17th Naples Workshop on Bioactive Peptides

EMERGING PEPTIDE SCIENCE IN 2022

BOOK OF ABSTRACTS

Co-Chairmen
Giancarlo Morelli, Michele Saviano, Menotti Ruvo, Paolo Grieco

June 16-18, 2022 - Naples, Italy
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Centro Congressi d'Ateneo "Federico II" – Via Partenope, Napoli

June 16-18, 2022

17th Naples Workshop on Bioactive Peptides

EMERGING PEPTIDE SCIENCE IN 2022

Organized by:

Centro Interuniversitario di Ricerca sui Peptidi Bioattivi (CIRPeB)
Università di Napoli "Federico II" – Dipartimento di Farmacia
Istituto di Biostrutture e Bioimmagini del Consiglio Nazionale delle Ricerche
Istituto di Cristallografia del Consiglio Nazionale delle Ricerche
Università della Campania "L. Vanvitelli"

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The Organizing Committee gratefully acknowledges the contribution given to the organization of the event by the following companies

PROGRAM

THURSDAY, JUNE 16TH

13.00-13.15 Welcome address

Session 1
Chairpersons: C.TONIOLO - K. HRISTOVA

13.15-14.00	**PL1 Ronald T. Raines** Massachusetts Institute of Technology Cambridge, USA	*Lessons from Collagen*
14.00-14.20	**O1 Lorenzo Stella** University of Roma Tor Vergata Roma, Italy	*Quantification of the association of antimicrobial peptides with live bacterial cells: what have we learned?*
14.20-14.40	**O2 Charles Chen** Massachusetts Institute of Technology Cambridge, USA	*In silico-guided design of membrane active peptide-based therapeutics for biomedical applications*
14.40-15.00	**O3 Enrico Federico Semeraro** University of Graz Graz, Austria	*Bridging the activity of lactoferricin derivatives in E. coli and lipid-only membranes: partitioning and kinetics*
15.00-15.20	**O4 Myriam Cotten** William & Mary Virginia, USA	*Piscidins: Anti-infective and Anticancer Copper-binding Peptides at the Crossroads of Neuro-immune Interactions*
15.20-15.40	**O5 Ines Neundorf** University of Cologne Cologne, Germany	*Multistep translation of a cell-penetrating peptide into an antimicrobial peptide*
15.40-16.00	**O6 Martin Ulmschneider** King's College London, United Kingdom	*Spontaneous assembly of functional membrane proteins from soluble membrane active peptides*
16.00-16.20	**O7 Burkhard Bechinger** University of Strasbourg/CNRS Strasbourg, France	*Antimicrobial peptides: Mechanism of action and lipid-mediated synergistic interactions within the membranes*

16.20-16.40 *Coffee Break*

Session 2
Chairpersons: L. STELLA - S. GALDIERO

16.40-17.00	**O8 Markus Weingarth** Utrecht University Utrecht, Netherlands	*The mechanisms of Lipid-targeting Antibiotics*
17.00-17.20	**O9 Chiara Falciani** University of Siena Siena, Italy	*Antimicrobial peptides against periodontal bacterial infections*
17.20-18.00	**PL2 Sam Gellman** University of Wisconsin Madison, USA	*Impact of Backbone Modifications on Informational Properties of Polypeptides*
18.00-18.15	**O10 Claudia Feurstein** Technische Universität Berlin Berlin, Germany	*Structure-activity analysis using computational mining of protein databases to assist design of antimicrobial peptides*
18.15-18.35	**O11 Toni Todorovski** Pompeu Fabra University Barcelona, Spain	*Peptide-porphyrin conjugates as promising antivirals against brain-targeting viruses*
18.35-18.55	**O12 Jíři Jiráček** Institute of Organic Chemistry and Biochemistry CAS Praha, Czech Republic	*Insulin analogues with altered insulin receptor isoform binding specificities and enhanced aggregation stabilities*
18.55-19.15	**O13 Carla Zannella** University of Campania L. Vanvitelli Napoli, Italy	*Host defence amphibian peptides and their potential as antiviral agents*
19.15	*Welcome Party*	

FRIDAY, JUNE 17TH

Session 3
Chairpersons: H. WENNEMERS - P. GRIECO

8.40-09.20	**PL3 Kalina Hristova** Johns Hopkins University Baltimore, USA	*Peptides for transport across biological barriers*
09.20-9.40	**O14 Rina Aharoni** The Weizmann Institute of Science Rehovot, Israel	*The story of the peptide-copolymer Glatiramer Acetate (Copaxone) in the treatment of multiple sclerosis: from Bench to Bed side*

9.40-10.00	**O15 William Wimley** Tulane University New Orleans, USA	*Using synthetic molecular evolution to identify host cell compatible antimicrobial peptides effective against drug-resistant and biofilm-forming bacteria, in vitro and in vivo*
10.00-10.20	**O16 Lucia Ferrazzano** University of Bologna, Italy	*AMP-based therapy for non-healing wound care: advanced trap&release methods for non-invasive delivery materials*
10.20-10.40	**O17 Alessandro Pini** University of Siena Siena, Italy	*A peptide-based medical device for the selective removal of LPS, LTA and living bacteria from the serum of sepsis patients*
10.40-11.10	*Coffee Break & Poster Session*	

Session 4
Chairpersons: M.L. MANGONI - R. RAINES

11.10-11.30	**O18 Gil Rosenman** Tel Aviv University Tel Aviv, Israel	*Bioinspired Materials: Physical Properties Governed by Biological Refolding*
11.30-11.50	**O19 Maria Moccia** National Research Council Bari, Italy	*The Peptide Nucleic Acid leitmotif and its application in miRNAs targeting.*
11.50-12.10	**O20 Francesco Merlino** University of Naples Federico II Napoli, Italy	*Pursuit of "Qualified" Antimicrobial Peptides to Challenge the Antibiotic Resistance*
12.10-12.30	**O21 Luigi Aloj** University of Cambridge Cambridge, United Kingdom	*Radiolabelled peptides in cancer diagnosis and therapy*
12.30-13.50	*Lunch on site*	

Session 5
Chairpersons: P. ROVERO - L. ADLER ABRAMOVICH

13.50-14.30	**PL4 Meritxell Teixido** IRB Barcelona, Spain	*Blood-Brain Barrier Shuttle Peptides, From Discovery to Applications*
14.30-14.50	**O22 Antonella Accardo** University of Naples Federico II Naples, Italy	*Peptide based hydrogels and nanogels for biomedical applications*

14.50-15.10	**O23 Maria Gallo** Pompeu Fabra University Barcelona, Spain	*A cognitive protection peptide for cannabis-based pain treatment*
15.10-15.30	**O24 Sharon Gilead** Tel Aviv University Tel Aviv, Israel	*Self-Assembled Peptides for Electronic and Piezoelectric Applications*

PEPTIDE SHOWCASE
Oral Communications presented by Companies
Chairpersons: F. FORMAGGIO - S. DE LUCA

15.30-15.45	**PS1 Antonio Ricci** Fresenius Kabi iPSUM Villadose, Italy	*Therapeutic peptide industrial chemistry and analytical consideration*
15.45-16.00	**PS2 Elisabetta Bianchi** IRBM Pomezia, Italy	*Development of Analogues of the Venom Toxin ProTx-II as Selective Blockers of the Ion Channel Nav1.7 for the Treatment of Pain*
16.00-16.15	**PS3 Raimund Maier** Iris Biotech GmbH Marktredwitz, Germany	*Peptide Optimization by Novel "Fatty" Amino Acids*
16.15-16.30	**PS4 Giorgio Marini** CEM Cologno al Serio (BG), Italy	*Optimized Synthesis and Purification Strategies for SPPS*
16.30-16.40	**PS5 Malachi Gillick-Healy** KelAda Pharmachem Ltd. Dublin Ireland	*International industrial collaborative opportunities with KelAda Pharmachem Ltd., Ireland*

16.40-17.20 *Coffee Break & Poster Session*

Session 6
Chairpersons: D. MARASCO - D. ANDREU

17.20-18.00	**PL5 Helma Wennemers** ETH Zurich, Zurich, Switzerland	*Synthetic Collagen – From Structure to Function*
18.00-18.20	**O25 Lihi Adler-Abramovich** Tel Aviv University Tel Aviv, Israel	*Designing New Bioinspired 3D Hydrogels for Tissue Regeneration*

