XIX Iberian Peptide Meeting (EPI 2025)

ABSTRACT BOOK

26-28 February 2025

Hospedería San Martín Pinario
Santiago de Compostela

Logo diseñado por Javier Montenegro y Yeray Folgar-Cameán y dibujado por Yeray Folgar-Cameán

Welcome

We are pleased to welcome you to the 19th Iberian Peptide Meeting (EPI) in Santiago de Compostela, a city that symbolizes both the end of a journey and the beginning of new opportunities.

Like the path taken by pilgrims on The Way to Santiago, EPI has come a long way since its first edition, establishing itself as a meeting point for the scientific community dedicated to Peptide Science. Over the years, it has provided a space to share advances in peptide synthesis, structural studies, computational and biophysical properties, as well as their pharmaceutical, materials, and food applications.

Faithful to our tradition, EPI remains strongly committed to supporting young researchers and pre- and postdoctoral students, offering them a space to present their work, exchange ideas and build new networks that will strengthen the future of our scientific community.

We hope this meeting will be an enriching milestone in your own journey, helping you discover new horizons. It has been a pleasure to organize this edition, and we hope you enjoy the meeting as much as we have enjoyed making it possible.

The organizing committee,

Ana Pina Javier Montenegro

XIX IBERIAN PEPTIDE MEETING

KN	Keynote	40+5	min
IL	Invited Lecture	22+3	min
OT	Oral Talk	13+2	min
ST	Short Talk	7+1	min
CT	Company Talk	13+2	min

Wednesday, 26th

11:45		**REGISTRATION**	
14:45		**WELCOME REMARKS**	
15:00	KN1	**Xavier Salvatella** ICREA Research Professor *Learning from nature: designing glutamine-based single α-helices to inhibit protein-protein interactions*	Chair: Eugenio Vázquez
15:45	OT01	**Paco Fernández-Trillo** Universidade da Coruña *Delivery of Antimicrobial Peptides through Peptide-Based Carriers*	
	ST01	**Christine Cruz Oliveira** Gulbenkian Institute for Molecular Medicine *Broad-spectrum antiviral peptide-porphyrin conjugates restrict Zika virus replication and mitigate disease in mice*	
	ST02	**Alba Ramil-Bouzas,** CICA *Cationic self-assembling lipopeptides as non-viral gene delivery vectors in mesenchymal stem cells*	
	ST03	**Vera Neves** Gulbenkian Institute for Molecular Medicine *PepH3-decorated nanoparticles for delivery of therapeutic peptides*	
	CT1	**Marisa Juanes** TraffikGene *TraffikGene: A Versatile Platform for Nucleic Acid Delivery Based on Amphiphilic Peptide Carriers*	
16:45		**Coffee Break**	
17:15	OT02	**Carles Mas-Moruno** Universitat Politècnica de Catalunya *Multifunctional and dynamic peptide-based hydrogels*	Chair: Diego Núñez
	ST04	**Cristina Montaner** Institut Químic Sarrià *De novo designed peptide masks enable ligand binding activation with different stimuli*	
	ST05	**Patricia Sanmiguel** Universidade de Santiago de Compostela *Selective recognition of three-way DNA junctions with designed α-helical peptides*	
	ST06	**Valeska Viereckt** University of Marburg *Bioluminescence-based Singlet Oxygen Generation*	
	ST07	**Manuel Pérez-Pérez** Universidade de Santiago de Compostela *Peptide-Coated Platinum Nanocages for Tumor Targeting*	
	OT03	**Ivo Martins** Gulbenkian Institute for Molecular Medicine *Peptide-based inhibition of Zika virus*	
	ST08	**Alberto Muñoz Villoria** Universidade de Santiago de Compostela *Toward tumor-targeted biorthogonal catalysis with a HER2 affibody*	
	ST09	**Bárbara Matos** i3S *PSA-responsive self-assembling peptides for prostate cancer drug delivery*	
	ST10	**Carlos Cuadros-Higueras** Instituto de Química Médica (IQM-CSIC) *Advances in cardiovascular deca-11 peptide analogues*	
	OT04	**Sónia Gonçalves** Gulbenkian Institute for Molecular Medicine *Silver nanostar-anticancer peptide conjugates: a novel approach to enhance anticancer efficacy*	
19:00			
19:30		**WELCOME COCKTAIL**	

Thursday, 27th

9:00	KN2	**Luc Brunsveld** Eindhoven University of Technology *A quantitative understanding of PPI stabilization by molecular glues – a crucial role for peptides*	
9:45	OT05	**Julian Bergueiro,** Universidade de Santiago de Compostela *Peptide assembly through exo-helical interactions*	Chair: Ana Pina
	ST11	**Joana Martins** Laboratório Associado para a Química Verde *P-Sulfonatocalix[4]arene-based ratiometric arrays for peptide sensing*	
	ST12	**Ion Turcan** Universidade de Santiago de Compostela *Screening of peptide amphiphiles with autocatalytic self-assembly*	
	ST13	**Diego Sánchez-Brunete** Universidade de Santiago de Compostela *A peptide-based receptor for polyoxometalates*	
	ST14	**Federica Souto-Trinei** CICA - Centro Interdisciplinar de Química e Bioloxía *Self-assembling lipopeptides as phospholipid membrane mimics*	
	ST15	**Pavel Zelenovskii** CICECO-Aveiro Institute of Materials *Self-assembling two-dimensional peptide nanostructures with piezoelectric properties*	
10:45		**Coffee Break and Posters**	
11:45	IL1	**Mohit Kumar** University of Barcelona *Active vesicle as synthetic cell and their role in peptide/protein self-assembly*	
12:15	OT06	**Roland Hellinger** Medical University of Vienna *Structure inspired grafting to design novel T-lymphocyte migration modulator peptides*	Chair: Myriam Royo
	ST16	**Román Fernández López** Universidade de Santiago de Compostela *Synthesis of 3-hydroxy-4-aminocyclohexanecarboxylic acid-based peptides*	
	ST17	**Alicia Moreno-Ainse** Center for Research in Biological Chemistry and Molecular Materials *The role of lysophosphatidic acid in cancer immune evasion*	
	ST18	**Carmen González-González** Universidade de Santiago de Compostela *Non-Aromatic Fluorescence in Zwitterionic Single α-Helical Peptides*	
	ST19	**Carina Esteves** UCIBIO *Peptide ionogels for artificial olfaction*	
	ST20	**José María Martínez Parra** Universidade de Santiago de Compostela *Exo-chirality of the α-helix in helical peptide assemblies*	
	OT07	**Iván Sasselli** CSIC-UPV/EHU *Converging experiments and computation for supramolecular peptide assemblies*	
13:30		**Lunch**	
15:00	KN3	**Olalla Vázquez** Marburg University *Peptides in Action! – unlocking their potential in chemical biology*	
	OT08	**Ignacio Insua** Universidade de Santiago de Compostela *Supramolecular peptide assemblies for structural and functional cell mimicking*	Chair: Marta Planas
	ST21	**Jianing Li** Imperial College London *Investigating how protecting groups affect physicochemical properties and purification of peptides*	
	ST22	**Axel Sarmiento** Universidade de Santiago de Compostela *DNA 3-way junction binding with Metal-stabilized collagen triple helices*	
	ST23	**Zoe Manglano Artuñedo** Autonomous University of Barcelona *A structure-based approach towards a new therapy for Parkinson's Disease*	
	ST24	**Joana Calvário** ITQB NOVA *Investigating Amino Acid Enrichments and Patterns in Phase-Separating Proteins*	
	ST25	**Adrián Sánchez-Fernández** Universidade de Santiago de Compostela *Modulating protein conformation and entanglement in compositionally designed deep eutectic solvents*	
16:45		**Coffee Break and Posters**	
17:45	IL2	**Benjamin Oller** ChemSynBio IQS Barcelona *How may peptides increase antibody tissue selectivity and brain transport?*	
18:15			
20:30		**SOCIAL DINNER**	

Friday, 28th

9:00	**KN4**	**Annemieke Madder** *Furan- and Triazolinedione-based Chemistries for Bio-orthogonal Protein Modification*	Chair: Paula Gómes
9:45	**CT2**	**Giorgio Marini** CEM (Alenium – Qlabo) *Innovations in Sustainable Peptide Production*	
	OT09	**María Angeles Jimenez**, IQF, CSIC *Understanding protein interactions using solution NMR and a "divide-and-conquer" approach*	
	ST26	**Jordi Lamata** Universidad de Girona *Novel peptaibol derivatives as promising candidates to target the plant quarantine pathogen Xylella fastidiosa*	
	ST27	**Jordi Pujols** Universidad Autónoma de Barcelona *Exploiting prion-like amyloid assemblies to produce functional nanomaterials*	
	ST28	**Ezequiel Silva-Nigenda** University of Windsor *Use of Computational modeling and Continuous-Flow Solid-Phase Peptide Synthesis for the design and synthesis of peptide ligands targeting HLA and Hsp90*	
	OT10	**Martin Calvelo Souto** Universidad de Santiago de Compostela *Influence of Lipid Composition on Amyloid Beta Aggregation*	
11:00		**Coffee Break**	
11:45	**IL3**	**Paula Ferreira** University of Minho *Supramolecular Peptide Hydrogels: Exploring the Role of Dehydroamino Acids*	Chair: Nuno Santos
12:15	**CT3**	**Valentina Stulberg** Iris Biotech GmbH *Building with precision - innovative tools in peptide synthesis*	
	ST29	**Sebastián Jiménez Millán** Centro de Física de Materiales (CSIC, UPV/EHU) *Interaction of SARS-CoV-2 Fusion Peptides with Lipid Membranes*	
	ST30	**Álvaro de la Cruz** CSIC - Institute of Medicinal Chemistry *Targeting the Weak Spot of Hemagglutinin: Toward Broad-Spectrum Influenza Therapeutics*	
	ST31	**Carmen Bretón Beltrán** Universidad de La Rioja *Development of Non-Natural MUC1 Glycopeptide Cancer Vaccines*	
	ST32	**Fabián Suárez-Lestón** Universidade de Santiago de Compostela *Coarse–Grained Molecular Dynamics Simulations of Antimicrobial Peptides*	
	ST33	**Inés del Barrio**, Institut de Química Avançada de Catalunya (IQAC-CSIC) *Cyclic iodonium salts as self-activated tags for primary aliphatic amines*	
13:15		**PRIZE CEREMONY AND CLOSING REMARKS**	
13:30		**Lunch**	

Keynotes

KN1 Learning from nature: designing glutamine-based single α-helices to inhibit protein-protein interactions

[1] **Prof. Xavier Salvatella**

1 ICREA Research Professor

The interactions between helical motifs and globular proteins offer opportunities for therapeutic intervention. Their modulation with small molecules is however challenging mainly because they bury large surfaces. Linear peptides that display the residues that are key for binding can be targeted to globular proteins when they form stable helices, which in most cases requires their chemical modification. Here we present rules to design peptides that fold into single α-helices by instead concatenating glutamine side chain to main chain hydrogen bonds recently discovered in polyglutamine helices(1,2). The resulting peptides are uncharged, contain only natural amino acids, and their sequences can be optimized to interact with specific targets. Our results provide design rules to obtain single α-helices for a wide range of applications in protein engineering and drug design (3).

1. Eftekharzadeh, B. et al; Biophys J. 2016, 110, 2361-2366.
2. Escobedo, A. et al.; Nat. Commun. 2019, 10, 2034.
3. Escobedo, A. et al.; Nat. Commun. 2022, 13, 7073.

KN2 A quantitative understanding of PPI stabilization by molecular glues - a crucial role for peptides.

[1] **Luc Brunsveld**

1 Chemical Biology at the department of Biomedical Engineering

Stabilization of protein-protein interactions (PPIs) with molecular glues is one of the most current and challenging topics in chemical biology and drug discovery. The three-body-problem (2 different proteins and a molecular glue) constitutes a conceptually completely fascinating and unique challenge both regarding its fundamental biophysical understanding and for the identification of novel molecular matter. Our group combines organic synthesis, protein chemistry, structural biology, and supramolecular chemistry to perform chemical biology studies on PPIs with the aim to enable innovative medicinal chemistry entries for 'molecular glues' for PPIs.

Intrinsically Disordered Proteins (IDPs) represent one of drug discovery's major challenges. Due to their high degree of conformational freedom, IDPs have no defined pockets for binding small molecules. Molecular glues that can strengthen protein-protein interactions (PPIs) are a revolutionary technology for drug discovery. The hub protein 14-3-3 regulates many IDPs and ID domains of multidomain proteins via phosphorylation-dependent PPIs. Stabilization of 14-3-3 PPIs with small molecular glues provides a unique entry point to render IDPs druggable and mitigate the aberrant behaviour of malfunctioning IDPs, for example in neurodegenerative diseases.

While inhibition of PPIs by small molecules has expanded the proteome suitable for therapeutic intervention, the opposite chemical-biology strategy of PPI stabilization by small molecular glues is, despite a recent surge of interest, remarkably underexplored. The lack of mechanistically understanding PPI stabilization impedes systematically identifying molecular glues and limits progress to drug IDPs.

Via a combination of mechanistic studies into 14-3-3 PPIs stabilization and the development of novel molecular concepts to drug the composite pockets of 14-3-3 PPIs, we aim to unlock the 14-3-3 interactome for novel drug discovery.

The general challenge centers around the three-body-problem (2 different proteins and a molecular glue). This unique element of PPI stabilization constitutes a conceptually fascinating and unique challenge both regarding its fundamental biophysical understanding and for the identification of novel molecular matter. Our group combines organic synthesis, protein chemistry, structural biology, and supramolecular chemistry to perform chemical biology studies on PPIs with the aim to enable innovative medicinal chemistry entries for 'molecular glues' for PPIs.

The presentation will highlight a combination of chemical biology and medicinal chemistry approaches to help to unravel the underlying complex interaction mechanisms. This conceptual approach to PPIs allows to recognize and apply supramolecular concepts such as multivalency and cooperativity within the context of drug discovery and as leading principles in for example compound optimization and selectivity towards specific PPI. Specific examples regarding the 14-3-3 PPI with the Estrogen Receptor, Tau and ChREBP will be

highlighted to illustrate the functionality of 14-3-3 molecular glues on the cellular level and beyond.

KN3 Peptides in Action! - unlocking their potential in chemical biology

[1] **Olalla Vázquez**

1 Marburg University, Marburg, Germany

Peptides have emerged as powerful tools in chemical biology and drug discovery because of their intrinsic characteristics that positioned them in the sweet spot between small molecules and biologics. Peptides act as key biological mediators with minimal toxicity. As biologics, peptides can acquire a particular secondary structure for improved molecular recognition. Yet, generally, peptides have lower cost and immunogenicity. Easily synthesised and modified, peptidomimetics can overcome challenges related to uptake and stability. The growing interest in synthetic peptides in drug research highlights their efficacy1 and their role in chemical biology as tools for modulating protein-protein interactions, target identification, and eventually deciphering complex biological processes to improve life.

In my group, we have intensively worked with peptides to manipulate biological processes. In this talk, I will provide a brief overview of our research program, highlighting the use of peptides as modular platforms for organelle-dependent activation of photosensitizers2 and optoepigenetic tools for in vivo control of hematopoiesis.3,4 Mainly, I will focus on our most recent work that demonstrates how peptides can assist us in the discovery of new therapeutic targets5 and beyond, including non-published results.

1. Muttenthaler, M.; King, G. F.; Adams, D. J.; Alewood, P. F. Trends in peptide drug discovery. Nat. Rev. Drug Discov., 2021, 20, (4), 309-325

2. Linden, G.; Vázquez, O., Bioorthogonal Turn-On BODIPY-Peptide Photosensitizers for Tailored Photodynamic Therapy. Chemistry 2020, 26 (44), 10014-10023

3. Albert, L.; Xu, J.; Wan, R.; Srinivasan, V.; Dou, Y.; Vázquez, O., Controlled inhibition of methyltransferases using photoswitchable peptidomimetics: towards an epigenetic regulation of leukemia. Chem. Sci. 2017, 8 (6), 4612-4618

4. Albert, L.; Nagpal, J.; Steinchen, W.; Zhang, L.; Werel, L.; Djokovic, N.; Ruzic, D.; Hoffarth, M.; Xu, J.; Kaspareit, J.; Abendroth, F.; Royant, A.; Bange, G.; Nikolic, K.; Ryu, S.; Dou, Y.; Essen, L. O.; Vazquez, O., Bistable Photoswitch Allows in Vivo Control of Hematopoiesis. ACS Cent. Sci. 2022, 8 (1), 57-66

5. Fischer, S.; Trinh, V. T.; Simon, C.; Weber, L. M.; Forné, I.; Nist, A.; Bange, G.; Abendroth, F.; Stiewe, T.; Steinchen, W.; Liefke. R.; Vázquez. O.* Peptide-mediated inhibition of the transcriptional regulator Elongin BC induces apoptosis in cancer cells; Cell Chem. Biol. 2023, 30, 766-79*

KN4 Furan- and Triazolinedione-based Chemistries for Bio-orthogonal Protein Modification

[1] **Annemieke Madder**

1 Organic and Biomimetic Chemistry Research (OBCR) group, Department of Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281, S4, 9000 Ghent, Belgium

Within OBCR, we have developed a highly selective and efficient singlet oxygen mediated crosslink technology which is applicable to peptide-protein, peptide-nucleic acid and nucleic acid interstrand crosslink scenarios.[1] For this purpose, a furan 'warhead' is introduced into one of the biomolecular partners and subsequently activated by means of an oxidation trigger such as singlet oxygen which induces generation of a nucleophile-sensitive keto-enal moiety.[2] The overall procedure allows spatiotemporal control of the crosslinking event.

In the context of peptide ligand-receptor interactions, we have described, in live cells under normal growth conditions, spontaneous enzymatic activation and crosslinking of furan-modified peptide ligands to their membrane GPCR with zero toxicity, high efficiency and spatio-specificity.[3] Furan introduction into peptide and protein ligands and subsequent covalent modification of their natural targets was achieved after triggering photocatalytic singlet oxygen generation.[4]

Different furan[5] and triazolinedione[6] based building blocks were further developed for versatile and site-selective modification of proteins and synthesis of bioconjugates. The talk will highlight selected specific examples of these cross-linking and conjugation methodologies.

The work was supported by the FWO-Vlaanderen, the BOF-UGent, the IOF-UGent and the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 721613 (MMBio ITN), No 956070 (OligoMed ITN), No. 665501 (Pegasus2 fellowship).

