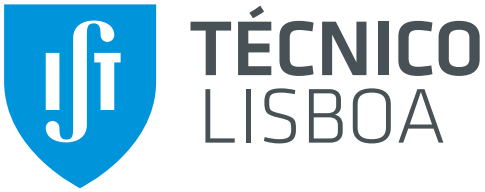
Program & Abstract Book



With the invaluable help of our institutional and corporate partners

Welcome

It is in the beautiful town of Sesimbra that we reconvene post-pandemic to continue the decades-long tradition of the EPI – Iberian Peptide Meeting.

We are confident this will be the perfect backdrop for three days of scientific exchange and collaboration, not only between the Portuguese and Spanish research communities, but also with the several participants hailing from beyond the Iberian borders.

As is tradition, the meeting covers varied fields of peptide science, ranging from peptide synthesis to structural characterization and to biological interactions and activity. As is also tradition, focus was put on facilitating attendance by young researchers, to boost exposure and networking, towards a stronger future community.

It has been a pleasure making this edition of the Meeting a reality, and we hope you share our enthusiasm for the upcoming days.

The organizers

Nuno C. Santos

João D. G. Correia

Manuel N. Melo

Contents

XVIII Iberian Peptide Meeting

Full Program

PL Plenary Lecture	52+6 min
KN Keynote	25+4 min
ST Short Talk	12+3 min
CT Company Talk	12+3 min
FT Flash Talk	3+1 min

Monday 27/11

12h00 — **Registration**

14h00 — **Opening remarks**

14h10

PL1 Maria Luisa Mangoni Sapienza University of Rome
Esc peptides for novel therapeutics against Pseudomonas aeruginosa *lung and ocular surface infections: beyond antimicrobial activity*

Chair: Rosario González-Muñiz

15h10

ST1 Helena S. Azevedo I3S, Universidade do Porto
Peptide building blocks to recreate basement membranes and develop in vitro *BBB models*

ST2 Douglas V. Laurents IQF Blas Cabrera, CSIC
Design principles for polyproline II helix peptides

CT1 Karin Rustler Iris Biotech
Novel protecting groups for side chain functional groups of amino acids

FT1 Marco Cavaco Instituto de Medicina Molecular
The use of a selective, nontoxic dual-acting peptide for breast cancer patients with brain metastasis

FT2 Pedro M. Alves I3S, Universidade do Porto
Dual-effect chitosan nanoparticles with conjugated pro-angiogenic and antimicrobial peptides

16h10 — **Coffee break & Posters**

17h00

ST3 Adam Carrera-Aubesart Universitat Pompeu Fabra
Topoisomers of a snake venom-derived antimicrobial peptide: lessons from in vivo *studies*

ST4 Bruna Costa I3S, Universidade do Porto
The Dhvar5-nanogels performance in combating orthopedic device-related infections

CT2 Alexandre Broges PARALAB-Bio
Pushing the boundaries in biomolecular interaction analysis with the WAVEsystem

FT3 Sofia A. Martins C2TN, Instituto Superior Técnico, Universidade de Lisboa
Bone-seeking peptides targeting the RANK-TRAF6 interface for bone metastases theranostics

FT4 Ana S. Silva-Herdade Instituto de Medicina Molecular
Bioactive peptides in functional foods can mitigate stress behaviors in zebrafish (Danio rerio)

Chair: Manuel N. Melo

18h00

19h00 — **Welcome cocktail**

Tuesday 28/11

9h00

KN1 Javier Montenegro CiQUS, Universidad de Santiago de Compostela
Supramolecular peptide assemblies for membrane transport and biomimetic systems

Chair: Helena S. Azevedo

9h30

ST5 Octavio L. Franco Universidade Católica de Brasília
In silico *optimization of antimicrobial peptide enables the development of bioinspired products*

ST6 Joana Calvário ITQB NOVA
Investigating amino acid enrichments and patterns: understanding biases in liquid-liquid phase separation

CT3 Luísa Aguiar Gyros Protein Technologies
An automated peptide synthesis and purification platform to overcome operational complexity

FT5 Christine Cruz-Oliveira Instituto de Medicina Molecular
Targeting arboviral infections in the central nervous system with peptide-porphyrin conjugates

FT6 Fernando Neiva Nunes ITQB NOVA
Room for improvement in the initial Martini 3 parametrization of peptide interactions

10h30

Coffee break & Posters

11h30

ST7 Vera Neves Faculdade de Medicina, Universidade de Lisboa
Peptide Shuttles to improve therapeutic action in the central nervous system

ST8 Salvador Ventura Universitat Autonóma de Barcelona
Exploiting α-helical peptides to block and unveil the structure of α-synuclein toxic oligomers

ST9 Jorge H. Fernandez Universidade Estadual do Norte Fluminense
Targeting integrin-mediated cell adhesion: unveiling the anti-metastatic potential of A9(x) cyclic peptides

ST10 Michael Timme University of Osnabrueck
Structural and topological determinants of Hexokinase-VDAC interaction

ST11 Beatriz L. Pires-Lima LAQV-REQUIMTE
Synthesis of pyridine-based melanostatin derivatives

FT7 José M. Coelho Instituto de Medicina Molecular
Antibiofilm effect of synthetic antimicrobial peptides on Gram-positive and Gram-negative bacteria

Chair: João Calamba Correia

13h00

Lunch

14h30

PL2 Norbert Sewald Bielefeld University
Cytotoxic peptides and conjugates for tumour targeting

Chair: Paula Gomes

15h30

ST12 Akhilesh Rai CNC, Universidade de Coimbra
Multifunctional antimicrobial coatings to prevent peri-implantitis

ST13 Ana Salomé Veiga Faculdade de Medicina, Universidade de Lisboa
Activity and mode of action of pepRF1, a new CXCR4-targeted HIV inhibitor

ST14 Lorenzo Cianni University of Antwerp
Exploring targeted autophagy modulation in pursuit of precision therapeutics for cancer and atherosclerosis

FT8 Catarina S. Lopes Instituto de Medicina Molecular
$\alpha_v\beta_3$ integrin peptide inhibitors impact on fibrinogen-erythrocyte binding

FT9 Cátia Rosa C2TN, Instituto Superior Técnico, Universidade de Lisboa
Design of novel peptides to target the intracellular G protein-dopamine receptor type 2 (D2R) interface

16h30

Coffee break & Posters

17h30

ST15 María Angeles Jiménez IQF Blas Cabrera, CSIC
Understanding protein interactions using NMR studies of peptide models

ST16 Ana Gomes LAQV-REQUIMTE
Peptide-based therapeutics towards the topical treatment of skin infections

ST17 Álvaro de la Cruz Instituto de Química Médica, CSIC
Design and synthesis of novel hemagglutinin fusion peptide inhibitors of H1N1 influenza virus

ST18 Rita Melo C2TN, Instituto Superior Técnico, Universidade de Lisboa
In silico *approach for the design of peptides targeting the RANK-TRAF6 interaction*

Chair: Nuno C. Santos

18h30

20h30

Meeting dinner

Wednesday 29/11

9h00 **KN2** Diana Lousa ITQB NOVA
Fusion peptides: Scenes from the lives of key players in viral infections

Chair: María Angeles Jiménez

9h30

ST19 Maria C. Lucana Institut Químic de Sarrià, Universitat Ramon Llull
BrainBike peptidomimetic enables efficient transport of antibody derivatives across brain endothelium

ST20 Sónia Gonçalves
Conjugation of nanostars with PaMAP1.9 to improve its anticancer activity

ST21 Miguel Maldonado
Novel leishmanicidal compounds as potent dimerization disruptors of LiTryR

FT10 Beatriz Vieira-da-Silva Instituto de medicina Molecular
Unravelling the factors that govern the ability of antiviral peptide-porphyrin conjugates to translocate the blood-brain barrier

10h30 **Coffee break & Posters**

11h30

Chair: Miguel A. R. B. Castanho

ST22 Diego Núñez-Villanueva Instituto de Química Médica, CSIC
A diastereopure azepane quaternary amino acid as effective inducer of β-turns and 3_{10}-helices

ST23 Sara C. Silva-Reis LAQV-REQUIMTE
Insights on the influence of proline residue on the biological effects of glypromate bioconjugates

ST24 Ana Alcalde-Ordóñez CiQUS, Universidad de Santiago de Compostela
Selective cleavage of DNA replication foci in mammalian cells by Cu^{II} peptide helicates

ST25 Ana C. Borges-Araújo ITQB NOVA
Study of alamethicin pore formation in lipid membranes using the Martini 3 coarse-grained force field

ST26 Rúben D. M. Silva C2TN, Instituto Superior Técnico, Universidade de Lisboa
Evaluating the activity of CXCR4 and D2R-targeting peptides using the TRUPATH platform

ST27 Ana Rita Ferreira LAQV-REQUIMTE
What can an ionic liquid do for the bioactivity of antimicrobial peptides? The case of W-BP100

13h00 **Closing remarks**

13h15 **Lunch**
**Attendance must be confrmed at registration time*

Poster list

(Posters P1 through P10 listed as Flash Talks FT1 through FT10)

P11 Anu Ploom University of Tartu
Kinetic study of aza-amino acid incorporation into peptide chain: Impact of steric effect of the side chain

P12 Axel Sarmiento Fuentes CiQUS, Universidad de Santiago de Compostela
Chemically fueled droplets for out-of-equilibrium sequence-specific RNA binding

P13 Carlos Cuadros-Higueras Instituto de Química Médica, CSIC
A "difficult sequence" cardiovascular peptide. Optimizing synthesis and purification

P14 Carmen González-González CiQUS, Universidad de Santiago de Compostela
Single α-helical peptide with conductive and fluorescence properties

P15 Carolina C. Buga Instituto de Medicina Molecular & ITQB NOVA
Molecular insights into membrane fusion mediated by the parainfluenza fusion peptide

P16 David Q. P. Reis ITQB NOVA
Peptide-based coacervates as catalytic microreactors

P17 Eduardo F. Vicente Universidade Estadual de São Paulo
Structural modifications of the antimicrobial peptide Ctx(Ile^{21})-Ha generate more potent peptide analogues with improved biological activities for animal production applications

P18 Patricia Sanmiguel CiQUS, Universidad de Santiago de Compostela
Helix-like peptides approach to three-way-junction binding

P19 Renato B. Pereira LAQV-REQUIMTE
Synthesis and antibacterial activity of Trichoderma longibrachiatum *longibramide E analogs*

P20 Ricardo Ferraz LAQV-REQUIMTE
Peptides derived from pet food protein ingredients: from proteomics to in vitro *bioactivity profiling*

Plenary Lectures

Esc peptides for novel therapeutics against *Pseudomonas aeruginosa* lung and ocular surface infections: Beyond antimicrobial activity

Maria Luisa Mangoni,[a] Mark Willcox,[b] Ivana d'Angelo,[c] Loretta Ferrera,[d] Y. Peter Di[e]

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[b] *University of New South Wales, Sydney, Australia;*
[c] *DiSTABiF, University of Campania Luigi Vanvitelli, Caserta, Italy;*
[d] *U.O.C. Genetica Medica, Istituto Giannina Gaslini, Genoa, Italy;*
[e] *Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, USA;*
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Antimicrobial resistance to existing antibiotics is an alarming threat to global human health and referred to as *the silent pandemic*. The Gram-negative bacterium *Pseudomonas aeruginosa* is a highly virulent and biofilm-forming "ESKAPEE" pathogen responsible for a variety of severe infections such as those found in the lungs of cystic fibrosis (CF) patients or associated with the ocular surface, like keratitis, especially in contact lens wearers. In this presentation, the *in vitro* and *in vivo* activities of the frog-skin derived antimicrobial peptide (AMP) Esc(1-21) and its diastereomer Esc(1-21)-1c (Esc peptides), will be shown. Esc(1-21) was discovered to have (i) rapid killing kinetics against *P. aeruginosa*, with a membrane-perturbing mode of action that limits the emergence of resistance; (iii) the capability to stimulate migration of bronchial epithelial cells and likely repair of the injured bronchial epithelium. Interestingly, the diastereomer Esc(1-21)-1c was less cytotoxic; more stable and with a better *in vivo* efficacy in reducing pulmonary bacterial load in a mouse model of *P. aeruginosa* lung infection. Furthermore, polymeric nanoparticles made of poly(lactic-co-glycolic) acid revealed to be a promising tool for pulmonary delivery of Esc peptides and to enhance their local antimicrobial activity. Recent studies have also highlighted their efficacy in restoring the function of defective CF transmembrane conductance regulator (CFTR) channel in CF patients[1], an unprecedented property of AMPs. In parallel, Esc(1-21)-1c was found to be more efficient than the all-L counterpart in treating *P. aeruginosa*-keratitis and with the capability to elicit corneal wound repair in appropriate mouse models. Yet, immobilization of these peptides to soft contact lenses revealed to be an effective strategy to produce an antimicrobial medical device able to hamper bacterial adhesion to it, and hopefully, bacterial transfer to the ocular surface. Overall, these data make Esc peptides encouraging alternatives to conventional antibiotics for the development of novel multifunctional drugs and nanotechnology approaches to treat lung pathology in CF and to fight eye dysfunctions like keratitis, characterized by both tissue injury and bacterial infection.