18.20-18.40	**O26 Evelien Wynendaele** Ghent University Ghent, Belgium	*Quorum sensing peptides as microbiota-derived mediators in different diseases*
18.40-19.00	**O27 Norman Metanis** The Hebrew University Jerusalem, Israel	*Chemoselective Modification of Cysteine and Selenocysteine in Peptides and Proteins*

Free evening

SATURDAY, JUNE 18TH

Session 7
Chairpersons: M. TEXEIDO - M. SAVIANO

8.20-9.00	**PL6 Alessandro Moretto** University of Padua Padua, Italy	*From sleepy to lively foldamers: using light to turn-on catalytic activity and programmable protein aggregation*
9.00-9.40	**PL7 Roger Cone** University of Michigan Ann Arbor, USA	*Development and application of new MC3R specific melanocortin peptides*
9.40-10.00	**O28 Raquel Lopez-Rios de Castro** King's College London, United Kingdom	*Finding an amyloid precursor protein occlusion peptide to reduce the production of toxic Aβ species*
10.00-10.20	**O29 Artur Krezel** University of Wroclaw Wroclaw, Poland	*Metal-coupled folding as the driving force for zinc proteins function - insight into sequence-structure-stability relationships in zinc finger, hook and clasp domains*
10.20-10.40	**O30 Carla Isernia** University of Campania L. Vanvitelli Caserta, Italy	*Influence of xenobiotic metal ions on structure and folding of the prokaryotic zinc finger Ros87*

10.40-11.10 *Coffee Break & Poster Session*

Session 8
Chairpersons: E. PEDONE - R. FATTORUSSO

11.10-11.50	**PL8 Christian Becker** University of Vienna Vienna, Austria	*New Tools for Protein Semi-Synthesis to study Posttranslational Modifications*

11.50-12.30	**PL9 Maurizio Pellecchia** University of California Riverside, USA	*Targeting protein-protein interactions by designing potent and effective peptide mimetics*
12.30-12.50	**O31 Aphrodite Kapurniotu** Technische Universität Munich Freising, Germany	*Designed peptides as potent inhibitors of amyloid self-assembly and cross-seeding of IAPP and Aβ42*
12.50-13.10	**O32 Nicolas Winssinger** University of Geneva Switzerland	*PNA to encode and fold peptide*
13.10-14.10	*Free Lunch*	

Session 9
Chairpersons: C. BECKER - C. ISERNIA

14.10-14.30	**O33 Luca Gentilucci** University of Bologna Bologna, Italy	*Hybrid α/β Peptide Ligands Targeting α4β1 Integrin: Therapeutic and Diagnostic Applications*
14.30-14.50	**O34 Pol Besenius** Johannes Gutenberg University Mainz Mainz, Germany	*Multicomponent Supramolecular Polymers as a Platform for the Design of Glycoconjugate Vaccines*
14.50-15.10	**O35 Alessandro Gori** SCITEC-CNR Milano, Italy	*Membrane-binding Peptides for Extracellular Vesicles On-chip Analysis*

Session 10
Chairpersons: A.M. PAPINI - M. RUVO

15.10-15.50	**PL10 Christian Heinis** Ecole Polytechinque Federale de Lausanne Lausanne, Switzerland	*New methods and attempts to develop orally available cyclic peptides*
15.50-16.10	**O36 Marina Rubini** University College Dublin Dublin, Ireland	*Semisynthetic approaches for studying post-translational modifications*
16.10-16.30	**O37 Claudia Tomasini** Università di Bologna Bologna, Italy	*Physical gels formed by Dopa-containing ultra-short peptides*

16.30-16.50	**O38 Giancarlo Terraneo** Politecnico di Milano Milano, Italy	*Halogenation as versatile tool for tuning structures and properties of amyloid peptides*
16.50-17.10	**O39 Giulia Marafon** ETH Zurich Switzerland	*Design of enzyme-inspired multivalent catalysts through functional patterning of nanosystems*
17.10-17.30	**O40 Manuela Grimaldi** University of Salerno, Salerno, Italy	*Tissue engineering and differentiation on neuronal stem cell targets through membrane active peptide*
17.30-18.00	*Coffee Break & Poster Session*	
18.00-18.20	**O41 Marta De Zotti** University of Padua Padua, Italy	*A linear antimicrobial peptide selectively detects sulfate anions*
18.20-18.40	**O42 Alice Romagnoli** Università Politecnica delle Marche Ancona, Italy	*Lost in translation: development of single and novel therapeutic peptidomimetic against multiple pathologies*
18.40-19.00	**O43 Elad Arad** Ben-Gurion University of the Negev Beersheba, Israel	*beta-amyloid fibrils catalyze neurotransmitter degradation*
19.00-19.10	Concluding Remarks	
21.00	***Gala Dinner***	

LIST OF POSTERS

P1	Semax, a synthetic regulatory peptide, affects copper-induced Aβ aggregation, amyloid formation and ROS production	*Francesco Attanasio*
P2	The syntetic eptapeptide Semax, a fragment of the ACTH hormone, sustains differentiated neuroblastoma, by stimulating the mitochondrial function and improving bioenergetic	*Francesco Attanasio*
P3	Peptide Nucleic Acid based "light up" probes for the early diagnosis of Celiac Disease	*Concetta Avitabile*
P4	Structure based development of HtpG-derived antigens against M. tuberculosis	*Giovanni Barra*
P5	Self-assembled peptide-based nanofibers for drug delivery in triple-negative breast cancer	*Rosa Bellavita*
P6	Antibacterial activity and serum stability of panusin, a lobster β-defensin, is conserved in the carboxy-terminal region	*Roberto Bello Madruga*
P7	Analogues of PMAP-36: synthesis, conformational analysis and bioactivity	*Barbara Biondi*
P8	Stability to proteolysis of GE11 analogues on gold nanostructures	*Francesca Biscaglia*
P9	Peptides with angiotensin I-converting enzyme inhibitory and antioxidant activity from raw and cooked trout meat protein hydrolysate	*Justyna Borawska-Dziadkiewicz*
P10	Barley (Hordeum vulgare L.) flakes as a source of antioxidant peptides	*Justyna Bucholska*
P11	The C-terminus of the GKY20 antimicrobial peptide, derived from human thrombin, plays a key role in its membrane perturbation capability	*Marco Campanile*
P12	Peptide encapsulation for structural and functional studies	*Carolina Cané*
P13	A Convenient Synthetic Route to (2S, 4S)-4-Methylproline	*Andrea Caporale*

P14 Lactoferrin-derived KDEON peptide and its biological properties *Floriana Cappiello*

P15 Topoisomers of a snake venom-derived peptide with anti-infective and antitumoral activity *Adam Carrera*

P16 Antibiofilm and antiviral properties of the antimicrobial peptide Temporin G *Bruno Casciaro*

P17 The peptide Hylin-a1 is able to inhibit Gram-positive bacterial infections *Annalisa Chianese*

P18 Carnosine-derived dipeptides as antineoplastic entites *Klaudia Chmielewska*

P19 Molecular mechanism of inhibitory effects of designed conformationally constrained peptides on amyloid self- and cross-assembly of IAPP and Aβ42 *Beatrice Dalla Volta*

P20 A straightforward chemical strategy for the triple functionalization of a peptide-based multimodal bioimaging probe *Lucia De Rosa*

P21 Metabolism and metabolites identification of selective αvβ3 [99mTc][Tc(N)PNP]-tagged RGDechi peptides *Annarita Del Gatto*

P22 Des3PI : A Computational Fragment-based Approach to Design Peptides Targeting Protein-Protein Interactions *Maxence Delaunay*

P23 Characterisation of the novel antimicrobial peptide B7-005: in vitro antimicrobial activity against ESKAPE pathogens and biocompatibility with human cells *Adriana Di Stasi*

P24 α-helix conformation and stability analysis of a VEGF mimetic peptide switched in the N-capping region from L- to D- amino acids: more than a preliminary view *Rossella Di Stasi*

P25 Cysteine-containing peptides for generation of modulable self-supporting hydrogels matrices *Carlo Diaferia*

P26 Solid-state optical properties of self-assembling amyloid-like peptides with different charged states at the terminal ends *Carlo Diaferia*