1. a) Carrette, L.L.G, Morii, T.; Madder, A. Bioconj. Chem. 2013, 24(12), 2008-2014; b) L. L. G. Carrette, E. Gyssels, N. De Laet and A. Madder. Chem Comm. 2016, 52, 1539. 2 a) Op de Beeck, M., and Madder, A. JACS 2012, 134, 10737–10740; b) De Laet, N., Llamas, E.M., and Madder, A. ChemPhotoChem 2018 2, 575–579. 3 a) Vannecke, Van Troys, Ampe & Madder, ACS Chemical Biology 2017, 2191; b) EP10196898.0.; c) EP 15176415.6. 4 a) Miret-Casals, L.; Vannecke, W.; Hoogewijs, Madder, A. et al. Chem. Comm., 2021, 57, 6054 – 6057; b) Miret Casals, L.; Van De Putte, S.; Madder, A. et al. Frontiers in Chemistry, 2022. 9:799706. 5 De Geyter, E.; Antonatou, E.; Kalaitzakis, D.; Madder, A. Chemical Science, 2021, 12, 5246 – 5252. EP 19160048.5. 6 a) Decoene, K. W.; Ünal, K.; Staes, A.; Zwaenepoel, O.; Gettemans, J.; Gevaert, K.; Winne, J. M.; Madder, A. Chemical Science, 2022, 13, 5390 – 5397

Invited lectures

IL1 Active vesicle as synthetic cell and their role in peptide/protein self-assembly

[1] **Dr. Mohit Kumar**

1 Assistant Professor at the Faculty of Chemistry, University of Barcelona

Chemical reactions in living systems are regulated by metabolic processes like the anabolic formation of bio-macromolecules and catabolic degradation of food into energy. These reactions often consume chemical energy and result in active self-assembled structures that exist under out-of-equilibrium (OOE) conditions, which facilitate unique functions of life. In specific, phospholipid molecules of cellular membranes are formed and sustained under OOE, which consume chemical energy to constantly form and breakdown the phospholipids. This provides unique properties like cellular plasticity, spatiotemporal control and regulate protein aggregation on cell surfaces.

In this talk, I will discuss a bio-inspired supramolecular system that forms transient lipid vesicle under OOE conditions, by consuming chemical energy, to result in vesicles with programmable lifetimes. We use simple imine chemistry for the formation of phospholipids and enzymatic ester hydrolysis for the degradation of lipids and the resultant vesicle. We will also demonstrate that the lifetime of these structures can be easily regulated based on the requirement. We are working towards using these active vesicles as an adaptive interface for targeted drug delivery. Finally, we will show how active vesicles can act as a scaffold for the self-assembly of peptides and proteins. These have implications in trying to understand how protein like Tau aggregates on active cellular surfaces like neuronal surface.

IL2 How may peptides increase antibody tissue selectivity and brain transport?

[1] **Benjamí Oller-Salvia**

1 ChemSynBio, IQS Barcelona, Ramon Llull University, Barcelona, Spain

Malignant brain tumors are severely impairing diseases and account for 4% of cancer-related deaths. The efficacy of current treatments is very limited and new therapies need to meet two main challenges: 1) overcoming the blood-brain barrier at the tumor periphery, and 2) selectively targeting tumor cells minimizing off-target toxicity. In our group (www.chemsynbio.iqs.edu), we combine chemical and synthetic biology to develop biotherapeutics aiming to meet these two challenges. One of the biggest issues in selectively targeting tumor cells resistant to chemotherapies is that the receptors they overexpress are also present on cells in healthy tissues. To address this issue, we have recently developed chemogenetic strategies to generate conditionally-active antibody mimetics that are able to engage the target receptor only when activated by tumor-specific proteases or other localized stimuli such as light.[i,ii] In order to enhance BBB penetration of antibody derivatives we utilize brain shuttle peptides that can hijack endogenous transport mechanisms on brain endothelium. We have recently generated new efficient shuttle peptides that have high resistance to proteases and can enhance the transport of protein therapeutics.[iii]

i. R Lucchi, J Bentanachs, B Oller-Salvia. 2021. The masking game: design of activatable antibody and mimetics for selective therapeutics and cell control. ACS Central Science. 7, 724-738.

ii. R Lucchi, M C Lucana, M Escobar-Rosales, C Díaz-Perlas, B Oller-Salvia. 2023. Site-specific antibody masking enables conditional activation with different stimuli. New Biotechnology. 78, 76-83.

iii. M C Lucana, R Lucchi, F Gosselet, C Díaz-Perlas, B Oller-Salvia. BrainBike peptidomimetic enables efficient transport of antibody derivatives across brain endothelium. RSC Chemical Biology. 2024, 5, 7. DOI: 10.1039/d3cb00194f

IL3 Supramolecular Peptide Hydrogels: Exploring the Role of Dehydroamino Acids

[1] **Paula M.T. Ferreira**

1 Centre of Chemistry, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal pmf@química.uminho.pt

In the last decades, supramolecular hydrogels, composed of amino acids and peptides, have revolutionized the field of biomaterials by providing unique advantages, including biological origin, bioactivity, biocompatibility, and biodegradability. These peptide-based hydrogels are particularly valuable in biomedical applications due to their low critical gelation concentration (CGC), which enhances efficiency, cost-effectiveness, and minimizes cytotoxicity. The inclusion of dehydroamino acids in the sequence of peptide hydrogelators endows them with proteolytic resistance and promotes self-assembly due to the conformational constraints in the peptide backbone introduced by the double bond. In this communication a library of peptide hydrogelators incorporating dehydroamino acids will be presented exploring the self-assembly characteristics of these dehydropeptides, focusing on their gelation behavior, mechanical properties, and responsiveness to external stimuli. The incorporation of dehydroamino acids imparts unique chemical properties to hydrogelators, resulting in improved stability and functionality, thereby making them promising candidates for various applications, including drug delivery.

Company talks

CT1 TraffikGene: A Versatile Platform for Nucleic Acid Delivery Based on Amphiphilic Peptide Carriers

[1] **Marisa Juanes**

1 TraffikGene

Despite the vast potential that nucleic acid therapeutics (NATs) hold to address the genetic basis of a wide variety of diseases, relatively few effective RNA-based treatments have been successfully developed due to the "delivery problem." To protect and transport RNA molecules, viral vectors, lipid nanoparticles (LNPs) and polymers have been tried in recent years. However, all these systems suffer from one or more limitations such as cost, toxicity, optimization challenges, and

difficult targeting to tissues of interest. All of these unsolved challenges limit the possibility to fully achieve the potential of RNA therapeutics.

TraffikGene's delivery platform allows for the development of safe, affordable and simple to formulate vehicles for nucleic acid delivery. In our approach, the reaction between short, reactive cationic peptides, with selected hydrophobic tails yields biodegradable amphiphilic peptide carriers. The modularity of peptide and tail components allows for high-throughput screening of libraries of delivery candidates. Carrier attributes can be chemically refined by means of structure- activity analysis to accelerate HIT-lead discovery. This unique platform approach has the potential to facilitate and de-risk advanced therapeutics' development by addressing the delivery problem.

These single component vehicles can easily complex genetic cargos to yield nanoparticles for in vivo delivery of mRNA, siRNA, pDNA or ribonucleoproteins. Due to the unique chemical properties of TraffikGene's carriers, after they reach the cell's interior, the nanoparticles are disassembled to promote endosomal escape, yielding innocuous metabolites which are easily processed by cells.

The functional versatility of this technology has been demonstrated in a variety of in vivo assays. Formulations of an mRNA encoding for a specific antigen with TraffikGene's carriers, resulted in strong and durable immune response against those antigens. These results open new possibilities to employ this delivery technology in the development of novel NATs in fields like cancer vaccination or rare diseases.

Horizon Europe-EIC Transition (Project 101113110), Horizon Europe-Path Finder (Project 101099867), Axencia Galega de Innovación (Project IN855A 2021/06)

CT2 Innovations in Sustainable Peptide Production

[1] **Giorgio Marini**

1 CEM(Alenium - Qlabo)

Increased global demand for peptide therapeutics has placed a renewed emphasis on improving the efficiency and sustainability of peptide production. This presentation will highlight workflow and methodological improvements for high-throughput peptide synthesis (96-well plates), enabling more efficient access to longer sequences at higher purities. In addition, recent improvements in microwave peptide synthesis related to the total elimination of all washing steps after each cycle (resulting in a massive waste reduction) will be highlighted, with application to both R&D and production scale synthesis1. Finally, a new HPLC process for peptide purification that completely eliminates the use of acetonitrile in place of ethanol will be demonstrated. This new process is based on a novel integrated heating system that not only improved peptide

recoveries by 50% on average, but also increased the final isolated purity.

CT3 Building with precision - innovative tools in peptide synthesis

[1] **V. Stulberg**

1 Iris Biotech GmbH, Adalbert-Zoellner-Str. 1, 95615 Marktredwitz, Germany

Iris Biotech offers a broad range of building blocks and reagents focused on peptide chemistry and related fields such as life sciences, drug delivery, and linker technologies (linkerology®) for conjugation. True to our slogan „Empowering Peptide Innovation“ we are dedicated to providing the latest technologies and innovative products from small scale to bulk production, for universities and pharma production, but also for areas such as biotechnology, food analysis, and material science.

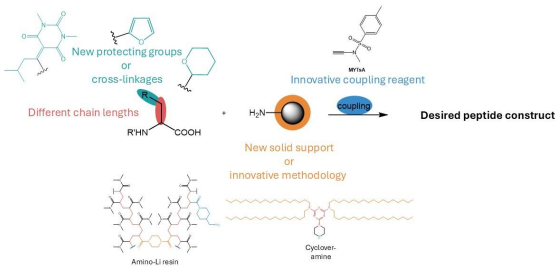
Our innovations include new protecting groups such as the tetrahydropyranyl (THP) protecting group [1] for serine, threonine, cysteine, and hydroxyproline or the 1-(1,3-Dimethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)-3-methylbutyl (ivDmb) protecting group[2] for lysine and ornithine. Both protecting groups are orthogonal to those commonly used in Fmoc-SPPS, offer superior properties, and provide multiple modification opportunities for these residues.

The extension of the side-chain´s carbon backbone by additional methylene groups (homo amino acids) is a known strategy to modulate the proteolytic and conformational stability of synthesized peptides, resulting in increased hydrophobicity or improved binding selectivity.[3] Our portfolio provides new derivatives of lysine, serine, glutamate and arginine in this area.

Another way to hot topics are cross-linking and conjugation of peptides to other (bio-)molecules. Here, we offer 2-furyl-L-alanine which is applied in oxidation-induced furan cross-linking[4] and diels-alder reactions and 5-hydroxy-1,5-dihydro-2H-pyrrol-2-one (5HP2O) building blocks[5] that serve as stable alternatives to maleimides avoiding classical drawbacks thereof, e.g., hydrolysis and thiol-exchange.

In the field of resins, we present the amino-Li resin, a cross-linked polyacryl amide-based solid support, that is compatible with both organic and aqueous solvents[6], as well as cyclover-amine, a novel soluble tag [7] for peptide synthesis, allowing to combine the advantages of both: liquid and solid-phase peptide synthesis.

MYTsA is an innovative ynamide coupling [8] reagent that can be used in both conventional (C to N terminus) or inverse chemical peptide synthesis (N to C terminus). This coupling reagent provides an improved atom economy and avoids racemization.



Overview of innovative tools in peptide synthesis provided by Iris Biotech

[1] Ramos-Tomillero I., Rodriguez H., Albericio F.; Org. Lett. 2015, 17, 7, 1680–1683.

[2] Ramkisson S., Al-Rasheed H. H., Dahlous K. A., De La Torre B. G., El-Faham A., Albericio F.; ChemistrySelect 2021, 6, 6626-6630.

[3] Proietti G., Wang Y., Rainone G., Mecinovic J.; Sci. Rep. 2020, 10, 13046.

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[7] Okada Y., Takasawa R., Kubo D., Iwanaga N., Fujita S., Suzuki K., Suzuki H., Kamiya H., Chiba K.; Org. Process Res. and Devel. 2019, 23, 2576-2581.

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Oral talks

OT01 Delivery of Antimicrobial Peptides through Peptide and Responsive Chemistry

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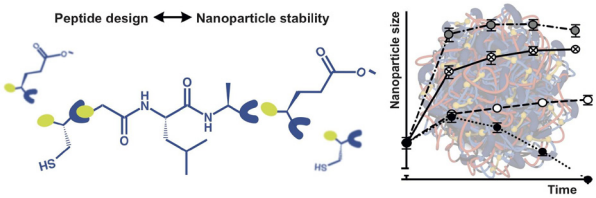
There are several strategies for the delivery of antimicrobial peptides and derivatives. Polyion complex (PIC) nanoparticles, formed by complexation of cationic and anionic polyelectrolytes, are particularly suited to deliver this type of antimicrobials.1 In this communication, we describe our efforts to prepare well defined PIC particles that exert an antimicrobial effect against bacteria. First, we will present our recent efforts to identify PIC particles that can efficiently deliver polymyxin B, a last resort antimicrobial against gram-negative bacteria. Using commercially available polymers, PIC particles with a range of stabilities and antimicrobial activities against P. aeruginosa have been identified.2,3 Moreover, we will present how particles that can respond to virulence factors can be prepared using enzyme-responsive peptides and polymers. In this approach, PIC particles are selectively degraded in the presence of pathogenic bacteria, releasing antimicrobials in a targeted way.4-6

We believe this approach can minimise off-target effects, including induction of resistance in non-pathogenic strains, often observed for antimicrobials.



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OT02 Multifunctional and dynamic peptide-based hydrogels with cell instructive and antibacterial properties

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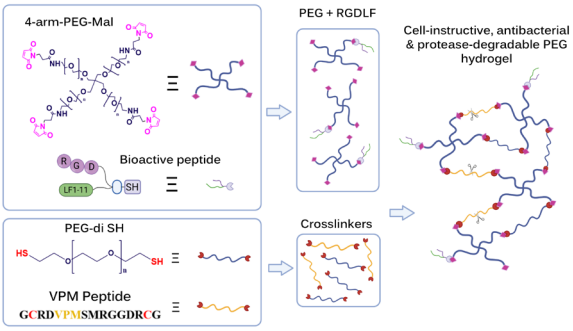
In recent years, the demand for multifunctional and stimuli-responsive hydrogels has intensified to simultaneously address the challenges associated with bacterial infections and inadequate tissue integration in various biomedical applications [1]. Therefore, in this work, we aimed to introduce a novel approach to design peptide-based hydrogels that integrate cell adhesive and antimicrobial properties together with stimuli-responsive cues in order to provide a dual, multifunctional system that dynamically provides effective antibacterial activity and cell instructive properties.

To this end, we have produced polyethylenglycol (PEG)-based hydrogels functionalized with the peptide sequences RGD, required for eukaryotic cell adhesion, and the antibacterial peptide LF1-11 [2]. The incorporation of these two bioactive moieties was done employing a custom-made branched peptidic platform, following a technology recently developed in our group [3]. Moreover, the system was crosslinked via maleimide-thiol chemistry with peptide sequences susceptible to degradation by matrix metalloproteases (MMPs) (i.e. the VPM peptide) [4], thus endowing a dynamic and controlled cell-mediated degradation of the hydrogel (Figure 1).

The multifunctional hydrogels showed excellent cell viability, and efficiently promoted the adhesion and differentiation of mesenchymal stem cells (MSCs). In addition, they displayed potent antibacterial effects against S. aureus and P. aeruginosa, chosen as Gram-positive and Gram-negative models of biomaterial-associated infections, respectively. Finally, the hydrogels were studied in a co-culture setting with MSCs and bacteria, mimicking a clinical scenario of tissue infection. Of note, the hydrogels were able to inhibit bacterial infection and provided protective effects for the cells in such a competitive and complex setting.

This work thus highlights the potential of these novel hydrogels as a promising platform in regenerative medicine, marking a significant advancement in the development of next-generation biomaterials.



Schematic representation of the peptide-based multifunctional and dynamic hydrogel

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OT03 Peptide-based inhibition of Zika virus

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Zika (ZIKV) and dengue (DENV) viruses are closely related flavivirus that have become a major global threat due to climate change and expansion of their mosquito vectors, as reviewed by us [1]. Their virion includes an outer lipid bilayer containing two viral proteins and, within, a nucleocapsid core with the viral RNA complexed with multiple copies of the capsid (C) protein [1-6]. This crucial structural protein is involved in viral assembly and encapsidation. As previously studied by us [2-6], DENV C binds host lipid systems, namely lipid droplets (LDs) and very low-density lipoproteins (VLDL). This study allowed us to develop pep14-23 [2,3], a peptide inhibitor of the binding to host lipid systems, based on a flavivirus capsid protein conserved segment. Following, we identified the targets of C protein within each host lipid system: apolipoprotein E (APOE) and perilipin3 (PLIN3), structurally analogous proteins found at the surface of, respectively, VLDL and LDs [4,5]. We then developed apoE-N2 as a potential peptide inhibitor, based on APOE [5]. Finally, using Vero cells infected with ZIKV, we evaluated the cytotoxicity and antiviral efficacy of both pep14-23 and apoE-N2. Cytotoxicity assays confirmed that the two peptides exhibited no significant toxicity at concentrations up to 100 μM. The antiviral assays demonstrated that while pep14-23 caused a slight reduction in viral titers, apoE-N2 significantly reduced the viral replication in a dose-dependent manner, achieving up to a 3.54 log reduction after 24 h of treatment, corresponding to >99.97% inhibition of viral replication. In contrast, a scrambled version of apoE-N2 showed no antiviral activity, confirming the sequence specificity of the observed effects. These findings show that apoE-N2 is a promising candidate for further development as an antiviral against ZIKV and related flaviviruses.

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OT04 Silver nanostar-anticancer peptide conjugates: a novel approach to enhance anticancer efficacy

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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 ACPs 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 ACP 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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OT05 Peptide assembly through exo-helical interactions

[1] **Julian Bergueiro**, [1] Jose María Martinez-Parra, [1] Rebeca Gómez-Ojea, [1] Alejandro Fiel, [1] Fernando Bordallo, [1] Javier Montenegro

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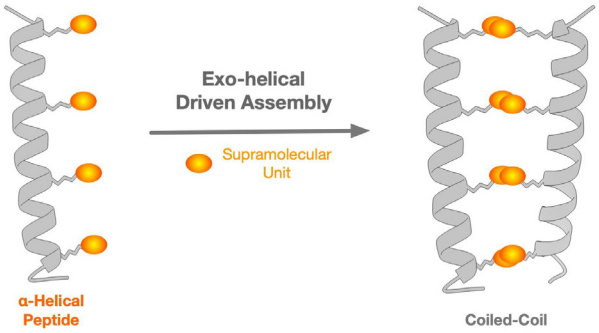
One of the most predominant peptide self assembly mechanisms is based on the α-helix (3.6-13) secondary structure.(1) The chemical information contained in the amino acid can be distributed on a helical fashion in the α-helical backbone exterior.(2) These chiral topologies generated by repetition patterns in the primary sequence can be utilized to master the assembly of α-helixes.(3, 4)

Non-proteinogenic amino acids were selected in this study to behave as a double function unit: i) As a structural reporter based on its chromophoric properties that will vary depending on their conformation in space in the folding or assembled state, ii) as a supramolecular interacting unit that will rule the assembly into coiled coil. By utilizing UV, CD and fluorescence spectroscopies, we firstly observe how the canonical patter can form coiled coil structures with new supramolecular units. Moreover, we investigate the dependence of these assemblies on the exo-helical topologies persistency. Interestingly we found that other topologies apart from the canonical heptad can be utilized to form assemblies. Molecular dynamic simulations supported by analytical ultracentrifugation, SAXS, and cryo-TEM helped us in the understanding of the assemblies formed.



