies with Circular Dicroism observed that substitutions made in the peptide sequence did not lead to significant conformational changes in the mimetic membrane environment.
Finally, interaction studies with artificial membranes were carried out for the peptide synthesized with nucleobases. Through DLS and TEM studies, it was inferred that the latter is able to promote fusion between both PE/PG-type and PC/Ch-type membranes.

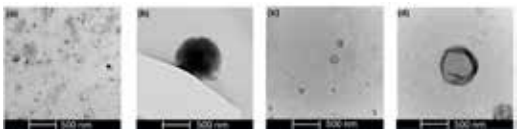

Figure 1. TEM images of Small Unilamellar Vescicles (SUVs) and the peptide A-TricKL-T solution in SUVs: (a) SUVs PC/Ch pristine, (b) A-TricKL-T/PC/Ch 1:6 , (c) SUVs PE/PG pristine, (d) A-TricKL-T/PE/PG 1:15

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Cyclic Antimicrobial Peptides Analogues of Temporin L: Design and Synthesis of Novel Guanidine-based Derivatives

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The increasing number of antibiotic-resistant bacterial strains has sustained the need to develop new antimicrobial agents. Antimicrobial peptides (AMPs) represent a viable alternative to classical antibiotics due to their properties.[1] Among them, temporins are cationic AMPs, derived from the frog skin secretions, and a notable isoform of this group is the 13-mer Temporin L (TL) peptide. TL exhibits a stronger and broader spectrum activity, but also a significant cell toxicity. It was designed and synthesized a library of macrocyclic peptide analogues characterized by non-canonical amino acids allowing different side-chain-to-side-chain crosslinking strategies (e.g., lactam bridge, guanidino-bridge). In particular, the incorporation of these linkers is also expected to impact on the physico-chemical (polarity contribution) and protease resistance properties. Furthermore, the effect of a new positive charge by the guanidine group as a cyclic motif or amino acid side chain group at a key position (Figure 1), has led to peptides with increased antimicrobial activity.[2,3] The most promising analogues, especially against Gram-negative bacteria, have showed a reduced cytotoxicity against human keratinocytes. Additionally, some of these showed an increase in pharmacokinetic properties. Preliminary results of antimicrobial activity and cytotoxicity obtained for these derivatives will drive the development of further analogues whose bacterial membrane interaction properties and their potential against multi-drug resistant infectious diseases will be investigated.

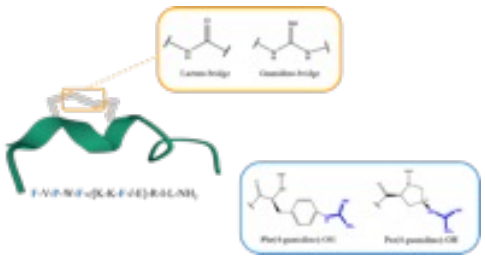

Figure 1. Structural modifications herein presented and applied to TL peptide sequence to improve antimicrobial properties.

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Ultrasonic-aided sustainable chemical synthesis of biologically active peptides

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The increasing demand for peptide-based therapeutics is forcing drug developers to address the sustainability challenges associated with their chemical production.[1,2] Among the various techniques for manufacturing peptide therapeutics, Fmoc-based solid-phase peptide synthesis (SPPS) stands out as the most effective but expensive strategy. However, it employs technologies that use large amounts of highly hazardous reagents and solvents, with minimal focus on green chemistry and engineering.[3]
The application of ultrasound (US) in such a heterogeneous system has been encouraged by our recent studies reporting significant material and reaction time savings when US irradiation efficiently supported two crucial SPPS reactions: amide bond formation and Fmoc deprotection.[4,5] Therefore, our presentation will focus on the US-assisted sustainable SPPS procedures that we have recently developed and applied to perform solid-phase reactions, including intramolecular cyclization, which are currently being explored to modulate both the pharmacodynamics and pharmacokinetic properties of peptide-based molecules.

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In Vitro Antimicrobial Activity of Bioactive Peptides on the Viability of Giardia duodenalis Cysts

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Giardia duodenalis, is a globally distributed protozoa widely reported in humans and many animals worldwide[1]. This study aimed to evaluate the in vitro activity of some antimicrobial peptides (AMPs) previously discovered in our lab, on the viability of G. duodenalis cysts isolated from naturally infected dogs. The antimicrobial peptides were synthesized by solid-phase peptide synthesis approach. Cysts were treated with six peptides (TL-34, TL-48, TL-42, TL-51, RB-71 and RB-58) at different concentrations (0.15 mg/ml, 0.3 mg/ml, 0.6 mg/ml, 1.2 mg/ml), metronidazole (50 µg/ml) (positive control) and phosphate buffer saline (PBS 1%) (negative control) for different contact times (30, 60 and 180 minutes). The viability of the cysts was evaluated by contacting the treated cysts with eosin stain 1% [2]. Fifteen minutes after exposure to the dye, the unstained cysts (which did not absorb the dye) were considered potentially viable and the stained cysts were considered non-viable. The results of study showed high efficacy at the two highest concentrations and after 180 minutes of exposure, four of the tested peptides (TL-34, TL-48, TL-42 and RB-58) inhibiting the viability of 100% of Giardia cysts. While the other two peptides (TL-51 and RB-71) were less effective, still giving moderate results inhibiting the viability of 100% of Giardia cysts only at the highest concentration after 180 minutes (Table 1). AMPs used in this study have in vitro antigiardial activity on cysts and seem to have potential for the treatment of giardiasis in dogs.

Bioactive Peptides	Time Exposure (Minutes)	Non-Viable Cysts (%) Concentration (mg/mL)			
		0.15	0.3	0.6	1.2
TL-34	30	0.0	21.0	36.0	74.8
	60	69.2	65.2	81.4	89.5
	180	70.0	87.5	100.0	100.0
TL-48	30	21.0	22.20	36.0	78.0
	60	21.20	68.9	94.3	100.0
	180	50.0	75.0	100.0	100.0
TL-42	30	8.0	11.1	59.0	100.0
	60	12.5	66.7	78.0	100.0
	180	50.0	100.0	100.0	100.0
TL-51	30	25.5	33.30	50.0	77.40
	60	52.5	60.0	89.8	100.0
	180	80.0	93.0	98.8	100.0
RB-71	30	0.0	33.3	40.0	70.0
	60	11.2	26.7	53.0	73.3
	180	56.0	78.8	89.0	100.0
RB-58	30	0.0	25.0	33.3	42.5
	60	15.0	39.0	52.9	61.5
	180	86.0	100.0	100.0	100.0
Positive Control Metronidazole (50 µg/mL)	30	81.5			
	60	89.8			
	180	100.0			
Negative Control (PBS 1%)	30	0.0			
	60	0.0			
	180	0.0			

1 Table 1. The results of in vitro antimicrobial activity of bioactive peptides on the viability of Giardia cysts.

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Keywords: Giardia duodenalis; in vitro test; antiprotozoal peptides.

Barley (Hordeum vulgare L.) proteins as a source of alpha glucosidase inhibitory peptides

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Cereal consumption has a beneficial effect on human health e.g. reduce the risk of many chronic diseases (type 2 diabetes, hypertension etc.). According to the literature, proteins can be a source of biologically active peptides in prevention of diet-related diseases [1]. Barley proteins may be a source of bioactive peptides. Peptides enzymatically released from food proteins, including those derived from barley, show variety of biological functions. The aim of the study was the determination of α-glucosidase inhibitory activity peptides derived from barley proteins. The analysis included an in silico analysis and in vitro protocols. The amino acid sequences of all proteins were acquired from the UniProt database [2]. The bioinformatic analysis was carried out by using BIOPEP-UWM database [3]. It included the calculation of profile of potential of α-glucosidase inhibitory activity of barley proteins, the frequency of α-glucosidase inhibitory fragments occurrence in protein sequence (A), simulation of proteolysis using human digestive enzymes to generate α-glucosidase inhibitors. Verification of in silico results included the extraction of barley proteins, enzymatic hydrolysis using the unified digestive protocol of Infogest [4] and the determination of α-glucosidase inhibitory activity of hydrolysates. The results showed that barley proteins may act as α-glucosidase inhibitors. This is indicated by the presence of fragments with α-glucosidase inhibitory peptides. Such peptides can also be released due to the action of digestive enzymes. Differences in the degree of α-glucosidase inhibition were demonstrated for samples of barley protein extracts and hydrolysates. Identification of α-glucosidase inhibitors in barley protein hydrolysates is yet to be determined.