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Acknowledgements

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Cytotoxic peptides and conjugates for tumour targeting

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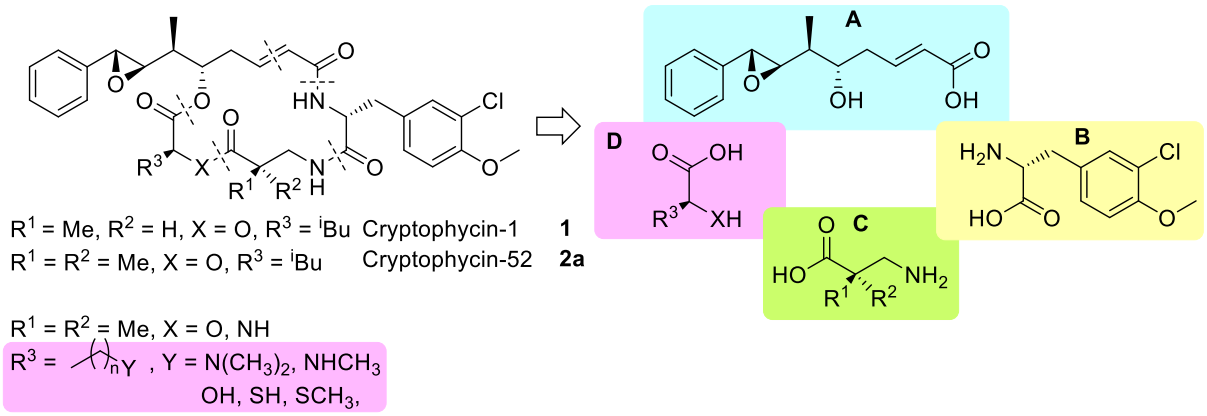
Many anticancer chemotherapeutics interfere with microtubule dynamics, which causes cell-cycle arrest and apoptosis. Cryptophycins are tubulin-binding 16-membered highly cytotoxic macrocyclic depsipeptides isolated from cyanobacteria. Strong antiproliferative activities with 100- to 1000-fold increased potency compared to paclitaxel and vinblastine have been observed. Cryptophycins are highly interesting drug candidates, since their biological activity is not negatively affected by P-glycoprotein, a drug efflux system commonly found in multidrug resistant cancer cell lines and solid tumors. These characteristics made the synthetic analog cryptophycin-52 (LY355703) a promising candidate for cancer treatment. However, the clinical trials had to be discontinued because of neurotoxic side effects and lacking efficacy in vivo.

We have developed efficient strategies for the synthesis of cryptophycins and their analogues taking specific emphasis on the synthetically most challenging unit A. In addition, new interesting functionalities have been introduced in different positions for SAR studies.[1] Novel unit D analogues with amino, hydroxy, mercapto and thioether moieties have been synthesized. Many of them are highly cytotoxic.

Cryptophycin conjugates with small molecules (folic acid), peptides, peptidomimetics, and antibodies have been developed for targeted delivery in tumor therapy.[2,3]



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Keynote Lectures

Supramolecular peptide assemblies for membrane transport and biomimetic systems

Javier Montenegro

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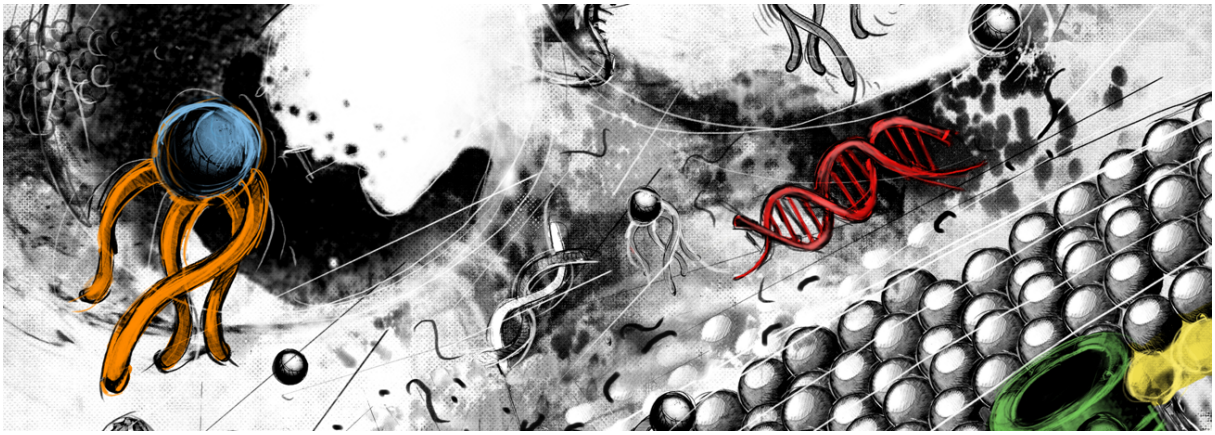
Our research group is interested in the application of supramolecular chemistry to understand and manipulate biology.[1,2] Our work philosophy is based in the importance of weak and non-covalent forces to control the shape and the topology of biomolecules, which are governed by the principles described by supramolecular chemistry. These supramolecular lessons can then be applied to control the properties and function of biomolecules. We believe that by modulating the shape we can mimic, control and improve functional behaviour. With focus in supramolecular interactions for artificial membranes and tubular composites, we investigate the construction of synthetic systems for controlling and emulating biology and life-like soft systems.[3-4]



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Fusion peptides: Scenes from the lives of key players in viral infections

Diana Lousa,[a] Mariana Valério,[a] Carolina C. Buga,[a,b] Ana M. Sequeira,[c] Bruno L. Victor,[a,d,e] Alessandro Laio,[f] Diogo A. Mendonça,[b] João Morais,[b] Manuel N. Melo,[a] Miguel Rocha,[c] Miguel A.R.B. Castanho,[b] Ana S. Veiga,[b] Cláudio M. Soares[a]

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Fusion peptides (FPs) are determinant for the infection process of enveloped viruses, which include major pathogens such as HIV, influenza and SARS-CoV-2. These peptides, located within viral fusion proteins, insert into the host membrane and promote fusion between the viral and host membrane, which is essential for the infection process. FPs are very promising therapeutic targets and, therefore, there is a tremendous interest in identifying these peptides and characterizing their properties.

FPs from different viral families are quite distinct at the sequence and structural level, although they are all hydrophobic and tend to have high Ala and Gly contents. In some cases (e.g. influenza, HIV and parainfluenza) the FP corresponds to the N-ter end of the fusion protein, whereas in other cases (e.g. dengue) it is internalized. This raises an important question: How can peptides with such distinct features execute the same function? In the last years, we have addressed this question using a combination of non-standard simulation techniques, machine learning methods and biophysical methodologies performed by collaborators. Using influenza and parainfluenza viruses as models, we show that these peptides use similar molecular mechanisms to promote fusion. We also shed light on important questions, such as the effect of pH and the impact of mutations. Currently we are investigating the effect of the putative fusion peptides from SARS-CoV-2, to determine which peptide(s) are used by this virus to promote fusion. Overall, these studies provide a global perspective of the molecular mechanisms of viral fusion peptides, which are privileged therapeutic targets to fight viral infections.

Company Talks

Novel protecting groups for side chain functional groups of amino acids

Karin Rustler,[a] Thomas Bruckdorfer,[a] Haixiang Zhang,[b] Udo Kalejs,[c] Vilnis Liepins,[c] Kai Holland-Nell,[d] Jacqueline Dalski,[d] Stephan Pritz[d]

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[b] *Iris Biotech Laboratories GmbH, Adalbert-Zoellner-Str. 1, 95615 Marktredwitz, Germany*
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We are presenting a **selectively cleavable protecting group for the epsilon-amino group of lysine**, which plays an important role during peptide synthesis, e.g., for modification of the lysine side chain on the synthesis resin. Trityl-based, mildly acidic cleavable protecting groups, e.g., Mtt or Mmt, are incompatible with strongly acid-labile resin linkers such as trityl. Amino acid derivatives, which carry the **hydrazinolysis-susceptible protecting group Dde**[1] **or ivDde**[2] are frequently used for the synthesis of branched, cyclic, or side chain-modified peptides. However, **Dde might migrate to free lysine epsilon-amino groups** (scrambling)[3,4] and, for the more robust ivDde, total removal is hardly possible.[5] In our study, we present the **possibilities and limitations of the new lysine Dmb** (dimethylbarbituric acid) derivatives (MeDmb, EtDmb, ivDmb) and compare their properties with the existing protecting groups Dde and ivDde.[6,7,8]

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 especially useful for masking functional groups which contain terminal atoms with free electron pairs (-OH, -SH, $-NH_2$).[9,10] Thus, they are suitable to protect, e.g., the side chains of the natural amino acids Lys, Tyr, Asp, Glu, Cys, Ser, and Thr, and of other building blocks which are used for peptide synthesis. Especially in the case of cysteines, these protecting groups are a valuable addition to the peptide chemist's toolbox for regioselective formation of disulfide bonds.

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Pushing the boundaries in biomolecular interaction analysis with the WAVEsystem

Alexandre Borges

Over the past few years, many techniques have been developed that have enabled the quantification of biomolecular interactions. Amongst those, label-free and real-time technologies are the highly desirable options, especially when they are versatile in experimental design and with a high degree of throughput.

GCI (Grating Coupled Interferometry), a proprietary technology developed by Creoptix AG and recently made accessible for research and industry applications, have revolutionized this field of interactomics in such a way that it combines the strengths of the current mainstream techniques (BLI and SPR), while reducing or eliminating their main drawbacks.

Due to its technology and chip design, GCI displays an extremely high sensitivity allowing for analyses with no lower size limit of the analyte compared to other techniques. Additionally, ultra-fast transition times of 150 ms can be monitored. Due to its no-clog microfluidics design, it also allows the direct study of biofluids and crude reaction mixtures.

An automated peptide synthesis and purification platform to overcome operational complexity

L. Aguiar, M. Muthyala, S. Lüdtke, R. Zitterbart

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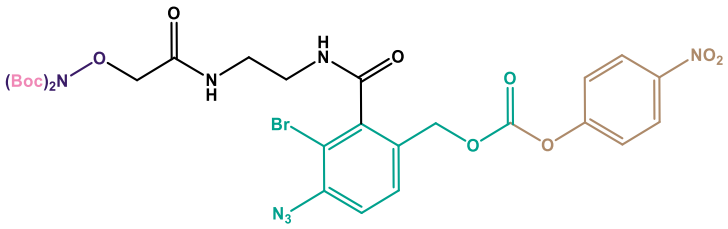
Purity no longer has to be at odds with speed and efficiency in early-stage drug development. Here we present the peptide synthesizer PurePep® Chorus and PurePep® Easy Clean (PEC) purification technology to address challenges faced in manufacturing novel peptide drug targets. Our PEC technology utilizes a catch and release approach, whereby a linker (picture below) is covalently attached during SPPS, immobilized onto agarose beads and released through a reduction reaction - yielding the purified PEC-grade peptide of interest to the user.[1]



When PEC is combined with the PurePep Chorus, users can have a faster and more flexible workflow from start to finish. The Chorus offers users with capabilities including (1) independent induction heating on each RV, that has adjustable deprotection and coupling temperatures, (2) reduced priming volumes, as expensive and sensitive monomers can be loaded in a SingleShot™ amino acids vial, and (3) live UV monitoring that takes the guess work out of achieving effective deprotection. Parallel synthesis and purification workflows have the capability of being achieved in a single day, as the PEC Auto Kit integrates well onto the Chorus, automating the parallel purification of six peptides in just over five hours. PEC provides an alternative approach to conventional HPLC, that can be labor intensive and have high solvent consumption. Binding assays have validated that PEC-grade peptides provide comparable results to HPLC-grade peptides.[2] Overall, we hope our PurePep solutions can be combined to reliably produce high quality peptides, on a shorter timescale.

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Short Talks

Peptide building blocks to recreate basement membranes and develop *in vitro* BBB models

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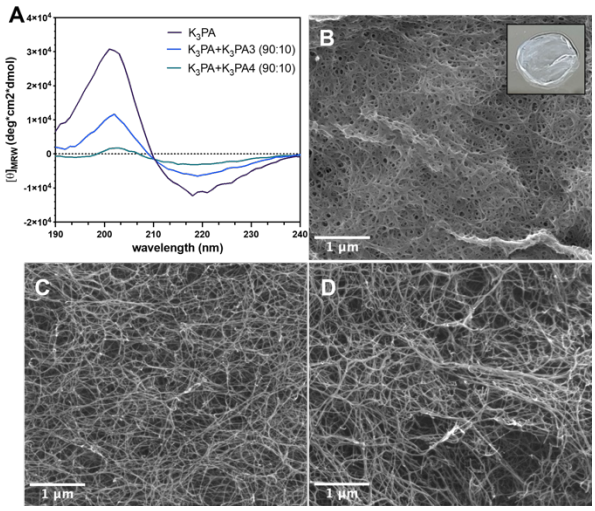
Basement membranes (BMs) are specialized extracellular matrices that provide tissue separation and barrier functions, but also serve as an instructive substrate for cell signaling. They are composed of various proteins (collagen type IV, laminin, nidogen, perlecan), displaying time- and tissue-specific expression patterns. For example, the BM contributes to the integrity of the blood-brain barrier (BBB), formed by brain endothelial cells (BECs), pericytes, embedded in the vascular BM, and astrocyte endfeet.

Our group has developed thin membranes by interfacial self-assembly between peptide amphiphiles (PAs) and hyaluronic acid (HA)[1] resembling the nanostructure of BMs. Currently, we are exploiting the use of cationic PAs (K_3PA: $C_{16}V_3A_3K_3$) incorporating sequences derived from BM proteins (Coll IV: $C_{16}V_3A_3K_3$- GEFYFDLRLKGDK, K_3PA3; LN: $C_{16}V_3A_3K_3$-RKRLQVQLSIR, K_3PA4:) to recreate the nanoarchitecture and composition of the BBB BM. K_3PA forms β-sheet structures (Figure A) and nanofibrous membranes in presence of HA (Figure B). Co-assembly of K_3PA with K_3PA3 or K_3PA4 does disturb significantly the β-sheet conformation of the co-assembled pair, neither the morphology of the membranes, when mixed at 90:10 ratio (Figure A, C, D). BECs will be cultured on the various membranes to identify bioactive signals capable of directing cellular processes and enhancing barrier properties.