Topologically driven supramolecular peptide self assembly into coiled coil structures

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OT06 Structure inspired grafting to design novel T-lymphocyte migration modulator peptides

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The migration of lymphocytes is essential in the progression of autoimmune and inflammatory diseases. The clinical efficacy of existing drugs is frequently constrained by adverse effects or insufficient of patient responses, underscoring the unmet medical need for better medications. The endogenous peptide inhibitor of transendothelial migration (TEM) (pepitem) controls lymphocyte migration (1). Due to its complex structure-function and sequence-activity relationships this prototype was unsuitable for conventional peptide drug developmental methods to date. Here we addressed these shortcomings by developing a new approach, termed structure-inspired grafting, for the design of stabilized peptide analogues (2). Based on, that native pepitem is endogenously embedded within a 14-3-3 protein, in silico analysis of parent molecule sequences across evolution in chordate identified a possible minimal active sequence. Next, we utilized structure-based similarity searches toward protein databases to select for the nearest structural match of the pepitem/14-3-3 epitope, resulting into the plant-derived peptide Veronica hederifolia trypsin inhibitor (VhTI) with a high confirmational fit. Guided by AI-based model predictions, the active sequence was integrated into this stable framework, yielding conformationally stabilized peptide probes of pepitem. Solid phase peptide synthesis produced selected molecules, and NMR spectroscopy confirmed the predicted models' high quality. Bioactivity validation experiments identified the best probe, designated VhTI-pep 2, which inhibited CD3+ T-lymphocyte migration in a TEM assay, and is ~2-fold more potent (EC50 of 2.5 ± 2.8 nM) as compared to pepitem (EC50 = 6.0 ± 6.4 nM). VhTI-pep 2 inhibited different T-cell subsets from healthy donors as well as from Multiple Sclerosis patients, highlighting the peptide's therapeutic potential. We demonstrate that accurate peptide-protein complex predictions are possible with the conformationally-stabilized probe, revealing the peptide pharmacophore's interaction with the proposed target protein cadherin (CDH) 15. Our work will guide future design of stabilized peptide, with the prospect of overcoming the challenges associated with flexible linear peptides.

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This work was supported by the project ZK-81-B (grant doi: 10.55776/ZK81) and the P36736B (grant doi: 10.55776/P36736 by the Austrian Science Fund (FWF).

OT07 Converging experiments and computation for symbiotic design of short peptide assemblies

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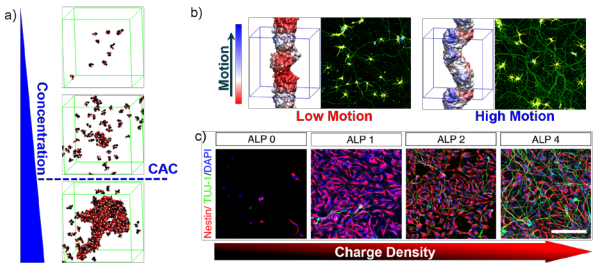
Supramolecular peptide assemblies (SPAs) hold great promise as multifunctional materials for applications in nanotechnology and biomedicine. Computational methodologies have become invaluable for uncovering the structural details of SPAs at the molecular level, helping to overcome the inherent limitations of traditional experimental techniques. By integrating computational simulations with experimental investigations, we can achieve a more comprehensive understanding of SPA self-assembly, accounting for factors such as amino acid sequences and environmental influences. This integrated approach not only enhances the interpretation of complex experimental data but also serves as an efficient tool for narrowing the experimental search space, streamlining the discovery process. However, challenges remain in aligning computational models with experimental observations. Key obstacles include: 1) accurately replicating experimental conditions specific to SPAs, particularly given the distinct effects of system size and concentration on SPA behavior compared to proteins (Figure 1a), and 2) identifying parameters that directly correlate with experimental features and the bioactivity of the materials (Figure 1b, c). In our work, we address these challenges by systematically exploring how critical parameters—such as concentration, system size, or mixing procedures in co-assemblies—affect SPA behavior. Additionally, we investigate the predictive power of coarse-grained simulations in determining intermolecular order, molecular mobility, and other key characteristics. Our findings underscore the essential role of a symbiotic approach that integrates computational and experimental methodologies to gain deeper insights into SPAs. By leveraging this interdisciplinary framework, we unlock previously inaccessible knowledge of SPA behavior, enabling the precise optimization of their properties for customized applications across diverse fields.



Computational parameters match experiments: (a) CAC; (b) molecular motion; and (c) charge density.

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Ramon y Cajal Program(RYC2021-033294-I), and Spanish State Research Agency (PID2022-136392NA-I00).

OT08 Supramolecular peptide assemblies for structural and functional cell mimicking

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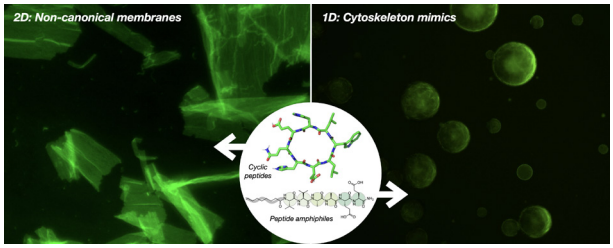
Rational peptide designs allow the simplified reproduction of cellular tasks and components through self-assembly. This communication will cover short peptides that mimic the behaviour of living matter in a minimalistic and simplified version towards the development of minimal synthetic cells (i.e. protocells): 1) Non-cannonical peptide functions, like cellular membrane formation, was attained by amphiphilic peptide monomers in two-dimensional packings. 2) Minimalistic cytoskeleton mimics allowed the reproduction of intracellular fibre scaffolds and derived life-like behaviour. 3) The autocatalytic self-reproduction of peptide amphiphiles imitates the perpetuation mechanisms of living matter. Combined, this work contributes to the synthetic engineering of minimal cell mimics - simplified versions of cells with functional capacity that may open exciting opportunities in cell and tissue engineering, biotechnology and advanced materials science.



Example one-dimensional (1D) and two-dimensional (2D) peptide assemblies obtained by rational design.

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This work was funded by the European Commission (ERC_StG_REPLICATE-101163359), Agencia Estatal de Investigación (RYC2021-031367-I, PID2023-147885OA-I00) and Xunta de Galicia (ED431F 2023/24, ED431C 2024/09).

OT09 Understanding protein interactions using solution NMR and a “divide-and-conquer” approach

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Understanding at the atomic level the intricate network of biomolecular interactions, which regulate biological processes in healthy living beings, still persists a challenge. Mis-regulation of protein interactions is at the origin of many diseases. Herein, we focus on two systems of relevance in human health: the cannabinoid receptor CB1R, and the intercellular adhesion molecule ICAM1.

CB1R is a G protein coupled receptor (GPCR) whose structure consists of an N-terminal region, seven transmembrane helices linked by extracellular and intracellular loops and a C-terminal intracellular domain. Distint signal cascades are triggered by activation of CB1R via either a canonical path by binding to G-proteins or via non-canonical ways upon binding to non-G-proteins, such as -arrestins, and CRIP1a. Insights into the interaction between CB1R and beta-arrestin have been provided by solution NMR studies on model peptides (1).

ICAM1 is a transmembrane glycoprotein of the immunoglobulin (Ig)-like superfamily, comprising five extracellular Ig-like domains, a transmembrane helix and a short cytoplasmic tail. Recently, ICAM-1 has been found to interact with NHERF-1, whose structure contains two PDZ domains followed by a long C-terminal tail.

To gain insights into the CB1R/non-G-proteins and ICAM1/NHERF1 interactions, the structural behaviors of model peptides derived from the cytoplasmic tails of CB1R and ICAM1, as well as their interaction with their respective partners, CRIP1a and NHERF1, are being examined using solution NMR. Current results will be described.

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Financial support from grants PID2020-112821GB-I00 (MCINN/AEI/10.13039/501100011033) and S2022/BMD-7232 (Spanish “Comunidad de Madrid”) is acknowledged. NMR experiments were performed in the “Manuel Rico” NMR laboratory (LMR) of the Spanish National Research Council (CSIC), a node of the Spanish Large-Scale National Facility (ICTS R-LRB)

OT10 Influence of Lipid Composition on Amyloid Beta Deposition: Insights from Coarse-Grained Molecular Dynamics Simulations

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The impact of lipid composition on amyloid beta (Aβ) accumulation was investigated through Coarse-Grained Molecular Dynamics (CG-MD) simulations using the Martini 3 force field.(1) Over 700 simulations were performed, starting from various initial Aβ secondary structures in the presence of lipid bilayers derived from a neuronal membrane model.(2)

To facilitate this study, our group developed Martini 3 parameters for different gangliosides, including monosialotetrahexosylganglioside (GM1) and monosialotrihexosylganglioside (GM2), complementing published parameters for monosialodihexosylganglioside (GM3), due to their prevalence in brain cell membranes.(3–5)

The simulations were initiated with the peptides in the aqueous phase to explore the potential spontaneous interaction with the bilayers (Figure 1). The results demonstrate that the presence of gangliosides, particularly GM1, significantly enhance Aβ deposition, increasing it tenfold compared to bilayers without gangliosides. This observation aligns with experimental findings and highlights the utility of computational models in studying Aβ-lipid interactions, as well as the methodology used.(6, 7) In contrast, no notable differences in Aβ aggregation were detected across lipid compositions, with aggregation levels averaging 90% in all cases.

Analysis of peptide-lipid interactions revealed that the sugar moieties in gangliosides, specifically N-acetylneuraminic acid (NANA), play a critical role in driving Aβ deposition. Furthermore, GM1 exhibited a greater number of interactions than GM2 and GM3, primarily due to its additional sugar units, which enhance contact frequency with Aβ peptides.

These findings highlight GM1's role as a key player in promoting Aβ deposition on lipid bilayers, suggesting its potential use as therapeutic target for amyloid-related conditions, such as Alzheimer's disease. Modulating GM1 levels in membranes, preventing its clustering in lipid rafts, or inhibiting its interactions with Aβ—particularly those mediated by NANA—could offer new strategies for therapeutic development. This study also highlights the importance of lipidomics in understanding amyloidopathies and developing novel treatment strategies.

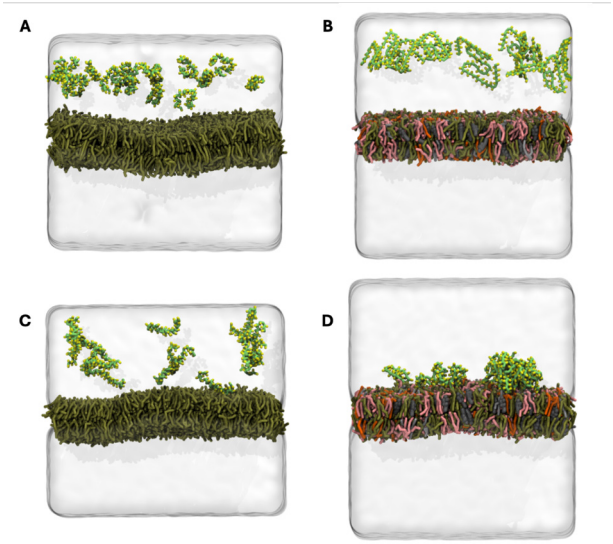

Figure 1. Initial (A, B) and final (C, D) snapshots from two of the CG-MD trajectories. Panels A and C show results for a pure POPC membrane, while panels B and D correspond to a POPC-POPE-POPS-CHOL-DPSM-GM1 (8:5:1:4:2:1) membrane.

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Short talks

ST01 Broad-spectrum antiviral peptide-porphyrin conjugates restrict Zika virus replication and mitigate disease in mice

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Viral infections pose a significant threat to human population worldwide. Virus emergence/re-emergence can lead to devastating consequences, e.g., Zika outbreak in Brazil in 2015 and COVID-19 pandemics in 2019. In light of this, broad-spectrum antivirals, such as porphyrins, represent a key tool for outbreaks/pandemic preparedness and fast response. Viruses, such as Zika virus (ZIKV) and SARS-CoV-2, can invade the central nervous system (CNS), causing neurological complications, such as ZIKV-induced microcephaly. However, the blood-brain barrier (BBB) hinders the effective delivery of antiviral drugs to the CNS. Additionally, drugs must also cross the blood-placental barrier (BPB) to prevent ZIKV-induced microcephaly.

In this work, using different porphyrins and BBB peptide shuttles (BBBpS), we developed peptide-porphyrin conjugates (PPCs) to translocate both BBB and BPB to target viral infection in the brain of adults and/or foetuses during pregnancy. PPCs were ranked according to their toxicity, BBB and BPB translocation capacity, and antiviral activity (against DENV, ZIKV, SARS-CoV-2 and HIV) in vitro and the most promising PPCs were tested against ZIKV in vivo. Viral load and cytokine expression were assessed in the brain of ZIKV-infected adult mice treated with BBB-translocating PPCs. Furthermore, to evaluate the effect of PPCs on the developing brain of ZIKV-infected mice, neonatal mice were infected and treated with PPCs capable of crossing both the BPB and the BBB. PPCs impact on brain viral load and disease progression was monitored. Overall, our results show that PPCs treatment reduced viral load and cytokine expression in mice brain, mitigating disease progression and mortality. These results suggest that the conjugation of BBBpS to antiviral porphyrins is a promising strategy to fight virus infection in the CNS of adults and in developing brains.

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ST02 Cationic self-assembling lipopeptides as non-viral gene delivery vectors in mesenchymal stem cells

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Mesenchymal stem cells (MSCs) possess great potential in regenerative medicine and gene therapy due to their unique properties, including self-renewal and differentiation into various mesodermal lineages. Consequently, human MSCs (hMSCs) are increasingly important in cell-based therapies for treating cartilage and bone injuries. [1]

The search for new non-viral vectors for the delivery of genetic material in MSCs remains a challenge in biomedicine. The development of efficient transfection systems for this key cell population is often hindered by the toxicity associated with transfection agents and the limited transfection efficiency achieved with current methodologies. [2]

Cationic lipid-based systems are extensively studied non-viral gene delivery agents. Their capability to spontaneously interact with negatively charged nucleic acids, combined with their ease of synthesis, excellent biocompatibility and low immunogenicity, positions them as promising candidates for gene delivery applications. [3]

In this study, we evaluated the potential of a novel class of self-assembling lipopeptides as gene delivery vectors in MSCs. The corresponding lipopeptide assemblies demonstrated a high capacity for genetic material complexation and achieved efficient transfection outcomes, underscoring their potential in advancing non-viral gene delivery strategies. [4] [5]

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ST03 PepH3-decorated nanoparticles for delivery of therapeutics across the blood-brain barrier

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Alzheimer's disease (AD) is the second most common neurodegenerative disease leading to cognitive decline that accounts for ~70% of all cases of dementia. Immunotherapy, particularly monoclonal antibodies designed to bind Aβ peptides have become a promising strategy for AD, but ensuring efficacy and safety is challenging and crucial for these therapies. We aimed to develop an innovative tagged nanoparticle (NP) that crosses the blood-brain barrier (BBB) and acts as a brain shuttle for the encapsulated single domain antibody (sdAb) recognizing Aβ oligomers. PepH3, a cationic BBB peptide shuttle (BBBpS) derived from Dengue virus type-2 capsid protein, was used to decorate the surface of NPs. PepH3 enhanced the uptake of the NPs into brain endothelial cells, and transcytosis of sdAb, as a potential therapeutic molecule, across both rat and human BBB culture models. The mechanism of transcytosis is a temperature-dependent active process that was reduced by metabolic and endocytosis inhibitors. The cellular uptake of the cationic PepH3-tagged NPs decreased when the negative surface charge of brain endothelial cells became more positive after treatments with a cationic lipid or with neuraminidase by digesting the glycocalyx. The NPs co-localized mostly with ER and Golgi and not with lysosomes, indicating the cargo may avoid cellular degradation. Our results support that a combination of nanoparticles with a BBBpS such as PepH3 peptide can improve the delivery of antibody fragments across the BBB.

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ST04 De novo designed peptide masks enable ligand binding activation with different stimuli

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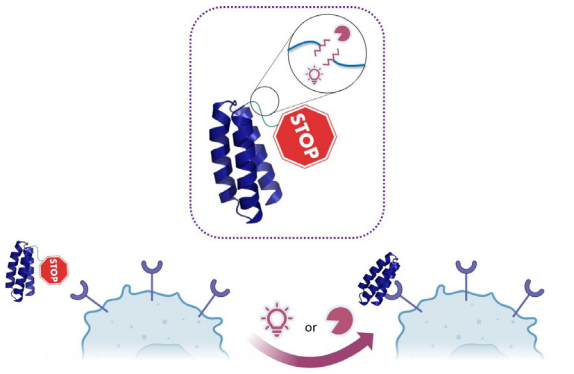
Protein therapeutics can be highly selective for their target. However, therapeutic targets are rarely expressed only in the diseased tissue. Conditional activation strategies aim to restrict the activity of protein therapeutics to the target site, thereby minimizing off-site effects. Most conditional activation strategies focus on antibodies since they are the most widely used class of biologics. However, de novo designed "minibinders" can be up to 20 times smaller than immunoglobulins, thus presenting advantages in terms of tissue diffusion, production, modification, and characterization.

Here we report a new class of conditionally-active minibinders with de novo designed minimal affinity masks. We generated protease- and light-labile versions of peptide masks. We confirmed that the masked minibinder displayed a binding decrease of roughly two orders of magnitude and near-complete recovery after the application of either light or proteases. Our results open new avenues for the design of targeted protein therapeutics with activity on demand.



ST05 Selective recognition of three-way DNA junctions with designed α-helical peptides

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Three-way junctions (3WJs) are symmetric assemblies formed by three intertwined duplex DNA segments. These 3WJs can appear because of the defective replication of repetitive DNA sequences called microsatellites [1]. Therefore 3WJ binding agents could found a possible application as selective drugs for targeting mismatch repair deficient phenotypes found in some cancer cells. A turning point in the development of therapeutic agents targeting non-canonical DNA structures, in particular 3WJs, was the discovery in 2006 by the group of Prof. M. Hannon that metal helicates bind to the branching point of 3WJs [2]. This was unexpected because researchers assumed that these helicates would interact with the major groove of DNA because their size is similar to that of the DNA-binding α-helices of natural transcription factors. So we asked ourselves, if helicates resemble α-helical peptides and display good binding properties, could then an α-helical peptide bind with good affinity to the central cavity of a 3WJ, provided that its size is similar to that of a metal helicate? Here, we describe the rational design of these peptide binders and explore the structural determinants of the binding. Moreover, we explore their biological properties, in vitro, against 3WJ's processing enzymes, and in vivo, against DNA repair deficient yeast strains.