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Prosystemin peptides as new promising tools for plant defense

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ProSystemin, an intrinsically disordered protein,[1] is the precursor of Systemin,[2] a proteolitically released C-terminal hormone peptide which activates plant defense responses. [3] We demonstrated that truncated ProSys, lacking Systemin, is not only a hormone scaffold, but it confers protection to tomato plants against biotic stressors when exogenously applied. By bioinformatics tools we identified repeated motifs (RMs) of different length, varying between 9 and 33 amino acids which are repeated 4 times along the whole tomato ProSys sequence. From a structural point of view, synthetic RMs revealed a variable degree of plasticity in different environmental conditions. Indeed, these peptides showed a disordered structure in aqueous solution consistently with our previous investigations carried out on the precursor protein. [1,4,5] RMs are mainly constituted by charged and polar residues, with a net negative charge given by Aspartic and Glutamic acids. The treatment of plants with RMs triggered the induction of defense mechanisms against S. littoralis larvae and B. cinerea fungus. These findings demonstrated that ProSys contains biologically active regions which can be used as natural plant protectors in agriculture.

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Protecting the environment with microalgae and carbonic anhydrases

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This work aims to develop a sustainable and innovative method for increasing carbon capture and microalgal biomass production. The goal is to contribute to the mitigation of climate change - for which CO2 is a major contributing factor [1] - and to enhance the production of renewable resources. The project's innovation lies in the use of Human Carbonic Anhydrase II (hCAII), an enzyme that catalyzes the conversion of CO_2 to bicarbonate and exhibits high catalytic activity.[2] This enzyme is immobilized within beads which are introduced into the microalgae culture medium. This process enhances the availability of carbon in a form that can be directly assimilated by photosynthetic microorganisms, thereby boosting biomass production. The resulting microalgae biomass can subsequently be extracted to obtain high-added value molecules in a cascade approach, thus contributing to the full exploitation of microalgae potential. Although CAs have been the subject of much research in the fields of CO_2 capture, their use in microalgae cultivation is still uncommon. Carbonic Anhydrases represent a biotechnological option to promote the direct capture of CO_2 from the air meanwhile increasing large-scale production of high-added value molecules.

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Design and Synthesis HA-based peptide vaccines against cancer and infectious diseases

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The COVID-19 pandemic has emphasized the importance of prevention and renewed trust in vaccines. Chronic diseases like cancer also remain a critical focus for healthcare systems. This work aims to validate and advance a recently patented technology that uses hyaluronic acid (HA) as an adjuvant for protein-based vaccines. These vaccines are safe and quickly produced but need effective adjuvants to enhance their immunogenicity. HA-conjugated protein/peptide antigens boost immunogenicity and promote strong, lasting immune responses without causing inflammation, making them suitable for diverse populations, including children and the elderly [1]. In particular, this work focused on HA-based anti-cancer and anti-viral vaccination settings. We designed and synthesized HA-based peptide vaccines targeting the human epidermal growth factor receptor 2 (HER2/neu) for HER2/neu-expressing breast cancer and the receptor-binding domain (RBD) of the Spike protein of SARS-CoV-2 for COVID-19[2][3]. Using X-ray structural analysis of HER2, we identified and synthesized various epitopes containing disulfide bridges, mimicking the extracellular region. Additional epitopes located beyond the extracellular part, which is interesting to analyze due to the limited knowledge of the transmembrane and intracellular regions, were also developed. Peptide sequences were obtained employing solid-phase synthesis, purified through RP-HPLC and characterized with mass spectrometry. Concurrently, studies are ongoing to identify RBD-derived peptide sequences from different SARS-CoV-2 variants. All peptide sequences will be conjugated with HA, subsequently tested for their mechanism of action and efficacy in various mouse and transgenic models and compared with existing vaccines.

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Synthesis of new potential bifunctional cyclopeptoid-based organocatalysts

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Over the past 20 years, organocatalysis covered a central role in organic synthesis because it allows the use of organic molecules as catalysts increasing the green aspects of organic reactions and decreasing the cost of the catalyst necessary to conduct the reaction. In addition, this type of catalysis gives the possibility of obtaining molecules in enantioenriched form in an easy way. However, to date the research towards the synthesis of new organocatalysts is still open because there are some limitations, such as the loading of the catalyst necessary to perform the reaction, the reusability and also because it is desirable obtain new structures that are more performing in terms of conversion and stereoinduction. A promising class of molecules are peptoids, oligomers of N-substituted glycines. In literature there are some examples of linear peptoids successfully applied as organocatalysts and these gave good results in terms of diastero- and enantioselectivity [1]. Moreover, cyclopeptoids, the cyclic form of peptoids, containing L-proline residues have been proven to be good catalysts in Phase-Transfer Catalysis (PTC), with good enantiomeric excesses [2]. An important feature of peptoids is their conformational instability. However, when the structure is cyclized, it gains rigidity (and conformational isomerism). In 2018 it was shown that the presence of a single stereogenic center on the peptoidic backbone blocks the conformation in a single diasteromeric form [3],[4]. This characteristic of cyclopeptoid makes them interesting as potential organocatalysts, where a well-defined spatial arrangement of the catalytic site play a central role. With this in mind, the synthesis of new cyclic trimeric peptoids is proposed. These macrocycles are appropriately functionalized with a primary amine and a tertiary amine in order to obtain new bifunctional organocatalysts (Figure 1).

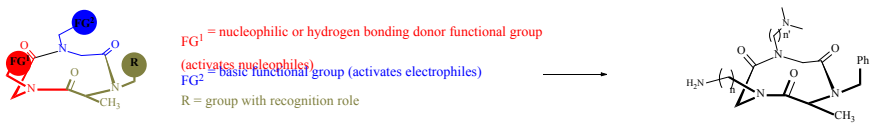

Figure 1. Proposed structure of bifunctional cyclic peptoids.

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Molecular insights on the amyloid aggregation process using a depsi-isomer of the Aβ peptide

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The amyloid beta (Aβ) peptide is the primary proteinaceous component of the amyloid plaques found in the brains of Alzheimer's disease (AD) patients and is widely recognized as a key factor in the disorder. However, the molecular details of the Aβ aggregation pathway remain elusive, largely due to the transient and unstable nature of the oligomeric states formed by Aβ peptide and the challenging preparation required for obtaining homogeneous, seed-free Aβ peptide samples [1]. We have recently developed an effective synthetic protocol, using solid phase peptide synthesis (SPPS), to prepare highly pure Aβ in the form of a depsi-peptide analogue, 26-O-acyl iso Aβ1-40. This non-aggregative precursor can be rapidly and quantitatively converted into the native peptide upon dissolution in a neutral pH buffer, thereby initiating the aggregation process. The depsi-Aβ analogue is a valuable tool for studying the Aβ aggregation process as it ensures the production of highly homogeneous, monomeric, and seed-free starting Aβ sample solutions [2]. We employed the 26-O-acyl iso Aβ1-40 analogue to investigate the oligomeric intermediates explored during the aggregation process using 1D 1H NMR experiments and 2D diffusion-ordered spectroscopy (DOSY) combined with the Inverse Laplace Transform (ILT) reconstruction method. The innovative DOSY-ILT approach allowed us to map the distribution of oligomers across a broad range of initial peptide concentrations, providing unique insights into the evolution of oligomeric species over time. These findings enhance our understanding of amyloid aggregation and offer a powerful strategy for the rapid screening of novel therapeutic drugs that specifically target distinct oligomeric species [3].

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Synthesis and Characterization of Nucleopeptide-Based Binders for ATP and GTP

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Nucleopeptides represent a class of synthetic biomolecules where the peptide backbone incorporates nucleobases in the side chains [1]. The versatility of nucleopeptides comes from the potential to manipulate both the peptide and nucleobase components. This allows for the tuning of desirable properties such as biostability, biocompatibility, multifunctionality, and the ability to spontaneously self-assemble in aqueous solutions [2]. Herein, we present an on-going investigation into the US-assisted [3] synthesis of tailored nucleopeptides aimed at identifying novel ATP and GTP binders. While these nucleopeptides feature diverse amino acid sequences, they share distinctive functional moieties: a nucleobase-bearing amino acid (NBA) in the C-terminus (Lys) to match Adenine or Guanine of the ATP or GTP, respectively; aromatic L-Phe and hydrophobic L-Leu, to enhance aggregation process after target recognition; three L-Arg residues to interact with the phosphate groups of ATP/GTP in cell and facilitate cell penetration through electrostatic interactions with negatively charged molecules on the cell surface; L-Ser as a hydrophilic spacer to balance the hydrophobic component and improve the solubility of the nucleopeptides in aqueous environments. Preliminary CD studies reveal nucleopeptide-ATP/GTP interactions by adopting distinct binding modes. NMR studies are in progress to gain further insights into their local conformational peculiarities and structural changes driving the target recognition process. Remarkably, some of these nucleopeptides, except for those bearing an acetyl group that replaced nucleobase on the side chain of Lys, also exhibited gel-forming abilities, highlighting the involvement of the nucleobase in sol-gel transition and opening new perspectives for drug delivery applications.

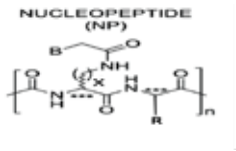

Figure 1: Nucleopeptide (NP) structure.

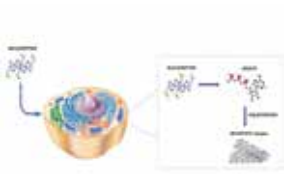

Figure 2: Nucleopeptide assemblies sequester ATP and GTP.