P27	All aromatic hydrogelators from a dopamine and 2-naphytlalanine hexapeptide library	*Carlo Diaferia*
P28	Applications of NMR spectroscopy for protein-peptide interaction studies on living cells	*Diana Donatella*
P29	Amyloid-like Prep1 peptides exhibit an intrinsic fluorescence signature in vitro and in living cells	*Nunzianna Doti*
P30	The interplay between carbonyl carbon pyramidalizations and n→π* interactions in biomolecules	*Luciana Esposito*
P31	Nanosystem for antiviral delivery	*Annarita Falanga*
P32	New hope in the war of antibiotic resistance: Antimicrobial Peptide	*Annarita Falanga*
P33	New structural determinants for antimicrobial peptides?	*Lucia Falcigno*
P34	Relationship excluded volume interactions/biological activity in D-leucine containing sequences	*Emma Fenude*
P35	Combining biological structures and molecular self-assembly lead to ordered nanostructures	*Emma Fenude*
P36	Self-assembly of short peptides into hydrogels	*Daniele Florio*
P37	Synthesis, spectroscopic and electrochemical studies of Dap homo-peptides, conjugated to ferrocene moieties	*Fernando Formaggio*
P38	Fluorescent nanospheres obtained by PEGylated tetratyrosine nanofibers heating process	*Enrico Gallo*
P39	Insulin conjugates for receptor studies and therapeutic	*María Soledad Garre Hernández*

P40	Self-assembled peptide gels for the release of active compounds	*Demetra Giuri*
P41	Development of Bombesin Based Peptide-Drug Conjugates and Combination with Conjugates Targeting Gonadotropin Releasing Hormone (GnRH), CD13 and Integrin Receptors	*Jacopo Gomena*
P42	Constrained α-helical macrocyclic peptides for enhancing the Antibiofilm activity on native Temporin L peptide.	*Nicola Grasso*
P43	Structure-based design of EGF-like bicyclic peptide inhibitors of protein Nodal activity	*Emanuele Iaccarino*
P44	Studying the structural determinants of aggregation in peptides of the Gadd45 protein family members	*Emanuele Iaccarino*
P45	Peptide-capped Gold Nanoparticles: a biomimicking approach towards organ cryopreservation	*Elisa Impresari*
P46	Design of short catalytic peptides inspired by hydrolases	*Patrizia Janković*
P47	The Proline hinge motif controls the immunomodulatory fucntion of bohevine herpesvirus 1-encoded inhibitor of TAP	*Natalia Karska*
P48	Structural vaccinology for the design of vaccine antigens against difficult infectious pathogens	*Eliza Kramarska*
P49	The Zn(II) binding properties of cysteine-rich cluster of hMTF-1: Relations between metal affinity and cellular free zinc fluctuations	*Artur Krężel*
P50	Proregenerative peptides as part of a composites for bone reconstruction	*Agnieszka Kubiś*
P51	Ability of glucosyl platinum(II) complexes to modulate the amyloid aggregation of the C-terminal region of the Aβ peptide	*Sara La Manna*
P52	Design, Synthesis, and Biological Investigations of New TSP 1 deriving Peptides as TGF-β Activators	*Patrycja Ledwoń*

P53	In depth analysis of the biological properties of the antimicrobial peptide Esc(1-21) and its one-residue-substituted analogs	*Maria Luisa Mangoni*
P54	Different loading and release of an antimicrobial peptide from alginate- and agarose porous scaffolds for bone regeneration.	*Mario Mardirossian*
P55	Metabolic syndrome preventing peptides derived from caseins (bovine, caprine and ovine) after in silico proteolysis	*Damir Mogut*
P56	Generation of a new AIF peptide targeting Human Cyclophilin A to inhibit AIF-mediated cell death.	*Alessandra Monti*
P57	The Thorpe-Ingold effect is highly efficient in promoting the β-turn conformation in an N^{δ}-acylated, (E) β, γ-olefin dipeptide amide isostere	*Alessandro Moretto*
P58	An improved application of the Carpino's acyl fluoride C activation methodology to the SPPS of C^{α}-tetrasubstituted α amino acid sequences	*Alessandro Moretto*
P59	Transforming a crude peptide sequence into valuable antimycobacterial products	*Gabriel S. Oliveira*
P60	AlphaFold predictions of peptide-MHC complexes	*Antonella Paladino*
P61	Small peptide conjugates for neuroprotection and Abeta detection	*Giuseppe Pappalardo*
P62	Potent and selective C-terminal carboxamide peptide inhibitors of Zika virus NS2B -NS3 protease	*Francesca Pavone*
P63	PMAP36 peptide analogues linked to polysaccharide scaffolds for new antimicrobial materials	*Peggion Cristina*
P64	Cyclic peptoids as a playground for studying weak interactions	*Giovanni Pierri*
P65	Design of helical foldamers for chemical catalysis	*Matteo Pollastrini*

P66	A new class of cell penetrating peptides and their binding affinity with nucleic acids	*Anita Romanowska*
P67	Soft materials as a novel diagnostic tool for MRI applications	*Elisabetta Rosa*
P68	The high-resolution structure of the human Annexin IV: insight into calcium-binding sites	*Alessia Ruggiero*
P69	Antimicrobial peptides can generate tolerance by lag and affect antimicrobial treatments	*Daniel Sandín*
P70	Site-Specific labelling of recombinant humanized antibody fragments	*Annamaria Sandomenico*
P71	From cyclic peptoids to azamacrocycles	*Rosaria Schettini*
P72	Viral weapons against bacteria: structural clues to fight antimicrobial resistance	*Flavia Squeglia*
P73	Delivery of inhibitors of the phosphatase SHP2 protein-protein interactions	*Claudia Storti*
P74	Plant peptides which modulate the immune system	*Iwona Szerszunowicz*
P75	Peptide interfaces for CRP molecular recognition – development, characterization and application	*Katarzyna Szot-Karpinska*
P76	Reactivity of vasopressin and its selenium analogues with mercury: LC-MS/MS investigation	*Diego Tesauro*
P77	A tribute to Prof. Louis A. Carpino: A statistical survey of geometry and conformation of the Fmoc-amino group in peptides from X-ray diffraction structures	*Claudio Toniolo*
P78	Native chemical ligation as a way for obtaining fully synthetic metallothioneins	*Józef Tran*

P79	Getting insights on structural and energetic properties of reciprocal peptide-protein interactions	*Daniela Trisciuzzi*
P80	Microfluidic impedance cytometry: a new tool to study antimicrobial peptides at the single-cell level	*Cassandra Troiano*
P81	Identification of peptide markers from allergenic seafood tropomyosins	*Marta Turło*
P82	Aggregation properties of a therapeutic peptide for rheumatoid arthritis: a spectroscopic and molecular dynamics study	*Venanzi Mariano*
P83	Zeolites employed as basic catalyst for nucleophilic substitution reactions: An analysis of the adopted approach and hypothesized new perspectives	*Valentina Verdoliva*
P84	Design and evaluation of peptides targeting Ship2-Sam	*Marian Vincenzi*
P85	Larazotide tripeptide derivatives as potential Main protease inhibitors	*Giovanni Vivenzio*
P86	Functional stapled fragments of human preptin of minimised length	*Lenka Zakova, Marta Lubos*
P87	Synthesis and nucleic acid binding evaluation of a thyminyl L-diaminobutanoic acid-based nucleopeptide	*Domenica Musumeci*
P88	Identification of a novel vasoactive pentapeptide through a bioassay-guided fractionation approach	*Giacomo Pepe*
P89	Discovery of a Novel Tetrapeptide against Influenza A Virus: Rational Design, Synthesis, Bioactivity Evaluation and Computational Studies	*Marina Sala*

PLENARY LECTURES

Lessons from Collagen

R. T. Raines

Massachusetts Institute of Technology, Department of Chemistry, 02139 – Cambridge, USA

The hydrophobic effect and hydrogen bonding have long been known to direct a polypeptide chain to assume a folded structure. Using collagen mimetic peptides (CMPs), we have shown that a previously unappreciated force—stereoelectronic effects—is responsible for the increased stability endowed upon the collagen triple helix by its prevalent (2*S*,4*R*)-4-hydroxyproline residues.[1–3] Exploiting these stereoelectronic effects (along with reciprocal steric effects and molecular self-assembly) enables the creation of synthetic collagens of unprecedented stability and length for myriad applications in biotechnology and biomedicine.[4]