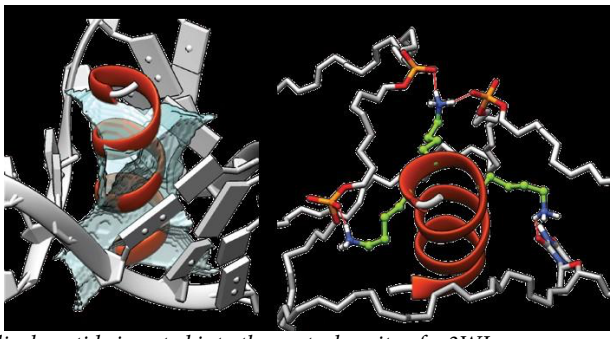

Figure 1. Model of a short α-helical peptide inserted into the central cavity of a 3WJ.

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ST06 Bioluminescence-based Singlet Oxygen Generation

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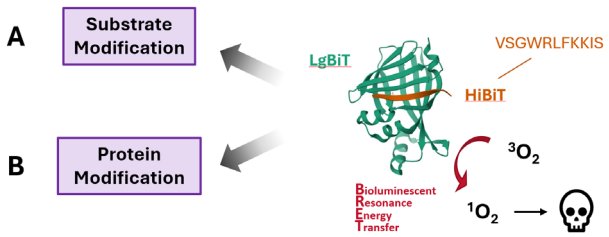
Bioluminescence – the emission of light by luciferase-like enzymes upon the oxidation of their substrates – has gained great interest as a tool for studying biological processes in the past years due to its high sensitivity and biocompatibility.[1] Along these lines, bioluminescence resonance energy transfer (BRET) has often utilized for the excitation of suitable acceptors.[2] This self-illuminating approach is independent of the light penetration in tissue, which provides advantages in biomedicine. Fluorophores such as fluorescein or BODIPY can be easily converted into photosensitizers upon halogenation, enabling the BRET-induced formation of singlet oxygen. Singlet oxygen is cytotoxic with a short lifetime of ~3-0.01 µs [3] and, therefore, a limited diffusion range (maximum of 134 nm in vivo [4]), minimizing dark toxicity. BRET-based singlet oxygen generation can either be utilized for the specific inactivation of biomolecules or even lead to cell death, which extends the scope of photodynamic therapy (PDT) owing to its independence from an external light source.[5]

Here, we report two novel self-illuminating systems based on a split version of the semisynthetic luciferase NanoLuc[6], consisting of the LgBiT protein and the 11 amino acid HiBiT peptide. In system A, the NanoLuc substrate coelenterazine (CTZ) was conjugated to a halogenated BODIPY moiety. Since BRET efficiency depends on donor-acceptor proximity and dipole orientation we envisioned BODIPY-CTZ conjugates with different linker lengths. We demonstrated that our best conjugates could be oxidized by the NanoLuc enzyme and, in turn, emitting light. Although this bioluminescence has a lower brightness than the unmodified substrate, the BRET could be successfully measured.[7] As the BRET-based generation of singlet oxygen could not be detected in cellulo, system B was designed. This approach deals with modifications over the NanoLuc, gaining brighter luminescence and, consequently, a cytotoxic effect in cancer cells.



NanoLuc-based generation of singlet oxygen via bioluminescence resonance energy transfer (BRET).

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ST07 Peptide-Coated Platinum Nanocages for Tumor Targeting

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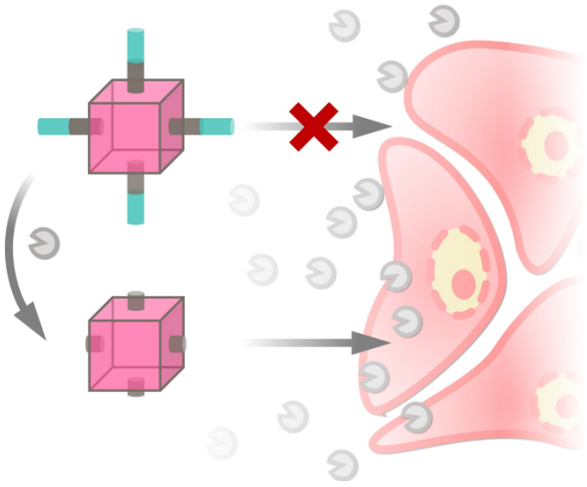
Despite the great advances in the use of nanostructured agents for therapeutics, their targeting to specific cells or tissues remains a challenge. Stimuli-responsive delivery systems can be designed for this purpose [1] and the particular properties of tumor microenvironment can be exploited to achieve tumor-targeted delivery [2]. In this work, we functionalized an organometallic platinum nanocage with a tumor microenvironment-responsive peptide (TMRP). Our objective is selective cell internalization in response to the presence of enzymes overexpressed and secreted by tumor cells. The TMRP was designed combining a sequence that reduces unspecific nanocage uptake with a cleavage site for certain tumor microenvironment enzymes. Then, the nanocages were coated with a stimuli-responsive peptide shell that would impede their internalization unless the coating is hydrolyzed by these enzymes in the presence of tumor cells. Pretreatment of the functionalized nanocages enzyme-rich media resulted in higher internalization rates, supporting that the TMRP is cleaved by the enzymes activating nanocages uptake. In addition, viability assays correlated higher enzyme levels in the pretreatment with higher cytotoxicity of the cages. In conclusion, the functionalization of a platinum nanocage with stimuli-responsive peptides allowed enhanced cellular internalization and toxicity upon exposure to tumor microenvironment enzymes in a promising approach for targeted delivery of antitumoral agents.



Peptide coating of platinum nanocages is cleaved by tumor enzymes, boosting uptake and cytotoxicity.

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ST08 Toward tumor-targeted biorthogonal catalysis with a HER2 affibody

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The promising potential of bioorthogonal catalysis in biomedicine is driving efforts to develop new strategies to regulate drug activity in living systems. To achieve this, it is essential not only to develop tailored inactive prodrugs and biocompatible metal catalysts, but also to implement strategies that allow drug production under spatial and/or temporal control.[1] In this context, we propose the development of palladium metallopeptides that can selectively release bespoke cytotoxic prodrugs via a bioorthogonal catalytic depropargylation reaction. As a proof-of-concept model, we will used modified affibodies to target breast tumors expressing the HER2 transmembrane tyrosine kinase receptor, which is overexpressed in 15-20% of all breast cancers and is associated with poor prognosis.[2] Affibodies are non-immunoglobulin, cysteine-free affinity proteins that have emerged as promising alternatives to traditional antibodies for biotechnological applications.[3] We have recently shown that grafting a pair of His residues in consecutive helical turns of a synthetic α-helical peptide creates a suitable coordination site for Pd(II), which can promote depropargylation reactions in living cells.[4] Building on this, we investigated the optimal positions for grafting pairs of coordinating histidine residues in the HER2 affibody (ZHER2:342). Preliminary catalytic assays have shown that the affibody with His residues at positions 40 and 44—ZHER2His2(40/44)[Pd(COD)]—preserves the overall HER2 fold and is able to promote the catalytic depropargylation of a fluorogenic probe. Current efforts are aimed at reproducing this reaction in cell culture and testing the depropargylation of other bioactive substrates to induce HER2-directed cytotoxicity.

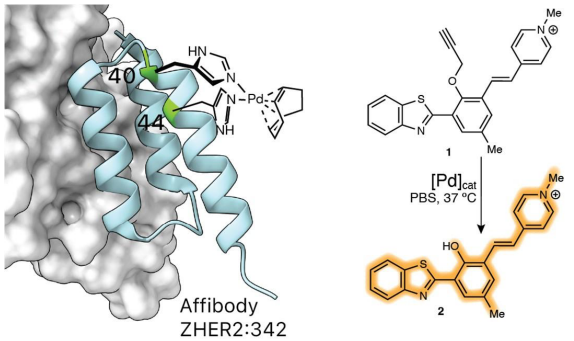

Figure 1. HER2 extracellular (grey) with affinity matured 3-helix affibody ZHER2:342 (light blue), modified with a Pd complex with His residues at positions 40 and 44. On the right, the fluorogenic reaction used to monitor the catalysis.

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ERDF). M.E.V is grateful to the Conselleria de Cultura, Educación e Universidades da Xunta de Galicia for their support through the GRC grant ED431C 2021/29. J. D. M. acknowledges the support of the Generalitat de Catalunya 2017SGR1323.

ST09 PSA-responsive self-assembling peptides for prostate cancer drug delivery

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Prostate cancer (PCa) remains a major global health concern, requiring innovative approaches to enhance therapeutic efficacy. Advanced drug delivery systems have emerged as a promising avenue to address the limitations of conventional chemotherapeutic drugs. Among these, self-assembling peptides have gained significant attention as versatile nanomaterials with potential application in drug delivery1. These peptides can be precisely designed to respond to stimuli present in the local microenvironment, such as aberrant enzyme activities, enabling the targeted delivery of anticancer agents. In the context of PCa, research on enzyme-responsive self-assembling peptides remains relatively underexplored. However, prostate specific antigen (PSA), a protease exclusively active in the prostate tumor microenvironment, holds significant promise. The enzymatic activity of PSA has been harnessed for drug delivery by conjugating anticancer agents with PSA-cleavable peptides, enabling targeted drug release2.

Here, we propose a self-assembling peptide nanobiomaterial incorporating a PSA-cleavable sequence. This innovative approach represents a compelling opportunity to enhance the therapeutic outcome of different anticancer agents in PCa by exploiting the prostate tumor-specific activity of PSA. For the design of the self-assembling peptide, a recently reported PSA-cleavable sequence (RSSYRSL)3 was used and coupled with a short β-sheet forming segment and an alkyl tail hydrophobic domain (Figure 1A), based on a typical structure of a peptide amphiphile (PA)1. The proposed PA was successfully synthesized and its cleavability by PSA is currently under evaluation, together with other molecularly engineered PAs, for obtaining PSA-sensitive delivery systems with tunable degradability and predictable drug release control (Figure 1B, C). This communication will present the results of these studies and the possibility to selectively deliver anticancer agents to prostate tumors.

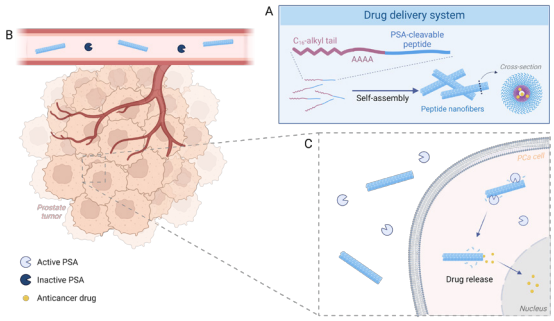

Figure 1: Representation of the self-assembling peptide drug delivery system proposed (A) and the respective prostate-specific PSA-induced drug release (B, C).

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ST10 ADVANCES IN CARDIOVASCULAR DECA-11 PEPTIDE ANALOGUES

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Ventricular arrhythmias -heart rhythm disturbances- can elicit ventricular fibrillation and sudden death (SCD), responsible for up to 50% of deaths in patients with heart failure (HF). Proarrythmogenic events involve a marked reduction in the expression of Nav1.5 and Kir2.1 channels, responsible of INa and IK1 currents, enhancing risk of arrythmia.1 Dapagliflozin and empagliflozin, two antidiabetic drugs, reduce morbidity and mortality in HF patients, and significantly increased INa and IK1 densities, as recorded in cardiomyocytes by the patch-clamp technique.2 Similarly, some of us discovered that the DNA-encoded peptide, DECA-11 (structure not disclosed yet), is able to increase the above mentioned currents.

To start a medicinal chemistry program based in DECA-11 it is critical to have a suitable synthetic method for analogue's preparation, to appropriately functionalize them to allow penetration through cadiomyocyte membrane, and to establish their preferred conformation and stability. During solid-phase preparation, using the Fmoc/tBu strategy, DECA-11 revealed as a "difficult sequence", therefore requiring extensive optimization of synthetic and purification methods. As for the permeability, different N-terminal functionalized DECA-11 derivatives have been explored. These derivatives contain an extra Lys residue, either at N- or C-terminal position with bear a fluorescent probe at the side-chain. Cell internalization was measured by confocal microscopy. Circular dichroism studies points to a feeble stabilization of an α–helical-type conformation in water that is clearly locked upon trifluoroethanol addition. Furthermore, some DECA11 modified peptides were synthesized to improve the mentioned helicity and their solubility indeed. The metabolic stability was studied in human serum, which certainly indicate high degrees of peptide remaining within 24 hours of incubation.

This communication will focus on the first stages of the medicinal chemistry program based on DECA-11 analogues, including synthetic and purification methods, membrane permeability, conformational CD studies and data about peptide stability in human serum. Promising preliminary activity on INa current will also be discussed.

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ST11 P-Sulfonatocalix[4]arene-based ratiometric array for sensing of cationic peptides in solution and different platforms

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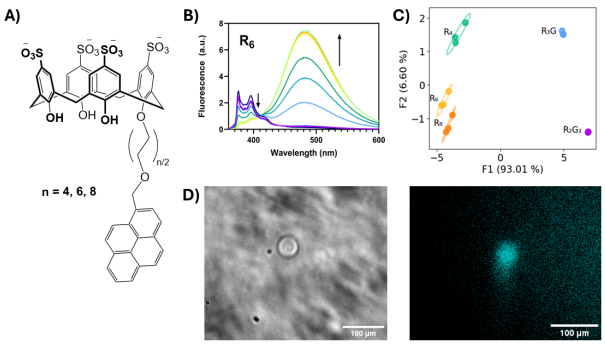
The study of peptides is of great interest, not only due to the variety of functions these molecules present in biological systems (e.g. signaling and neuromodulation) but also as tools in biotechnology and as pharmacological agents.1,2 Supramolecular receptors have had a rising application in the transport, modulation and sensing of these biomolecules, due to the strong interactions they can establish.3 Furthermore, the application and study of these sensing systems in membranes helps better understand and develop efficient sensing platforms for applications in biological environments, where compartmentalization with biological membranes is prevalent.4

Our work focused on the development of a family of supramolecular sensors for polycationic peptides (SCnPy), an amphiphilic system based on a p-sulfonatocalix[4]arene receptor, monosubstituted in its lower rim with a variable length aliphatic chain (n=4, 6, 8), with a pyrene fluorescent moiety at its end (Fig. 1A). Using oligoarginines as model analyte, this sensor was shown to shift from monomer to ground-state dimer emission upon addition of an analyte with sufficient cationic charges and present dual emission both in solution and when inserted into phospholipidic membranes. The emission of ground-state dimers is detectable in Egg Yolk Phosphatidylcholine Large Unilamellar Vesicles (EYPC-LUVs) and Giant Unilamellar Vesicles (EYPC-GUVs) by fluorescence spectroscopy and microscopy, with UV excitation (Fig. 1D).



A) Structure of SCnPy B) Titration of SC6Py with R6 C) PCA of SCnPy, 5 µM, and peptide, 10 µM D) Microscopy images of SC4Py-R6 in EYPC-GUVs

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ST12 Screening of peptide amphiphiles with autocatalytic self-replication

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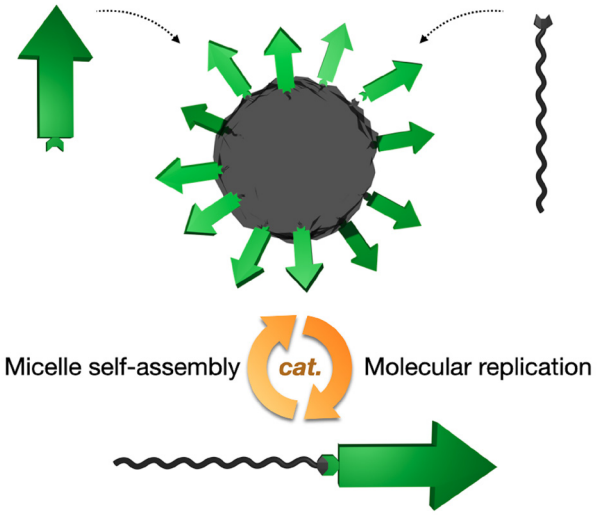
Molecular replication, a hallmark of life, enables the transmission of both structural and functional information across generations of living beings. Mimicking molecular replication synthetically with much simpler molecules than those evolved by Nature is a key step towards creating artificial "living" matter[1]. In this study, we describe the structural simplification of self-assembling peptide amphiphiles with autocatalytic self-replicating behaviour, which mimics the perpetuation mechanisms of living matter. This system uses two reactive peptide amphiphile precursors to generate in situ the self-assembling product, forming autocatalytic micelles that accelerate this reaction to produce molecular copies. A collection of precursors with varying sizes was screened combinatorially, revealing a minimal tripeptide amphiphile required to trigger autocatalytic self-replication. These findings will contribute to the advance of supramolecular systems with cooperative life-like behaviour.



Two reactive precursors generate in situ autocatalytic micelles that accelerate this reaction to produce molecular copies if itself.

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ST13 A PEPTIDE-BASED RECEPTOR FOR POLYOXOMETALATES

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Polyoxometalates (POMs) are a family of inorganic oxide clusters composed of transition metal ions (Mo, V, W, etc.) in their highest oxidation states. These compounds have potential applications in chemical biology as antiviral,[1] antibacterial [2] or anticancer drugs.[3] In this context, the covalent or non-covalent association of POMs with biomolecules, such as peptides, is a key factor for the successful development of these systems in this research field.[4].

Here, we describe the design and synthesis of a peptide platform which can bind with high affinity to some POMs under physiological conditions through non-covalent interactions. The versatility of the SPPS methodology has allowed us to functionalize the peptide receptor in order to improve its biological properties. Moreover, several studies were carried out to determine the cancer cell selectivity and citotoxicity of these novel POM-peptide hybrids.

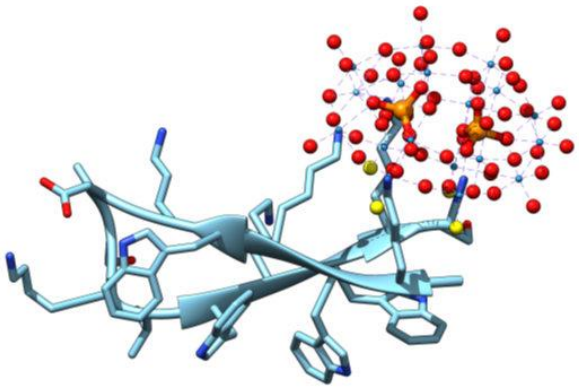

Figure 1. Tentative model of a peptide – POM hybrid made with Chimera software.