(A) *CD spectra of PA solutions (0.05 µM, pH 7). SEM micrographs showing the surface of membranes fabricated by self-assembly of 1% (w/v) HA and 2% (w/v)* ***(B)*** *K_3PA,* ***(C)*** *K_3PA/K_3PA3, (90:10 ratio) and* ***(D)*** *K_3PA/K_3PA4 (90:10 ratio).*

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Design principles for polyproline II helix peptides

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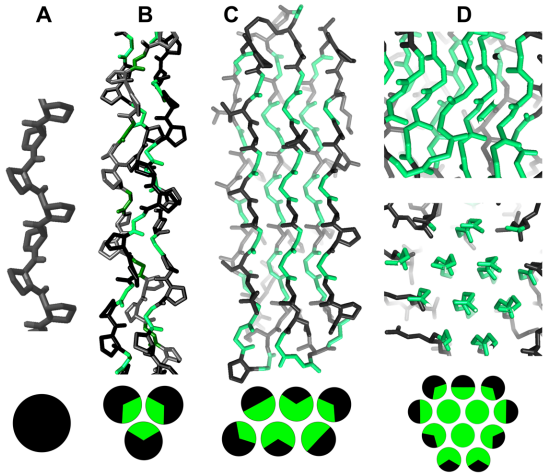
The polyproline II (PPII) helix is the protein secondary structure adopted by $(Pro)_N$ peptides in aqueous solution. This structure is also adopted by $(Pro\text{-}Pro\text{-}Gly)_N$ peptides whose tiny glycine allow PPII helices to form the collagen triple helix. Moreover, peptides with $(Xaa\text{-}Gly\text{-}Gly)_N$ sequences adopt PPII helices assembled into bilayers[1] and $(Gly)_N$ peptides adopt PPII helices organized as a 3D honeycomb[2]. These glycine-rich PPII helices have been recently identified in natural proteins[3], but the bases of their stability is a subject of active research[4]. Our analyses suggest that Cα-H···O=C H-bonds contribute to conformational stability. We find PPII helices are arranged to promote favorable macrodipole interactions. Finally, we observe great variety in the connecting loops, which suggests tolerance of flanking sequences. These results suggest improvements for designing Gly-rich peptides to adopt PPII helical bundles for biotech.



***Top**: PPII helices adopted by **A** $(Pro)_N$ peptides, **B** $(Pro\text{-}Pro\text{-}Gly)_N$ peptides in collagen, **C** $(Xaa\text{-}Gly\text{-}Gly)_N$ in antifreeze proteins and **D** $(Gly)_N$ segments in anaplastic lymphoma kinase. **Bottom**: Pro (black) versus Gly (green) content.*

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Topoisomers of a snake venom-derived antimicrobial peptide: lessons from *in vivo* studies

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To combat antimicrobial resistance (AMR), it is essential to have a good understanding of the infection mechanisms, particularly those concerning the interaction between anti-infective agents and pathogen targets. In this context, antimicrobial peptides (AMPs) are emerging as promising therapeutic alternatives in the global issue of AMR. Ctn[15-34][1], a segment of crotalicidin (Ctn), a cathelicidin from a South American rattlesnake, was shown as a promising candidate for its antimicrobial and antitumoral properties as well as its remarkable stability in human serum. These preliminary data led to studying topoisomers (retro, enantio, and retroenantio versions) of both Ctn and Ctn[15-34] to unveil structural features responsible for their activity[2]. All topoisomers demonstrate *in vitro* activity against gram-negative bacteria and tumor cells comparable to the cognate sequences, albeit slightly elevated toxicity toward normal cells. Still, increased stability in human fluids encouraged further exploration. Hence Ctn[15-34] and Ctn retroenantio, the best *in vitro* performers, were selected for an *in vivo* efficacy evaluation in a murine model of *Acinetobacter baumannii* systemic infection. Unexpectedly, all animals treated with retroenantio peptide succumbed within 8 h. For Ctn[15-34]-treated animals, divergent survival outcomes between genders were found, females outlasting males by ~ 1 day. These results once more serve as cautionary note to researchers in (peptide) medicinal chemistry, to treat encouraging *in vitro* data as an incentive to necessary *in vivo* studies but with the caveat that, at current levels of understanding, straightforward translation may not always be the case.

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The Dhvar5-nanogels performance in combating orthopedic device-related infections

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Orthopedic Device-Related Infections (ODRIs) are a major medical challenge, particularly due to the involvement of biofilm-encased and multidrug-resistant bacteria[1]. Current treatments, based on antibiotic administration, have proven to be ineffective. Consequently, there is a need for antibiotic-free alternatives[2]. Antimicrobial peptides (AMPs) are a promising solution due to their broad-spectrum of activity, high efficacy at very low concentrations, and low propensity to induce resistance[3]. We aim to develop a new AMP-based chitosan nanogel coating to prevent ODRIs. Chitosan was functionalized with norbornenes (NorChit) and then, a cysteine-modified AMP Dhvar5 was covalently conjugated to NorChit (NorChit-Dhvar5), through a thiol-norbornene photoclick chemistry. NorChit-Dhvar5 nanogels were produced using a microfluidic system used and were characterized using Transmission Electron Microscopy (TEM) and Nanoparticle Tracking Analysis (NTA). The nanogels antibacterial properties were assessed in Phosphate Buffer (PBS) for 6 h, against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. aureus* methicillin-resistant (MRSA), and in Muller-Hinton Broth (MHB), 50% (v/v) in PBS, for 6 and 24 h against MRSA. The obtained NorChit-Dhvar5 nanogels, presented a round-shaped and ~100 nm. NorChit-Dhvar5 nanogels in a concentration of 10^{10} nanogels/mL in PBS were capable of reducing the all bacteria up to 99%. These results were corroborated by a 99% *S. aureus* MRSA reduction, after 24 h in medium and medium supplemented with human plasma proteins. Furthermore, no signs of cytotoxicity against osteoblastic MC3T3-E1 cells after 14 days were verified, having high potential to prevent antibiotic-resistant infection in the context of ODRIs.

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In silico optimization of antimicrobial peptide enables the development of bioinspired products

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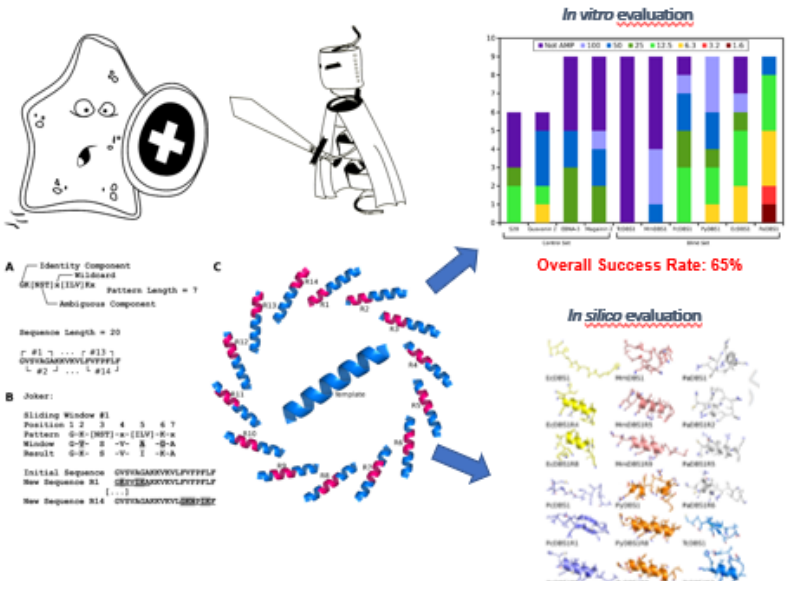
Antimicrobial peptides (AMPs) have attracted considerable attention because of their multiple and complex mechanisms of action toward resistant bacteria. However, reports have increasingly highlighted how bacteria can escape AMP administration. Here, we have described the use multiple strategies including genetic and Joker algorithms to design synthetic AMPs derived from bacteria, plants and animals. This approach yielded different peptides classes including mastoparans, glycin-rich and clavanins that possess an unusually high proportion of cationic residues and use tyrosine residues as hydrophobic counterparts. At least dozens of peptides emerged as a prototype AMP, among fifteen guavanin analogues screened for their activity against an engineered luminescent strain. Similarly, clavanins and mastoparans derivated were also selected. Peptides were further characterized in terms of structure, activity and biotechnological potential for development new compounds useful for human and animal health. Most of those novel peptides were unstructured in water and underwent a coil-to helix transition in hydrophobic environments. This conformation was corroborated by NMR analysis in dodecylphosphocholine micelles, which revealed an α-helical structure. Peptides generated caused a bactericidal effect at low micromolar concentrations to several resistant bacteria, causing membrane disruption, without triggering depolarization but rather hyperpolarization. Finally, here the strategies for production in large scale was also discussed in order to prepare such peptides for the market. In summary the present work presents a computational approach to explore natural products for the design of short and potent peptide antibiotics that could be used against resistant bacteria.



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Investigating amino acid enrichments and patterns: Understanding biases in liquid-liquid phase separation

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Our research focuses on the exhaustive analysis of amino acid enrichment and patterning within IDPs that undergo liquid-liquid phase separation (LLPS). This study encompasses an examination of 198 distinct proteins representing a wide array of functional protein families, spanning from RNA and DNA binding proteins to hydrolases and structural proteins. The amino acid enrichment analysis revealed pronounced variations in regions that promote droplet formation, referred to as Droplet Promoting Regions (DPRs). Notably, these regions exhibit a prominent enrichment of Glycine (G), Serine (S), Proline (P), and Alanine (A). Furthermore, we have observed that polar amino acids play a pivotal role in promoting disorder within intrinsically disordered proteins, while aromatic and charged residues, though present in lesser quantities, confer the multivalent properties essential for the 'stickers' component crucial to LLPS. While these overall findings align with previous literature, our research reveals family-specific variations in amino acid composition. Notably, we observed significant differences in the percentage of polar residues, with 56% in RNA-binding and 36% in chromatin-binding proteins, thus illustrating the distinct characteristics of different protein families concerning LLPS.

Additionally, amino acid patterning within DPRs revealed repeating motifs in such regions, with residues appearing in triples being particularly prevalent, such as GRG in RNA binding proteins, PAP in regulatory proteins and SAS in hydrolases. Charged amino acids, represented in pairs (e.g. DD, KK) were identified, as well as clusters of the previously mentioned enriched residues (e.g. GGG, PPP). Furthermore, rare (less than 1%) but significant motifs, such as YSPTSPSY, YGGDRGG, and YGQQSS, were found to repeat perfectly along DPR sequences, emphasizing their relevance to LLPS.

Hence, we are actively pursuing a comprehensive understanding of the relationship between sequence disorder and a protein's ability to undergo LLPS, in order to elucidate on the chemical principles governing LLPS in biological systems. This knowledge will have profound implications for controlling and harnessing this phenomenon, and to develop artificial systems with broad applications in, for instance, drug discovery and biocatalysis.

Acknowledgements

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Peptide Shuttles to improve therapeutic action in the central nervous system

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The field of Blood-brain barrier (BBB) peptides shuttles (BBBpS) has advanced rapidly in recent years. The application of peptides to improve the transport of therapeutics to the brain is extremely important for brain pharmacology and medicinal chemistry. However, little is known about BBBpS properties that enable brain delivery. In fact, the development of these molecular tools depend on the hypothesis that cell-penetrating peptides (CPPs), with proven capacity to traverse cell membranes, will traverse other barriers (e.g., behave as BBBpS). While CPPs have indeed been efficiently used to deliver cargoes such as proteins, nucleic acids, small drugs, or nanoparticles into cells, success in delivering these cargoes to the brain is very limited. Thus, the possible relationship between CPPs and BBBpS remains unclear and has not been subject to thorough experimental scrutiny. In the present work, we have applied a quantitative methodology that combines meta-analysis and statistical reasoning, to demonstrate that, based on intrinsic structural features, i.e., size, few aromatic residues, slightly hydrophobic and slightly cationic nature, very few CPPs are indeed BBBpS. To support and validate our hypothesis, 10 peptides were selected and tested in an in vitro BBB model and in vivo. Most BBBpS candidates were able to cross the BBB, displaying similar or improved brain penetration, when comparing with PepH3, a well-characterized and successful BBBpS[1-4]. On the other hand, non-BBBpS displayed poor BBB permeability, thus corroborating the methodology adopted.

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Exploiting α-Helical Peptides to Block and Unveil the Structure of α-Synuclein Toxic Oligomers

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Alpha-synuclein aggregation plays a pivotal role in driving neurodegeneration observed in Parkinson's disease. The quest for molecules capable of specifically targeting the toxic oligomeric forms of α-synuclein with high affinity has been a long-standing objective. Leveraging the biophysical properties of toxic oligomers, we have identified a family of α-helical peptides, including human endogen molecules, with remarkably low nanomolar affinities for these α-synuclein species, while preserving the functionality of the monomeric protein. These peptides exhibit potent anti-aggregation capabilities, effectively mitigating oligomer-induced neuronal damage[1,2,3].

Exploiting these peptidic nanomolar binders, and integrating SSNMR, CryoEM, HX-MS, SAXS and kinetic analysis we have uncovered a symmetric and well-defined internal architecture within α-synuclein oligomers. The NAC domain emerges as the oligomer's rigid core, while the N- and C-termini adopt partially collapsed and disordered states, demonstrating their nonessential role in oligomer formation. Intriguingly, the transition from oligomers to fibrils is orchestrated by a short α-synuclein N-terminal region, spanning residues 36 to 57. Notably, hereditary mutations associated with early-onset Parkinson's disease, induce conformational fluctuations in this precise N-terminal region, leading to the accumulation of toxic oligomers that resist molecular chaperone-mediated remodeling[4].

These findings not only advance our comprehension of the mechanisms governing oligomer-to-amyloid conversion but also propose a novel pathogenic mechanism underlying α-synuclein mutations, thereby establishing a robust foundation for novel, peptide-based, therapeutic strategies targeting oligomer-associated pathology in Parkinson's disease.