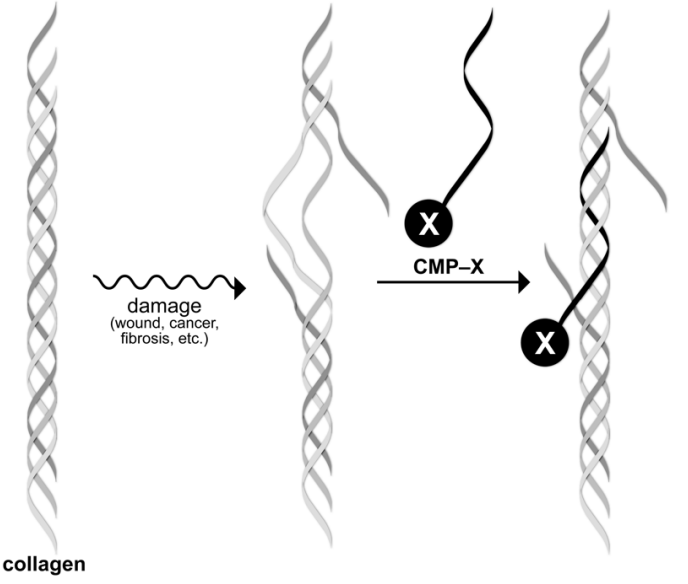


Figure 1. Scheme for the use of a CMP to anchor a molecule (X) to damaged collagen. A CMP does not bind to an intact collagen triple helix. (That is, a "quadruple helix" is not stable.) In contrast, a damaged triple helix provides a binding site for a CMP. This strategy is intrinsically theranostic—combining the potential for imaging and therapy. For example, a pendant probe could enable assessment, while a pendant drug elicits healing.

References

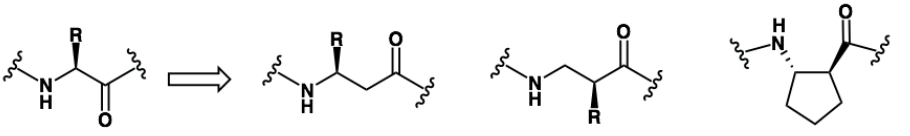
1. M. D. Shoulders, R. T. Raines, *Annu. Rev. Biochem.* (2009), *78*, 929–958.
2. K. L. Gorres, R. T. Raines, *Crit. Rev. Biochem. Mol. Biol.* (2010), *45*, 106–124.
3. R. N. Newberry, R. T. Raines, *Top. Heterocycl. Chem.* (2017), *48*, 1–26.
4. S. Chattopadhyay, R. T. Raines, *Biopolymers* (2014), 101, 821–833.

Impact of Backbone Modifications on Informational Properties of Polypeptides

S. H. Gellman

University of Wisconsin-Madison, USA, Department of Chemistry, Madison, WI 53706

This lecture will describe recent progress in development of backbone-modified peptides intended to mimic information-rich surfaces displayed by specific conformations of natural polypeptides. Backbone modification is achieved through replacement of one α-amino acid residue or more with a β-amino acid residue. The β residue can display the natural side chain, or this residue can be preorganized via a ring. The resulting α/β-peptides can inhibit specific protein-protein interactions, or they can augment signalling through polypeptide-activated receptors. Merits of the α/β-peptides include enhanced resistance to proteolysis and the ability to transmit signals that differ subtly from those of a prototype α-peptide. Similar effects can be observed for heterochiral polypeptides containing L-to-D replacement at selected positions.



Peptides for transport across biological barriers

Kalina Hristova[1], Alexander Komin[1], Raleigh Linville[1], Piotr Walczak[2], and Peter C. Searson[1]

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The blood-brain barrier (BBB) tightly controls entry of molecules and cells into the brain, restricting the delivery of therapeutics. One strategy to enhance delivery across endothelial or epithelial monolayers is conjugation to cell-penetrating peptides (CPPs). We showed that a CPP, called the CL peptide, can increase the delivery of small-molecule cargoes across model epithelium approximately 10-fold. The delivery of the cargo involves peptide cleavage by cells, which releases the cargo prior to transport out of the epithelium. Given the limitations of this approach, we also explored the reversible disruption of cell-cell junctions between brain microvascular endothelial cells to enable transient entry into the brain. We demonstrated that melittin, a membrane active peptide present in bee venom, supports transient blood-brain barrier opening (BBBO). We used a tissue-engineered model of the human BBB to optimize dosing and elucidate the mechanism of opening. Melittin and other membrane active variants transiently increase paracellular permeability via disruption of cell-cell junctions that result in transient focal leaks. We further demonstrated that transient BBBO can be reproduced in a mouse model. Peptide-induced BBBO represents a novel technology for delivery of therapeutics into the brain.

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Blood-Brain Barrier Shuttle Peptides, From Discovery to Applications

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

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

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

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

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Synthetic Collagen – From Structure to Function

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Collagen, the most abundant protein in mammals, is a key contributor for the strength and stability of skin, bones, and connective tissue. Collagen formation is thus vital for the integrity of skin, tendons, and the tissue in essentially any organ. Excessive collagen formation is, however, characteristic of fibrotic and malignant diseases, which include major global health issues.

The Wennemers group has used collagen model peptides (CMPs) to understand the stability of collagen at the molecular level and to establish functional synthetic collagen triple helices.[1] These include pH-responsive synthetic collagen,[2] hyperstable triple helices,[3] and heterotrimeric collagen.[4] Building on these data, we designed and synthetized a chemical probe for the simultaneous monitoring and targeting of lysyl oxidase (LOX)-mediated collagen cross-linking.[5] The probe allows for the detection of LOX activity *in vivo* and in tissue sections.

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From sleepy to lively foldamers: using light to turn-on catalytic activity and programmable protein aggregation

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In recent years chemists developed protein secondary structure mimics to achieve some desirable features of proteins, which are able to interfere with the biological processes. Such non-natural oligomers, so called foldamers,[1] can adopt highly stable and predictable architectures and have extensively demonstrated their attractiveness for widespread applications in fields from biomedical to material science. Foldamer science was more recently considered to provide original solutions to the de novo design of artificial enzymes.[2,3] In this presentation we report our progress on this field, where foldamer are accurately designed in the way to achieve chemical and biochemical consequence in virtue of their primary and secondary structures. In particular, we made use of secondary structure of foldamer to run: (i) programmable and enantioselective amide bond formation,[4] (ii) the hydrolyses of stable esters,[5](iii) the formation of "hard to achieve" intramolecular C-C bonds,[6] and finally to induce a spatiotemporal protein aggregation.[7-9]

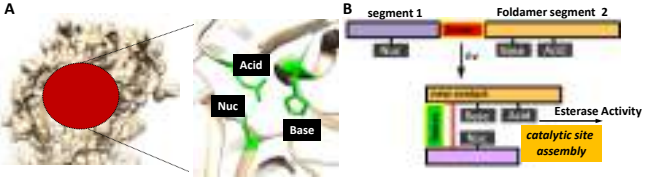

Figure 1. The catalytic action of serine peptidases depends on the interplay of a nucleophile, a general base and an acid. In the classic trypsin family this catalytic triad is composed of serine, histidine and aspartic acid residues. According to the chymotrypsin X-ray diffraction structure, the geometric relationship of Asp^{102}, His^{57} and Ser^{195} led to the hypothesis that His^{57} assists in relocating the proton from Ser^{195} to Asp^{102} in a charge relay mechanism. B: Our approach to achieve a functional photochemical response from an appropriately designed helical foldamer that aims to mimic the catalytic triad motif present in peptidases. In our strategy, two peptide segments are connected by an isomerizable linker which, upon absorption of light, re-folds the whole foldamer in a way that guarantees the contact between the two segments and allows assembly of the catalytic site.

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Melanocortin Peptides and the Mechanics of Energy Homeostasis

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Variations in melanocortin peptide signaling are responsible for some of the most observable human phenotypes, including red hair and early onset obesity. In the nervous system, study of melanocortin and agouti signaling, and their GPCRs, have elucidated many novel aspects of the dialectic of neuropeptidergic signaling. Examples include the first discovery of an endogenous GPCR antagonist (AgRP - possibly also a biased agonist), one of the rare examples of a neuropeptidergic GPCR exhibiting a gene dosage effect fundamental to the mechanics of energy homeostasis, and one of the rare examples of a GPCR capable of G-protein independent coupling to an ion channel.