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ST14 Self-assembling lipopeptides as phospholipid mimics: Construction of functional synthetic cells

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An essential characteristic of living cells is their ability to self-organize in a spatiotemporal manner, a property that determines the functionality of cellular compartments. Phospholipids, as the primary building blocks of these membrane-bound compartments, have extensively captivated the attention of scientists. Despite their key role in relevant biological processes, generating natural phospholipids still remains challenging [1]. Therefore, there has been an enormous interest in the straightforward fabrication of biomimetics that emulate phospholipid properties while expanding their functionality [2].

In this study, we describe the synthesis, characterization, and applications of a new class of functional lipopeptides that mimic the structural attributes of natural phospholipids. These lipopeptides possess the ability to self-assemble into well-defined compartments, emulating natural cells. The corresponding artificial cells effectively encapsulate relevant biomolecules (i.e. proteins, DNA, RNA), delivering them to predetermined locations [3, 4].

Our findings highlight the potential of synthetic lipopeptides to emulate the structural complexity of natural phospholipids, while offering a versatile platform for functionalization [5]. This work contributes to the broader field of synthetic cells and the development of innovative materials for biological and therapeutic applications.

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ST15 Self-assembling two-dimensional peptide nanostructures for energy storage

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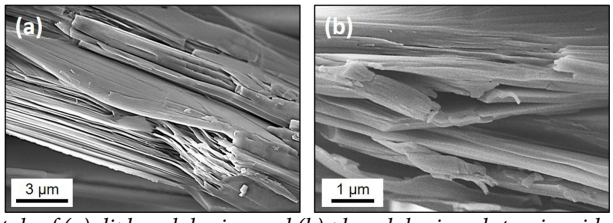
Supramolecular peptide-based materials are promising building blocks and functional elements for a wide range of bioelectronic devices, such as biosensors, energy harvesters, and energy storage devices [1,2]. These materials are inherently eco-friendly and offer a remarkable chemical diversity and ability to self-assemble into hierarchical supramolecular nanostructures with unique physical properties. Though two-dimensional (2D) peptide crystals and thin films have the greatest practical importance [3,4], they still remain the least studied structures. In this work, we used antisolvent crystallization to obtain crystals based on several dipeptides: diphenylalanine (FF), dileucine (LL), phenylalanine-glutamic acid (FE), and tryptophan-glycine (WG), and studied their structure, morphology, and physical properties. These peptides self-assemble into the crystals consisting of 2D layers holding together by weak non-covalent bonds. Then, we adopted spin-coating technique to create large-area (c.a. 500 mm2) thin films made of these dipeptides. The films demonstrate high degree of the crystallinity, thickness below 400 nm, and the piezoelectric coefficient d33 c.a. 18 pm/V comparable to that of other organic materials.



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ST16 Synthesis of 3-hydroxy-4-aminocyclohexanecarboxylic acid for the preparation of self-assembled cyclic peptide dimers with functionalized internal cavity

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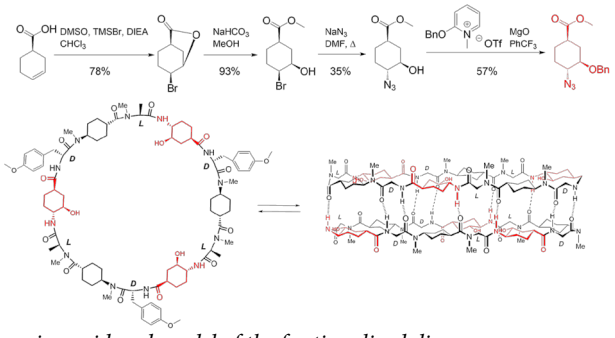
Cyclic peptides (CPs) formed by α-amino acids of alternating chirality form nanotubes because adopting a planar conformation in which the amide groups lay perpendicular to the disc-shaped structure. This feature predisposes to CPs to stack by forming hydrogen bonds through these groups to generate tubular structures known as nanotubes.[1] On the other hand, the selective alkylation of certain amides of the peptide backbone restricts the capability for hydrogen bond formation to only one of its faces and, consequently, these peptides can only give rise to dimeric structures.[2]

Currently, nanotubes and dimers have also been synthesized using CPs in which β, γ, δ or ε and α amino acids are combined, giving them new properties. Some of the dimers formed by different types of amino acids have already shown interesting properties as ion transporters, photo- and electroactive species or for the encapsulation of molecules.[3]

In this work, we present our preliminary work towards the synthesis of a new δ-functionalized amino acid and a dimer forming CP that contain α and δ-amino acids with a large internal cavity. The used of the new amino acid will provide hydroxyl groups in the internal cavity of the resulting assembly, providing it with unique properties that will be used in the recognition of complex biomolecules such as oligosaccharides.



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ST17 The role of lysophosphatidic acid in cancer immune evasion mediated by antimicrobial peptides.

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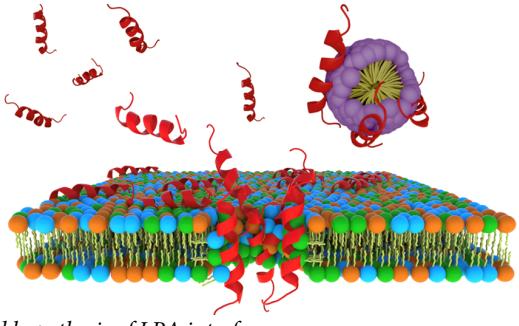
One of the earliest hallmarks of cancer transformation is the alteration of the lipid composition of the cell membrane. These changes, including the externalization of phosphatidylserine (PS), create a unique lipid pattern that is typically recognized by the innate immune system.[1] Antimicrobial peptides (AMPs), key components of innate immunity, can selectively bind to these altered membranes, disrupting them and leading to cancer cell death.[2]

However, cancer cells can evade this innate immune response, avoiding membrane disruption by AMPs. [3] This work explores the hypothesis that lysophosphatidic acid (LPA), a lipid released during tissue injury and a biomarker for various cancer types, plays a crucial role in this evasion. LPA, unique for its dual deprotonation capability at physiological pH, is capable of forming micelles and interacting with AMPs, potentially interfering with their ability to recognize and disrupt cancer cell membranes.[4]

To test this hypothesis, we combined experimental and computational approaches. Fluorescence spectroscopy, circular dichroism (CD), dynamic light scattering (DLS), and molecular dynamics simulations were employed to investigate the action of two AMPs, melittin and LL-37, against model membranes mimicking healthy (zwitterionic POPC) and cancerous (anionic POPC:POPS 3:1) cells, in the presence and absence of LPA. This integrated approach aims to provide a deeper understanding of how LPA modulates AMP-membrane interactions, shedding light on its role in cancer progression and immune evasion.



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ST18 Non-Aromatic Fluorescence in Zwitterionic Single α-Helical Peptides

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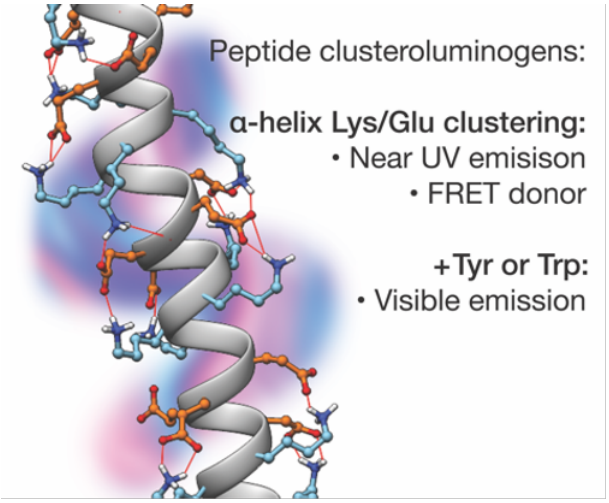
Besides GFPs and proteins incorporating extrinsic chromophores, it is generally accepted that the UV/vis absorption and fluorescence of proteins originates from the presence of aromatic residues in their sequence.[1,2] However, in addition to the conventional fluorescence from intrinsic chromophores, researchers have reported fluorescence from peptides lacking aromatic residues or prosthetic groups, such as concentrated solutions of poly-lysine, or amyloid assemblies [3,4]. Despite the fundamental interest and potential applications of this anomalous luminescence, our understanding of this phenomenon is still imperfect, and no model system or general design rules to obtain non-aromatic peptide luminogens are available.

Here, we show that short peptides derived from zwitterionic single α-helices (SAHs), formed exclusively by non-aromatic lysine and glutamic acid residues are UV-active and luminescent at near-UV wavelengths in solution. We demonstrate that their emission depends on the α-helical folding of the SAHs, which promotes the intramolecular aggregation of the Lys/Glu side chains, leading to non-aromatic fluorescence (NAF). We also show that the introduction of Trp or Tyr residues within the helical peptide framework produces long-wavelength luminescence, red-shifted from the characteristic emission of the aromatic residues in the sequence.



α-helical zwitterionic peptides display in solution non-aromatic emission in the near UV. Introduction of aromatic residues in the sequence leads to new long-wavelength clusteroluminescent emission bands in the visible region of the spectrum.

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ST19 Peptide ionogels for artificial olfaction

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Ionogels bear tunability, non-volatility, high ionic conductivity and good thermal, chemical and electrochemical stability [1,2]. The molecular self-assembly mechanisms of biomolecules in ionic liquids are complex and still poorly understood, as ionic liquids themselves present self-assembling properties. The versatility of the ionic liquids can be tailor-made in relation to the properties of gelator molecules, leading to rationally designed structured materials [3]. In this work, we designed tripeptide ionogels, previously shown to self-assemble in aqueous solvent [4]. Inspired by the peptide's self-assembly propensity in aqueous environments, we designed an ionic liquid solvent that could facilitate the formation of ionogel. The tripeptide ionogels presented unique properties not shown in the corresponding hydrogels, namely high air-stability and ionic conductivity, which were explored as gas sensitive layers in electronic noses. We show the potential of tripeptide ionogels to act as humidity sensors and to discriminate at the single carbon alcohol level and between twelve volatile organic compounds under environmental conditions. This new class of stable conductive soft biomaterials unlocks a wide range of applications within biological, medical and industrial fields.

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ST20 Exo-chirality of the α-helix in helical peptide assemblies

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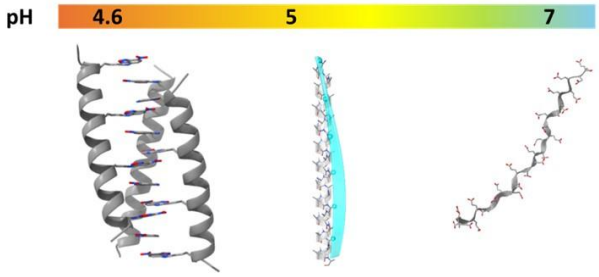
The spatial distribution of chemical functional groups has been exploited using artificial helical polymers in order to promote specific properties. In nature, helical macromolecules like proteins and polynucleotides encode chemical information in helical environments (DNA, peptide helices) that is crucial for their ubiquitous biological functions. Proteins are potential scaffolds that can be used to develop new biomaterials and therapeutics due to their stability and biocompatibility. The structural parameters of peptides α-helix (3.6/13) secondary structure are well described, so the three-dimensional disposition of the lateral chains of amino acids in the helical surface can be predicted. Using periodic repetition patterns, residues will be inserted in a concrete chiral disposition (exo-chiral topologies), with potential undescribed properties in peptides functions.

In this work we aim to elucidate the chiral topologies that arise from the different repetition patterns in α-helical peptide sequences. To achieve our goal, a high helical peptide and a good characterization technique are required. A pH responsive oligoglutamic acid model is proposed, which contains a high helical content at acidic pH (1). To elucidate the exo-chiralities, non-canonical chromophore nitrobenzofurazan (NBD) is attached to the different repetition patterns of the peptide sequence. This sophisticated design allows the elucidation of the folding dynamics monitored through spectroscopic techniques (2). Circular dichroism experiments confirmed the exciton couplings of the different exo-helical topologies, matching the different chiral parameters (handedness, pitch) studied through Molecular Dynamic calculations. Additionally, the assembling properties of each topologic repetition were evaluated in aqueous solution, relating the specific exo-chiralities to their tendency to form non canonical peptide assemblies. The results proved that non-canonical repetitions were able to induce assembly, remaining as the first example of chiral repeats driving lateral interactions in protein self-assembly.



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Xunta de Galicia Predoctoral Fellowship ED481A 2021/136. Ministerio de ciencia, innovación y universidades.

ST21 Investigating how protecting groups affect physicochemical properties and purification of peptides

[1] **Jianing Li**, [2] Vivek Verma, [1] Nicholas M Harrison, [1] Jerry Y Y Heng

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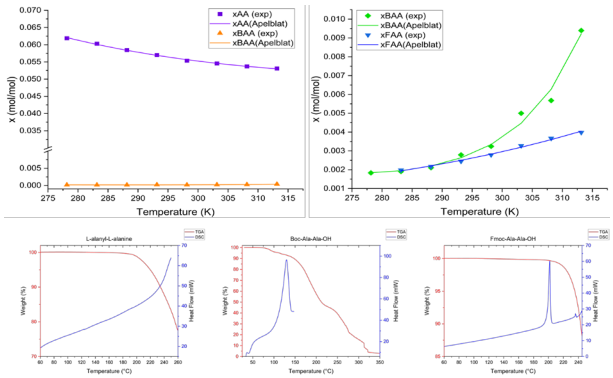
Nowadays, over 85% of therapeutic peptides are chemically synthesised via SPPS and LPPS (1) which involves an excess amount of protected amino acids to enable correct elongation. Consequently, the removal of amino acid residues becomes a demanding section of the downstream purification process accounting for more than 80% of the production cost (2). Peptide crystallisation, as a sustainable alternative to chromatographic purification (3), can circumvent the drawbacks of chromatography: low throughput, energy-intensive, and substantial toxic organic solvent consumption. An accurate solubility profile is a prerequisite of peptide crystallisation, as it serves the purpose of designing crystallisation under reasonable supersaturation.

To meticulously examine how protecting groups affect physicochemical properties and the crystallisation of peptides, N-carbamate-protected alanine homopeptides were selected as a model compound to be studied. The solubility of unprotected, -Boc, and -Fmoc L-alanyl-L-alanine (AA) in water, ethyl acetate, and acetone from 278.15K to 313.15K were measured using gravimetric and spectroscopic methods. At 313.15K, the hydrophobicity of Boc reduces the solubility of Boc-AA in water to 0.7% of unprotected AA (Fig. (a)). Compared with Boc-AA, the aromatic rings in Fmoc further inhibit its solubility at higher temperatures (Fig. (b)). Powder X-ray diffraction (PXRD) was conducted to confirm if the morphology changes before and after the solubility experiments. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) have been carried out to reveal the effect of protecting groups on the melting temperature, decomposition temperature and fusion energy (Fig. (c), (d), (e)). Compared to unprotected peptides, the stochastic nature of nucleation and the low solubilities make the determination of metastable zone width (MSZW) of protected peptides extra challenging and further experiments are ongoing.

In summary, protecting groups greatly impact the physiochemical properties of peptides. The fundamental study of protected peptides is essential to offer insights into the design of downstream purification strategies.



Experimental/correlated solubility and thermal analysis of 3 dipeptides in water and ethyl acetate

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ST22 DNA 3-way junction binding with Metal-stabilized collagen triple helices

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Collagen is the most abundant protein in the human body, providing structural stability to tissues. Types I, II, III,

V, and XI are the most common and form a triple helix structure [1]. Each chain in this helix has a repetitive

sequence (XYG)n, where X and Y can be Pro-Pro or Pro-Hyp, with Gly every third residue to form a compact

structure. Polymeric Collagen Mimetic Peptides (CMPs) have been extensively used as models of natural collagen

and as the basis of biomaterials [2]. Researchers have shown that model collagen peptides featuring terminal

metal-chelating bipyridines self- assemble into stable right-handed superhelix of polyproline II-type fibers in the

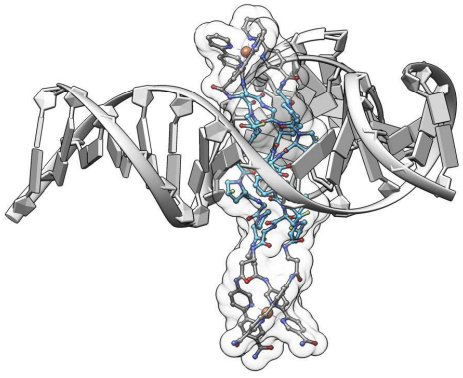
presence of appropriate metal ions [3]. The diameter of the collagen fiber is 15 Å, which could potentially fit inside

the central cavity of a three-way (3WJ). Based on these precedents, we describe a novel discrete metallo-CMP

structure where metal coordination to both C- and N-terminal ends enhance the stability of a short triple helical

collagen assembly. Moreover, we demontrate that the resulting discrete metallo-CMP displays selective binding

to DNA 3WJ [4].



Model of the complex between a DNA 3WJ and the metal-stabilized CMP.

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2021/29, and by PID2021-127702NB-I00 and TED2021-131641B-C42, funded by MCIN/AEI/10.13039/501100011033 and by ERDF A way of making Europe. A.S.F. thanks the Ministry of Education for his FPU fellowship FPU20/00944

ST23 A structure-based approach towards a new therapy for Parkinson's Disease

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide, and it remains incurable despite the extensive research. PD is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta, leading to the manifestation of movement-related symptoms such as rigidity, tremor, or bradykinesia. The major pathological hallmark of PD is the accumulation of protein inclusions in the brain, named Lewy bodies and Lewy neurites, primarily composed of aggregated α-Synuclein (αSyn). Of all the species formed during the amyloid aggregation process, αSyn oligomeric species are currently considered the main culprits for the collapse of neuronal homeostasis and disease propagation throughout the brain.

Toxic αSyn oligomers present hydrophobic and anionic surfaces, distinguishing them from αSyn monomers. We have exploited these differential structural properties to identify a family of peptides that bind to these αSyn species with low nanomolar affinity, without interfering with the monomeric functional protein (1,2). This binding is translated into an anti-aggregation activity and the ability to abrogate oligomer-induced cell damage (1,2). With a structure-function relationship in hand, we identified a human neuropeptide (NP) that showed a remarkable anti-aggregation activity in vitro. Importantly, we have tested the effect of NP in a PD mouse model overexpressing human αSyn in dopaminergic neurons using a non-invasive route of administration. Behavioural assessment showed a re-establishment of motor coordination and significant improvements in motor skills and balance after treatment with NP. Altogether, these results suggest that NP might open the way to a promising strategy for the development of a disease-modifying therapy for PD.

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This work was funded by the Spanish Ministry of Science and Innovation (PID2022-137963OB-I00) and by the Department of Research and Universities of the Government of Catalonia (LLAV00027 and 2024PROD00124).