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Targeting integrin-mediated cell adhesion:Unveiling the anti-metastatic potential of A9(x) cyclic peptides

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Integrins, integral membrane proteins, embrace pivotal roles in cell adhesion, migration, proliferation, survival, and intracellular signaling processes. Integrin subunits, including α4, α5, α6, αV, β1, and β3, are fundamental in facilitating cell adhesion during metastasis. Disrupting their functionality presents an inventive possibility for metastatic tumor treatment. Leveraging the interactive loop of desintegrin-like domains, we have engineered the A9(x) cyclic peptides based on the interactive loop of human "A Desintegrin and Metalloprotease" (ADAM) proteins through *Coputer Aided Molecular Desing* (CAMD) methods. These cyclic peptides are designed to interact with the MIDAS site of integrins, effectively disrupting cell-matrix communication. To gauge the inhibitory potential of the A9(x) peptide structures in impairing various aspects of metastatic cell lineage B16F10 behaviour, including adhesion, proliferation, and migration in fibronectin, collagen, and laminin-coated dishes. In comparison to fibronectin (control) B16F10 cells displayed reduced to laminin (26%) and collagen (35%) adhesion. In adhesion assays, the A9s peptide demonstrated its capacity to inhibit cell adhesion to fibronectin, achieving a remarkable inhibition (68%) at a concentration of 1uM, with an estimated IC_{50} of 200 pM. In growth assays, concentrations of 0.1nM and 100nM of A9s led to notable growth inhibition (55%) between 24 and 72 hours. The addition of peptide (100nM) at 24 and 48 hours further escalated growth inhibition to 78%. Scratch assays uncovered a proliferation inhibition in B16F10 cells (35%) treated with 100nM of A9s at 6 and 12 hours of incubation. Simultaneously, cell migration assays exhibited a remarkable inhibition (56%) of the B16F10 cell line migration process at a peptide concentration of 0.1nM. Low toxicity of the peptide was confirmed at mM concentrations in MTT test. Notably, when compared to Cilengitide (EMD 121974), the A9(x) peptides consistently demonstrated superior inhibitory efficacy. Together, these findings support the A9s peptide's potential to disrupt adhesion and migration processes in B16F10 cells, acting as an effective integrin antagonist at nM concentrations without apparent cellular toxicity. These results strongly suggest a promising anti-metastatic role for this cyclic peptide structure.

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Structural and topological determinants of Hexokinase-VDAC interaction

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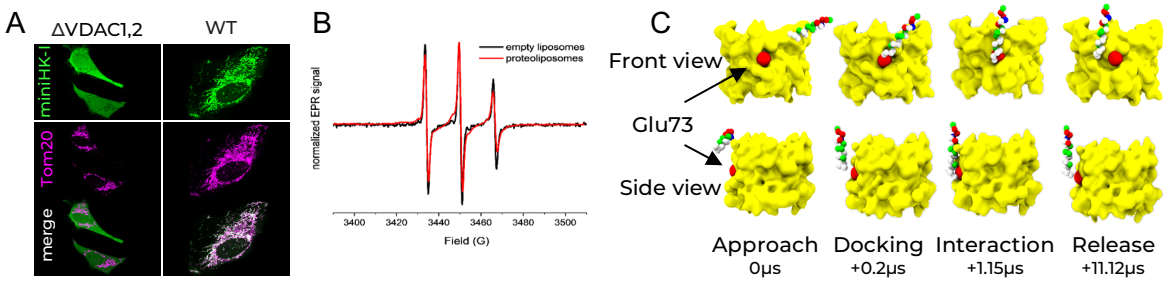
Ceramides are central intermediates of sphingolipid metabolism that can activate a variety of tumor suppressive cellular programs, including cell cycle arrest, senescence and apoptosis. Leakage of ceramides from the ER to mitochondria induces the mitochondrial pathway of apoptosis via the pore forming Bcl-2 protein Bax. Our group has previously identified an isoform of the Voltage Dependent Anion Channel (VDAC), VDAC2, as a direct effector of ceramide-induced apoptosis[1]. Ceramides can directly bind to VDACs via a membrane facing glutamate, Glu73 in VDAC1 and Glu84 in VDAC2, respectively. Importantly, this residue is also a direct and essential binding partner for the amphipathic, N-terminal helix of Hexokinase-1 (HK-1), the entry point enzyme of glycolysis. In contrast to ceramide binding to VDACs, HK-1 binding to VDACs promotes cell survival and growth and is heavily upregulated in glycolytic cancer cells. Here we show, that VDACs need to be specifically oriented in the membrane, with Glu73/84 in a deprotonated state, to be able to recruit HK-1. Our findings mark a first step in unraveling the complex interplay of interactions between HK-1, VDACs and ceramides which has the potential to decide the fate of the cell.



A Live-cell imaging of GFP-tagged miniHK-1 (Residues 1-17 of human HK-1) and Tom20, an Outer Mitochondrial Membrane marker. B EPR-spectroscopy of MTSSL-labelled HK-1 peptide (Residues 1-25 of human HK-1) incubated with empty liposomes or VDAC1-containing proteoliposomes. A shallower increase in the EPR-signal and a lower overall signal amplitude indicate immobilization of the labelled peptide onto the proteoliposomes. C Coarse-grain MD simulation snapshots showing the interaction of a N-terminal helix peptide of HK-1 with VDAC1.

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Synthesis of pyridine-based melanostatin derivatives

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Melanostatin (MIF-1) is an endogenous hypothalamic neuropeptide that acts as a positive allosteric modulator (PAM) of the D_2 receptors (D_2R). By increasing the affinity of these receptors to dopamine, MIF-1 enables their activation at lower concentrations,[1-3] being potentially relevant in Parkinson's Disease therapy. Despite its potent PAM activity, MIF-1 exhibits low gastrointestinal absorption and reduced metabolic stability.[1-3] Previous studies using heteroaromatic scaffolds led to MIF-1 analogs with improved PAM activity in comparison with the parent neuropeptide.[1-3]

In this work, 12 novel pyridine-based MIF-1 derivatives were synthesized, chemically characterized, and pharmacologically evaluated by functional assays at the D_2R. In the functional assays, one MIF-1 analog promoted a 3-fold increase of dopamine potency at 0.01 nM, while the parent neuropeptide was devoid of PAM activity at this concentration. Toxicological assays using human differentiated SH-SY5Y cells demonstrate that this compound does not exhibit significative toxicity at 100 μM (MTT reduction and neutral red uptake assays). This research opens one avenue for the discovery of novel anti-Parkinson hits with potent PAM activity and safe toxicological profiles.

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Multifunctional antimicrobial coatings to prevent peri-implantitis

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The placement of dental implants is widely known as reconstructive treatment to replace missing or damaged teeth[1]. The bacterial infection leads to inflammation in gingiva followed by bone loss around the dental implants, which is called peri-implant diseases. The implant failure due to peri-implantitis has been increasing at significant rate of 8%, which corresponds to more than 1 million failures globally[2]. Here, we demonstrate the coating of near-infrared (NIR) light-responsive polydopamine (PDA) on Ti surfaces followed by the covalent conjugation of LL37 peptides. LL37-PDA-Ti surfaces have antimicrobial property and are biocompatible to human gingiva fibroblasts (HGFs) and human oral keratinocytes (HOKs) cells.

A very thin layer (5 ±2 nm) of PDA is coated on Ti surfaces. Photothermal measurement indicates that the exposure of NIR light for 5 min increases the temperature of Ti surfaces to 15 ±3°C. Zeta potential measurement shows that LL37-PDA-Ti surfaces are positively charged compared to negatively charged PDA-Ti surfaces. FTIR and XPS studies also indicated the conjugation of LL37 peptides on PDA-Ti surfaces. Importantly, there is no leaching of LL37 peptides from the surfaces after incubation in PBS for 24h. LL37-PDA-Ti surfaces are potent to kill *S. aurues*, *E. faecalis* and *E. coli* bacteria in 10% HS upon the exposure of NIR light for 2 min. Moreover, LL37-PDA-Ti surfaces destroy *E. faecalis* biofilm after exposure to NIR light. There is no cytotoxicity induced by LL37-PDA-Ti surfaces to HOKs and HGFs after exposure to NIR light compared to control. LL37-PDA-Ti surfaces promote adhesion and proliferation of HOKs.

In summary, we show that LL37 peptides can be conjugated on PDA-Ti surfaces. LL37-PDA-Ti surfaces kill Gram-positive and Gram-negative bacteria without inducing major cytotoxicity to human cells.

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Activity and mode of action of pepRF1, a new CXCR4-targeted HIV inhibitor

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We report the anti-HIV-1 peptide pepRF1, a human serum-resistant peptide derived from the Dengue virus capsid protein. In vitro, pepRF1 shows a 50% inhibitory concentration (IC_{50}) of 1.5 nM with a potential therapeutic window higher than 53,000. This peptide is specific for CXCR4-tropic HIV-1 strains, preventing viral entry into target cells by binding to the viral co-receptor CXCR4. pepRF1 is more effective than T20, the only peptide-based HIV-1 entry inhibitor approved by FDA for clinical use, and excels in inhibiting an HIV-1 strain resistant to T20 (HIV-$1_{NL4.3}$ DIM) with an IC_{50} of 2.8 nM. Overall, our study led to the discovery of a peptide highly active against HIV-1, serum-stable, and with low toxicity. Potentially, pepRF1 can be used alone or in combination with other anti-HIV drugs to fight AIDS. Furthermore, one can also envisage its use as a novel therapeutic strategy for other CXCR4-related diseases.

Exploring targeted autophagy modulation in pursuit of precision therapeutics for cancer and atherosclerosis

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Autophagy, a ubiquitous cellular process, serves as the primary detoxification and recycling mechanism within cells. It plays a dual role in health and disease. In tumorigenesis, autophagy functions as a survival mechanism, shielding cancer cells from the hostile tumor microenvironment and cytotoxic agents.[1] Conversely, in atherosclerosis, autophagy proves indispensable for preserving vascular health and mitigating plaque progression. Likewise, autophagy impairment has been linked to instability and rupture of atherosclerotic plaques.[2] Of note, autophagy can be targeted pharmacologically to influence disease processes. **A main challenge with this respect lies in the lack of specificity of current autophagy-modulating agents. Chronic administration in humans can reasonably be anticipated to cause stress and/or toxicity.** In our current study, we have addressed this challenge by **designing and synthesizing autophagy modulators that are specifically targeted towards disease-relevant tissues**, thereby circumventing systemic exposure concerns. Our approach involved the screening of various fluorescent endothelial cell (EC)-specific **homing peptides**, culminating in the selection of one with exceptional internalization capabilities within TNFα-activated vascular ECs. Additionally, we have demonstrated the selective binding of the **RGR peptide** to tumor vasculature *in vivo*. Utilizing these targeting agents, we have conjugated potent autophagy modulators such as rapamycin and ULK1 inhibitors to different linkers, yielding a novel set of eight compounds capable of targeting either ECs or tumor vasculature. *In vitro* characterization has revealed that this innovative set of **autophagy modulators can robustly influence autophagy within the nanomolar range, all without inducing cytotoxicity.** Furthermore, we are now evaluating the most promising compounds *in vivo*. **This research holds considerable potential for advancing precise therapeutic interventions in both cancer and atherosclerosis.**

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Understanding protein interactions using NMR studies of peptide models

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The main challenge of structural biology is the understanding at atomic level the intricate network of biomolecular interactions, which regulate all the biological processes in healthy organisms, being many diseases caused by mis-regulation of protein interactions. Herein, we focus on two systems involved in physiological processes of relevance in human health: the cannabinoid receptor CB1R, and the intercellular adhesion molecule ICAM-1.

CB1R is a G protein coupled receptor (GPCR) whose structure is that characteristic of GPCR receptors, which consists of a N-terminal region, seven transmembrane helices connected by extracellular and intracellular loops and a C-terminal intracellular domain. Different signal cascades are triggered by activation of CB1R via either a canonical pathway by binding to G-proteins or via non-canonical pathways upon binding to non-G proteins, such as β-arrestins and the cereblon. NMR characterization of peptide models has provided insights into the interaction of CB1R with β-arrestin.[1]

ICAM-1 is a transmembrane glycoprotein of the immunoglobulin (Ig)-like superfamily, consisting of five extracellular Ig-like domains, a transmembrane domain and a short cytoplasmic tail. Recently, ICAM-1 has been found to interact with NHERF-1, whose structure consists of two PDZ domains followed by a long C-terminal tail.

To get insights into the CB1R/non-G proteins and ICAM-1/NHERF-1 interactions, we proceeded to design peptide models of the cytoplasmic tails of CB1R and ICAM-1. Solution NMR is being used to examine the structural behavior of these peptides, as well as their interaction with their protein partners.

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Peptide-based therapeutics towards the topical treatment of skin infections

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Due to widespread multidrug-resistant (MDR) microbes, efficient treatments for infected wounds are being exhausted. The current standard of care requires oral antibiotics and other measures, often complex and distressing (e.g., amputations). A perfect treatment should promote both antimicrobial protection and fast tissue regeneration, to improve the inefficient healing in elderly people affected with, e.g., diabetes.[1] Considering the above, we advance peptide conjugates as potential active pharmaceutical ingredients for topical formulations to tackle skin infections, combining antimicrobial peptide and a short cosmeceutical peptide. The best chimera constructs exhibited: (i) antibacterial and anti-biofilm activity against Gram-positive and Gram-negative bacteria, including MDR clinical isolates; (ii) retained activity against *S. aureus* in simulated wound fluid; and (iii) antifungal activity.[2] The replacement of the antimicrobial peptide by an ionic liquid afforded a new conjugate, a peptide-ionic liquid construct, with *in vitro* broad-spectrum antibacterial activity, antifungal action, collagen-inducing effect and promising preliminary *in vivo* effect.[3] These results will be shown alongside the most recent findings that provide deeper insight into their mode of action.

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Design and synthesis of novel hemagglutinin fusion peptide inhibitors of H1N1 influenza virus

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Each year, influenza A virus cause millions of severe cases worldwide. The recent outbreaks of highly pathogenic (re)emerging influenza viruses, combined with the shortage of drugs in clinical use and their low effectiveness, and the appearance of viral resistance, emphasize the need of new novel influenza drugs acting through novel mechanisms of action different from those exploited so far. Hemagglutinin (HA) is an homotrimeric glycoprotein which is an attractive target for the design of novel anti-influenza compounds, due to its essential role in the virus entry into the host cell.[1]

We have previously identified a unique class of *N*-benzyl-4,4-diubstituted-piperidines as specific H_1N_1 influenza A virus fusion inhibitors. Mechanistic and computational studies followed with the peptidomimetic **DICAM180** prototype show that the inhibitory activity is mediated through binding to, a so-far unexplored, pocket in the HA_2 subunit, close to the highly conserved fusion peptide. The proposed binding mode of these compounds involves a direct π-stacking interaction with the Phe9 HA_2 residue of the fusion peptide and a stable salt bridge of the protonated piperidine N with the Glu120 HA_2 of the protein, as mayor interactions.