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Paper-based electrochemical peptide nucleic acid (PNA) biosensor for detection of miRNA-492: a pancreatic ductal adenocarcinoma biomarker

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Pancreatic ductal adenocarcinoma (PDAC) is the main neoplastic and lethal disease of the pancreas; it's sometimes called "silent" disease because symptoms are rarely noticeable in its early stages and become evident only when cancer has become very large or has already spread to other organs [1]. The lack of nonspecific symptoms causes delays in diagnosis and doesn't allow effective screening strategies. For a more accurate and timely diagnosis, it's important to identify new PDAC biomarkers.

Recently miRNAs, a class of small non coding RNAs, have gained interest as promising candidates for non-invasive circulating biomarkers, being stable in bodily fluids, expressed at detectable levels and representative of many diseases, including PDAC [2]. We report a cost-effective Peptide Nucleic Acid (PNA) paper-based biosensor for the detection of miRNA-492 (Figure 1), which is recognised as a biomarker for PDCA [3].

To design a miniaturised, sensitive and robust paper-based platform, an electrochemical sensor was screen-printed on office paper and then engineered with a highly specific thiolated PNA as the recognition element. Taking advantage of the versatile properties of PNAs, a signal on strategy was employed for miR-492 detection and the formation of PNA/miRNA-492 adduct was evaluated by monitoring the interaction between the positively charged ruthenium (III) hexamine with uncharged PNA and/or negatively charged PNA/miRNA-492 duplex by differential pulse voltammetry. The paper-based biosensor provided a linear range up to 100 nM, with a LOD of 6 nM. Excellent selectivity towards one- and two-base mismatches (1mM, 2mM) or scrambled (SCR) sequences was highlighted and the applicability for biomedical analyses was demonstrated, measuring miRNA-492 in undiluted serum samples.

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Novel Aβ/Prion chimera peptides: studies on the inhibition of the Aβ fibrillogenesis and the role of transition metal ions

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Alzheimer's disease (AD) is a progressive neurodegenerative condition affecting 50 million people worldwide. Despite pathogenesis of this neurodegenerative disease remains unclear, increasing evidences point out a critical molecular process involving the aggregation of tau and β-amyloid (Aβ) proteins.[1] Promising strategies toward pathological amyloid proteins and deposition include inhibitors able to interfere with the early stage of the Abeta's aggregation process. Recent advances in peptide-based inhibitors involve peptides derived from the amyloid sequence.[2] In particular, the peptide KLVFF corresponding to the hydrophobic core of Aβ42, because of its strong affinity for Aβ42, exhibits advanced inhibitory activities against fibrous aggregation.[3] Peptides derived from other amyloid protein sequences can also disrupt amyloid aggregation. In particular, the recombinant full-length PrP (PrP23–231) and/or its N-terminal fragment, inhibit the formation of Aβ42 amyloid fibril.[4] Moreover, the interaction between the Prion peptide fragment and Aβ42 can be also assisted by Cu(II) ions by means of the direct involvement of the histidine residues present in the N-terminal domain of Aβ42 and Prion proteins, in the formation of macrochelate complexes.[5] In this scenario, the use of di-functional molecules, capable of synergic and/or additive actions to recognize different regions of the amyloid beta, represents a promising approach for the development of new class of therapeutic agents for AD. In this work, we synthetized and characterized two peptides, namely KTNMKHMAGKLVFF and PHGGGWGQKLVFF, bearing the Aβ42 recognition group KLVFF, combined with amino acid sequences from Prion protein (chimera peptides). The interaction between the peptide conjugate and Aβ42 was studied by using an array of different biophysical techniques including Circular Dichroism (CD), thioflavin T (ThT) Fluorescence assay and high resolution electrospray mass spectrometry (HR-ESI-MS). Copper(II) coordination properties of the chimera peptides were also investigated by pH-potentiometry and spectroscopic techniques. The results observed revealed the ability of chimera peptides to interact with Aβ42. This interaction can be affected by the presence of copper ions.

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Binding properties of the peptides mimicking the Viral Envelope protein to second PDZ domain of ZO1 protein

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The Envelope (E) protein is one of the main structural proteins encoded by the genome of SARS-CoV, SARS-CoV-2 and MERS-CoV Coronaviruses and participates in many aspects of the viral life cycle such as virus maturation, assembly, and virulence mechanisms [1]. The E protein is characterized by the presence of a PDZ-binding motif (PBM) at its C-terminus, which allows it to interact with several PDZ-containing proteins in the host cell [2]. One of the main binding partners of the Coronavirus E protein is the PDZ2 domain of ZO1, a protein with a crucial role in the formation of epithelial and endothelial tight junctions (TJs) [3]. To date the molecular details of the interaction between PDZ2-ZO1 and the E protein have not been established.

Here, we report the structural characterization, by solution NMR spectroscopy, of three peptides mimicking the C-terminal portion of E protein from SARS-CoV, SARS-CoV-2 and MERS-CoV and their interaction with the ZO1-PDZ2. The results could provide novel insights for the elucidation of the molecular mechanisms involved in the insurgence of the pathology.

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Experimental and computational methods to explore non covalent interactions in model pentapeptides/b-cyclodextrin complexes

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Cyclodextrins (CDs) are oligosaccharides constituted by units of D-glucopyranose organized in a cyclic structure with a hydrophobic cavity that shows peculiar inclusion properties for different compounds. b-CDs are currently the most studied CDs, commonly used in pharmaceutical formulations for the excellent pharmacokinetic properties and ability to improve solubility of hydrophobic species[1].

Considering that, in the case of peptide-cyclodextrin and protein-cyclodextrin host-guest complexes, the aromatic amino acids are reported to be the main responsible of the interaction, in a previous work we studied the b-CD inclusion properties of nine model tripeptides. These peptides were obtained permuting the position of two L-alanines with that of one L-tryptophan, or phenylalanine, or L-tyrosine, respectively[2]. Interestingly, we found that the tripeptide Ac-AYA-NH_2 is the most favorite for the interaction, evidencing that the position of the aromatic side-chain in the sequence modulates the formation of the inclusion complexes.

Here, to further clarify the conformational aspects of the peptide-cyclodextrin host-guest formation we report the results of the study extended to five model pentapeptides constituted by four L-alanines and one aromatic permuting tyrosine. The pentapeptides:b-CD host-guest inclusion properties have been analyzed experimentally by Uv-Vis and NMR and by computational methods such as molecular docking and molecular dynamics.

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Association of self-assembled structures and their material function: the way by which this class of building blocks bears the potential for diverse applications

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Rationally designed materials that exploit specific, directional, tunable and reversible non-covalent interactions offer unprecedented advantages: they enable modular and generalizable platforms with tunable mechanical, chemical and biological properties. Indeed, the reversible nature of supramolecular interactions gives rise to biomaterials that can sense and respond to physiological cues, or that mimic the structural and functional aspects of biological signalling. Self-assembly is defined as the ability of a molecule, without guidance of external factors, to associate through non-covalent interactions to form highly ordered 3-dimensional structures. One of the main driving forces of self-assembly is molecular amphiphilicity, which can drive formation of complex and stable nanostructures. Self-assembling of peptide and peptide conjugates have attracted great attention due to their biocompatibility, biodegradability and biofunctionality. Understanding assembly mechanism enables the better design of peptides which may form useful and functional nanostructures. On the other hand, the design of peptides able to fold into β-hairpin or β-sheet has been much less satisfactory, mainly because of their high tendency to aggregate and low solubility. Both disadvantages are due to the amphipathic character of the β-structure and to the high content of hydrophobic residues, the ones with highest β-sheet propensities. Similar to β-sheet structure, short helical peptides have been recently discovered to possess a diverse set of functionalities with the potential to fabricate artificial self-assembly materials. Here we outline the functional roles of self-assembled peptides and their potential as artificial materials. The peptides here presented are: i synthetic homologues of natural, metabolically stable bioactive sequences and, therefore, able to form specific secondary structures; ii composed of hydrophobic amino acid with specific non-covalent interactions (hydrogen bonds, hydrophobic interactions); iii used as building blocks in order to build oligopeptides which assume new, regular secondary structure. The understanding of sequence-to-structure relationship of the observed peptides and their functional roles opens a new direction of molecular engineering and development of future functional materials.

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Synthesis, conformation, and electrochemical behaviour of peptides functionalized with ferrocene moieties

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We recently synthesized a series of peptides characterized by the presence of ferrocene (Fc) moieties, covalently bound at the N and/or C-terminus or at an amino acid side chain.[1] The main purpose was to investigate the influence exerted by the macrodipole of helical peptides on charge/electron transfer processes. Indeed, Fc is a reliable and easy to handle electrochemical probe. In addition, Fc is known for its ability to promote the generation of reactive oxygen species (ROS) under physiological conditions. ROS concentrations higher than normal favour carcinogenesis, but above a certain value a cytotoxic effect is observed. Interesting results are reported in the literature about Fc-conjugates displaying anticancer activities. [2-4] We then decided to extend our studies to the design, synthesis and characterization of new Fc-peptide constructs with effective antitumor action.
The peptide should selectively address the drug toward over-expressed cancer cell receptors, where Fc can then display its anti-proliferative activity through the production of (ROS). To fine tune the Fc oxidation potential, we linked to Fc an aromatic unit. Finally, the Fc-aromatic moiety was bound to the peptide through a spacer, to increase flexibility and, consequently, to improve peptide adhesion to cell receptors (Figure 1).