ST24 Investigating Amino acid Enrichments and Patterns in Phase-Separating Proteins: Understanding Biases in Liquid-Liquid Phase Separation

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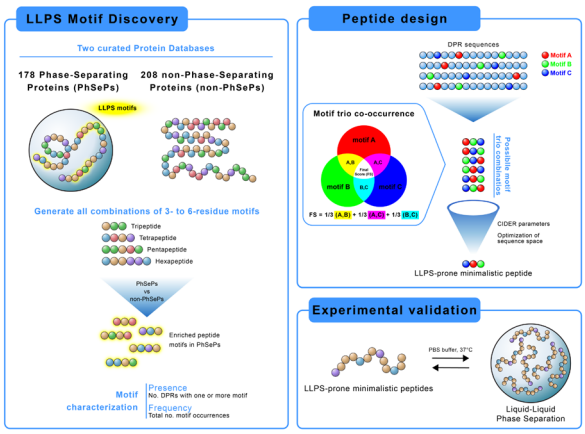
1 ITQB NOVA

Liquid-Liquid Phase Separation (LLPS) forms membraneless organelles, enhancing biochemical processes. The stickers-and-spacers model explains LLPS but is mainly validated in Prion-like RNA Binding Proteins. We explore peptide motifs in LLPS in broader protein contexts. We developed a computational approach for motif discovery, implemented in 178 Phase Separating Proteins (PhSePs), complemented by the FuzDrop and CIDER servers, which identified droplet-promoting regions (DPRs) and examined disorder-related characteristics. Our database of PhSePs was analyzed against proteins with low propensity for LLPS. This comparative analysis revealed 129 enriched peptide motifs with folds higher than 0.2, consisting of 3 to 6 residues, with tetrapeptides being the most prevalent. Key features of the enriched motifs included Gly-rich sequences punctuated with aromatic, charged, and polar residues, as well as homopeptide repeats (e.g., GGDR, SRGG, YGGG, QQQQ, PPPP). Analysis of motif presence, frequency, and co-occurrence revealed widely distributed motifs across different DPRs, identified motifs with significant repetitive patterns, and highlighted motif trios that are more likely to co-occur within a sequence. By harvesting this analysis, we developed a data-driven approach for minimalistic peptide design with LLPS propensity, further using the CIDER server for peptide characterization and peptide design refinement. We designed 8 peptides with various motif combinations and amino acid distributions, which were experimentally validated to undergo LLPS, exhibiting liquid-like behavior with diverse molecular mobility patterns and droplet dynamics. Our approach bridges a non-biased computational approach with experimental validation, offering insights into sequence determinants of phase separation, with the potential for designing minimalistic synthetic condensates with tailored properties.



Graphical abstract showcasing our computational approach for LLPS motif discovery and peptide design

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ST25 Modulating protein conformation and entanglement in compositionally designed deep eutectic solvents

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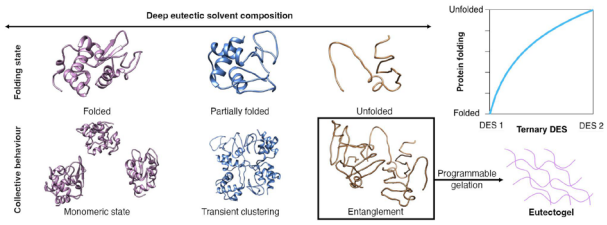
The emergence of neoteric solvents has opened new realms in science and technology. By exploiting their "design" character, by which the physicochemical properties can be tailored (e.g., polarity and charge density), supramolecular interactions and macroscopic responses can be now controlled through solvent effects. In recent years, deep eutectic solvents (DESs) have breakthrough as task-specific “cocktails” for biomolecules in enzymology, synthesis of biomaterials, and drug delivery [1]. DESs are anhydrous solvents obtained through the combination of simple organic compounds (e.g., choline chloride and urea at a 1:2 mole ratio), where a depression in the melting point allows the mixture to remain liquid at room temperature.

Recently, we have shown the ability of DESs to control solvophobic sequestration and protein folding [2]. These findings open the possibility of developing new protein-based materials, where the behaviour of biomolecules can be tailored through changes in the DES composition. Herein, we will present the fundamentals aspects that control protein conformation and collective behaviour in these solvents. By tailoring solvent properties, we demonstrate the ability of DESs to modulate the conformational landscape of proteins. This results in a range of stable folding intermediates between a globular fold and an unfolded polypeptide chain (see Fig.). Notably, each conformational intermediate can prompt different responses in the system, from the stabilisation of folded proteins to the formation of supramolecular protein-based gels [3]. Overall, we demonstrate the vast array of possibilities for protein behaviour in compositionally designed DESs, where these alternative solvents could open the avenue for the next generation of molecular materials.



Schematic representation of the modulation of protein behaviour through the compositional design of DESs.

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ST26 Novel peptaibol derivatives as promising candidates to target the plant quarantine pathogen Xylella fastidiosa

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Xylella fastidiosa is a Gram-negative phytopathogen that poses a major threat to agriculture worldwide due to the significant economic losses it causes to important crops such as grapevines, olive and almond trees.1 Antimicrobial peptides (AMPs) are considered to be suitable for plant disease control because of their high bactericidal and/or antibiofilm efficacy, low toxicity and high biodegradability.2,3 As a result, they meet the requirements of recently established legislation regarding phytosanitary products, providing a more environmentally friendly substitute for pesticides.

A specific class of AMPs are peptaibols which are characterized by the presence of a C-terminal aminoalcohol and α-aminoisobutyric acid (Aib) residues. They have attracted great interest due to their potential in plant disease management. For instance, trichogin GA IV is a peptaibol that has been described to display activity against plant pathogens, including Botrytis cinerea and Fusarium oxysporum.4

In the present study, we designed a collection of 26 analogs derived from trichogin GA IV and a set of 23 new peptaibols based on AMPs with activity against X. fastidiosa. The structural modifications introduced were: (i) acylation of the N-terminus with an acetyl, butanoyl or octanoyl group; (ii) substitution of the C-terminal amino alcohol with an amino acid amide; (iii) incorporation of one to three Aib residuies; and (iv) incorporation of D-amino acids.5 These peptaibols were synthesized on solid phase which required the optimization of the Aib coupling as well as of the cleavage conditions. All the sequences were screened for their antibacterial and antibiofilm activity against X. fastidiosa, and for their hemolysis and phytotoxicity.

This communication will focus on the synthetic strategy followed as well as on the discussion of the biological activity results obtained. Our primary objective is the identification of promising candidates to contribute to the ongoing search for novel drugs to control X. fastidiosa.

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ST27 Exploiting prion-like amyloid assemblies to produce functional nanomaterials

[1] **Jordi Pujols**

1 -

Although traditionally associated with pathological conditions, protein self-assembly into amyloid fibrils is also linked to essential biological functions. Functional amyloids have been described in organisms ranging from prokaryotes to eukaryotes, where they support crucial cellular processes. For instance, the curli system facilitates bacterial biofilm formation (1), while the Orb2 system in Drosophila plays a vital role in memory formation (2). Similarly, the yeast prion protein Sup35 undergoes a reversible self-assembly process to regulate genetic inheritance (3). Organisms exploit the controlled and reversible self-assembly capacity, and the inherent amyloid mechanical stability to display specific functions. By leveraging these properties, amyloid-based structures have recently been proposed as building blocks for the development of protein-based biotechnological and biomedical applications.

In this project, we harness the molecular grammar governing amyloid formation to develop novel nanomaterials. By modulating their assembly kinetics and structural organization, we demonstrate how these materials can be tailored for a variety of biotechnological and biomedical purposes. Specifically, we employ an engineered version of the prion-like domain of Sup35 (4, 5), and a series of short minimalistic amyloidogenic peptides (6, 7). These self-assembly templates are fused to functional proteins to create amyloid-based materials with practical functions. Examples include fibrils with enzyamatic activity (PMID: (8), agents that promote cell-to-cell interactions (9), and neutralizers of SARS-CoV-2 viral particles (10, 11). Altogether, this series of studies underscore the versatility of amyloid assemblies as a new frontier in protein-based nanomaterials, bridging the gap between protein self-assembly and functional material design.

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ST28 Use of Computational modeling and Continuous-Flow Solid-Phase Peptide Synthesis for the design and synthesis of peptide ligands targeting HLA and Hsp90

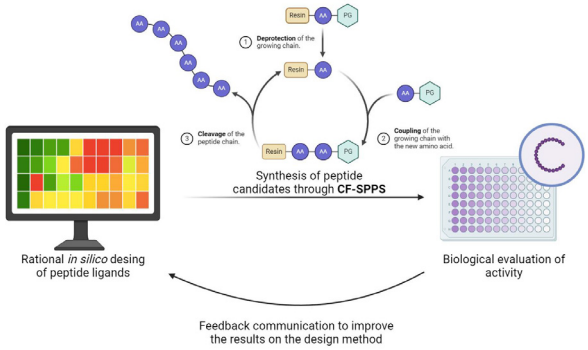
[1] **Ezequiel Silva-Nigenda**, [1] John F. Trant, [1] Sarfraz Ahmad, [1] Usman M. Mirza, [1] Olena Tykhoniuk

1 University of Windsor

Most drug candidate molecules being developed focus on their ability to bind to a protein thus altering its function. Small organic molecules achieve this by binding to small cavities in desired targets, inhibiting catalytic centers or blocking the binding of the natural substrate in such pockets. However, these small molecules fail to inhibit protein-protein interactions, which have garnered significant attention in the pharmaceutical industry in recent years. Peptides possess attractive features, including high structural compatibility with the targeted proteins and their ability to disrupt the protein-protein interfaces. Efficient in silico design of high-affinity peptide ligands is an ever-growing field that still demands the synthesis of such ligands to confirm their desired activity.1

Batch-mode solid-phase peptide synthesis has been the standard for drug discovery; however, synthesizing a library of candidates can be time- and resource-consuming, as well as considered less “green” due to the significant amount of waste produced. Strategies such as the search for new environmentally friendly solvents or processes that reduce the use of materials during the synthesis and purification of potential candidates allow easier activity screening, complimenting the computational design.2

In this work, we present our efforts for the rational design of two libraries of peptides targeting HLA-DR (autoimmune diseases) and Hsp90 (cell cycle control), as well as the use of CF-SPPS for synthesizing potential candidates to evaluate both the design model and the synthetic feasibility of the peptide ligands.



Workflow of computational modeling, synthesis and evaluation of peptide ligands

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ST29 Interaction of SARS-CoV-2 Fusion Peptides with Lipid Monolayers by Molecular Dynamics Simulations

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Membrane fusion between the viral envelope and host cell is a key stage during the infectivity of SARS-CoV-2 that enables the release of the viral genetic material into the target cell. Within the region of the Spike protein implicated in this process, several peptides have been identified to interact with the cell membrane. We aim to understand the viral infection role of these fusion peptides at a molecular level by studying their interaction with biomimetic model membranes via Molecular Dynamics (MD) simulations. We first use all-atom (AA) MD simulations to obtain a stable conformation for each peptide in physiological conditions. The resulting structures are then mapped into a coarse-grained (CG) model in order to increase the length and timescales for the studied systems. The CG model we employ, Martini 2, developed to simulate the behaviour of a lipid membranes and proteins, has been proven to be transferable to a wide range of biological systems. We explore the effect that peptide concentration has on the interaction with biomimetic lipid monolayers under varying surface pressure as well as how lipid composition affects their binding to the membrane.

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2. Spanish State Research Agency (PID2022-136392NA-I00)

ST30 Targeting the Weak Spot of Hemagglutinin: Toward Broad-Spectrum Influenza Therapeutics

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Mutations in the Influenza virus represent a significant challenge in the fight against this pathogen. The continuous emergence of viral resistant strains compromises the efficacy of existing antiviral therapies, resulting in scarce reliable and effective antivirals. This fact leads to substantial morbidity and mortality rates, alongside considerable economic burdens from increased hospitalizations and healthcare costs. Therefore, there is an urgent need for innovative strategies aimed at developing broad-spectrum anti-Influenza agents.

One promising approach involves the design of drugs that directly interact with highly conserved regions across various viral strains. In line with this concept, our research group has identified a novel class of N-benzyl-4,4-disubstituted-piperidines that specifically inhibit H1N1 influenza A virus fusion process by interacting directly with the fusion peptide of hemagglutinin (HA). These fusion inhibitors are so far, the only molecules that interact with the fusion peptide. Mechanistic and computational studies utilizing the peptidomimetic prototype DICAM180 demonstrated that its inhibitory activity is mediated by binding to a previously unexplored pocket in the HA2 subunit. This interaction involves a direct π-stacking with the Phe9 residue of the HA2 fusion peptide.

This binding mode has served as a starting point for the design of new derivatives. Taking advantage of the trimeric structure of HA, we have conducted structure-activity relationship (SAR) studies, supported by molecular dynamics simulations with the objective to simultaneously target the second fusion peptide. This approach is intended to enhance antiviral activity and broadening the anti-influenza spectrum.

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ST31 Development of Non-Natural MUC1 Glycopeptide Cancer Vaccines

[1] **C. Breton**, [1] P. Oroz, [2] Y. Méndez, [2] A. V. Vasco, [1] A. Avenoza, [1] M. M. Zurbano, [1] J. H. Busto, [1] F. Corzana, [1] J. M. Peregrina, [2] G. J. L. Bernardes

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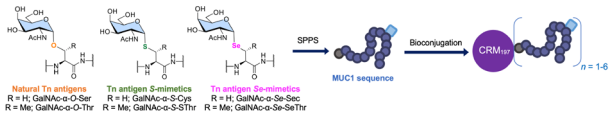
One of the most recent approaches to treating cancer is immunotherapy, which relies on the ability of the immune system to recognize and effectively destroy malignant cells. The development of immunotherapy requires the study of new biomarkers, as in the case of the glycoprotein MUC1 mucin. In cancer cells, unlike in healthy cells, alterations occur in its glycosylation, exposing different antigens that can trigger an immune response, such as the Tn antigen (GalNAc-α-O-Ser/Thr) [1]. Thus, the Tn antigen has been incorporated into peptides and used to generate therapeutic vaccines against cancer. However, the therapeutic use of O-glycopeptides is sometimes limited since they are easily hydrolyzed in biological systems. To overcome this problem, a plethora of structural mimetics of the Tn antigen has been used, including those that involve changes in the O-glycosidic bond [2].

In this work, the oxygen atom of this bond has been replaced by a sulfur or selenium atom. These Tn antigen mimetics have been incorporated into the MUC1 tandem repeat peptide sequence using the SPPS (Solid-Phase Peptide Synthesis) methodology. With the aim of designing a new generation of chemically defined therapeutic cancer vaccines, the glycopeptides with the highest affinity to the 5E5 anti-MUC1 antibody have been selected to be bioconjugated into the CRM197 carrier protein. The immune response of these anti-cancer vaccines will be studied in vivo in future work.



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ST32 Coarse–Grained Molecular Dynamics simulations of short helical peptides. Towards a connection with antimicrobial activity

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Antimicrobial peptides are a promising candidate for the development of new, more efficient therapies. The discovery of new sequences with therapeutic power are however limited by the high cost of the trial–and–error approach commonly followed. In this regard, computational methods may be an effective way to filter the vast space of candidates into a smaller set of sequences with promising antimicrobial activity, but the availability of a large and reliable dataset of antimicrobial peptides is a bottleneck towards the effective implementation of these approaches.

To overcome this limitation, data can be generated through simulation, namely Molecular Dynamics (MD) simulations. In this study, we explored the potential of coarse–grained (CD) MD simulations to analyze peptide-lipid bilayer interactions across different membrane compositions. While these simulations inherently lack the resolution to fully capture the complexity of such interactions, our analysis revealed distinct behavioral patterns associated with active antimicrobial peptides compared to non-active sequences. Moreover, we were able to develop a predictive model using features derived solely from these simulations which successfully classifies peptide sequences based on their antimicrobial activity with a reasonable degree of accuracy. These findings highlight the potential of CG MD simulations as a cost-effective and scalable tool for prioritizing sequences in the search for novel antimicrobial peptides.

This work was supported by the Spanish Agencia Estatal de Investigación (AEI) and the ERDF (PID2019-111327GB-I00, PDC2022-133402-I00, PID2022-141534OB-I00, CNS2023-144353), by the Xunta de Galicia (ED431C 2021/21, Centro de investigación do Sistema universitario de Galicia accreditation 2023-2027, ED431G 2023/03) and the European Union (European Regional Development Fund – ERDF). F. S.-L. thanks the Xunta de Galicia and the Axencia Galega de Innovación for funding of his predoctoral contract (02_IND606D_2022_2667887). All simulations were conducted at the Centro de Supercomputación de Galicia (CESGA).

ST33 Cyclic iodonium salts as self-activated tags for primary aliphatic amines

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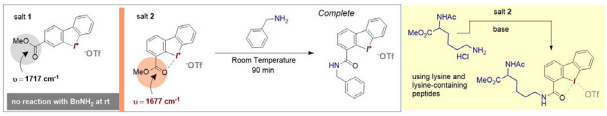
Diaryliodonium salts are hypervalent organohalogen derivatives distinguished by the presence of an iodine(III) center flanked by two aromatic moieties, and a highly polarized bond to a third electronegative substituent. They are generally air- and moisture-stable, and are widely employed as electrophilic arylation agents.[1] In particular, cyclic diaryliodonium salts are used in a wide variety of transformations always involving the breakage of the iodine(III)-carbon bond. This usage thus includes ring-opening and ring expansion processes,[2] as well bond-forming reactions via intervening aryne formation.[3] Beyond their use in synthesis, cyclic iodonium salts are drawing attention in the fields of crystal engineering, molecular design,[4] and in biochemical contexts, for example as go-to inhibitors of NADPH oxidase (NOX).

Once again, the presence of a highly reactive (electrophilic) C-I moieties have thus far limited the transformations of the cyclic iodonium scaffold to those involving the breakage of this bond, either thermal, metal-catalyzed or radical. This has led us to consider the question of whether we could perform “peripheral” chemistry with these salts without touching the C-I bond? If so, what types of transformations?

And so, in this project, we are developing routes for facile and rapid attachment of a diaryliodonium fragment to molecular targets, with the aim to subsequently exploit the iodine(III) center in molecular engineering or as potential pharmaceutical warhead. Here, we will discuss an exceptionally facile formation of an amide linkage in an ester-containing iodonium precursor thanks to a close interaction between iodine(III) center and the ester carbonyl group. The carbonyl activation with iodine(III), in fact, could be readily observed through an impressive decrease in the carbonyl IR stretching frequency when compared to the “normal” ester stretching band. This strategy is compatible with polyfunctionalized amines, including short oligopetides.[5]



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Posters

P01 Sequence optimization of cathelicidin PMAP-36 for protease resistance and grafting on cotton-based wound dressings

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The overspread use of antibiotics and the consequent development of antibacterial resistance calls for new agents with a mechanism of action that is less easily circumvented. The porcine myeloid antimicrobial peptide PMAP-36 has a broad spectrum-activity that includes P. Aeruginosa and S. Aureus, and its membranolytic mechanism of action makes it a great candidate for further improvement [1]

In this contribution, we report the synthesis of a series of shortened and modified analogues of PMAP-36 and their characterization in terms of structure, protease resistance and biological activity. The shortening of the sequence, the substitution of some residues with unnatural or D-amino acids, and the introduction of lipid moieties at the N-terminus was done to make the synthesis simpler, enhance the helical structure of the peptides and make them less susceptible to protease degradation, respectively [2, 3] Moreover, the most promising peptide was considered as the antimicrobial agent in wound healing dressings and herein we show two different strategies to conjugate the peptide on cotton substrates by chemoselective ligations [4].