These novel dialectics all play an important role in the regulation of energy homeostasis, however much remains to be understood. While much is known about the role of the MC4R in energy homeostasis, the MC3R has been an enigma. Recent work has begun to provide a framework: the MC3R is a negative regulator of MC4R neurons, and is critical for protecting both the upper and lower boundaries of energy homeostasis, rather than set point itself[1]. One mechanism we have identified is a role of the MC3R in the sensing of energy deficits by the AgRP neurons. Additionally, the MC3R is widely expressed in the CNS, and provides state-specific inputs to energy homeostasis. For example, MC3R provides bidirectional communication between energy state and reproductive state, being required for the dependence of reproductive competence on nutritional state[2], and for the increased food intake and energy storage during pregnancy[1]. Lastly, the recently reported case of a patient with homozygous loss of the MC3R demonstrates the conserved role of the MC3R in mouse models and human[2].

Three melanocortin peptide drugs have now been approved by the FDA for syndromic obesity (Imcivree), erythropoietic protoporphyria (Scenesse), and female hyposexual disorder (Vyleesi), however all these compounds are pan-agonists of the melanocortin receptors. In order to advance our understanding of the role of the MC3R in particular, we have sought to create potent, receptor subtype-specific MC3R and MC4R compounds[3]. After 8 rounds of SAR, and synthesis and pharmacological characterization of close to 500 different peptides, we are now able to report compounds with sub-nanomolar EC_{50} values and 1000X specificity for the hMC4R, and low nanomolar EC_{50} values and 1000X specificity for the hMC3R.

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New Tools for Protein Semi-Synthesis to study Posttranslational Modifications

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At least 50% of all human proteins are predicted to experience one or more posttranslational modification (PTM) during their life cycle.[1] These PTMs can result from enzymatic or non-enzymatic reactions and enzymatic PTMs are well-known for being involved in regulating many cellular events such as gene expression, intracellular and extracellular signal transduction, protein-protein as well as cell-cell interactions.[2, 3] Non-enzymatic posttranslational modifications (nPTMs) are increasingly recognized to affect such events as well, with a special emphasis on age-related, metabolic and neurodegenerative diseases.

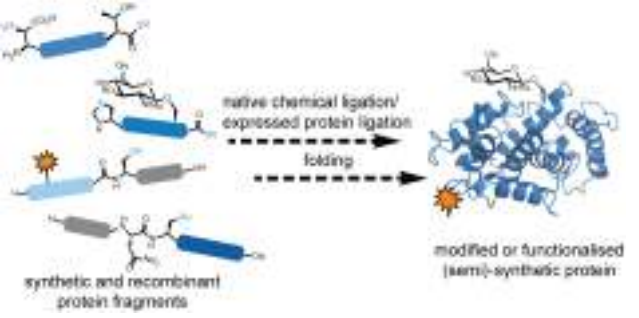

Figure 1. Ligation approaches to assemble semi-synthetic proteins.[4]

Here several examples will be discussed that involve different semi-synthesis routes to access site-specific PTMs within proteins such as Hsp27, G-CSF and Tau. For the small heat shock protein Hsp27, which has been shown to carry argpyrimidine (Apy) modifications, we can demonstrate that Apy modifications significantly influence chaperon function for a variety of client proteins.[5] Furthermore, new tools to access challenging protein targets via (semi-)synthesis will be discussed.[6, 7]

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Targeting protein-protein interactions by designing potent and effective peptide mimetics

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The identification of potent and pharmacologically viable agents mimicking protein-protein interactions (PPIs) remains a challenging task, despite PPIs represent a viable and largely untapped therapeutic target space. We recently proposed the HTS by NMR[1-2] and the focused-HTS by NMR approaches [1, 3-6] that consist of combining principles of positional scanning combinatorial chemistry and protein-based NMR screening to identify, within libraries of > 100,000 peptide-mimetics, possible initial binders to a protein target. In our experience, the approach can result in the identification of initial tri- or tetra-peptides with affinities in the high (HTS by NMR) to low (*f*HTS by NMR) micromolar range. Subsequently, structure-based iterative optimizations of the initial hits can lead to potent and selective agents. The approach was proven very successful in the rapid identification of ligands with low nanomolar affinities for various targets including EphA2, EphA4, IAPs, and MMPs. While the optimization of ligands is possible based on the structure, we also found that introducing properly placed electrophiles such as aryl-sulfonyl fluorides or aryl-fluorosulfates [7-9] can more rapidly identify covalent agents that target binding site Lys or Tyr residues. Finally, extending the strategy to a E3 ligase, we propose the design of E3-Lys-covalent bi-functional agents that work as protein degraders (LYCODE). I will report on examples related to these recent strategies.

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New methods and attempts to develop orally available cyclic peptides

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My laboratory is engaged in the discovery and development of cyclic peptides for therapeutic application. We generate the cyclic peptides by screening large combinatorial libraries against disease targets using phage display and microplate-based screens. The libraries are synthesized by efficient macrocyclization reactions applied to either phage-displayed peptides or synthetic peptides. We have demonstrated the pharmacological activity of several of the cyclic peptides in vivo.

In recent years, we have started to address the long-standing goal of developing orally available peptides. In my talk, I will present how we used phage display to enrich peptides that bind to targets of interest, and that resit proteases in the gastrointestinal tract of mice upon oral application.[1,2] In a second part of my talk, I will present an approach for the high-throughput screening of sub-kDa cyclic peptides that we have developed with the aim of generating cell permeable and orally available cyclic peptides.[3,4]

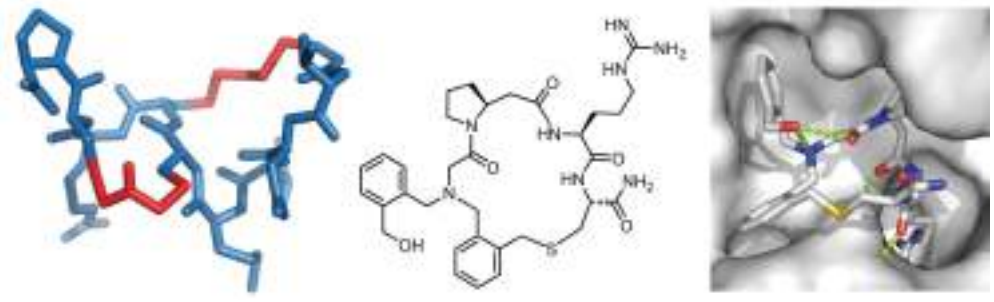

Figure 1. Double-bridged peptide (left), sub-kDa cyclic peptide (middle) and cyclic peptide bound to target (right)

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ORAL PRESENTATIONS

Quantification of the association of antimicrobial peptides with live bacterial cells: what have we learned?

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Antimicrobial peptides (AMPs) are crucial effectors of innate immunity and promising molecules to fight drug-resistant microbes. They usually kill bacteria by perturbing their cellular membranes. Therapeutic applications and a full understanding of the biological functions of AMPs have been hampered by a chasm between biophysical studies on model bilayers and microbiological experiments. For instance, the affinity of AMPs for their target membranes is an essential determinant of peptide activity and selectivity. This property is well characterized using artificial bilayers but is essentially unexplored for real cells. We developed a spectroscopic assay allowing the quantitative determination of peptide association to live bacterial cells. Characterizing this basic aspect has led to multiple insights into the function of AMPs.[1-7] Our data showed that millions of peptides must bind to each cell to cause its death. This number even exceeds the complete coverage of cell membranes. Peptide affinity for bacteria whose membrane has already been perturbed is an order of magnitude higher than for live, healthy cells. After membrane perturbation, AMPs accumulate inside the cell, binding to intracellular components. Peptide sequestration by dead cells can protect the remaining bacteria from AMP activity. Based on cell-binding results, we predicted and observed a specific trend for the cell-density dependence of AMP activity. The inoculum effect is significant for cell densities above 5×10^5 cells/mL, while for lower densities the active concentrations are essentially constant, in the micromolar range. As a consequence, AMP selectivity depends on the relative concentrations of target and host cells. These results question the clinical utility of activity and selectivity determinations performed at fixed, standardized cell densities. Overall, our findings clarified some key aspects of AMP function but also led to several new questions, which will be addressed during the presentation.