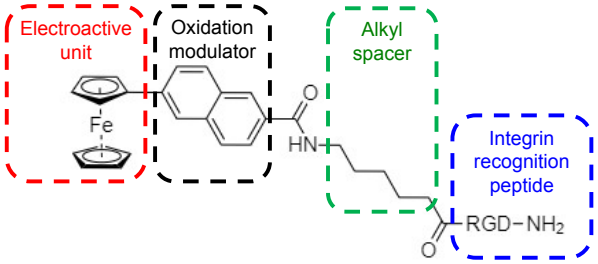

Figure 1. One of the synthesized constructs with potential antitumor activity. The four modules are highlighted.

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Development of peptide-based molecules as novel tools for prion diseases treatment

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Prion diseases are a class of lethal, neurodegenerative and transmissible disorders affecting both humans and animals. The key molecular event responsible for the onset of prion diseases is the conformational conversion of the cellular prion protein (PrP^{C}) into its misfolded isoform, called scrapie (PrP^{Sc}), which is prone to aggregation and forms fibrils that accumulate in the brain of affected individuals.[1] The human prion protein (HuPrP) is a glycoprotein ubiquitously expressed in the central nervous system, consisting of an intrinsically disordered N-terminal domain (NTD) and a C-terminal globular domain (CTD) that contains two short β-strand and three α-helices. The formation of the $HuPrP^{Sc}$ is triggered by a drastic structural change in the native conformation, which rearranges into a misfolded isomer with a high β-sheets content involved in prion fibrillation.[2] Currently, the molecular mechanism leading to this structural transition is still poorly understood, and no effective therapy is available for these pathologies. Recently, it was shown that the intrinsically disordered NTD (23-89) of HuPrP establishes a set of transient electrostatic interactions with the globular CTD (90-231), which play a crucial role in stabilizing the native fold of HuPrP, counteracting the structural transitions that lead to the misfolded β-rich intermediate state.[3] This remarkable observation suggests a novel route for the design of drugs against human prion diseases by targeting the pathogenic structural conversion of HuPrP with peptides or peptidomimetics able to stabilize the native physiological conformation of HuPrP by mimicking the NTD and reproducing the interaction surface that it establishes with the CTD. Here, we report an experimental protocol for the expression in E. coli and the purification of recombinant NTD-HuPrP(23-89) enriched with NMR - active isotopes and its preliminary NMR characterization, aimed at identifying the key residues involved in the stabilizing interactions with the CTD. We also report the design, synthesis via solid-phase peptide synthesis (SPPS), and NMR characterization of a twenty-one amino acid peptide, encompassing the region from Lys23 to Ser43 of the NTD, called MANTRAP 1. NMR data indicate that MANTRAP 1 displays a high degree of conformational flexibility without adopting any preferential secondary structure and, notably is able to transiently interact with CTD.

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Development of novel peptide-based radiopharmaceuticals for targeted cancer diagnosis: structure-activity studies

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RGDechi is a bifunctional chimeric peptide composed of a cyclic RGD containing pentapeptide covalently linked by a spacer to an echistatin domain.[1] This peptide is able to selectively modulate $\alpha_V\beta_3$ function and its antiangiogenic activity, antiadhesive, antiproliferative, and pro-apoptotic effects on human malignant melanoma cells were demonstrated. RGDechi can be considered a good candidate as a non-invasive diagnostic tracer in tumor imaging.[1,2] ^{99m}Tc ($t_{1/2}$=6.02 h and E_γ=140 keV) is the workhorse radionuclide in Nuclear Medicine for SPECT imaging applications. To prepare the [^{99m}Tc]-tagged RGDechi derivative peptides the labeling approach which involved the use of the [^{99m}Tc(N)(PNPn)]$^{2+}$-system (PNP= bis-phosphino-amine) was investigated.[3] By exploiting this technology, both the molecular weight and lipophilicity of the radiolabeled peptides were easily modified by varying substituents of the P atoms of PNP in [^{99m}Tc(N)(PNP)] scaffold; this allows for a fine modulation of their stability and pharmacological properties. Taking advantage of the [^{99m}Tc(N)(PNPn)] technology, a Cys residue was coupled on the side chain of the Lys1, obtaining RGDechi-Cys analogues. A Cys residue was selected as a bifunctional chelating agent (BFCA), to allow the coordination to the [^{99m}Tc][Tc(N)(PNPn)]-synthon, through the negative sulfur (S^-) and the neutral nitrogen (NH_2) atoms affording the final monocationic complex. Moreover, a spacer between the peptide recognition sequence and the radioactive probe was introduced to evaluate its influence on the peptide activity. The cellular uptake and internalization studies of the obtained radiolabeled peptides were performed.

Figure 1. Schematic representation of our project.

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Peptide-based approach to target G-quadruplex DNA: from discovery to anticancer potential

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G-quadruplexes (G4s) are non-canonical secondary structures formed in G-rich DNA and RNA sequences, particularly enriched in cancer-related genes and regions predisposed to cancer amplification. G-quadruplexes represent promising targets for therapeutic intervention. In particular, the G4 structures formation is tightly controlled by several proteins that bind and stabilise or unfold them.[1] The main challenge in anticancer research is to identify drugs with high selectivity and minimal side effects. The traditional small molecule drugs are not able to fully guarantee these aspects. Moreover, even though protein-based drugs can interact with high specificity, their high molecular weight and limited permeability limit their anticancer efficacy. In this context, peptides can offer a unique combination of protein and small drug advantages, representing a new avenue for the development of anticancer agents. A novel approach involving the alignment of several human G4- binding proteins was employed, identifying a common peptide motif named NIQI (Novel Interesting Quadruplex Interaction motif) consisting of a 20-mer R/G-rich sequence.[2] Herein, we synthesised this peptide and characterised its ability to bind different G-quadruplexes.[3,4] Several biophysical techniques were employed to evaluate the interactions between peptide and G4 structures.[4] Circular dichroism (CD) was used to analyse the secondary structures of the peptide and to evaluate induced G4's conformational changes. Moreover, CD-melting experiments were performed to assess the ability of NIQI to thermally stabilise the G4s. Microscale thermophoresis (MST) and isothermal titration calorimetry (ITC) experiments were carried out to quantify the binding affinity and stoichiometry of the peptide's interaction with the various G4s and to verify its selectivity for G4s over duplex DNA. Additionally, nuclear magnetic resonance (NMR) experiments were recorded to get more information about the binding mode and identify the DNA regions affected by peptide binding. Finally, using the Ala-scan approach, the impact of each amino acid residue on binding to various G4s was systematically evaluated, identifying key and less relevant amino acids in the interaction. These findings lay the foundation for developing a new class of peptide-based G4 ligands, highlighting their potential as effective tools to disrupt DNA-protein interactions and offering promising therapeutic applications.

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Parallel phage display selection of peptides enabled by his-tag purification of phage

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Application of chemical reactions to phage-displayed peptide enables the in vitro evolution of chemically modified (e.g. cyclized) peptides. However, the process involves labour-intensive experimental steps such as PEG precipitation and centrifugation, which limits the number of selections (e.g. different targets, different libraries) that can be performed in parallel.

We have proposed a novel phage display procedure that incorporates automated bead-based phage purification to bypass the lengthy purification process and to enable multiple phage display selections in parallel. To replace the conventional purification method, an octa-histidine tag, that allows the phage to ne directly purified by cobalt-carboxylmethylaspartate (Co-CMA) magnetic beads, is inserted into pIII of the phage. Moreover, a magnetic bead processor, KingFisher, is applied to automate the purification scheme in a high-throughput manner.

With this procedure, we could achieve the parallel selection of up to 96 different chemically-modified peptide libraries or 96 different targets. Together with the robust chemical diversification strategies developed in our laboratory, we foresee to maximize the potential of phage display for drug development.