DICAM180 interacts with only one of the fusion peptides of the monomers of the homotrimeric HA.[2] This binding mode has been used as a starting point for the design of new derivatives able to establish an additional interaction with the fusion peptide of the second monomer of HA. The aim of the modifications is to obtain a simultaneous interaction with the Phe9 of both monomers to improve the antiviral activity and to obtain wide spectrum anti-influenza compounds. Several chemical modifications of **DICAM180** have been designed with the aid of a combination of docking studies and Molecular Dynamics simulations to obtain compounds that could potentially establish those additional interactions and to improve the activity data.

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In silico approach for the design of peptides targeting the RANK-TRAF6 interaction

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Breast and prostate cancer have high recurrence rates in bone, with around 70% of patients having a high probability of developing bone metastases[1]. Metastization has a major impact on patients' quality of life, causing bone pain, pathological fractures, hypercalcaemia and spinal cord compression, which leads to increased morbidity and decreased survival. Since the drugs available for bone metastases treatment mainly provide palliative care, innovative therapeutic approaches, and drugs with different mechanisms of action are urgently needed to address this unmet medical need. Tumor necrosis factor receptor associated factor 6 (TRAF6) is the critical signaling molecule associated with the receptor activator of nuclear factor κ B (RANK) that regulates osteoclast differentiation and activity. Inhibiting RANK-TRAF6 binding could potentially affect osteoclast function. *Ye et al.* identified a P-X-E-X-X-(aromatic/acidic residue) binding motif to RANK-derived TRAF6 that inhibits TRAF6 signaling[2]. *Chen et al.* showed that peptides with motif RQMPTEDEY inhibit TRAF6 by reducing osteoclast formation and bone resorption in multiple myeloma bone marrow monocytes[3]. In this context, we explored *in silico* tools to understand in atomistic detail the interactions of these peptides with TRAF6. Homology modelling studies have been carried out to generate human TRAF6 model and 3D structures of peptides. Later, docking studies were used to study the binding affinity of these peptides with TRAF6, as well as to analyze the key interactions established at the RANK/TRAF6 interface.

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BrainBike peptidomimetic enables efficient transport of antibody derivatives across brain endothelium

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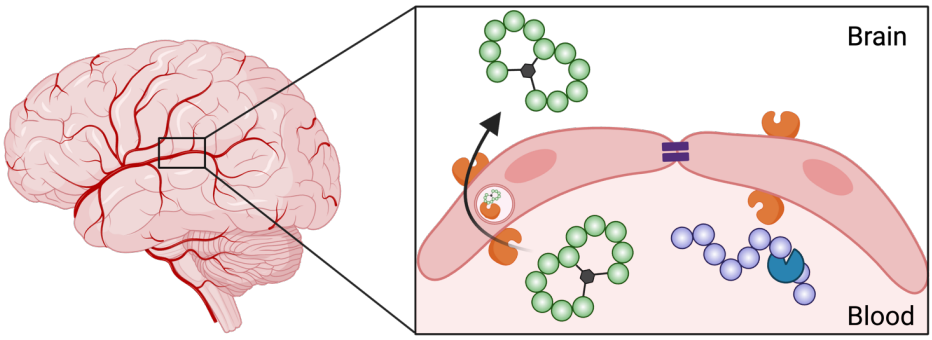
Protein therapeutics cannot reach the brain in sufficient amounts because of their low permeability across the blood-brain barrier (BBB).[1] Although brain shuttle peptides may enhance BBB transport of large therapeutics via receptor-mediated transport, high lability to proteases limits their efficiency. We have previously shown that increasing protease resistance generates more efficient peptide shuttles. However, the capacity of these shuttles to transport proteins was limited.[2] Here we develop BrainBikes, a novel family of bicyclic protease-resistant peptide shuttles capable of increasing transport of antibody derivatives. Utilizing a chemical linker, we generate several bicyclic analogs from a linear peptide targeting the transferrin receptor (TfR1).[3] All analogs have increased metabolic resistance and one of them, BrainBike-4, displays 7-fold higher affinity for cells with high levels of TfR1. We site-specifically conjugate BrainBike-4 to a single chain variable antibody fragment (scFv) utilizing a chemoenzymatic approach. Conjugation of BrainBike-4 increases 5 times the transport of the scFv in a human cell-based model of the BBB, which represents a substantial enhancement with respect to previously reported peptide shuttles. Our results show the potential of bicyclic peptidomimetics as brain shuttles and open up new opportunities for the transport of biotherapeutics.



BrainBikes can efficiently transport protein therapeutics across brain endothelium.

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Conjugation of nanostars with PaMAP1.9 to improve its anticancer activity.

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Breast cancer is rising among women worldwide and represents a major challenge to current therapies. Challenges to be overcome include the lack of alternative treatments and the aggressiveness of available chemotherapeutic strategies. Antimicrobial peptides (AMPs) have been identified as promising alternatives to conventional molecules used today against infections. Some of them have been shown to have dual activity, both as antimicrobial and anticancer peptides (ACPs). The use of nanocarriers in combination with AMPs has drawn attention to the development of an improved drug delivery system. Silver nanoparticles (AgNPs) have special properties and can act selectively on negatively charged breast cancer cell membranes. From the perspective of a combinatorial drug therapeutic, binding AMPs to the surface of silver nanoparticles is a strategy that could successfully improve selectivity and specificity toward breast cancer cells. In this work, the capabilities of silver nanostars (AgNSs) conjugated with the AMP PaMAP1.9 (AgNSs-PaMAP 1.9) were evaluated in three different types of human breast cells (MCF 10A, MCF7 and MDA-MB-231). Confocal microscopy, flow cytometry and scanning electron microscopy showed that AgNSs-PaMAP 1.9 have enhanced anticancer activity in MCF 7 and MDA-MB-231, compared with Pa-MAP 1.9 alone. In MCF 10A cells, this conjugate acted intracellularly without damaging the membrane structure. AgNSs-PaMAP 1.9 exhibits different mechanisms of action depending on the breast cell subtype, being more effective against MDA-MB-231. Overall, the results show that the combination of the PaMAP1.9 peptide with AgNSs improves its anticancer activity.

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Novel leishmanicidal compounds as potent dimerization disruptors of *Li*TryR

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Although leishmaniasis is the second-largest deadly parasitic disease, only a limited and outdated number of drugs are available for the treatment. The search for new drugs against leishmaniasis, less toxic and acting by mechanisms of action different from those of the currently available drugs, is a global necessity. Trypanothione Reductase of Leishmania infantum (*Li*-TryR) is an essential and exclusive enzyme for the antioxidant defenses of these parasites, and is a validated target for the rational design of drugs against leishmaniasis. In our group we have developed an alternative inhibition strategy of the enzyme which consist on the disruption of the homodimeric interface of the *Li*-TryR.[1] The proof-of-concept of this approach was performed by using peptides and peptidomimetics that mimic 'hot spots' at the homodimeric interface.[2,3]

In the search for new dimerization inhibitors with improved activity /toxicity profile, we have recently reported a symmetrical peptidomimetic based on 1,2,3-triazole-phenyl-thiazole scaffold.[4] By molecular modelling studies we identified, an almost unexplored hydrophobic region as putative binding site for this inhibitor located at the central interfacial domain of *Li*-TryR. In order to determine the potential as leishmanicidals and to carry out SAR studies we report here the design and synthesis of novel symmetrical triazole-phenyl-thiazole compounds of general structure **I** modified at R_1, R_2 and R_3 positions (Figure 1).

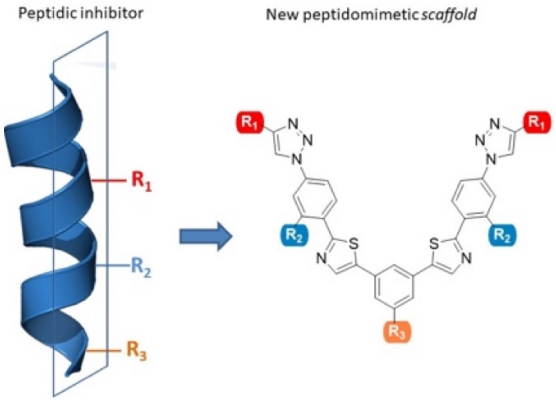

Figure 1. Target symmetrical triazole compounds **I**.

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A diastereopure azepane quaternary amino acid as effective inducer of β-turns and 3_{10}-helices

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The use of constrained amino acids is a well-known strategy to induce secondary structure elements in peptide chains, of interest to achieve fundamental understanding of complex protein-protein interactions.[1] We have reported the stereoselective synthesis of a novel azepane quaternary amino acid (Aze) and its ability to promote the folding of peptides into β-turns and 3_{10}-helices (Fig. 1).[2] These peptides also showed interesting supramolecular self-assembly properties, providing the basis for the development of novel azepane-derived bioactive molecules, catalysts and biomaterials.[3]

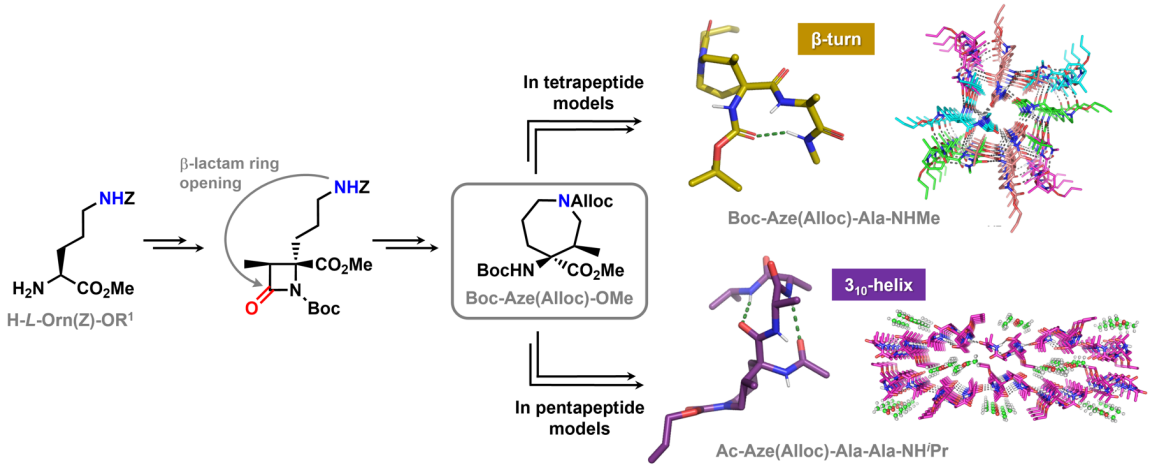

Fig 1. Stereoselective synthesis of azepane quaternary amino acid Aze and X-ray structures of Aze-containing tetrapeptide (CCDC 2281591) and pentapeptide (CDCC 887956) models.

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Insights on the influence of proline residue on the biological effects of glypromate bioconjugates

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Glypromate is a short endogenous neuropeptide that displays neuroprotective activity in several *in vitro* and *in vivo* models of neurological conditions of the central nervous system (CNS).[1] However, despite its therapeutical potential in CNS-related disorders, this neuropeptide exhibits an unfavorable pharmacokinetic profile.[1]

Herein, following a diversity-oriented synthesis strategy, a total of 36 new Glypromate bioconjugates were synthesized and biologically evaluated in human SH-SY5Y cells (differentiated and non-differentiated states, 100 μM) using the MTT reduction assay. In SH-SY5Y cells injured by $A\beta_{25-35}$, some Glypromate conjugates reduced the formation of $A\beta_{25-35}$ aggregates up to 40%, outperforming the activity of the parent neuropeptide (around 12%). In SH-SY5Y cells injured by 6-hydroxydopamine (6-OHDA), one of these conjugates showed a two-fold increase in the neuroprotective effect when compared with the parent neuropeptide and exhibited a synergistic effect.

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Acknowledgements

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Selective cleavage of DNA replication foci in mammalian cells by Cu^{II} peptide helicates

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Recently, we reported the synthesis of oligocationic peptide ligands containing artificial bipyridine amino acid residues, which fold predictably into self-assembled helicates in the presence of Fe^{II} ions. These peptides helicates show a high affinity and selectivity towards DNA Three Way Junctions (DNA-3WJ) over B-DNA both in vitro and in vivo, being able to selectively label DNA replication foci in cell nuclei.[1] As a continuation of this work, here we report the design and synthesis of a series of peptides helicates that exhibit high and extremely selective nuclease activity towards DNA-3WJ in vitro. We also show that these helical peptides selectively cleave DNA replication foci in functional nuclei, therefore acting as selective metallonucleases for DNA-3WJ in vivo. To our knowledge, this is the first example of a nuclease agent selective for DNA-3WJ, both in vitro and in vivo. Considering that DNA replication is deregulated in cancer cells, these novel artificial metallonucleases should be, in principle, much more active in cancer cells than in healthy ones. We believe that we may be on the verge of discovering a new family of anticancer drugs with extraordinary cancer cell selectivity.

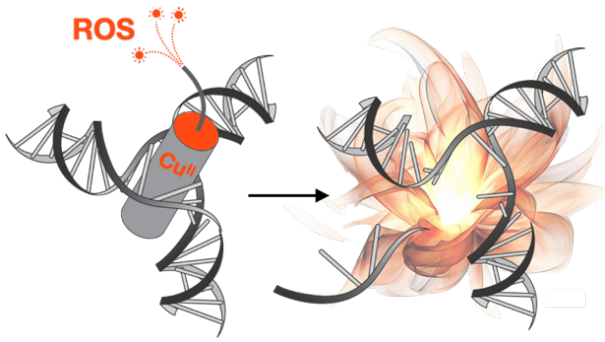

Illustration of the 3WJ-specific metallonuclease approach.