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In silico-guided design of membrane active peptide-based therapeutics for biomedical applications

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Membrane-lytic peptides offer broad synthetic flexibilities and design potential to the arsenal of anticancer therapeutics, which can be limited by cytotoxicity to noncancerous cells and induction of drug resistance via stress-induced mutagenesis. Despite continued research efforts on membrane-perforating peptides for antimicrobial applications, success in anticancer peptide therapeutics remains elusive given the muted distinction between cancerous and normal cell membranes and the challenge of peptide degradation and neutralization upon intravenous delivery. Using triple-negative breast cancer as a model, the authors report the development of a new class of anticancer peptides. Through function-conserving mutations, the authors achieved cancer cell selective membrane perforation, with leads exhibiting a 200-fold selectivity over non-cancerogenic cells and superior cytotoxicity over doxorubicin against breast cancer tumorspheres. Upon continuous exposure to the anticancer peptides at growth-arresting concentrations, cancer cells do not exhibit resistance phenotype, frequently observed under chemotherapeutic treatment. The authors further demonstrate efficient encapsulation of the anticancer peptides in 20 nm polymeric nanocarriers, which possess high tolerability and lead to effective tumor growth inhibition in a mouse model of MDA-MB-231 triple-negative breast cancer. This work demonstrates a multidisciplinary approach for enabling translationally relevant membrane-lytic peptides in oncology, opening up a vast chemical repertoire to the arms race against cancer.

Figure 1. Figure caption example

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Bridging the activity of lactoferricin derivatives in *E. coli* and lipid-only membranes: partitioning and kinetics

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Novel antibiotics based on membrane active antimicrobial peptides (AMPs) are promising candidates for defending the spread of diseases caused by multi-resistant pathogenic bacteria. Notwithstanding the number of works that explore the relationship between AMP activity and membrane architecture, the full mechanism that lead to cell death is currently not clear. This prompted us to investigate the mode-of-action of lactoferricin derivatives on both live *E. coli* and biological lipid-membrane mimics [1-3].

In particular, we explored AMP partitioning in bacteria [4] and lipid-only vesicles revisiting susceptibility and leakage assays, respectively, and zeta-potential and Trp-fluorescence spectroscopy [1]. The structural rearrangement in vesicles and bacteria upon mixing with AMPs was probed by transmission electron microscopy and small-angle neutron and X-ray scattering [2-3]. The latter permitted to access the kinetics in live cells with an unprecedented time (milliseconds) and length (nanometre to sub-micrometre) scales.

To name but a few, results suggest that these AMPs strongly partition into the lipid membranes, quickly translocating into the cytoplasm/lumen (<1 s) and, simultaneously, causing weak leakage and negligible lipid flip-flop. Nevertheless, membrane remodeling in both membrane mimics and live cells is significant but, strikingly, only incidental to bacterial killing.

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Piscidins: Anti-infective and Anticancer Copper-binding Peptides at the Crossroads of Neuro-immune Interactions

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Piscidins, which were discovered in the mast cells of fish, are host defense peptides (HDPs) with antimicrobial, antiviral, anticancer, and anti-inflammatory properties.[1,2] It was recently reported that they are expressed in not only immune but also neural cells, and thus there is growing interest in their possible participation in neuro-immune interactions. Given that piscidins are pH resilient and potent *in vitro* and *in vivo*, they constitute promising templates for biomedical applications. The research presented here focuses on our discovery that piscidins can use their amino-terminal copper-and-nickel (ATCUN) motif to bind to Cu^{2+}, which is a strong redox center. We present biological data demonstrating that Cu^{2+}-binding to the piscidin isoforms P1 and P3 enhances their antimicrobial and anticancer activities. We investigate the underlying mechanism of these effects by using complementary biophysical and biochemical methods, including solid-state NMR, density functional theory calculations, mass spectrometry, and permeabilization assays on P1, the more potent isoform, and several of its mutants. We find that: 1) once bound to Cu^{2+}, piscidin can form radicals, and oxidize lipids and DNA; 2) the stronger membrane permeabilization that piscidin achieves upon metallation correlates with a deeper bilayer insertion of the amino-terminal region; 3) several aromatic sidechains at the amino end of piscidin are within reach to interact with Cu^{2+} via cation-π interactions, and therefore can help stabilize the metal ion within hydrophobic bilayers.[2,3]

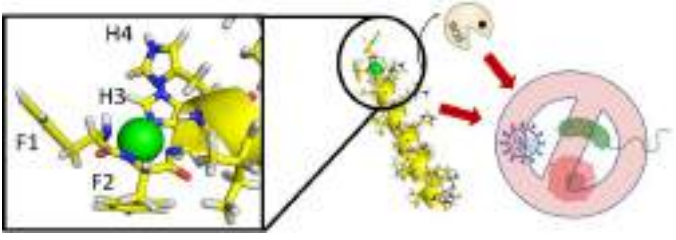

Figure 1. Piscidin exerts antimicrobial, antiviral, and anticancer activities. Biological activity can be enhanced through metallation and structural changes in the amino-terminal region. Some of the cartoons were obtained from Biorender.

Our work also shows that P1 is active against SARS-CoV-2. Using dye leakage assays, solid-state NMR, calorimetry, and cryo-EM, we characterize the ability of the peptide to alter the stability of viral envelope mimics constituted of the liquid ordered and disordered phases.

Important ramifications of our findings are discussed. For instance, we highlight the ability of piscidin to leverage multiple mechanisms, including metal binding, to kill pathogens where it is expressed, including within vital organs and their network of nerves. Furthermore, we discuss that Cu^{2+} helps enhance the biological activity of piscidin through both physical (e.g., peptide charge and conformation) and chemical (e.g., radical formation) changes. These results could be relevant to other membrane-binding peptides such as amyloids and neuropeptides that also bind to Cu^{2+}, are enriched in aromatic residues, and exist in tissues that perform immune and neural functions.

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Multistep translation of a cell-penetrating peptide into an antimicrobial peptide

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Since some time, antimicrobial peptides (AMP) have been evolved as promising alternatives to commonly used antibiotics. These peptides act by permeabilizing pathogen cell membranes, whereas being harmless to mammalian cells. Contrarily, another class of membrane-active peptides, namely cell-penetrating peptides (CPPs), is known to translocate in eukaryotic cells without substantially affecting the cell membrane. CPPs and AMPs share several physicochemical characteristics, therefore, in this study we aimed to direct the activity of a CPP towards antimicrobial activity.
Recently, we have developed a promising new cell-penetrating peptide, namely sC18, which we highlighted as efficient transporter in various applications. Within this work, we generated and screened a synthetic library based on the peptide sC18 to identify the active residues within the CPP sequence and to discover novel AMPs with high activity. Peptides with increased hydrophobicity were tested against various bacterial strains, and hits were further optimized leading to four generations of peptides, with the last also comprising fluorinated amino acid building blocks. We highlight new candidates, particularly those from generation 4, with promising antimicrobial activities against pathogenic strains and when immobilized on titanium surfaces. Since for some of them we also detected anticancer activity, these new peptides may present a valuable and promising source for the development of future therapeutics with antibacterial activity and beyond.

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Finding an amyloid precursor protein occlusion peptide to reduce the production of toxic Aβ species

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Alzheimer's disease (AD) affects 10% of the population over the age of 65 and 50% of the population over the age of 85. The most accepted theory behind AD is the amyloid cascade hypothesis (ACH), which focuses on the overproduction of toxic amyloid-β (Aβ) peptide species that forms the bulk of the senile plaques in brain, and these plaques are the definitive diagnostic marker of AD.
The amyloid precursor protein (APP) is a single-span membrane protein, and Aβ production depends crucially on γ-secretase cleavage of APP near a GxxxA transmembrane dimerisation motif. Experimental evidence suggests that the APP membrane domain forms an equilibrium of monomeric and homodimeric species, with the dimeric form resistant to γ-secretase proteolysis, as the amino acids targeted by γ-secretase lie in the buried interface of the dimer. Unfortunately, wild-type APP does not form a strong homodimer, and is predominantly in its monomeric protease susceptible form.
We have developed a multi-stage virtual assay based on all-atom and coarse-grain molecular dynamics (MD) simulations to find an APP mutant (occlusion peptide) that forms a highly stable heterodimer with APP to protect the GxxxA motif from γ-secretase, and thus avoiding the high production of toxic Aβ species with the ultimate goal of preventing the ACH from starting.