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Structure-based design and testing of a bicyclic peptide mimicking the EGF-like domain of Cripto-1

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Cripto-1 (known as TDGF-1, Teratocarcinoma Derived Growth Factor) is a small protein identified as the progenitor of EGF-CFC cell growth factors (EGF-CFC protein superfamily).
Cripto-1 is an oncofetal factor overexpressed in several types of cancers cells including also the CSCs component. Its multifunctional roles in neoplastic settings are attracting an increasing interest for developing therapies to block its functions [1].
Cripto-1 cellular signalling pathways are triggered by synergistic or distinct interactions of its two structurally functionally independent domains named EGF-like and CFC, with several different molecular players [1].
Molecules of synthetic or biological origin that target Cripto-1 are needed to both investigate its mechanism of actions and to obtain new potential therapeutics tools.
We report here a small bicyclic peptide mimicking the Cripto-1 EGF-like domain encompassing residues 82-106 of the protein. This synthetic bicyclic surrogate (named Bi-EGF peptide) has been successfully prepared following the TBMB approach [2] and tested via BioLayer Interferometry for binding to several Cripto-1 ligands and receptors including Nodal, Glypican-1 and GRP78. The biological properties of this molecule are being further investigated in terms of functional effects in Cripto1/Nodal positive cancer cells in combination with a previously reported [2] similar bicyclic molecule named B3 which is a surrogate of the protein CFC domain. A structure of the Bi-EGF peptide without the TBMB constraints, as obtained by the server Alpha Fold 3, shows that in its linear form the peptide has a tendency to adopt a closed but not fully ordered conformation which has been confirmed by a CD analysis of the molecule in solution at neutral pH.

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Exploring Fe(III) coordination and membrane interaction of a siderophore-peptide conjugate: Enhancing synergistically the antimicrobial activity

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Bacteria have developed various methods to acquire iron, a crucial nutrient for their growth, from mammalian cells. One prominent strategy is the secretion of siderophores, small high-affinity iron chelators that facilitate the uptake of iron(III) from the extracellular environment. These siderophores sequester iron ions from human tissues, fluids and cells, transferring them into bacterial cells via specialized porins. The conjugation of antimicrobial peptides (AMPs) with iron chelators represents a promising approach to reducing iron availability, triggering bacterial death, and enhancing the overall effectiveness of AMPs. Recently, we designed a multi-block molecule featuring an antimicrobial peptide, whose efficacy has been gradually improved through specific sequence modifications. This molecule also incorporates a flexible bio-linker, optimized in length, and terminates in a hydroxyamide unit, a potent metal chelator. Consequently, the molecule integrates two components that work synergistically to combat infection. To assess whether the peptide unit retains its structural integrity and antimicrobial activity despite the addition of a long tail, and to characterize its interaction with bio-membrane models mimicking Gram-negative membranes, we conducted fluorescence-based experiments and circular dichroism studies. These investigations supported our design, which effectively combines two distinct components. Iron(III) paramagnetic NMR spectroscopy and ultraviolet-visible (UV-Vis) spectroscopy validated the peptide's chelating activity and binding affinity. Spectrophotometric methods were employed to determine the complexation parameters, including Michaelis constant (K) and the number of binding sites (n), which were corroborated by the iron (III) paramagnetic NMR data. Our results demonstrate that integrating antimicrobial properties with iron-trapping capabilities is effective for treating infections caused by Gram-negative pathogens.

Recombinant Ig1 Axl receptor domain molecular characterization for application in drug discovery

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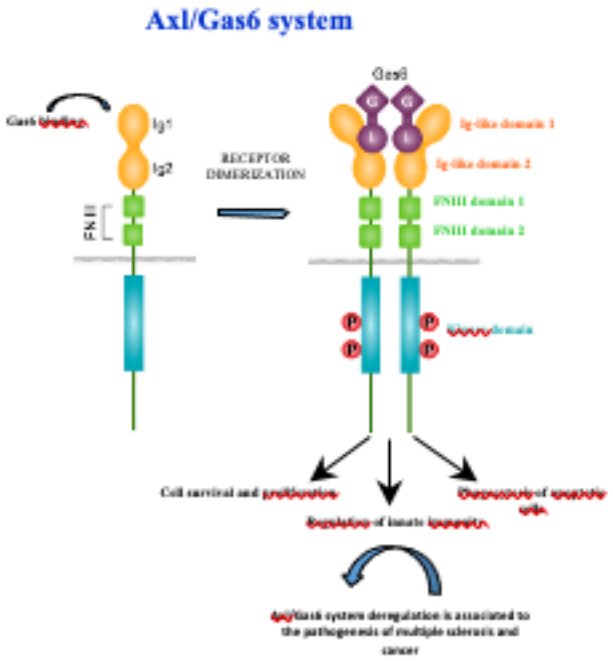
Axl receptor tyrosine kinase and its natural ligand, the growth arrest-specific protein 6 (Gas6), regulate several biological processes such as cell proliferation, survival, adhesion, migration, invasion and angiogenesis. Deregulation of Axl/Gas6 axis signalling is involved in both the onset and progression of a range of malignancies, autoimmune disorders and viral infections [1]. Based on its key role in these settings, Axl is considered as a novel and attractive prognostic biomarker in high impact diseases but also as a promising target in drug discovery for the development of molecules with therapeutic and diagnostic purposes [1, 2]. Structural organization of Axl receptor consists in three regions: an extracellular portion composed of two immunoglobulin (Ig)-like repeats, Ig1 and Ig2, and two fibronectin type III (FN III)-like repeats, a transmembrane region and an intracellular kinase domain [3]. Upon Gas6 binding to its extracellular portion, Axl undergoes dimerization and subsequent trans-autophosphorylation of its intracellular kinase domain, activating downstream signalling pathways [4]. Here, we report the cloning, expression and purification of Axl Ig1 domain and its spectroscopic and biochemical characterization, including the interaction analysis with its natural ligand Gas6 performed by Bio-layer interferometry.



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Peptide nucleic acid (PNA) through sensing platform to detect miRNAs biomarkers

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A recent development in carbon nanomaterials involves the use of a new type of fluorescent carbon nanoparticles known as carbon nanodots (CNDs). These possess excellent properties, such as good water solubility, low toxicity, and high chemical stability.[1] We have combined the specific characteristics of CNDs with peptide nucleic acid (PNA),[2] a mimic of natural nucleic acid with unique features, to create a sensing platform to detect nucleic acid biomarkers such as miRNA, mRNA,[3] etc. Water soluble CNDs were synthesized by thermal decomposition of citric acid, carried out by slightly modifying the protocol reported by Hu.[4] This method was selected for its use of a relatively low reaction temperature through an affordable synthetic route. CNDs display the typical emission excitation-dependent behaviour ranging from 360 nm to 540 nm. They show a more intense peak in photoluminescence at 504 nm when the excitation wavelength is 400 nm.

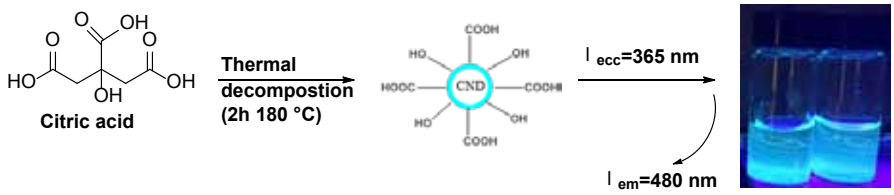

Figure 1. Synthesis of carbon nanodots

The detection of levels of miRNAs in biological fluids has the potential for an earlier disease diagnosis and to predict prognosis and response to therapy. [3] The sensing platform was built using a PNA probe designed to detect a specific miRNA. The probe was absorbed onto the surface of carbon nanodots, producing distinct signals for the single-stranded PNA and the double-stranded hybridized target. This method ensures highly sensitive, rapid, direct, and straightforward detection of trace biomarkers.

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Food derived appetite inhibiting peptides. In silico analysis

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Research on the relationships between food-derived biopeptides and key gastrointestinal hormones such as ghrelin, leptin, cholecystokinin (CCK), and peptide YY (PYY) underscores their significant role in hunger and appetite regulation. Biopeptides influence the secretion and activity of these hormones, potentially enhancing satiety and energy balance through direct effects on gastrointestinal cells or indirect modulation of appetite-regulating pathways.

The goal of this study was to analyze bioactive peptides in the BIOPEP-UWM database (available at https://biochemia.uwm.edu.pl/biopep-uwm/) [1] and predict protein sources with given peptides, as well as the possibility of their enzymatic release. At the time of the research, the database contained 773 protein sequences and 4,879 biopeptide sequences, with 62 types of biological activities.

The discovery of peptides like DLVDK from Oreochromis niloticus (Nile tilapia), which stimulates CCK release, and other peptides with inhibitory effects on enzymes such as dipeptidyl peptidase 4 (DPP-IV) and angiotensin-converting enzyme (ACE), highlights the potential of biopeptides in improving metabolic health through appetite control and metabolic processes. Additionally, tilapia proteins serve as precursors to four peptides—GPFPLLV, VAPEEHPT, VADTMEVV, and FAMD—that act as DPP-IV inhibitors, thereby prolonging the incretin effect and PYY activity. Furthermore, the identification of the dipeptide GW, exhibiting “multi-activity” as an inhibitor of ACE, DPP-IV, and TPP-II (tripeptidyl peptidase 2), extends CCK activity and also possesses antioxidant properties.