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Study of alamethicin pore formation in lipid membranes using the Martini 3 coarse-grained force field

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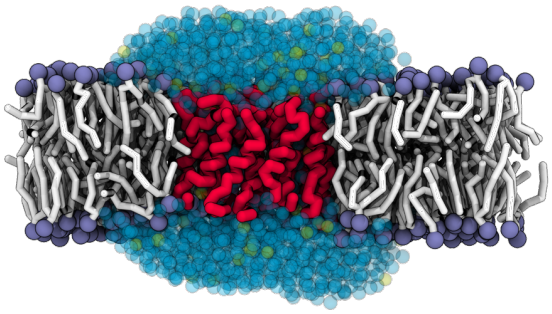
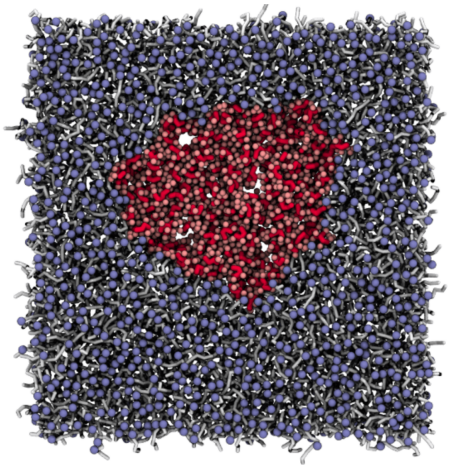
Alamethicin (Alm) is an antimicrobial peptide known for its activity against Gram-positive bacteria and fungi. Alm aggregates to form ion-conducting channels according to the barrel-stave model — in which the peptides' hydrophilic face is aligned towards the centre of the pore. Coarse-grained (CG) molecular dynamics (MD) simulations allow us to study systems at time and size scales otherwise inaccessible, at the expense of some atomic detail. Alm pore formation could not be faithfully reproduced in CG simulations using the Martini 2 model, due to the establishment of continuous interpeptide contacts between Alm residues that collapsed any would-be pore lumen. With the new Martini 3 and its improvement on protein-protein interactions, we hoped that these pores could now be represented. Indeed, we were able to use Martini 3 to simulate Alm behaviour in lipid membranes and observed Alm pores with water and ion transport. We also found that the peptides had a tendency to aggregate into clusters until they formed a large raft. Although the discrete levels of conductance were not recovered, our work is still an improvement over past CG studies.



Selected snapshot of the side view of an alamethicin pore structure. Lipid heads are represented in lilac, tails in white, Alm backbone in red, water in blue, and ions in yellow.



Selected snapshot of the formation of the alamethicin raft. Lipid heads are represented in lilac, tails in white, Alm backbone in red, side chains in pink, water in blue, and ions in yellow.

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Evaluating the activity of CXCR4 and D2R-targeting peptides using the TRUPATH platform

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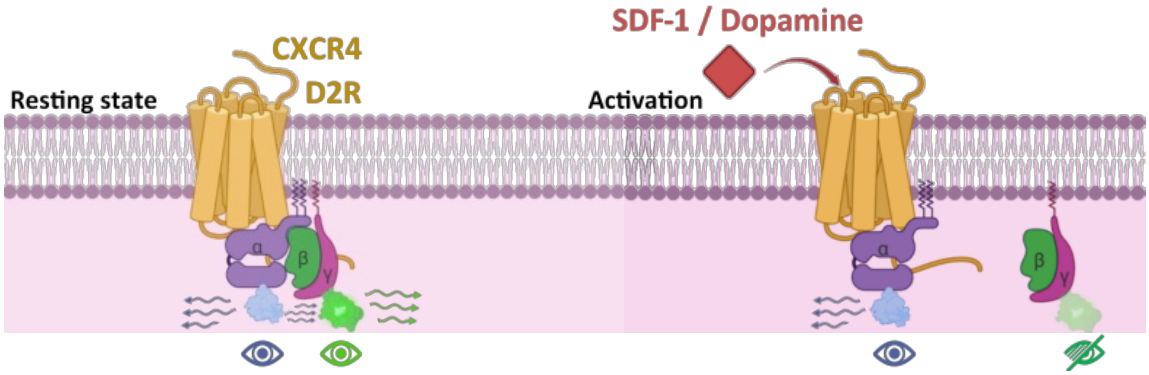
G-Protein-Coupled Receptors (GPCRs) are the largest family of membrane receptors that are targeted by FDA and EMA approved drugs. These seven-transmembrane-domain proteins transduce extracellular signals into intracellular responses through the coupling to 16 different heterotrimetric G-proteins (constituted by Gα, Gβ and Gγ subunits), resulting in a multitude of biological and pathological responses. Due to GPCR pathway complexity, GPCR-targeted drugs might produce unexpected side effects *in vivo*. Considering the complex signaling mechanism of GPCRs, the design of new, biased therapeutic drugs, with high selectivity and fewer side effects as proven challenging. Within this context, an innovative, open-source platform – TRUPATH – was developed using bioluminescence resonance energy transfer 2 (BRET2) based biosensors.[1] TRUPATH allows the direct evaluation of G-protein heterotrimer activation through specific single pathway resolution. Aimed at the development of new therapeutic targeted peptides, we will present our work with two distinct GPCRs, namely CXCR4 and dopamine receptor D2 (D2R), using the TRUPATH methodology. Evaluation of the small protein Stromal cell derived factor 1, CXCR4 agonist, revealed different activation strength and EC_{50} values depending on the chosen G-protein. Additionally, we will also present and discuss the inhibitory effect of Gα-mimicking peptides on D2R.



Graphical representation of the BRET2 methodology used to evaluate GPCR (CXCR4 or D2R) activation.

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What can an ionic liquid do for the bioactivity of antimicrobial peptides? The case of W-BP100

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Antimicrobial peptides (AMPs) are being largely explored as an alternative to antibiotics in the fight against antimicrobial resistance. Conjugation with antimicrobial ionic liquids (ILs) is a recently proposed option to improve the antimicrobial potency of AMPs[1]. Herein, we propose to boost the antibacterial activity of W-BP100[2], and its parent peptide BP100, using dodecylimidazolium-based ILs through: (i) covalent or (ii) non-covalent (equimolar mixtures) conjugation. Covalent conjugation, through click chemistry[1], improved the antibacterial activity of BP100, but not of W-BP100, yielding compounds that were more haemolytic than the parent AMPs. Opposingly, non-covalent conjugates retained antimicrobial activity, but were less haemolytic than both the parent AMPs and their respective covalent conjugates (Figure 1). Also, the W-BP100:IL noncovalent conjugate showed to substantially reduce the viability of preformed *S. aureus* biofilms, similarly to the IL but better than the free AMP. Results support the use of AMP and IL conjugates, particular non-covalent conjugates, as an alternative approach to address bacterial infections, positioning ILs as possible adjuvants in AMP-based therapies.

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Flash Talks

The use of a selective, nontoxic dual-acting peptide for breast cancer patients with brain metastasis

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Triple-negative breast cancer (TNBC) is an aggressive subtype defined by the lack of commonly therapeutically targeted receptors. Chemotherapy is the main therapeutic option, with poor results. Another major challenge is the frequent appearance of brain metastasis (BM) associated with a significant decrease in patient overall survival[1]. The treatment of BM is even more challenging due to the activity of the blood-brain barrier (BBB). Here, we present a dual-acting peptide (PepH3-vCPP2319) designed to tackle TNBC/BM, in which a TNBC-specific anticancer peptide (ACP) motif (vCPP2319)[1] is joined to a BBB peptide shuttle (BBBpS) motif (PepH3)[2]. PepH3-vCPP2319 demonstrated selectivity and efficiency in eliminating TNBC both in monolayers (IC_{50}≈5.0 μM) and in spheroids (IC_{50}≈25.0 μM), with no harsh toxicity toward noncancerous cell lines and red blood cells (RBCs). PepH3-vCPP2319 also demonstrated the ability to cross the BBB *in vitro* and penetrate the brain *in vivo*, and was stable in serum with a half-life above 120 min. The interaction with tumor cells is fast; with a quick peptide internalization *via* clathrin-mediated endocytosis without membrane disruption is obsevred. Once inside the cells, the peptide is detected in the nucleus and the cytoplasm, indicating a multi-targeted mechanism of action that ultimately induces irreversible cell damage and apoptosis. In conclusion, we have designed a dual-acting peptide capable of brain penetration and TNBC cell elimination.

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Dual-effect chitosan nanoparticles with conjugated pro-angiogenic and antimicrobial peptides

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Skin chronic wounds are often infected and show deficient vascularization, hindering cell proliferation and tissue repair. Antimicrobial peptides (AMP) are less likely to induce resistance compared to antibiotics/antiseptics, but are prone to proteolytic degradation, as is the vascular endothelial growth factor (VEGF), commonly used to stimulate angiogenesis. Dhvar5 (LLLFLLKKRKKRKY) is a synthetic peptide, derived from the histatins family, with a cationic C-terminus and a hydrophobic N-terminus. QK peptide mimics the receptor binding region of $VEGF_{165}$ and assumes an α-helical conformation in solution, essential for its biological activity. Since peptide conjugation to biomaterials is effective to enhance resistance towards proteases, herein, both Dhvar5 and QK were modified with a cysteine and an aminohexanoic acid spacer, at either the C- and N-terminus, and conjugated onto norbornene-chitosan nanoparticles (NorChit NP) via thiol-ene chemistry. NP production and peptide conjugation occurred in one step, in a microfluidics device coupled to an UV LED (λ=365 nm). Dhvar5-NorChit NP were tested on Staphylococcus epidermidis (ATCC 35984) and Pseudomonas aeruginosa (ATCC 27853). Moreover, QK-NorChit NP were evaluated in a 3-day proliferation assay using Human Umbilical Vein Endothelial Cells (HUVEC). NP were also assessed in vivo in a mice abrasion model (Dhvar5-NP) and in a Chorioallantoic Membrane Assay (CAM) assay (QK-NP). Produced NP (100 - 150 nm; 10^9 NP/mL) had 40% and 80% of initial Dhvar5 and QK, respectively. Dhvar5-NorChit NP (10^8 NPs/mL) showed a potent bactericidal effect in vitro, which was reduced in vivo, probably due to the complex environment present in the exudate. Both bare and NorChit-QK NPs promoted HUVEC metabolic activity and angiogenesis in the CAM model. Overall, NorChit NP conjugated with bioactive peptides could tackle two hallmarks of chronic wounds — bacterial infection and impaired angiogenesis —, showing potential to improve current therapies.

Acknowledgements

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Bone-seeking peptides targeting the RANK-TRAF6 interface for bone metastases *theranostics*

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The bone is one of the most frequent sites for metastases in breast and prostate cancer, and innovative agents for the treatment of bone metastasis are required. The receptor activator of nuclear factor kappa B (RANK)/tumor necrosis factor receptor associated factor 6 (TRAF6) axis plays a pivotal role in bone metastization as it is involved in osteoclast activation, cancer cell migration, and invasion[1]. We thus propose novel molecules with therapeutic potential comprising a bone-seeking unit (bisphosphonate), a cathepsin K-cleavable linker (Fragment A), a cell penetrating peptide (CPP) (Fragment B) and TRAF6-binding sequences (Fragment C). Upon accumulation on the bone, the cathepsin K-sensitive linker will be cleaved and release Fragments B and C, which will be internalized by the cells and inhibit osteoclastogenesis and reduce metastatic potential. In this work, we report on the construction and characterization of a set of peptide sequences that contain two different types of CPPs with three different cathepsin K-sensitive linkers to select suitable candidates for subsequent construction of the complete multifunctional peptides. The peptides were prepared by Solid-Phase Peptide Synthesis, purified by RP-HPLC (> 95%) and characterized by ESI-MS. The CPPs without linkers were also characterized by Circular Dichroism (CD), and fluorescently labeled for internalization studies by flow cytometry and confocal microscopy. Lastly, the sensitivity of the three linkers to cathepsin K was assessed through an enzymatic cleavage assay. The results have allowed us to select a specific CPP and a specific linker to be incorporated into the final multifunctional peptides.

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Bioactive peptides in functional foods can mitigate stress behaviors in zebrafish (*Danio rerio*)

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The development of functional additives that improve the well-being and immune condition of fish exposed to stress factors in aquaculture is being investigated. Functional feeds are designed to fulfil nutrient requirements for growth, but also reinforce immunity and stress resistance. The research of functional feeds to reduce stress-related losses in aquaculture creates the basis for the development of a feed that softens stress and prepares zebrafish (*Danio rerio*) for stress situations. The present work aims to assess the effects of protein hydrolysates (PH) and a bioactive peptide (BP) has functional additives, on stress resilience of adult zebrafish prior and after an acute stress stimulus.

Using a commercial-like diet as control (ZF), 4 other functional diets were formulated: a diet containing 3% inclusion level of fish PH (CTR), a diet with 3% inclusion level of a short chain bioactive peptide (BP), a diet with 6% inclusion of the same bioactive peptide (BP2) and a diet with 3% shrimp PH (HP1). Diets were randomly assigned to groups of 90 fish. After 3 weeks of feeding, fish were subjected to an acute stress stimulus (crowding during 3h). A behavior test (novel tank diving) was performed in a group of animals prior and immediately after stress. A skin swab for cortisol quantification was performed in another group of animals also prior and after the stress stimulus.

The results show no differences in fish weight between groups, indicating that all the diets fulfill the growth requirements at this stage of development. In the novel tank diving test, the animals were placed individually in a transparent tank and the behavioral activity was analyzed for 5 min. Distance traveled and mean velocity were measured. The ZF diet shows a significant increase of the distance traveled and mean velocity after acute stress, which is correlated with higher levels of anxiety. BP and BP2 diets show decreased traveled distances and suggest that peptide concentration influences positively the reduction of the stress level. In conclusion, the functional diets containing the bioactive peptide are more efficient in mitigating the stress level when compared to the other diets. Analysis of immune response and gene transcription are being performed to obtain a more complete characterization of zebrafish stress response.