CD and NMR analysis showed that the analogues with shorter lipid chains retain a stable helical conformation, and the substitution with Aib or D-amino acids resulted as the most effective strategy for enhanced protease stability. In particular, the short peptide PMAP-36(12-24) and its D-enantiomer remained active at μM concentrations against the tested strains: E. coli, A. baumannii, K. pneumoniae, P. aeruginosa, and S. epidermidis, while showing low cytotoxicity against eukaryotic cells. Also, the conjugation PMAP-36(12-24) was successful and produced a with antibacterial properties.

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P02 Functionalization of supramolecular peptide materials for electrochemical applications

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Peptides are renowned for their self-assembly capabilities, forming supramolecular structures with tunable mechanical properties and enhanced biocompatibility. These characteristics can be precisely engineered at the nanoscale, making peptides promising biomaterials [1]. Bioelectronics, particularly electronic skin, represent a significant advancement in healthcare. However, current bioelectronics primarily utilize non-stretchable and non-degradable materials like metals. The integration of self-assembling peptide materials could potentially address these limitations, if we consider these materials would need to possess key electrochemical properties such as conductivity and electrochemical reversibility. Peptides often exhibit ionic conductivity naturally, and their electronic conductivity can be enhanced through strategic incorporation of aromatic residues and design of secondary structures that facilitate electron transport. However, their electrochemical reversibility can vary depending on their composition and design. The addition of electroactive groups is common, but it often raises concerns about their sustainability and functionalization protocols [2], showing the need for innovative strategies to enhance the electrochemical properties of supramolecular peptide-based biomaterials.

Herein, we introduce PSF-1, a collagen mimetic peptide capable of self-assembly into a hydrogel, and R, a flavin-like molecule, as our sustainable electroactive group. Through cyclic voltammetry assays it was revealed that PSF-1 possesses ionic conductivity but lacks electrochemical reversibility, while R demonstrated pH-dependent electrochemical reversibility. A novel co-assembly method was also tested to address the incorporation of R into PSF-1. During the self-assembly process of PSF-1, a R stock solution was added, achieving a co-assembled peptide biomaterial (PSF-1R) with a final concentration of 20mM of PSF-1 and 2.5mM of R. Subsequent solid-state cyclic voltammetry evaluations of PSF-1R exhibited pH-dependent oxidation and reduction peaks. These experiments established pH 6 as the ideal threshold for the optimal function of PSF-1 and R, solidifying their potential as viable biomaterial candidates for bioelectronic applications. Our research offers new insights towards the development of sustainable and high-performance bioelectronics.

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P03 Backbone Modification in Peptide Nucleic Acids: Impact on Helical Structure

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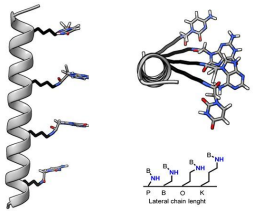
Peptide nucleic acids (PNA) are synthetic polymers conformed by an amino acid backbone and nucleobases attached in the lateral chains1. In the last years several applications have been described based on the recognition between PNA and nucleotides2, 3. However, few examples exploit the topological possibilities of the secondary structures that peptide backbones can adopt4. The alpha helix which displays controllable exo-helical topologies is a promising skeleton for control the PNA interactions5. This study is focused on the synthesis of alpha helicoidal PNA by exploring different backbone topologies to evaluate the solubility and helical folding. Furthermore, different linkers (P, B, O or K) were evaluated to change the distance between the backbone and the nucleobase. Nucleobases were attached by amide bond between acetic acid nucleobase (B) and amine in the peptide lateral chain. The pH dependence in the secondary conformation of the peptide was also considered in this study.



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P04 Psysol 2 as a Novel Research Tool to Study Human POP Inhibition

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Synucleinopathies, including Parkinson's disease, are a class of neurodegenerative diseases characterized by the accumulation of alpha-synuclein fibrils within Lewy bodies. Prolyl oligopeptidase (POP) has been demonstrated to enhance αSyn aggregation in Parkinson's disease models, whereas small molecules inhibitors were reported to reduce aggregation, induce autophagy, and limit ROS production. While active-site-directed small molecule inhibitors often struggle to modulate protein-protein interaction, which has been associated with the neuroprotective effects of POP inhibition, psysol 2, a cyclic cystine knot peptide, offers potential as a novel tool to address this limitation. This project aims to unravel the structural and functional mechanisms of POP inhibition by using this novel type of macrocyclic modulator.

Enzyme kinetics studies revealed that psysol 2 modulates POP activity through a mixed-type inhibition mechanism, reducing the enzyme's catalytic efficiency and substrate affinity in a concentration-dependent manner. A more detailed interaction model, however, remains elusive to date, as the peptide/protein complex has been difficult to crystallize. To address this limitation, we resorted to molecular dynamics simulations. These computational approaches identified a new binding site on POP, which aligns with the mixed-type inhibition mechanism observed in our kinetic studies, supporting the hypothesis of an allosteric modulation of the enzyme by psysol 2. To validate the computational model, we will perform amino acid mutation studies and site-specific mutagenesis to identify key interacting sites in peptide and protein, guiding structure-activity relationship work toward designing more potent POP inhibitors.

In conclusion, this study will explore the molecular details of a novel class of peptide modulators targeting POP and their role in functional cellular assays, including models of αSyn aggregation and autophagy. At a more general level, psysol 2 and its derivatives may serve as probes to investigate POP's enzymatic mechanisms and its interactions within protein networks, providing deeper insights into its functional and mechanistic properties.

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P05 Peptides targeting protein aggregation in biomolecular condensates

[1] **Carla Garcia-Cabau**, [1] Anna Bartomeu, [1] Jesús García, [1] Raúl Méndez, [1] Xavier Salvatella

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The inclusion of microexons by alternative splicing occurs frequently in neuronal proteins. The roles of these sequences are largely unknown, and changes in their degree of inclusion are associated with neurodevelopmental disorders. Decreased inclusion of a 24-nucleotide neuron-specific microexon in CPEB4, an RNA-binding protein that regulates translation through cytoplasmic changes in poly(A) tail length, is linked to idiopathic autism spectrum disorder (ASD) [1]. Neuronal CPEB4 forms condensates that dissolve after depolarization, a transition associated with a switch from translational repression to activation [2]. Heterotypic interactions between the microexon and a cluster of histidine residues prevent the irreversible aggregation of CPEB4 by competing with homotypic interactions between histidine clusters. The microexon is therefore required in neuronal CPEB4 to preserve the reversible regulation of CPEB4-mediated gene expression in response to neuronal stimulation [3]. In order to reduce the rate of aggregation, we designed synthetic peptides based on the sequence of the microexon and studied their effect on the condensates formed by CPEB4. The peptides partitioned into the condensates, increased their thermodynamic stability and decreased aggregation in a concentration-dependent manner. Our observation that the normal activity of the microexon can be restored in trans, opens up an opportunity to treat ASD on the basis of regulation of the dynamics of biomolecular assemblies by drug-like small molecules and peptides.

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P06 Supramolecular self-assembling peptides for antimicrobial application.

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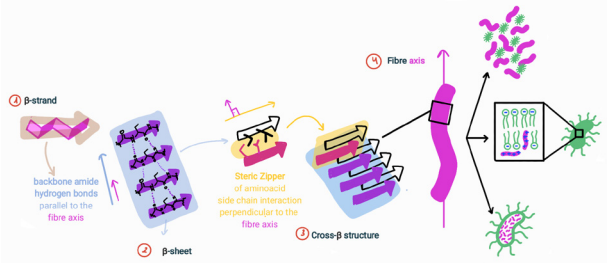
Antimicrobial resistance (AMR) is leading to a worldwide healthcare crisis by dramatically restricting effective treatments against infection (1). Driven by the need for new strategies to target microbes beyond traditional single-molecule antibiotics, supramolecular self-assembling peptides offer an alternative approach to overcome AMR by cytotoxic nanostructures targeting bacteria. (2–4)

We have synthesised and characterised two self-assembling peptide sequences derived from the amyloid β-42 peptide: Ac-GGVVIAK-NH2 and Ac-FFK-NH2. Here we report the characterisation of the resulting nanostructures and screening of self-assembling conditions. Their supramolecular self-assembly in aqueous buffer produced the expected nanofibres that will be studied as cytotoxic agents, aiming for a selective action against bacteria.



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P07 Antibiofilm effect of synthetic antimicrobial peptides on Gram-positive and Gram-negative bacteria

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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 bioactive molecules that have attracted interest due to their high potential as alternative treatments for infectious diseases. Not only are they easier to synthesize, but they are also generally composed of short amino acid sequences and can bypass the resistance mechanisms observed for conventional antibiotics, making them less susceptible to antimicrobial resistance. The development of alternative strategies, such as AMPs and silver nanoparticles (AgNPs), is crucial to address multidrug-resistant infections. AgNPs not only serve as effective antimicrobial agents but also act as delivery systems for AMPs, enhancing their stability and reducing toxicity. Among those, silver nanostars (AgNSs) are particularly advantageous due to their unique shape, which increases binding efficiency to bacterial cells and facilitates interactions with other molecules, like AMPs, due to the increased surface-to-volume ratio. These innovations can help alleviate pressure on conventional antibiotics, emphasizing the need for research investments, appropriate usage guidelines, and infection control measures to combat the global antimicrobial resistance crisis. The aim of this work was to investigate the effect of star-shaped silver nanoparticles conjugated to antimicrobial peptides to improve their antibiofilm potential. Unconjugated cationic AMPs: PwAMP1B5 and PyAMP1B5 were tested against E. coli, P. aeruginosa, S. aureus, S. epidermidis, and E. faecium. Our results show that PwAMP1B5 and PyAMP1B5 have an extended activity, affecting both the Gram-negative and Gram-positive bacteria tested. Unconjugated AgNSs were tested against planktonic E. coli, S. epidermidis and E. faecium, showing that they have a high ability for inhibiting bacterial growth at low concentrations. All these results suggest that both peptides and the AgNSs can be considered effective antimicrobial agents, with the potential for enhanced efficacy when conjugated.

P08 Targeting the SARS-CoV-2 main protease with peptidomimetic inhibitors

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The COVID19 pandemic highlight the need for effective antiviral treatments, leading to the exploration of various biological targets. Among them, the SARS-CoV-2 main protease (Mpro), which plays a critical role in processing viral polyproteins, has emerge as a key target for therapeutic intervention (1,2). Nirmatrelvir, a peptidomimetic inhibitor of Mpro, became the first oral treatment approved for COVID19 in combination with ritonavir (3).

The present work aims to identify novel covalent inhibitors of SARS-CoV-2 Mpro, taking advantage of the known 3D structures of this protein. To this aim, we have first generated a virtual library, based on conformationally restricted amino acids, whose synthetic methodology has been developed in our group. This library was screened by Induced fit docking and molecular dynamic studies to identify the most promising compounds. Selected peptidomimetics were then synthesized through multistep solution-phase methods.

Compounds were evaluated to assess their capability to bind the Mpro, and those exhibiting good enzymatic inhibition were further tested in cell culture infected with a clinical isolate strain of SARS-CoV-2 to determine their antiviral potency. Notably, a few compounds showed one or two digits nanomolar activity in the isolated enzyme, and were able to prevent the viral replication in A549-ACE2 cells, with micro- and submicromolar EC50 values. Regarding selectivity, these compounds were able to inhibit SARS-CoV-1 Mpro and MERS-CoV replication, albeit with lower potency. On the contrary, they did not exhibit inhibitory activity against several RNA viruses from the Rhabdoviridae and Flaviviridae families. The covalent mode of action was confirmed through X-ray crystallography, that lead to the resolution of several protein-inhibitor complex structures, showing the formation of a covalent bond with Cys145.

Overall, this collaborative effort, integrating expertise from several scientific groups result in the identification of a novel family of potent, covalent Mpro inhibitors with promising pan-coronavirus activity.

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P09 Novel spiroheterocycles obtained via the Groebke-Blackburn-Bienaymé Reaction: versatile synthons in peptidomimetic chemistry

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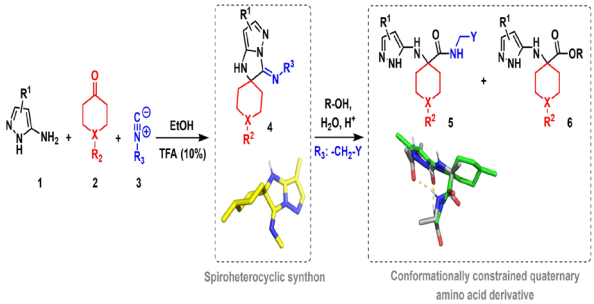
Spiroheterocycles are a class of organic compounds comprising two or more saturated rings connected at a single carbon atom, often incorporating heteroatoms such as nitrogen, oxygen, or sulfur. Their pharmacological significance was first recognized in the 1960s, with a substantial increase in interest during the 2000s due to their favorable bioavailability and pharmacokinetic properties.[1] Among these, nitrogen-containing spiroheterocycles are particularly noteworthy for their diverse biological activities.[2]

This study aimed to explore the utility of the three-component Groebke-Blackburn-Bienaymé (GBB) reaction (3CR) for synthesizing spiroheterocycles (Fig. 1). The GBB reaction was originally described as a method for combining aminoazoles, aldehydes, and isonitriles to produce aromatic imidazo[1,2-b]pyrazoles. In this work, we investigated the use of cyclic ketones to synthesize spiroheterocyclic derivatives. Specifically, we reacted 2-aminopyrazoles 1 with cyclohexanones 2 and various isonitriles (3) in the presence of trifluoroacetic acid as a catalyst, yielding spiropyrazoles 4 with diverse substitution patterns. The reaction typically proceeded efficiently at room temperature. Interestingly, when the isonitrile group was attached to a secondary carbon, α-N-(3-pyrazole)amino-α-carboxamides 5 and ester derivatives 6 were also identified/isolated, being the spirocycle cleavage facilitated under acidic aqueous condition. The newly synthesized spiro[cyclohexanoimidazo[1,2-b]pyrazoles] and the quaternary amino acid derivatives represent structurally diverse compounds with potential applications in medicinal and peptidomimetic chemistry. Preliminary modelling studies suggest that carboxamide derivatives could serves as mimics of peptide 310-helices (Fig 1).



Synthesis of spiropyrazoles 4 via a Groebke-Blackburn-Bienaymé reaction and derivatization to yield carboxamides 5 and ester derivatives 6 (minimum energy conformer of a simple spiropyrazole in yellow along with the superposition of a simple carboxamide in green and a 310-helix in grey; MMFFs, CHCl3 solvation).

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P10 Sustainable biocontrol of Esca disease in kiwifruit using Trichoderma-derived peptaibols

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Modern kiwifruit cultivation is increasingly threatened by idiopathic wood diseases such as Esca (also known as "Esca disease" or "Wood decay of kiwifruit"), elephantiasis, and silver leaf [1, 2]. These diseases significantly affect the productivity and quality of kiwifruit by damaging the wood of trunks and branches, which leads to gradual plant weakening [2]. Esca, a particularly complex and destructive disease, is caused by a consortium of fungal pathogens [3] and shares similarities with Esca disease in grapevines, manifesting chronic and acute symptoms, severely impairing the health of infected plants. Current control measures for Esca rely heavily on copper-based pesticides. While somewhat effective, these pesticides pose significant challenges, including environmental harm, phytotoxicity, and increased pathogen resistance. The growing restrictions on agrochemical use in the EU have highlighted the urgent need for sustainable, environmentally friendly alternatives to conventional agrochemicals.

The KIWIBOL project was established to address this challenge by identifying new agents to combat Esca. To achieve this, Trichoderma species were isolated from soils and rhizospheres of both symptomatic and asymptomatic orchards. These isolates were screened for antibacterial activity (against plant-pathogenic bacteria) and antifungal activity (targeting the fungi associated with Esca) using an in vitro dual-culture method. Among the isolates, T003 and T033 demonstrated significant biocontrol potential and were selected for further analysis.

The extracts obtained from these promising Trichoderma isolates exhibited potent antimicrobial properties, making them highly promising sources of bioactive molecules for combating Esca. Chemical characterization of the crude extracts was performed using liquid chromatography-mass spectrometry (LC-MS), revealing the presence of peptaibols, a class of linear, amphipathic polypeptides synthesized through the fungal non-ribosomal peptide synthetases (NRPS).

This research underscores the potential of Trichoderma-derived peptaibols as natural, eco-friendly alternatives to synthetic agrochemicals for wood disease control in kiwifruit cultivation.

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This research was funded by Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES, Portugal), through project KIWIBOL (PTDC/ASP-PLA/2440/2021), and received support from FCT/MCTES (LA/P/0008/2020 DOI:10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI:10.54499/UIDP/50006/2020 and UIDB/50006/2020

DOI:10.54499/UIDB/50006/2020), through national funds. RBP thanks FCT and LAQV/REQUIMTE for the contract (REQUIMTE 2023-05). EG is grateful to FCT for the PhD grant (2021.07616.BD). LP-D is grateful to the Spanish Ministerio de Universidades for a Margarita Salas postdoctoral grant funded by the European Union NextGenerationEU plan.

P11 Moving away from solid phase: the solution for the synthesis of the γ-peptide P33

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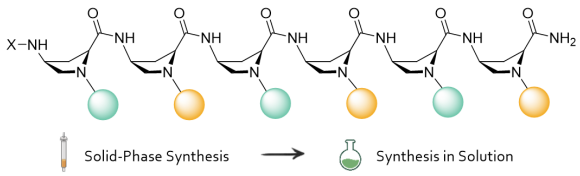
Cell-penetrating peptides (CPPs) are commonly used as vehicles to facilitate the delivery of therapeutic molecules across the cell membrane. Their ability to be internalized into different cell lines, together with their capacity to deliver a wide range of cargoes into the cells and their low cytotoxicity are some of the advantages that have made CPPs popular.[1] However, the low enzymatic resistance of the peptides due to the lack of secondary structure results in poor bioavailability profiles for these CPPs.[2] This problem is circumvented by using peptide foldamers that adopt a specific conformation in solution. In this context, our group studied a library of γ-peptides based on a cis-γ-amino-L-proline backbone functionalized at the α-amino.[3] Fortunately, some of these examples have shown medium to high cell uptake capacities and intrinsic therapeutic potential in various diseases that cannot yet be disclosed. This is the case of P33, a hexapeptide with two substituents on the α-amine, one of which is common to all residues. This work describes the attempts to define an optimized route to P33 and the setbacks encountered with each approach. Starting with a solid phase approach, the amino acids were functionalized prior to their introduction into the peptide chain. Unfortunately, the low solubility of some residues and a problematic introduction of the common substituent at the very end of the synthesis made this strategy unfeasible. The synthesis of P33 in solution was then considered a realistic approach due to the small size of this γ-peptide. However, the epimerization of the α-carbon of each residue and the incorporation of one of the substituents were some of the major problems to be overcome. In the end, the synthesis of P33 proved to be quite challenging but was achieved and optimized in an efficient and elegant 12-step route, and several derivatives were prepared.