The mutants resulting from this virtual assay are synthesised in the solid-phase and evaluated experimentally. The results of the experimental analysis allow us to identify correlations between the experimentally observed results and various metrics which we measured from the simulations (hydrogen bonding, hydration, buried area of peptides, etc.) in order to develop predictive models, which in the future we could use to identify other mutants we may have not previously considered as well as for similar investigations of other transmembrane peptide oligimerisation studies.

Antimicrobial peptides: Mechanism of action and lipid-mediated synergistic interactions within the membranes

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

Interestingly mixtures of peptides such as magainin 2 and PGLa which are stored and secreted naturally as a cocktail exhibit considerably enhanced antimicrobial activities when investigated together in antimicrobial essays but also in pore forming experiments applied to biophysical model systems. Our investigations reveal that these peptides do not form stable complexes but act by specific lipid-mediated interactions and through the nanoscale properties of phospholipid bilayers. Notably, a quantitative idea about the strength of the lipid packing interactions can be obtained when comparing the peptide topologies in DMPC and POPC bilayers.

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The mechanisms of Lipid-targeting Antibiotics

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Antimicrobial resistance is a major threat to global health. To combat this threat, new antibiotics are urgently needed. Ideal candidates could be antibiotics that target special lipids that only exist in bacterial, but not in human cell membranes. These drugs kill refractory pathogens without detectable resistance. This has generated huge interest.

So far, the molecular mechanisms of lipid-targeting antibiotics have proven elusive due to technical challenges. Here, we determine the killing mechanism of teixobactin[1], considered the first new antibiotic in 30 years, on several length- (Å to mm) and time-scales (min to hr). We show that teixobactin kills bacteria by the formation of amyloid-like fibres that disrupt the bacterial membrane, which is a new type of antimicrobial action[2,3].

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Antimicrobial peptides against periodontal and periradicular bacterial infections

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Periodontal and periradicular diseases are inflammatory conditions of infectious origins that can lead to tooth loss and can develop into a systemic condition of hyper-inflammation possibly leading to broad range of diseases, including cardiovascular diseases (1-2).

Figure 1. Relationship between the infectious and inflammatory features of periodontitis and cardiovascular deseases.

Antimicrobial peptides M33D and M33Dl/i are tetra-branched nanopeptides active against Gram-negative and Gram-positive bacteria (3-4). They feature a notable long-life, unusual for peptides, due to the branched form that provides resistance to plasma, serum and bacteria proteases (3). Both peptides proved to act on the membrane of bacteria producing impairment of membrane functionality and death. The two peptides were also useful as bactericidal in dentin slices washings, abolishing bacteria regrowth. M33D also neutralized lipopolysaccharide (LPS) and lipoteichoic acid (LTA), thus exerting an anti-inflammatory activity. Chronic inflammation like that caused by periodontitis accelerates the progress of cardiovascular diseases (5). The use of antimicrobial peptides in tooth cavities washings and fillings is a very promising new field of development that provides tools to fight dental infections and their severe consequences and, at the same time, preserves standard antibiotics from resistances onset.

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Structure-activity analysis using computational mining of protein databases to assist design of antimicrobial peptides

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Antimicrobial peptides (AMPs) are produced by organisms from all kingdoms of life. AMPs can be ribosomal or non-ribosomal and contain various structural features [1-3]. They are a promising alternative to antibiotics to combat multi resistant pathogens. An analysis of existing AMPs is vital to understand (i) the relationship between the producing organisms and the peptide structure and (ii) if structural properties of AMPs correlate with their activity against a taxonomic group.

We leveraged over 10,000 peptides of the three antimicrobial peptide databases APD, DRAMP and DBAASP for computational analysis. After the application of stringent quality control criteria 3,828 peptides formed the basis for the analysis. The examination of structural characteristics of AMPs covered their molecular weight, charge at physiological pH, length, grand average of hydropathy (GRAVY), amino acid composition, and the occurrence of a γ-core motif. Additionally, a potential bias considering the choice of tested target organisms and the AMP producer was evaluated.

In this study we were able to show that the majority (62%) of AMPs possess the classic AMP characteristics of an average length of 40 amino acids, a positive net charge, a hydrophobic character, and a molecular weight higher than 2.5 kDa. Thus, up to 38% of AMPs deviate from this pattern. In particular, Gram-negative bacteria show a strongly deviating AMP pattern. Moreover, the γ-core motif is present in only 12.9% of analysed AMPs, challenging the hypothesis to be a unifying structural signature in all cysteine stabilized AMPs. Additionally, by correlating the producing organism of an AMP and the target organisms tested a bias in the choice of the latter could be revealed.

The understanding of AMP structure and the correlation of this structure with the producing organism(s) and their efficacy against specific taxa will accelerate the development of novel antimicrobial peptides. Furthermore, the generated insights advocate for improved standardization regarding the activity testing of AMPs.

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Peptide-porphyrin conjugates as promising antivirals against brain-targeting viruses

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Anti-infective drugs (antibacterials, antivirals, antifungals and antiparasitics) have been successfully used over the last century to fight a multitude of infectious diseases in humans, animals and plants. However, according to the World Health Organization (WHO), antimicrobial resistance is now one of the top ten global public health threats that humanity is facing. Viral infections are a specific case in point, not only because the current number of antiviral drugs is low, but also because their therapeutic spectrum is narrow, often restricted to the virus they were developed for. Among human-targeting viruses, brain-penetrating ones such as Zika (ZIKV) or HIV pose serious therapeutic challenges in that antiviral drugs with promising in vitro profiles, such as porphyrins, become ineffective in vivo due to the restrictive permeability of the blood-brain barrier (BBB). To face such difficulties, in the last two decades substantial efforts have been devoted to develop BBB shuttles, able to pass BBB and translocate various payloads. As most of these molecules are peptide-based the term "BBB peptide shuttle (BBBpS)" has been coined.

We hypothesized that, by conjugating a porphyrin antiviral to a BBBpS, one might circumvent the BBB challenge to fighting viral central nervous system (CNS) diseases. Therefore, we performed a comparative study of various on-resin conjugation strategies to generate peptide-porphyrin conjugates (PPCs) with potential antiviral activity and ability to cross BBB with good translocation rate. We found that carbodiimide activation produced highest yields independently of the BBBpS-porphyrin combination used. In total, sixteen novel conjugates, combining six BBBpS (P1-P6), two porphyrins (MPIX and PPIX) and one PEG-like linker (O_2Oc), were obtained with >90% purity. Most conjugates showed high serum stability and did not alter cell viability at broad range of concentration tested. Eight of them were active against ZIKV, while four also inactivated HIV, both in a micromolar range. Altogether, current results portray peptide-porphyrin conjugation as a promising strategy to tackle brain-residing viruses.

Acknowledgements
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Insulin analogues with altered insulin receptor isoform binding specificities and enhanced aggregation stabilities

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Insulin is a lifesaver for millions of diabetic patients. There is a need for new insulin analogues with more physiological profiles and analogues that will be thermally more stable than human insulin. Here, we describe the chemical engineering of 48 insulin analogues that were designed to have changed binding specificities toward isoforms A and B of the insulin receptor (IR-A and IR-B). We systematically modified insulin at the C-terminus of the B-chain, at the N-terminus of the A-chain, and at A14 and A18 positions. We discovered an insulin analogue that has Cα-carboxyamidated Glu at B31 and Ala at B29 and that has a more than 3-fold-enhanced binding specificity in favor of the "metabolic" IR-B isoform. The analogue is more resistant to the formation of insulin fibrils at 37 °C and is also more efficient in mice than human insulin. Therefore, [AlaB29,GluB31,amideB31]-insulin may be interesting for further preclinical evaluation.

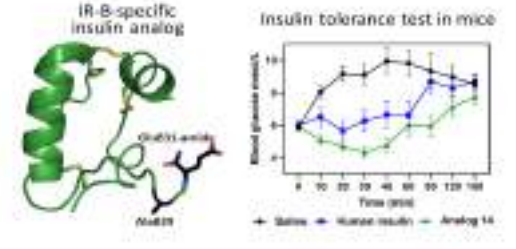

Figure 1. Structure of insulin analogue **14** (left) and results of insulin tolerance test in mice (right).