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Targeting arboviral infections in the central nervous system with peptide-porphyrin conjugates

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The global incidence of arboviral infections has increased dramatically over the years with climate changes threatening outbreaks in previously unaffected areas. Arboviral infections can often affect the central nervous system (CNS), causing severe neurological complications, such as encephalopathy, encephalitis, Guillain-Barré Syndrome and microcephaly. Currently, there are no specific drugs available to treat arboviral diseases, which treatment faces several challenges, including co-infection with different co-circulating arboviruses and difficulties in early clinical diagnosis due to the overlap of symptoms. Tetrapyrrolic macrocycles, such as porphyrins, present broad-spectrum antiviral activity targeting the envelope of several viruses, including dengue (DENV) and zika (ZIKV) viruses. However, antiviral drug delivery to the CNS is prevented by the highly selective blood-brain barrier (BBB). Moreover, in order to prevent ZIKV-induced microcephaly, drugs must also cross the blood-placental barrier (BPB). In the attempt to deliver antiviral porphyrins to the CNS to treat neurological complications, including ZIKV-induced microcephaly, we developed peptide-porphyrin conjugates (PPCs), using different porphyrins and BBB peptide shuttles (BBBpS). In this work, we evaluated the PPCs ability to translocate both BBB and BPB *in vitro*, PPCs cytotoxicity and PPCs antiviral activity *in vitro* and *in vivo*. First, PPCs translocation was measured using human BBB and BPB *in vitro* models. Next, DENV and ZIKV inactivation by the PPCs was evaluated by plaque assay, while PPCs cytotoxicity was assessed by resazurin-based assays in several pharmacological relevant cell lines. Finally, the best performing PPCs were tested *in vivo* using a neonatal mice model of ZIKV infection. Overall, our results show that PPCs are able to target arboviral infections in the CNS. These results suggest that the conjugation of BBBpS to antiviral porphyrins is a promising strategy to fight CNS arboviral infection.

Acknowledgements

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Room for improvement in the initial Martini 3 parametrization of peptide interactions

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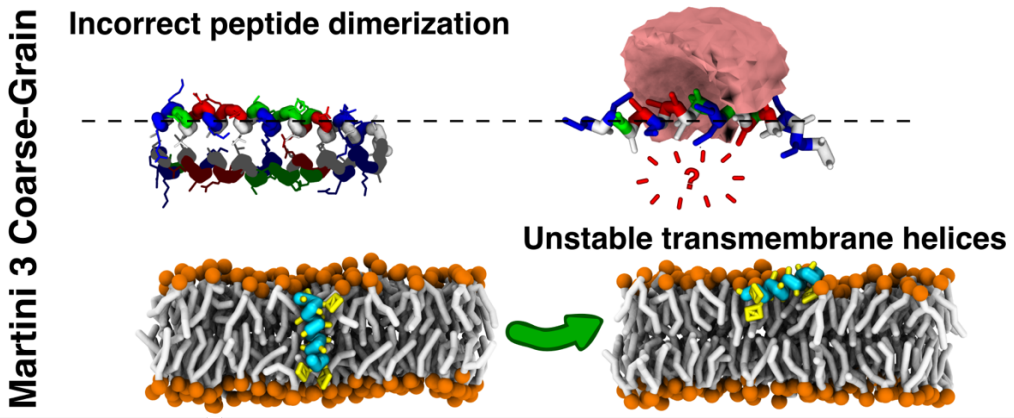
Coarse-grained simulation rose in popularity during the last decade, and one of the most widespread forcefield is the general purpose forcefield Martini. That currently is in its third iteration, Martini 3, and has been used with great success in various applications. However, this work focus on inconsistencies of the initial Martini 3 forcefield regarding peptide interactions in biologically relevant systems. These inconsistencies can be explained by the incorrect depiction of hydrophobic/hydrophilic balance in this initial parametrization. Firstly, coiled coil dimers were represented using heptad repeats which are drivers of coiled coil, not only by a mere polarity matching but leverages from the spatial fitting of each helix's side chains into inter-residue gaps in the other helices. However, Martini 3 failed to have any kind of propensity to dimerize, even when dimerized preferred to cluster around polar/charged residues. Transmembrane peptides, using WALPs a series of single pass transmembrane peptides which has a well described tilt angle behavior in response to membrane thinning. Although Martini 3 was able to represent the tilting behavior of the WALPS, the smaller WALPs were consistently ejected from the membrane showing a preference for the adsorbed. With this report we show there is still much work left in protein parametrization in Martini 3 and these results may be used as steps towards a more robust forcefield.



Depiction of the incorrect peptide dimerization and ejection of transmembrane helix

Acknowledgements

We thank T. Cordeiro for bringing to our attention the coiled coil system that motivated part of this study. M.N.M. thanks Fundação para a Ciência e a Tecnologia, Portugal for fellowship CEEC-IND/04124/2017, and for funding project MOSTMICRO-ITQB with references UIDB/04612/2020 and UIDP/04612/2020.

Antibiofilm effect of synthetic antimicrobial peptides on Gram-positive and Gram-negative bacteria

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One of the leading causes of death worldwide are infectious diseases. In the 20th century, improvements in sanitation and the development of medical treatments such as antibiotics led to a dramatic decrease in deaths from infectious diseases. However, the overuse of antibiotics in recent years has led to the development of antibiotic resistance, which threatens the effectiveness of antibiotics. Antimicrobial peptides (AMPs) are a class of peptides found in living organisms as an essential component of their innate immune systems. These small bioactive molecules have attracted interest due to their high potential as alternative treatments for infectious diseases. Not only they are easier to synthesize, but they are also generally composed of short amino acid sequences and can bypass the resistance mechanisms observed in conventional antibiotics, making them less susceptible to antimicrobial resistance.

The aim of this work was to investigate the antibiofilm potential of newly designed cationic AMPs: *Pa*MAP1.9, *Pa*MAP2, *Pw*AMP1B5 and *Py*AMP1B5. To test their antimicrobial activity, minimal inhibitory concentrations (MICs) and confocal microscopy images were used against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, and *E. faecium*. Our results show that *Pa*MAP1.9 has the potential to inhibit *E. coli* biofilms, while *Pa*MAP2 shows a high affinity for *S. epidermidis* biofilms and can prevent biofilm formation even at sub-inhibitory concentrations, with low biofilm cell viability. *Py*AMP1B5 and *Pw*AMP1B5 showed the most extended activity, generally affecting both Gram-negative and Gram-positive bacteria tested. All these results suggest that these peptides can be considered as bioactive molecules that can be used as antimicrobial agents.

Acknowledgments

Fundação para a Ciência e a Tecnologia – Ministério da Ciência, Tecnologia e Ensino Superior (FCT-MCTES, Portugal) project 2022.01991.PTDC.

$\alpha_v\beta_3$ integrin peptide inhibitors impact on fibrinogen-erythrocyte binding

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Integrins play a crucial role in linking the cytoskeleton to the extracellular matrix[1,2]. Previous studies identified the presence of the $\alpha_v\beta_3$ integrin on the erythrocyte membrane, acting as receptor for fibrinogen and contributing to increased cell-cell adhesion[3,4]. This integrin specifically recognizes the Arg-Gly-Asp (RGD) domain present in extracellular matrix proteins and fibrinogen. To investigate the impact of $\alpha_v\beta_3$ integrin inhibitors on fibrinogen-erythrocyte receptor interaction, four integrin inhibitor peptides were tested: cilengitide, 29[1], FRX025, and an $\alpha_v\beta_1$ ligand used as control. The interaction and its inhibition were assessed using atomic force microscopy (AFM)-based force spectroscopy and zeta potential measurements. A significant reduction of fibrinogen-erythrocyte binding frequency was observed in the presence of these inhibitors. Notably, peptide 29 exhibited enhanced selectivity and efficacy compared to cilengitide. In the presence of 29 and fibrinogen, the erythrocyte surface charge is more negative than without this inhibitor. This result indicates its efficacy on inhibiting fibrinogen-erythrocyte binding, which may mitigate erythrocyte aggregation and improve microcirculation flow conditions. The targeting of the $\alpha_v\beta_3$ integrin may be used to develop potential therapies to reduce hypercoagulation in cardiovascular patients.

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Design of novel peptides to target the intracellular G protein-dopamine receptor type 2 (D2R) interface

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The dopamine receptor type 2 (D2R) is a G-protein coupled receptor (GPCR) that interacts with G proteins triggering conformational changes that leads to a cascade of signaling pathways involved in key biological functions and neurological diseases.[1]

The main goal of this project was the development of peptides with therapeutic potential that may interfere with the G protein-D2R interface.

Previous computational studies have predicted the G protein-D2R interface,[2] which resulted in the design of Gi protein-based peptide sequences. Since the interactions take place in the cell cytoplasm, some of the peptides contain also a cell penetrating sequence (CPP).

Herein, the 3D structures of the peptides were predicted and molecular docking studies with D2R were developed through the HADDOCK webserver. Two approaches were considered in the molecular docking studies: 1) the active site was defined based on the residues described in the literature, and 2) all the residues in the sequence were in the active site. Additionally, detailed evaluation of HADDOCK score, interface residues and hydrogen bonds allowed to select the most stable peptide-D2R complexes. Apart from the computational studies, we will also report on the preparation of the corresponding peptides by solid-phase peptide synthesis (SPPS) under ultrasonic agitation, purification by RP-HPLC (≥ 60%), and characterization by MS-ESI. Biological evaluation of the peptides is currently underway.

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Unravelling the factors that govern the ability of antiviral peptide-porphyrin conjugates to translocate the blood-brain barrier

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Zika virus (ZIKV) and Dengue virus (DENV) are able to cross the blood-brain barrier (BBB), reach the central nervous system (CNS), and cause severe neurologic diseases. There are no antivirals or efficacious vaccines that can treat or prevent the diseases caused by ZIKV and/or DENV. One of the main challenges in antiviral drug development is the design of drugs that cross the BBB and fight infections directly in the CNS. Recently, *de novo* designed peptide-porphyrin conjugates (PPCs) have shown both activity against enveloped viruses, and ability to cross a cellular model of the BBB.[1] Here, seven PPCs were studied in order to unravel the influence of factors such as concentration, incubation time, and the presence of serum proteins, on the extent of PPCs translocation across a human *in vitro* cellular model of the BBB. In addition, the PPCs association with bovine serum albumin (BSA), and the size of PPC aggregates in the presence and absence of BSA were studied. All PPCs efficiently translocate the cellular *in vitro* BBB model without significant damage to the integrity of the barrier at 10 μM. The PPCs reach the maximal percentage of translocation after 24 hours of incubation. Translocation in medium without serum is decreased for five conjugates, and it is not affected for the other two. The seven PPCs bind to BSA, forming aggregates. BSA affects the size of the aggregates of the PPCs except in one case.

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Posters

Kinetic study of aza-amino acid incorporation into peptide chain: Impact of steric effect of the side chain

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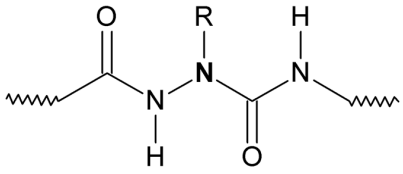
Aza-peptides are peptidomimetics developed to avoid drawbacks of peptides as drug candidates.[1] However, the information concerning their bioactivity is quite limited due to the absence of efficient synthesis strategies of these compounds by SPPS method. Our group has developed a method to quantitatively determine the azapeptide bond formation reaction rate and yield to study the suitability of the SPPS protocol for aza-peptide bond formation.[2–5] Additionally, we have investigated with this method, how the bulkiness of various aza-amino acids (AzAA) influences their reactivity with the *N*-terminus of amino acids in synthesis of model aza-peptide H-AzAA-Ala-Phe-NH_2 using BTC (Bis(trichloromethyl)carbonate) as activator for aza-amino acid precursor.[6] The results of kinetic measurements showed that the same method can be used for aza-amino acid incorporation to the peptide sequence regardless of its or preceding amino acid structure while utilizing BTC for activation.



Aza-peptide

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Acknowledgements

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Chemically fueled droplets for out-of-equilibrium sequence-specific RNA binding

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The chemical reaction cycle consists of two chemical reactions. Thus, a precursor is temporarily fuel-driven activated to perform a function, and that function is regulated through the kinetics of activation and spontaneous deactivation. In one chemical reaction cycle we developed a C-terminal aspartic acid is converted into its corresponding anhydride at the expense of a condensing agent. That anhydride spontaneously deactivates through hydrolysis to its precursor with a half-life of roughly 30 seconds. We designed a peptide such that, upon activation, it non selectively binds RNA and can form a coacervate droplets. These droplets spontaneously emerge in response to chemical fuel, they will decay without the fuel and thus have a finite lifetime. While this is exciting, these droplets are not catalytically active and do not perform any function other than being a compartment. The next big step towards synthetic life is to functionalize the droplets with catalytically active RNA.