Structure of P33 and its derivatives

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P12 Synthesis and evaluation of novel peptide ligands for triple negative breast cancer

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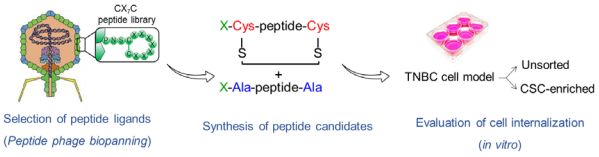
Triple-negative breast cancer (TNBC) is a heterogeneous sub-type of cancer which accounts for 15-20% of newly diagnosed breast cancers [1]. Clinically, TNBC is defined by the absence of expression of estrogen and progesterone receptors and human epidermal growth factor receptor 2 and, consequently, hormone therapy and anti-HER2 drugs are not effective choices for this type of tumors. TNBC is considered an aggressive cancer and is associated with a poor prognosis because of this lack of recognized molecular targets for therapy, the higher rates of relapse and risk of metastasis. Increasing evidences point to a particular type of subpopulation of cancer cells with stem cell-like properties and typically resistant to conventional chemotherapeutic agents, named cancer stem cells (CSC), as the responsible to sustain the tumor growth, incidence of metastasis and relapse of patients [2]. For these reasons, to eradicate cancer, new therapeutic approaches should be developed focusing on this CSC subpopulation, not only in terms of CSC-selective drugs but also in terms of CSC-targeting to optimize drug delivery. A potential tool for the identification of homing peptides for specific targets is the use of phage display peptide libraries, a technique that offers the opportunity to screen a countless number of potential ligands simultaneously [3].

This work describes the use of peptide phage biopanning on a TNBC cell model, both on unsorted cell population and CSC-enriched population, to identify novel targeting peptides and the subsequent synthesis and modification of the selected candidates. Finally, it is also described the preliminary in vitro evaluation of these peptides to assess their ability to interact and/or internalize in both cell populations (unsorted and CSC-enriched) of the TNBC model.



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P13 Multi-routed Dissipative Self-assembly of Dithioketene Derivatives

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Nature demonstrates remarkable control over self-assembly, maintaining dynamic structures far from equilibrium through continuous energy dissipation as shown by microtubule [1] formation during cell division. Inspired by these biological systems, we aim to develop reaction cycles that drive dissipative self-assemblies using dithioketene-based fuels and deactivated peptide monomers as substrates in aqueous environments. In our approach, peptide monomers will be activated by attaching a dithioketene fuel, leading to the formation of assemblies such as coacervates or hydrogels. [2] When an external charged molecule such as a surfactant is introduced, it will interact with the activated monomers, causing them to coalesce into oily droplets that rise to the surface, rendering the solution colourless. To complete the cycle, a second fuel initially present as an inactive pre-fuel is gradually released, deactivating the assemblies and restoring the peptide monomers to their original state. We aim to create versatile forward and backward reaction cycles that transiently activate and deactivate these self-assembling dithioketene-functionalized peptide monomers (Figure 1). This approach will yield dynamic architectures which are particularly relevant to prebiotic chemistry and protocell models.

Our approach will utilize a unique 'click-de-click' strategy [3] to precisely modulate the assembly process. We emphasize on high fuel efficiency, fine-tune reaction kinetics, and strategies for waste management via gas-phase or insoluble by-products. This innovative approach aims to minimize waste accumulation, extend system longevity, and enable recyclability. By achieving transient and reversible self-assemblies that cycle between different states, we unlock the potential to mimic life-like behaviours in synthetic materials.

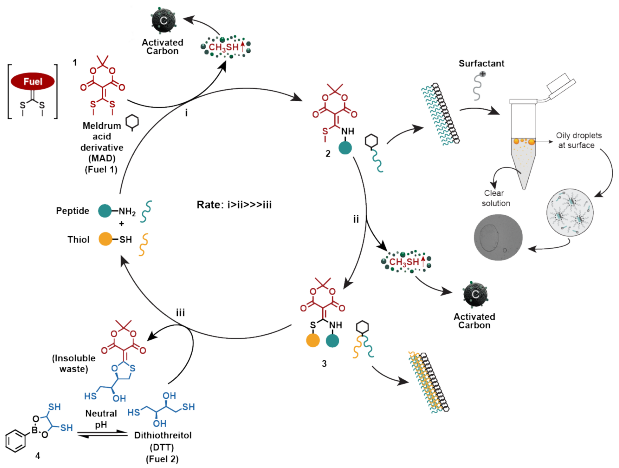

Figure 1: The process of coacervation involving fuel and peptide with a surfactant.

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P. Ranasingh and N. Singh acknowledge the Generalitat Valenciana for the CIDEGENT PlaGenT grant (CIDEXG/2022/16) for the PRONESS project, as well as to the Ministerio de Ciencia y Técnica y de Innovación for the new grant for Project MASS-UP under the Plan Estatal de Investigación Científica y Técnica y de Innovación (PID2022- 143047NA-I00). We also appreciate the assistance of Maruxa Peiró Álvarez and Cristian Vincent Barrera at Universitat Jaume I, Spain, for their contributions to the TEM, ESI-MS, and NMR measurements.

P14 Engineering Dynamic ATP-Based Coacervates via Peptide-Nucleotide Complex Coacervation

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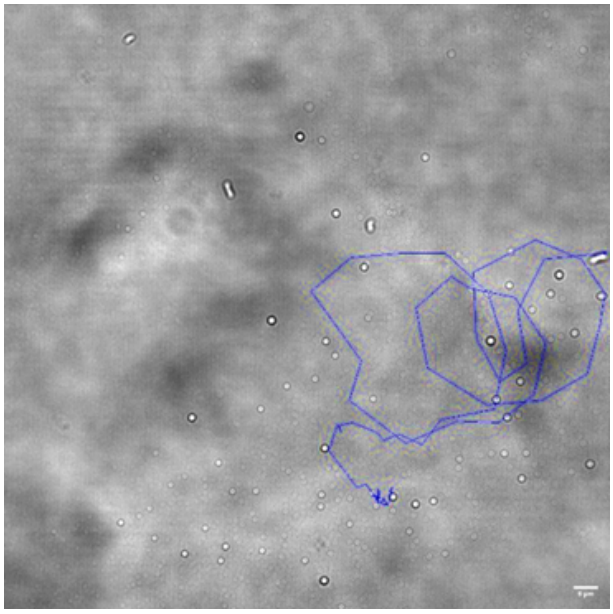
Adenosine triphosphate (ATP) is a vital molecule in living cells, serving both as a chemical signal and a molecular fuel1. This dual functionality is exemplified in actin filament dynamics, where ATP binding induces polymerization and ATP hydrolysis, catalyzed by ATP-actin units, leads to depolymerization. These processes create an intermediate out-of-equilibrium state until ATP is depleted.

Coacervates are micro-sized, membraneless droplets formed via liquid-liquid phase separation (LLPS)2. They are believed to have played a crucial role as protocells in the origin of life by selectively concentrating molecules and enhancing reaction rates through compartmentalization3. Designing ATP-based coacervates enables the creation of functional and dynamic structures, paving the way for engineering out-of-equilibrium protocells4.

In this study, we report the formation of dynamic condensates through associative LLPS of the peptide sequence P29 (KDFLPSPQTAW) with ATP. Over four days, we observed a significant increase in inorganic phosphate (Pi) concentration, indicating accelerated ATP hydrolysis. Remarkably, the coacervates exhibited motility and developed elongated structures. We hypothesize that this motion is driven by energy released during ATP hydrolysis, while the formation of elongated structures is due to the ATP responsiveness of these coacervates.Our findings provide new insights into creating complex systems with diverse functionalities and highlight potential applications in developing novel peptide-nucleotide compartments. By leveraging ATP's dual role and the unique properties of coacervates, this research advances our understanding of early life forms and opens new avenues for bioengineering and synthetic biology. This work not only sheds light on the potential mechanisms of primitive cellular life but also offers innovative strategies for designing dynamic, self-organizing systems that mimic aspects of living organisms.



Brightfield microscopy and tracking of the movement of an individual coacervate on the third day of experiment with the sample 5mM ATP: 15mM P29 in a scale of 5µm. In blue is the line with the course taken by the coacervate made with the tracking tool from the ImageJ program. Scale bar 5µm.

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P15 A Metal Catalysed Cyclic Peptide Synthesis Strategy

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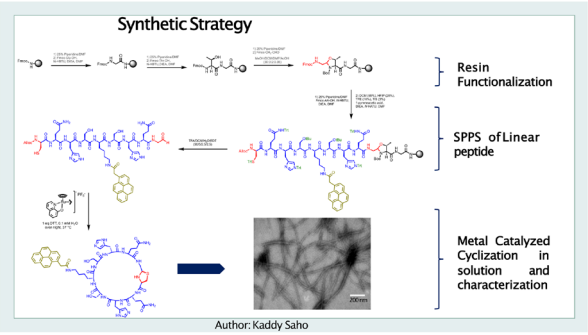
As the need arises for more effective drug delivery to increase the effectiveness of conventional drugs for various diseases such as cancer, the controlled generation of bioactive molecules and drugs emerge as an alternative of great interest. In this regard, the in-situ formation of self-assembly cyclic peptide nanotubes appears as a powerful strategy due to their remarkable therapeutic capabilities[1,2]. The objective of our work is the development of a simple chemical protocol that allows the catalysed formation of cyclic peptides that could self-assemble into SCPNs.

In this project, we begin by studying all elementary steps that can trigger the selective cyclisation of linear peptides and the subsequent nanotube formation. Thereafter, we design and synthesised peptides which were protected at their N-terminal ends with Alloc groups and a carbonyl aldehyde at the C-terminus for which a solid phase methodology was developed. These synthesised linear peptides in their native form are unable to cyclise, however, using a Ruthenium (III) complex {[Cp(allyl)(HQ)Ru]+PF6-} as an activator removes the Alloc protecting group leading to the fast formation of thiazolidine within the micro cycle in solution. Additionally, these cyclic peptides were characterised and proven to self-assembly into nanotubes in solution.



Synthetic Strategies

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P16 BLOOD BRAIN BARRIER – PEPTIDE SHUTTLES FOR DRUG DELIVERY INTO THE CNS

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The permeability of the blood-brain barrier (BBB) is crucial for drugs targeting the central nervous system. However, most molecules are unable to cross the BBB due to its rigorous transport regulation (1). The 'Trojan Horse' approach exploits receptor-mediated transcytosis to carry cargos across the BBB. Despite promising advances in peptide-based BBB shuttles, the proteolytic instability of peptides remains a substantial problem in the development of peptide therapeutics. Utilizing natural peptides as scaffolds in drug design can enhance stability, affinity to a target or cell penetration (2,3,4). In this project, we used the molecular grafting approach to design and synthesized a set of peptide probes by modifying proteolysis-stable cyclic sunflower trypsin inhibitor 1 (SFTI-1) to include peptides with reported transport across the BBB, in particular peptide 22 and MiniAp-4. We implemented a brain endothelial cell transport assay for bioactivity testing of probes, quantifying transport with LC-MS. The most active probe in the in vitro transport assay, SFTI-1-peptide 22, obtained a considerable permeability (Papp 9x10^-6) compared to peptide 22 (Papp 3.5x10^-6) alone and SFTI-1 alone (Papp 5.3x10^-6). Further, we evaluated pharmacokinetic (PK) parameters for the determination of the unbound brain-to-plasma ratio (Kp,uu,brain) of SFTI-1-peptide 22. Pharmacokinetic characterization with the brain slice method, plasma and brain tissue binding assays predicted a Vu,brain of 0.175, fu,plasma of 0.587 and a fu,brain of 0.120. Next, we will assess in vivo BBB permeability by intravenous administration of SFTI-1-peptide 22 to a rat and measurement of the concentration in the brain to calculate Kp and to ultimately determine the Kp,uu,brain. Our findings will demonstrate that a nature-derived peptide scaffold can enhance the stability of an incorporated bioactive peptide while facilitating transcellular transport, offering proof-of-concept for designing stabilized peptide BBB shuttles.

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P17 Collagen Mimetic Peptides Reimagined: The Role of Non-Biased Sequences in Structural Integrity

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Collagen, the most abundant protein in animals, is a vital structural component of connective tissues, providing strength and support to maintain their integrity and mechanical properties. Collagen structure is formed by the folding of three polypeptide chains with polyproline II PPII) conformation, that further assembles into a PP-II triple helix, leading to higher order assemblies of fibers and networks. The main interactions involved in the self-assembly process are hydrogen bonding, hydrophobic forces, and electrostatic attractions [1].

Extracting and synthesizing natural collagen is costly and complex. The intricate purification processes and challenging recombinant techniques increase production costs and time, limiting its widespread use [2].

Collagen mimetic peptides (CMPs) are being developed as a promising alternative to natural collagen. CMPs are designed to recapitulate the self-assembly mechanism of collagen, featuring repeating Gly-X-Y sequences that mimic collagen's triple helical structure [3].

Our study focuses on the development of a novel peptide that, while classified as a CMP because it mimics collagen's self-assembly mechanism (PPII-triple helix-Hydrogel) deviates from the conventional sequence of the collagen. Our data suggest that the self-assembly is potentially achieved through aromatic interactions.

Circular dichroism spectroscopy results demonstrate that this peptide forms PPII structures, which subsequently organize into triple helices, mimicking the behavior of traditional CMPs. Nuclear Magnetic Resonance techniques not only corroborate these structural data but also elucidate the structural properties and molecular interactions enabling the peptide's assembly into complex supramolecular structures.

The discovery that non-canonical sequences can maintain the structural integrity characteristic of CMPs paves the way for developing peptides with adaptable properties across diverse scientific contexts and fields.

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P18 RATIONAL DESIGN AND SYNTHESIS OF UNNATURAL GLYCOPEPTIDES FOR DETECTING TUMOR-ASSOCIATED AUTOANTIBODIES IN PANCREATIC CANCER

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Cancer remains the second leading cause of death worldwide, with nearly 20 million new cases and 9.7 million cancer-related deaths reported in 2022 alone. By 2040, the annual incidence is projected to rise to 29.9 million. The ongoing need for innovative and effective treatment strategies is compounded by the critical importance of early detection, especially for aggressive cancers such as pancreatic cancer, where survival rates are significantly improved through timely diagnosis.

Mucin-1 (MUC1) is a heavily O-glycosylated glycoprotein expressed on the epithelial cell surface. In healthy tissues, the peptide backbone is adorned with complex oligosaccharides, but in cancer cells, aberrant glycosylation results in truncated carbohydrates, exposing tumor-associated carbohydrate antigens (TACAs) like the Tn determinant (α-O-GalNAc-Ser/Thr).1 These antigens are immunogenic and recognized by anti-MUC1 antibodies, making them promising biomarkers for early-stage cancer. However, current diagnostic methods often rely on natural antigens, which may lack the specificity and stability required for accurate differentiation.2 Furthermore, anti-MUC1 antibodies are found in both cancer patients and healthy individuals, though at markedly lower levels in the latter, emphasizing the need for precise synthetic antigen models that faithfully mimic tumor-associated epitopes of MUC1.

In this work, we developed a diagnostic assay for pancreatic cancer that leverages tumor-associated autoantibodies against MUC1 using engineered glycopeptides displayed on nanoparticle probes. By employing a structure-guided approach, we designed non-natural glycopeptides based on the MUC1 scaffold with enhanced binding affinity for specific anti-MUC1 antibodies. These glycopeptides were tailored to recognize distinct tumor-associated epitopes, as targeted by monoclonal antibodies SM3 and 5E5. The synthetic glycopeptides were conjugated onto gold nanoparticles and integrated into a dot-blot assay to detect autoantibodies in serum samples from pancreatic cancer patients and healthy controls. This approach highlights the potential of engineered peptide-based diagnostics in improving the specificity and sensitivity of cancer detection.3

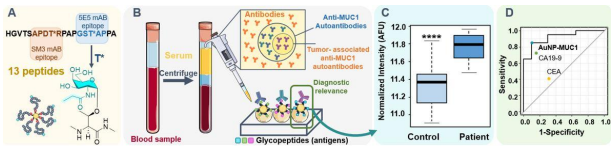

Figure 1. A. Glycopeptides with modification in the SM3 or 5E5 epitope. Structure of antigen with the best outcomes. B. Diagnostic assay principle. C. Antibody levels in healthy volunteers (control) and in pancreatic cancer patients. D. ROC curve.

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P19 Engineering Adaptive Vesicles: Fuel-Driven Self-Assembly of Biomimetic Lipids and Lipopeptides

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Living systems uniquely form active self-assembled structures, which consume chemical energy for their adaptive, and autonomous structural & functional responses [1]. These dynamic structures can form and break by maintaining out-of-equilibrium processes through chemical transformations that drive dissipative adaptation and self-assembly [2]. At the cellular level, membranes are not static boundaries but metabolically active interfaces that undergo continuous assembly and disassembly to maintain homeostasis and respond to environmental cues. However, creating synthetic vesicles that replicate the dynamic behaviour of cellular phospholipid membranes remains a significant challenge and is rarely reported in the literature [3]. Here, we present a bioinspired strategy for the in-situ synthesis and fuel-driven self-assembly of biomimetic phospholipids that replicate the dynamic metabolism of cellular membranes. The design employs an amino-ester bond to form lipids (anabolic reaction) via imine bond formation, which self-assemble into vesicles under physiological conditions. Simultaneous hydrolysis of the ester bond by lipase (catabolic reaction) leads to vesicle disassembly, mimicking the cyclical processes observed in cellular membranes. To further understand these systems functional capabilities and to emulate membrane complexity, we have synthesized lipopeptides and are investigating their self-assembly properties and interactions with OOE lipid vesicles. By integrating principles of metabolic mimicry, self-assembly, and dynamic regulation, these biomimetic systems represent a significant step toward creating synthetic structures that emulate the functional and dynamic nature of cellular membrane and their potential application as an adaptive interface for targeted drug delivery.

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