The in silico comparison of bioactive sequences with different proteins showed that peptides stimulating GLP-1 secretion, identified in Bos taurus (bovine) hemoglobin, are also present in the sequences of Sus scrofa (porcine) hemoglobin (ANVST), Capra hircus (goat) hemoglobin (KAAVT), and Oryza sativa (rice) proteins (KAAVT). Another identified hexapeptide (DLVDK) from tilapia exerts CCK-stimulating activity. Additionally, a GW dipeptide was identified, which has multi-activity: it is an inhibitor of ACE, DPP-IV, and TPP-II (prolonging the action of CCK), and also has an antioxidant effect, improving many aspects of the so-called metabolic syndrome.

This in silico analysis supports the concept that food-derived peptides can induce appetite control and prevent metabolic diseases. The potential sources of food biopeptides as precursors for agents regulating appetite and metabolism include proteins from Oreochromis niloticus (Nile tilapia), Bos taurus (cattle), Capra hircus (goat), and Oryza sativa (rice).

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Structural and biophysical studies of key pathogen targets for drug development

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Biophysical methods and structural biology, especially X-ray crystallography, are well established as key components of the early-stage drug discovery project. In the first stages of drug design, the biophysical methods allow to identify small molecules binding to a target (hit identification). Successively, structural information provided by X-ray crystallography permit to draw a Structure- Activity Relationship (SAR) analysis which will allow the rational design of derivatives with improved properties enabling the hit-to-lead progression.
We combine molecular biology with biophysics and x-ray crystallography to support the development of molecules of therapeutic interest, including Proteolysis Targeting Chimera (PROTAC)- based anti- infective derivatives in collaboration with UNIBO and UNICA. In this work we present a structural biology approach to drug development using as targets the recombinant NS2B-NS3 of the west nile virus (WNV) and protein targets from other human pathogens.

Cyclic Peptides blocking the interaction of Tissue Factor with Factor VII

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The major mechanism of blood clotting is triggered by exposure of tissue factor (TF) to coagulation factor VII (FVII) which, upon binding to it, becomes enzymatically active (FVIIa) and activates in turn factors IX and X, leading to thrombin generation and clot formation. Excessive slowing or acceleration of this mechanism leads to alterations in the delicate balance of hemostasis that, if not corrected endogenously, must be treated pharmacologically. To shift the balance toward reducing clotting one possible approach is therefore to prevent TF binding to FVII.
We have explored the possibility of obtaining inhibitors of the TF:FVII interaction using peptides mimicking regions of TF at the interface with FVII. Starting from a crystallographic structure of TF in complex with FVIIa (pdb: 1dan, [1]), we have designed and prepared a set of cyclic peptides containing loops and b-strands of TF in contact with FVIIa (loop 27-38, loop 49-62, b-strand 74-78 and b-strand 91-96). The two loops have been separated by a GGCGG motif and supplemented at the N- and C-terminus with two additional cysteines needed to achieve bi-cyclization (folding) through the well-known reaction with 1,2,3-tris(bromomethyl)benzene (TBMB, [2]). The linear 74-78 and 91-96 b-strands have been instead fused to obtain polypeptides of sequence CEPLYENXXRVFSYC where XX are either L-Pro-L-Pro, L-Pro-D-Pro, D-Pro-L-Pro, or D-Pro-D-Pro required to modulate the conformation of the final cyclic peptides obtained by closure of the N- and C-terminal cysteines. The cyclic peptides do bind FVII with an affinity in the μM range and prevent its activation in a functional in vitro assay. Prediction of the peptide conformations with the Alpha Fold 3 server affords well-folded β-strand-containing structures for all mono- and bicyclic peptides, with the 2 or 3 cysteines spatially very close together. CD spectra of the polypeptides collected in aqueous buffer show that the molecules adopt the expected ordered conformations that likely favor their binding and inhibition properties.

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Nanofibers based on Self-Assembling Peptide for SiRNA Delivery

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Peptide-based self-assembling nanosystems have shown significant potential as non-viral vectors for the delivery of gene and small interfering RNA (siRNA) therapies. siRNA has demonstrated great promise in selectively silencing genes implicated in disease, offering a possible targeted therapeutic strategy. However, clinical application is hindered by challenges such as poor stability in biological fluids, limited cellular uptake, and non-specific tissue targeting. To face these obstacles, we developed nanofibers (NFs) made up of amphiphilic peptides with several surface-decorated moieties, as therapeutic, targeting, and penetration-enhancing ones. We have synthesized and used two structural peptides P1 and P2 specifically designed with a balanced hydrophilic-hydrophobic profile and an optimized electrostatic distribution. This design ensures the spontaneous self-assembly process, driven by both hydrophobic interactions and controlled electrostatic forces. The surface of the nanofibers was functionalized with various biological motifs, playing a crucial role for siRNA delivery. We bound to P2 a cell-penetrating peptide, well-known as gH625, the targeting peptide, an arginine derived-peptide able to bind siRNA via electrostatic interactions. In this study, structural and biophysical characterization of selected nanofibers was performed to gain insights into their interaction with nucleic acids. The methods employed for characterization included dynamic light scattering (DLS), zeta potential measurements, and circular dichroism (CD) spectroscopy. Additionally, the effects of temperature and pH on the conformation of nucleic acids were investigated.

Design and preparation of functionalized nanoparticles for cancer treatment

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Current therapeutic regimens based on poorly specific cytotoxic drugs show limited outcomes and severe related toxicities. Innovation in the field of oncology therapy has led to the development of advanced molecular systems named Antibody Drug Conjugates (ADCs) that combine the potency of cytotoxic drugs with the precision of targeting antibodies. New therapeutic strategies to further improve tumor targeting and decrease the off-target effects, are however still needed. Nanotechnology is entering this field offering new strategy to deliver therapeutics to tumors [1]. This study focuses on the generation of multifunctional nanoparticles (NPs) designed to enhance therapeutic efficacy and reduce the side effects of conventional chemotherapeutic drugs using nanoparticles functionalized with antibody fragments (AFs), in most cases antibody Fabs, that target specific biomarker overexpressed on cancer cells, and loaded with the drugs. The conjugation of AFs is achieved through specific chemoselective reactions on chemical linkers introduced on the C-terminus of their heavy chain that connect it to the surface of NPs ensuring stability in circulation and orientation of the AFs itself.

Initial tests have been carried out using the Fab' of the antibody Trastuzumab, a human IgG1 targeting the Her2 receptor. Different linkers compatible with the chosen conjugation chemistry have developed. Some include short peptide substrates of a microbial trasglutaminase [2] which can thus be exploited to both link the AFs to NP surfaces or to anchor additional chemical groups. Others include chemical moieties needed for direct embedding on the NPs.

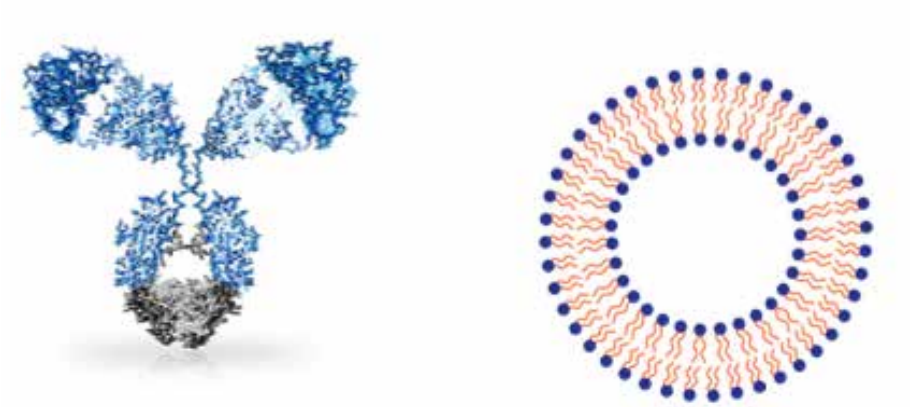

Figure 1 Example of an Antibody and liposome Nanoparticles

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Understanding PE_PGRS, cell wall proteins promoting mycobacterial survival in TB

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Tuberculosis, caused by Mycobacterium tuberculosis (Mtb), has been known to mankind since ancient times, and yet it is the leading cause of death by a single bacterium, infecting over 10 million and killing more than 1 million annually. A remarkable feature of TB is the dormancy, where Mtb establishes a dynamic equilibrium with the host immune system that lasts for a lifetime, although the immunological mechanisms governing the host-pathogen interaction remain poorly understood as are the virulence factors responsible for these distinct features [1].
The complete sequencing of the Mtb genome revealed the existence of a unique family of genes, the PE_PGRS family, containing about 65 members, 51 of which are thought to express functional proteins. PE_PGRS proteins play critical roles in bacterial pathogenesis and immune evasion. Their high abundance in pathogenic mycobacterial strains and the diversification of their functional roles are central elements in the evolution and virulence of Mtb [2].
The PE_PGRS family shares a peculiar modular structure constituted by a conserved N-terminal PE domain, a polymorphic glycine-rich domain (PGRS), and a C-terminal domain that varies in length and uniqueness among different PE_PGRS proteins. Yet, the lack of structural data of these proteins has so far hampered a satisfying understanding of their role in Mtb pathogenesis. Recently, we proposed the "sailing" model to describe how PE_PGRS proteins navigate the mycobacterial membrane exposing structural motifs for host interactions and/or deliver functional protein modules at their C-terminus [3]. This model, supported by AlphaFold 2.0 predictions, offers new insights into their dynamic behavior.
In collaboration with the Catholic University of Rome, we are investigating a panel of PE_PGRS proteins to tackle general features of these important virulence factors [4]. To this end, we integrate structural and biochemical approaches to characterize these modular domains and explore the structure-function relationship of this enigmatic family. Based on bioinformatic analysis, we have recombinantly produced both the PE domains and the C-terminal domains of selected PE_PGRS proteins. Our ongoing research is aiming to deepen the understanding of the PE_PGRS protein family and their interactions with host molecules, offering key insights into Mycobacterium tuberculosis pathogenesis.