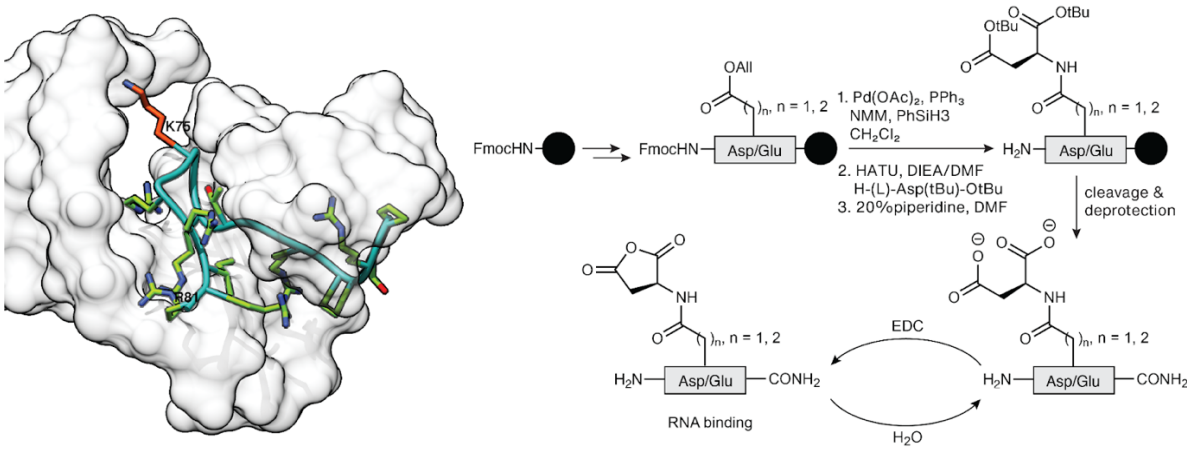

Fig. 1. Left. Detail of the structure of the BIV Tat-TAR peptide hairpin and the RNA trans-activation response element highlighting the position of Lys[75] in orange, and the C-terminal Arg[80]. Right: Synthesis of the dynamic ***R80D*** *peptide by orthogonal modification of a Glu (4).*

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A "difficult sequence" cardiovascular peptide. Optimizing synthesis and purification

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Ventricular arrhythmias — heart rhythm disturbances — can trigger ventricular fibrillation and sudden death (SCD), responsible for up to 50% of deaths in patients with heart failure. Proarrythmogenic events include a marked reduction in the expression of Nav1.5 and Kir2.1 channels, responsible of I_{Na} and I_{K1} currents.[1] The UCM group discovered a peptide (DECA11) able to increase the above mentioned currents, as they do some antidiabetic drugs.[2]

In order to initiate a medicinal chemistry program based in DECA11, we need efficient methods for the synthesis and purification of this peptide and related analogues. The first attempt to prepare DECA11 used a typical rink amide polystyrene resin (PS) and a Fmoc/^{t}Bu solid-phase strategy. After cleavage from the resin, this experiment afforded the desired peptide in less than 50% purity, with four main HPLC byproduct peaks, corresponding to deletions of different amino acid residues, which also complicated the purification step. DECA11 has a so called "difficult sequence". To avoid the possible aggregation of the growing peptide on the polymeric PS, we studied different commercially available resins, both PS with grafted polyethylene glycol (PEG), and 100% PEG based resins. In addition, we have contemplated the use of pseudoproline residues, and diverse coupling agents and swelling solvents.

This communication focuses on different synthetic approaches for the preparation of DECA11, suitable purification methods and preliminary conformational CD studies.

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Single α-helical peptide with conductive and fluorescence properties

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Traditional organic fluorophores are characterized by extended π-electron systems that emit light upon returning to their ground state after excitation with light of appropriate wavelength; unlike them, nonconventional luminophores, or clusteroluminogens (CLgens), such as polyethylenimine, carbohydrates, or nonaromatic protein crystals and aggregates, display luminescent emission despite lacking aromatic or conjugated systems.[1,2] CLgens contain electron-rich functional groups featuring π and/or lone-pair (n) electrons (e.g., R–NH_2, –OH, –C≡N, –$CONH_2$, or –COOH), which can come close to each other, leading to significant intra/intermolecular n-π*, π-π, and dipole–dipole interactions, giving rise to extended electron delocalization and through-space conjugation as clustered chromophores with enriched energy levels and lowered energy gaps. CLgens display concentration-enhanced and aggregation-induced emission, excitation-dependent emission, and prevailing triplet emission. In the context of proteins, it has been shown that Lys, Lys dendrimers, Lys-rich proteins, amyloids, and poly(amino acids) show clusteroluminescence in highly concentrated solutions or in the solid state, but not as dilute solutions.[3] Herein we report a designed single α-helical peptide (SAH) composed of repeating sets of non-aromatic Glu and Lys residues that displays clusteroluminescent emission at c.a. 415 nm upon excitation at 325 nm.

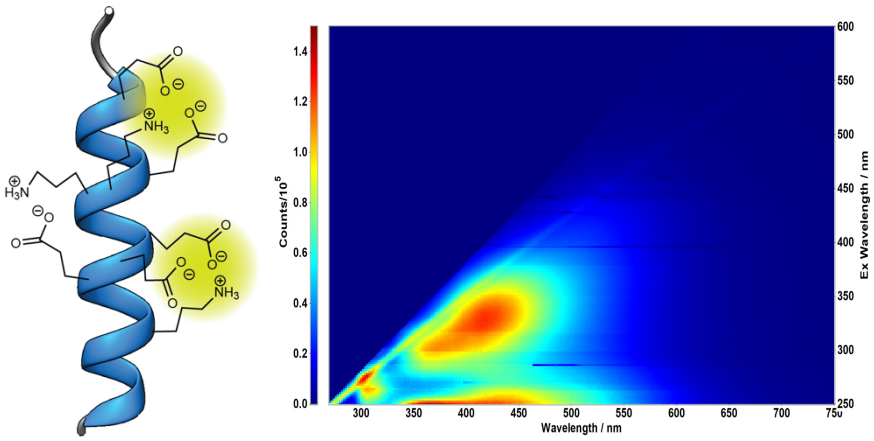

Figure. *a) Schematic representation of the single α-helix (SAH) peptide with emissive Glu/Lys clusters; b) EEM (excitation-emission matrix spectra) measurement of the SAH clusteroluminescent peptide.*

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Molecular insights into membrane fusion mediated by the parainfluenza fusion peptide

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The parainfluenza viruses cause a significant number of infections worldwide and pose a major disease burden on children. These viruses enter host cells through the fusion of the viral envelope with the cell membrane mediated by the fusion glycoprotein. The fusion peptide, exposed upon proteolytic cleavage of the fusion glycoprotein, is believed to play a crucial role in the fusion process. However, the exact mechanism by which it promotes membrane fusion is not yet fully comprehended.[1] In a previous study, we found that the parainfluenza fusion peptide (PIFP) assembles into porelike structures in the membrane, leading to lipid head intrusion and lipid tail protrusion as well as promoting membrane leakage and fusion. Additionally, we have identified PIFP amino acid residues essential for membrane fusion.[2] Here, we investigate the impact of those residues on the membrane fusion process using a combination of experimental and computational approaches. Our findings demonstrate the critical role of the PIFP N-terminus for membrane interaction and induction of leakage and fusion of lipid vesicles. Moreover, we propose that the Q120 residue, located in the hydrophobic core of the membrane, potentially mediates water passage by establishing key peptide-peptide interactions. Overall, this work enhances our understanding of the PIFP-induced membrane fusion process and provides valuable insights for the development of viral entry inhibitors.

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Peptide-based coacervates as catalytic microreactors

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In this work, we show the creation of dynamic micro-sized liquid condensates formed by a catalytic peptide, whose primary sequence is composed of phase-separating residues (Arg, Lys, Ser, Pro) and ability to hydrolyze phosphate ester compounds and bind to phosphotyrosine assemblies[6].

Turbidity measurements and optical microscopy revealed that the peptide could phase separate and form coacervates, however, a delicate balance between concentration andenvironmental conditions could also lead to peptide aggregates. Circular dichroism and NMR revealed that the peptide presents a fully folded β-hairpin structure only in the coacervate phase, and the liquid-liquid phase separation (LLPS) driven by cation-π and aromatic interactions. The partitioning of the molecules was shown to be controlled by charge interactions. Nevertheless, the hydrophobicity character of the catalytic peptides seems to play a rolein mediating the partitioning process. The P7 inherent encoded functionality of having affinity towards phosphorylated assemblies, seems to accelerate the sequestration of the BSAp inside coacervates compared to its non-phosphorylated counterpart, BSA.

Ultimately, these catalytic coacervates-based reactors' present a catalytic efficiency for standard phosphatase substrate with a 15,000-fold higher compared with P7 in a bulky solution. This work provides a substantial opportunity to leverage the field of catalytic peptides through compartmentalization in aqueous media.

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Structural modifications of the antimicrobial peptide Ctx(Ile^{21})-Ha generate more potent peptide analogues with improved biological activities for animal production applications

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The antimicrobial resistance is an urgent world health problem and can be tackled by developing new and efficient antimicrobial molecules. Antimicrobial peptides have shown a promising class of potent antibiotic molecules. Particularly, the antimicrobial peptide Ctx(Ile^{21})-Ha, has been extensively studied by our research group and exhibited great biological activities against bacteria and fungus, mainly to those pathogenic for animal production.[1] In this way, this study proposes the structural modification, such as C-terminus truncation and strategic amino acid replacement to improve the biological activity of analogues designed. CD spectroscopy was evaluated to analyze the secondary structure and enzymatic degradation essays were performed to examine the peptides' stability, as well their biological activities. The results showed the peptide analogues present a disordered structure in aqueous solution, but in presence of membrane mimetics (micelles and liposomes) they clearly present an α-helical conformation in different contents, even the 12-mers peptide analogue. Stability essays with trypsin and chymotrypsin revealed that the Ctx(Ile^{21})-Ha peptide analogues did not degraded in intestinal fluid simulation for at least two hours. Finally, the biological essays displayed that the most cationic analogues showed the lowest MICs against pathogenic bacteria, with 10-fold lower than the original Ctx(Ile^{21})-Ha peptide.

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Helix-like peptides approach to three-way-junction binding

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Therapeutic DNA targets have been known for a long time and pharmacological agents targeting it, such as cisplatin, are powerful weapons for fighting diseases like cancer[1,2,3]. In addition to B-DNA, non-canonical DNA structures present in uncontrolled, rapidly dividing cells are hot targets for the design of new chemotherapeutic agents[4]. Modeling, synthesizing, and characterizing these molecules, as well as measuring their DNA binding properties through their dissociation constants (K_D) are the first steps in the path to new lead compounds. On the other hand, biocompatibility, the capacity of the molecule to permeate cell membrane so it can reach the intracellular target, and its biological effects, derived from its interaction with the cellular machinery are key parameters to the final use and therapeutic potential of the treatment. In this project it will be described: the synthesis of a new specific ligand of non-canonical DNA structures, its binding ability and its biological effects.

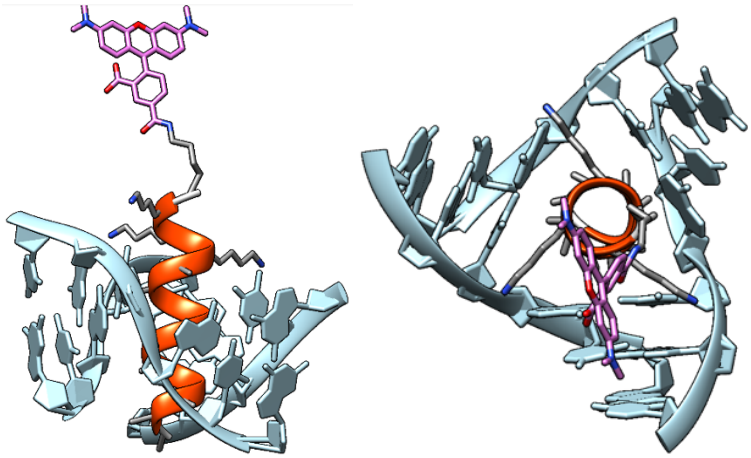

Fig. 1. Hypothetical complex of the proposed new 3WJ binder based on Ac-Ala10-Lys3-Lys(TAMRA)-NH2. Side and top view of this complex are shown for clarity.

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Synthesis and antibacterial activity of *Trichoderma longibrachiatum* longibramide E analogs

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Antibiotic resistance is a spiraling global health concern that requires the development of new classes of antibacterial drugs. In this context, peptaibols are promising membrane-active peptides since their antibacterial action does not involve specific cellular targets, reducing the likelihood of bacterial resistance development. Peptaibols are natural linear peptides with 5–20 amino acids (AAs) that are synthesized by non-ribosomal peptide synthetases and possess some unusual features, such as: a) rare AAs like α-aminoisobutyric acid and isovaline; b) one *C*-terminal alcohol, and c) an acetylated *N*-terminus. These characteristics make peptaibols less prone to lose antimicrobial activity via proteolytic degradation, which is a key asset when developing peptide-based therapeutic candidates. Recently, Zhang et al. 2022 [1] isolated a series of new 11-residue peptaibols, denominated longibramides, from *Trichoderma longibrachiatum*. Among them, longibramide E, which contains an unusual hydroxyproline in its sequence and an L-leucinol in its *C*-terminus, sparked us particular interest due to its activity against *S. aureus* MRSA T144 (MIC value of 30 μg/mL) [1]. Based on this finding and the growing body of evidence that acylation of the *N*-terminus with a fatty acid increases peptide antimicrobial properties [2], a longibramide E analog featuring a *n*-octanoyl instead of an acetylated *N*-terminus was synthesized. In view of the possible industrial production of these analogs, the relatively expensive *C*-terminal leucinol moiety was replaced by a leucine amide. The synthesis of target peptaibols was carried out both manually and automatically, using Symphony® X, to compare synthesis method efficiency for this class of metabolites. Moreover, PurePep® Easy Clean was used to purify some of these oligomers, whose antibacterial activity will be determined against susceptible and multidrug resistant strains of Gram-positive/negative bacteria.

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Peptides derived from pet food protein ingredients: from proteomics to *in vitro* bioactivity profiling

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Peptides derived from protein ingredients have been characterized as having several important biological activities, such as antimicrobial, antihypertensive, antioxidant, anti-inflammatory, analgesic and immunomodulatory. Having this in mind and in line with the One Health approach, investment is increasing on ways to promote the good health and well-being of pets. Sustainable functional ingredients for pet food are therefore needed.

In view of this, we have been exploring peptides derived from pet food protein ingredients, as nutraceuticals for high quality pet food formulations. As such, we have used a combination of proteomics, bioinformatics, chemical synthesis, and in vitro assays, to identify non-toxic oligopeptides (up to 15 amino acids) which might possess antimicrobial, antioxidant, anti-inflammatory, and antihypertensive activity. In addition, we have also explored the nutraceutical potential of milk-derived peptides, namely, bioactive peptides from lactoferrins formerly reported to possess antimicrobial activity.

Results from these studies will be addressed in this communication. Overall, most of the peptides investigated displayed antioxidant activity, though less potent than that of typical non-peptide antioxidants like, e.g., quercetin. Similar findings were made regarding antihypertensive action, as some peptides showed some degree of inhibition of angiotensin I-converting enzyme (ACE-1) but much weaker than that of the reference drug captopril. Still, it remains to be established whether some of the tested peptides may have better pharmacokinetics than that of the bioactive compounds taken as reference (e.g., quercetin). Therefore, further studies are necessary to establish the real potential of these peptides as nutraceutical agents.

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