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Structure-based design of mini-enzymes effective against Klebsiella pneumoniae

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Antimicrobial-resistance (AMR) stands nowadays as a pressing concern in public health. A group of six pathogens is named with the acronym 'ESKAPE' because of their ability to evade the biocidal action of traditional clinically used antibiotics [1,2]. Structural insight of molecular factors which play a key role in AMR is fundamental to deeply understand their mechanisms on and develop effective new drugs. The bacterium Klebsiella pneumoniae exploits various instruments to evade the immune system response and antibiotic attacks during infection. One such strategy involves its cell envelope, that includes polysaccharide layers acting as protective shield against stress and external substances.

Virion-associated depolymerases are large trimeric and multi-domain proteins that constitute the phage arsenal to degrade the polysaccharidic barriers in outer membrane of their bacterial host [3]. Thus, as recombinant proteins, they are endowed with outstanding potential in biotechnology and medicine [4]. In this study [5], we elucidated the structural and functional features of the capsular depolymerase KP34gp57 from the Klebsiella phage KP34. Based on the crystal structure and site-directed mutagenesis, we localized the key catalytic residues in an intra-subunit deep groove. Moreover, we engineered several N- and C-terminally truncated versions of KP34gp57 to dissect the role of each domain in the enzyme stability and catalytic activity. Serendipitously, our studies revealed C-terminally trimmed KP34gp57 variants that did not trimerize and were sufficiently stable to preserve full catalytic activity as monomers [6]. The development of trimmed monomeric and fully active phage depolymerases is innovative in the field, as no previous example exists apart from bacterial enzymes. Mini phage depolymerases can be optionally combined within chimeric enzymes to extend their activity range, facilitating their use in standalone treatments.

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Optimization of liposome nanocarriers for peptide delivery to activate LCAT in the treatment of neurodegenerative diseases

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The Lecithin Cholesterol Acyltransferase (LCAT) is an enzyme that catalyzes the esterification of cholesterol for their removal from the brain.[1] LCAT requires the stimulating action of apolipoprotein A-I (apoA-I). Oxidative stress impairs the activity of apoA-I, inducing the onset of neurodegenerative diseases.[2] On this premise, a new therapeutic strategy could focus on stimulating LCAT by means of a peptide that mimics the amino acid sequence of native apoA-I[3]. Additionally, peptide penetration into the brain is inhibited by the blood-brain barrier, which prevent most drugs from reaching the brain[4]. So, drug administration faces issues with opsonization of nanocarriers, as proteins like fibrinogen and immunoglobulins bind to their surface when nanoparticles enter the body. This process triggers the immune system to remove the nanoparticles, leading to their rapid elimination from the bloodstream.[5] PEGylation may obstruct this process and enhance the therapeutic efficacy of drugs, as it increases the likelihood of reaching the target before being eliminated from the body.[6] The study uses liposomes as drug carriers due to their physicochemical and biological advantages, prepared using a supercritical CO2 assisted method (SuperSomes), overcoming conventional production methods. SuperSomes allows an easy process scale-up, nanometric dimensions of the obtained liposomes, negligible solvent residues, and higher drug encapsulation efficiencies (up to 90%). Additionally, it enables to produce liposomes in a single step process and in continuous-mode characterized by a unimodal particle size distribution, ensuring reproducibility and high-quality of the results.[7] This study investigated the interactions between produced liposomes and plasma proteins, to compare differences between healthy individuals and neurodegenerative-affected patients. This research could significantly enhance the development of effective therapeutic strategies using peptide nanocarriers.

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Designing self-assembling bioactive lipopeptides

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Molecular self-assembly, driven by weak non-covalent interactions, enables the formation of robust and highly ordered nanostructures. Understanding the principles of supramolecular chemistry and the mechanisms underlying this process is crucial for designing dynamic and stable supramolecular systems, which can be controlled through external stimuli such as temperature, pH, and concentration.[1] In this context, self-assembling peptides (SAPs) have garnered significant attention due to their unique physicochemical properties, including biocompatibility, biodegradability, low cytotoxicity, and synthetic flexibility.[2] Among these, lipopeptides—a distinctive class of SAPs—rely on the hydrophile-lipophile balance and non-covalent interactions between peptide components to drive self-assembly.[3] In this study, we have developed a library of self-assembling lipopeptides by conjugating fatty acids with bioactive peptides tailored for tissue regeneration applications. We investigated the impact of different fatty acids on the self-assembly behaviour of these lipopeptides across various environments, thereby paving the way for the development of advanced composites suitable for 3D-printed scaffolds.[4]

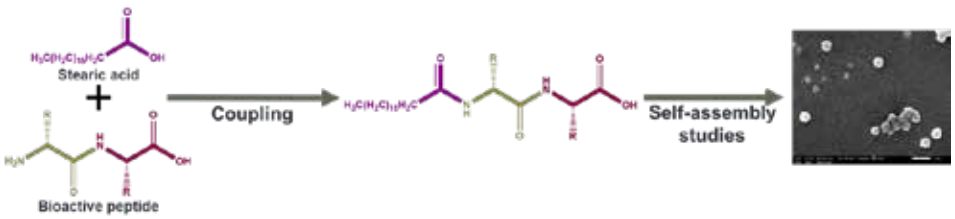

Figure 1. Synthesis of self-assembling lipopeptides

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Design and synthesis of peptidomimetic inhibitors of the TRIP8b-HCN interaction to prevent oxaliplatin-induced peripheral neuropathy

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Oxaliplatin-induced peripheral neuropathy (OIPN), a frequent side effect in patients receiving oxaliplatin to treat metastatic colorectal cancer, has a negative impact on quality of life and may lead to discontinuation of treatment. To date there is no treatment available, hence a strong unmet medical need. Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are widely expressed along the pain pathway and play an important role in the development and maintenance of neuropathic pain.[1] Nonselective HCN blockers have been shown to alleviate pain symptoms in acute OIPN models. Their cardiac side effects, however limit the clinical translation of their use.[2] Interestingly, the function of HCN is tightly regulated by its auxiliary subunit, tetratricopeptide repeat-containing Rab8b interacting protein (TRIP8b) which is not expressed in the heart.[3] In this context, disrupting HCN-TRIP8b protein-protein interaction could decrease OIPN symptoms, without inducing any cardiac effect. Based on the cocrystal X-ray structure of the tetratricopeptide repeat region (TPR) of TRIP8b with the C-terminus sequence of HCN[4] and MD simulations, we have designed peptidomimetic molecules targeting the TRIP8b-HCN interaction. These are mimics of the SNL peptide corresponding to the C-terminus of HCN channels. The efficacy of the compounds on behavioural pain tests were verified in OIPN animals.[5] Among the synthesized compounds, a hit peptoid demonstrated a dose-dependent antihyperalgic effect correlated with a modulation of the ionic current (Ih) triggered by HCN channels expressed in peripheral sensory neurons. More importantly, we did not observe any modulation of cardiac HCN currents highly suggesting the innocuity of our compound on the cardiac function.

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Solid-State Strategy for the S-Conjugation of Peptides Catalyzed by Zeolites and Promoted by Microwave Radiation: A Green Approach

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A green synthetic protocol to add a chemical function to a fully deprotected peptide to obtain a bioactive and/ or fluorescent-labeled conjugate is reported. A range of S-conjugation reactions promoted by the commercially available LTA zeolite to introduce different substituents on peptide cysteine residues [1,2] has been shown to take place in the solid state or in the presence of minimal amounts of organic solvent, with yields that are comparable to those of standard solution methods. The additional advantage of the procedure consists of easing the work up, for which green solvents, such as aqueous systems, can be employed. The protocol is implemented with microwave irradiation to shorten the reaction time as dielectric heating increases the diffusion rates of the mechanically milled reactants. [3]

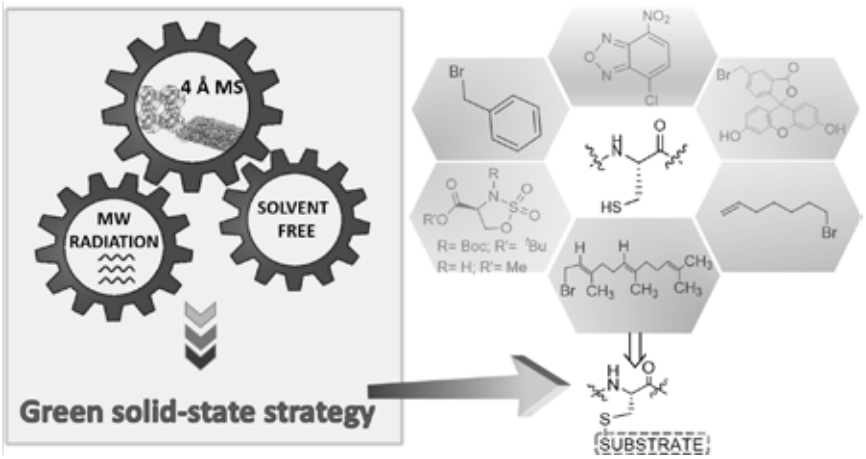

Figure 1. S-conjugation reactions performed by using a green solid state strategy

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Novel Carbonic Anhydrases in P. aeruginosa with antibacterial potential

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Given the urgent need to discover novel antimicrobial targets to fight bacterial infections, bacterial Carbonic Anhydrases (CAs, E.C 4.2.1.1) have emerged as a promising area of research.[1] Targeting bacterial CAs offers a novel and innovative approach to antimicrobial drug development: the inhibition of these enzymes can disrupt bacterial homeostasis and metabolic functions, thereby reducing virulence and inhibiting bacterial growth.[2,3] Focusing on the Pseudomonas aeruginosa genome, three novel potential γ-CAs were identified (PA-CAs). Synthetic DNA genes were created and utilized for heterologous expression in Escherichia coli, successfully purifying high-quality samples for biochemical and structural analyses, including studies on oligomeric state, secondary structure composition, and thermal stability. Kinetic investigations confirmed that PA-CAs exhibited CA activity and X-ray studies their belonging to the γ-class. Inhibition assays with various inhibitors were performed, yielding promising results. In conclusion, identified PA-CAs showed in vitro potential as molecular targets for the development of novel antibacterial drugs.

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Structural analysis of carbonic anhydrases from Acinetobacter baumanii for the development of novel antibacterial agents

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Acinetobacter baumannii is an invasive Gram-negative bacterium that causes a range of infections, including urinary tract infections, pneumonia, meningitis, and skin/wound infections, determining 929,000 deaths annually attributable to antimicrobial resistance (AMR). One of the main features of this pathogen is its ability to evade the host immune response and survive in harsh environmental conditions, including the presence of multiple antibiotics. A. baumannii microorganism is able to form biofilms on biotic and abiotic surfaces to enhance its survival in the host or a nosocomial environment with desiccation stress and starvation.[1-2] In addition, it protects itself against the immune system of the host by means of a lot of virulence factors and resistance mechanisms including surface glycoconjugates, secreted proteins, and metabolic pathways.[3-4] In this scenario, improving the pharmacological arsenal against A. baumannii pathogen is of fundamental importance.[5]

An emerging strategy for the treatment of bacterial infections to overcome the serious AMR problem is based on the identification of new molecular targets. Members of the carbonic anhydrase (CA) enzyme families have recently emerged as potential and novel antibacterial drug targets, since compelling data in literature strongly indicate that interference with CA activity leads to an impairment of bacterial growth and virulence, which in turn leads to significant antibacterial effects.[6]

Here, we report preliminary structural studies on two CAs, namely gAbCA and bAbCA, in order to provide a solid background for the rational design of new drugs targeting these enzymes.

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Screening of different green bases in Fmoc solid-phase peptide synthesis

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In recent years, the ACS Green Chemistry Institute has been promoting the use of more environmentally friendly solvents in all synthetic processes.[1] However, the solid phase peptide synthesis (SPPS) is the main technique for producing biologically active peptides, but is not regarded as environmentally sustainable due to the large amounts of toxic solvents and reagents employed. In this context, efforts are being made to replace piperidine in the Fmoc deprotection step. Different green bases [2] have been tested in various solvents with the aim of making the deprotection phase more environmentally friendly and minimizing side reactions.

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Effect of leptin on nitric oxide synthase activity in the hypothalamus of rats

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Introduction: Leptin is an adipokine, involved in the regulation of energy homeostasis. Leptin binds to the functional form of its receptor, which is expressed throughout the central nervous system but particularly in neurons of several regions of the hypothalamus, such as the arcuate nucleus, supraoptic nucleus, paraventricular nucleus and lateral hypothalamic area. Nicotinamide adenine dinucleotide phosphate-diaphorase [NADPH-d] staining method is widely used as a histochemical marker for nitric oxide synthase activity. In rat brain, abundant NADPH-d activity has been demonstrated in the hypothalamus.

Objective: To investigate NADPH-d activity in the arcuate nucleus, supraoptic nucleus, paraventricular nucleus and lateral hypothalamic area after intraperitoneal administration of leptin in rats.

Materials and methods: The study was carried out on 6 male Wistar rats with body weight 350 g, divided into 2 groups of 3 rats each: first group - injected i.p. with leptin (0.5 mg/kg) and second group (control group)- injected with saline (0.2 ml/100g). The animals were anesthetized 45 minutes later with thiopental (40mg/kg) and transcardial perfusion was performed using 4% paraformaldehyde. Serial coronal sections were cut on a freezing microtome (Reichert-Jung) at a thickness of 40 μm. Sections were then stained with the NADPH-d-technique using 0.1–0.2 mg/ml of nitroblue tetrazolium, 1 mg/ml β-NADPH and 0.3–0.5 % Triton X-100 in 0.1 M TRIS– HCl buffer (pH 7.4) at 37°C for 30–60 min. Data were statistically assessed by two-tailed Student's t-test and presented as mean ± standard error of the mean (SEM).

Results: A large number of NADPH-d positive neurons were present in the hypothalamus of both leptin- and saline-treated rats. As was often seen, NADPH-d reaction product diffusely filled the cytoplasm of neurons, with the nucleus remaining stain-free. Peripheral administration of leptin resulted in a significantly higher NADPH-d positive cell number in the supraoptic nucleus, paraventricular nucleus and lateral hypothalamic area as compared with the control animals. Additionally, relative to saline-treated rats, leptin produced more intense NADPH-d staining in the supraoptic nucleus and lateral hypothalamic area. The average optical density of NADPH-d positive neurons in the supraoptic nucleus and lateral hypothalamic area was significantly higher in leptin-treated animals as compared with saline-treated animals. By contrast to saline-treated rats, where nucleus arcuatus was unstained, leptin-treated rats showed a few NADPH-d positive neurons in the arcuate nucleus [1, 2, 3].

Conclusion: We demonstrated that systemic administration of leptin led to an increased NADPH-diaphorase activity in the arcuate nucleus, supraoptic nucleus, paraventricular nucleus and lateral hypothalamic area in rats. We suggest that these hypothalamic regions may be important centers in the brain for the leptin action, mediated by increased nitric oxide synthesis.

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Peptide-based soft materials paving the way to access higher contrast efficiency in MRI scans

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The use of Gd (III) complexes as T1 MRI contrast agents (CAs) has been questioned because of the evidence of ions accumulation in body districts of patients receiving multiple doses. For this reason, soft materials like Peptide-based Hydrogels and Nanogels (PBHs and PBNs) have been proposed to encapsulate Gd (III) complexes with the aim to protect them from transmetallation phenomena occurring in vivo and being responsible of Gd (III) release.[1] Moreover, the generation of supramolecular CAs can allow to increase the efficiency of the contrast, in terms of relaxivity value, in order to reduce the amount of the administered dose. This strategy has been exploited also to improve the performance of highly stable Fe (III) complexes and Chemical Exchange Saturation Transfer (CEST) agents,[2] recently emerged as valuable and non-toxic alternatives to the classical Gd (III) based contrast.

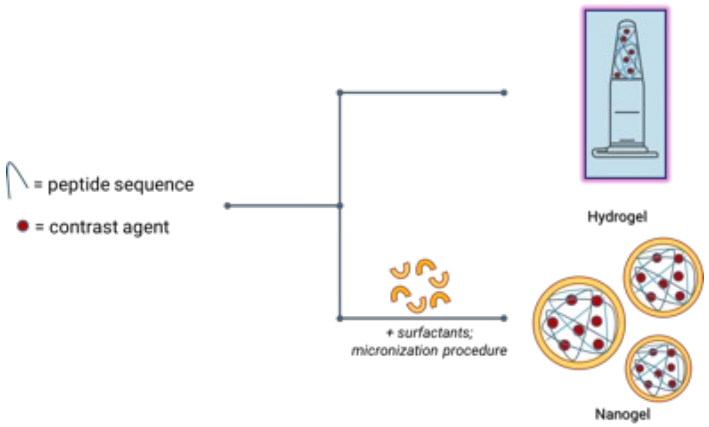

Figure 1. Schematic representation of supramolecular Cas formulation methods

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