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Host defence amphibian peptides and their potential as antiviral agents

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Nowadays antimicrobial drugs are lacking of innovative therapies leading to the huge development of resistance. In this scenario the World Health Organization (WHO) ruled as priority the research of new agents able to fight the superbugs that are emerging. Not only bacteria, parasites and fungi are acquiring new escape mechanisms, also the viruses adopt new strategies in order to survive. Then the characterization and the development of new antiviral therapies are mandatory. Antimicrobial peptides (AMPs) could represent an important source of new anti-infective agents. They represent the most ancient and fast-acting elements of the host's innate defence system against microbial pathogens[1]. Concerning the fact that several AMPs were full characterized for their antibacterial activities, we investigated the potential of a member of temporin group, the temporin L (TL). The amphibian temporins belong to one of the largest families (more than 100 members) and are among the smallest-sized AMPs (10-16 amino acids) found in nature to date[2]. TL is the only temporin acting also against Gram-negative bacteria but it has, unfortunately, a relevant haemolytic activity[3]. Thus several modifications in the primary structure of TL were achieved in order to increase the antimicrobial activity and reduce the toxicity. Here we reported for the first time TL derivate, its analogues and lipidated peptides' antiviral evaluation as inhibitors of different enveloped viruses infections. Furthermore, other frog-derived peptides have been analysed for their antiviral effect, indicating a very broad range of activity against several viruses, including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Our results are promising for novel possible applications of amphibian peptides in the field of antiviral agents.

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The story of the peptide-copolymer Glatiramer Acetate (Copaxone) in the treatment of multiple sclerosis: from Bench to Bed side

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Multiple sclerosis (MS) is a complex disease in which inflammatory autoimmune reactivity in the central nervous system (CNS) results in demyelination, axonal and neuronal pathology. Treatment strategies aim to reduce the detrimental inflammation and induce neuroprotective repair processes.

Glatiramer acetate (GA, Copaxone) was originally designed as a peptidomimetic based on the myelin basic protein (MBP) autoantigen in attempt to induce experimental MS, and was found to be a disease-modifying therapy that reduces relapse rate and disability. GA is the first, and so far the only, therapeutic agent to contain a copolymer as its active ingredient. Using the animal model of MS, experimental autoimmune encephalomyelitis (EAE), we found that GA-treatment affects various levels of the innate and the adaptive immune response, generating immunomodulatory shift from the pro-inflammatory to the anti-inflammatory pathways. This includes downregulation of T- helper (Th) -1 and Th-17 cells and induction of Th2 and T-regulatory cells. Furthermore, T-cells specific to GA cross the blood brain barrier (BBB) and secrete *in situ* anti-inflammatory cytokines, inducing bystander effect on the CNS resident cells.

The consequences of GA-treatment on the CNS injury inflicted by the disease were studied using immunohistochemistry, electron microscopy, and magnetic resonance imaging. These analyses revealed reduced demyelination and neuro-axonal damages, as well as neuroprotective repair processes such as neurotrophic factors secretion, remyelination and neurogenesis. These combined findings indicate that a synthetic peptide-copolymer can counteract the inflammatory/neurodegenerative disease course, supporting linkage between immunomodulation, neuroprotection and therapeutic activity in the CNS.

Using synthetic molecular evolution to identify host cell compatible antimicrobial peptides effective against drug-resistant and biofilm-forming bacteria, *in vitro* and *in vivo*

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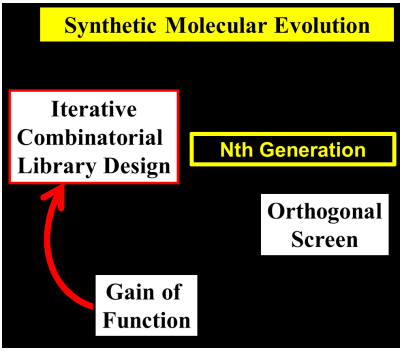

Membrane permeabilizing antimicrobial peptides have not succeeded in the clinic, in part due to impediments that limit their applications *in vivo*. These include low solubility, residual toxicity, susceptibility to proteolysis, development of resistance, and loss of activity due to binding to host cells, tissue, and serum proteins. We have used synthetic molecular evolution to evolve and optimize peptides that lack these impediments**.** The lead peptides we have discovered have broad-spectrum sterilizing activity against all ESKAPE pathogens, including biofilm-forming pathogens, both *in vitro* and *in vivo*. They are not inhibited by host cells, tissue, or serum proteins and retain activity in the protein- and cell-rich environment of a purulent, infected wound. The lead peptides block biofilm formation *in vivo* and are highly active against existing biofilms *in vitro*. The lead peptides are highly active against several independent collections of clinical isolates of drug-resistant bacteria, including pan drug resistant organisms. Perhaps most importantly, the lead peptides do not induce the development of resistance in Gram negative bacteria under conditions that drive rapid evolution of resistance to conventional antibiotics.

AMP-based therapy for non-healing wound care: advanced trap&release methods for non-invasive delivery materials

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Antibiotic treatments based on preventing the development of antibiotic-resistance are the focus of skin chronic non-healing infected wound care. Skin damages are often accompanied by bacterial contamination, whose care is further complicated in bedridden and elderly patients who have developed pressure sores and gangrenous wounds. If the use of systemic antibiotics is not advisable due to the presence of comorbidities which worsen the clinical picture,[1] the common approach for wound care is mechanical/surgical cleansing to remove the necrotic tissues, followed by medication for re-epithelization induction: a topic antibiotic can be used for this purpose, despite its activity can be reduced by dilution in wound exudates or partial removal during dressing change.
To contain the main drawbacks of this approach, the development of easy-to-use medical tools for fast and early mapping of dermal bacterial infections is the focus of this project, where an antimicrobial peptide (AMP) conjugated with a NIR-tag is loaded on a biomaterial.[3] The pH of the wound allows the release of the conjugate on the infection site, which can be visualized by a NIR-lamp to correlate the intensity of the signal with a quantitative evaluation of the bacterial colonization.

Figure 1. General scheme of AMP-NIR loaded biomaterial.

The antimicrobial peptide is Lytixar (LTX-109), an investigational tripeptide[2] displaying bactericidal lytic mechanism of action, proved by in vitro and in vivo models. In the current study, structural modifications on the original peptide have been introduced to proceed with covalent conjugation with NIR-tags, namely IR-783 and MHI-148, belonging to the family of cyanine dyes.[4] Currently, data on release from biomaterials and antibiotic activity are under evaluation.

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A peptide-based medical device for the selective removal of LPS, LTA and living bacteria from the serum of sepsis patients

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Lipopolysaccharide (LPS) from Gram-negative bacteria and, in rarer cases Lipoteichoic acids (LTA) from Gram-positive ones, are responsible for the development of local inflammatory response and, in extreme case of sepsis and septic shock. Sepsis is the leading cause of death in intensive care units (ICUs) throughout the globe[1]. Unfortunately, despite substantial advances in the pathophysiology of sepsis there is no efficacious therapy against this syndrome.[2] The use of antibiotics is often inefficacious[3], so alternative strategies are urgently necessaries. Here we report the use of the antimicrobial peptide SET-M33[4] in a medical device capable to remove selectively LPS, LTA and living bacteria from human serum and blood of animals inoculated with toxins. The medical device, constructed with a biocompatible matrix covalently linked to the peptide, is able to remove from sera more than 85% of LPS and LTA and about 99% of *Pseudomonas aeruginosa* or *Staphylococcus aureus*, two among the most important bacteria involved in the onset and evolution of sepsis. Sera processed in this medical device were also analysed for its possible modification of protein content resulting in no changes of serum α, β, and γ globulins in the electrophoresis profile.
This medical device is thought to be applied in an extracorporeal machine to be used in ICUs through a circulation system exemplified in the picture below.

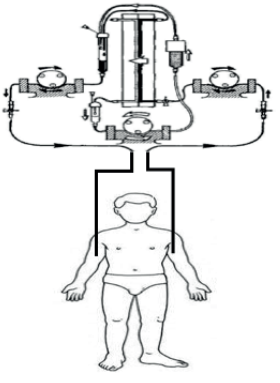

Figure 1. The extracorporeal circulation containing the peptide-based medical device for toxins and bacteria removal

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Bioinspired Materials: Physical Properties Governed by Biological Refolding

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Peptide and protein biomolecules, folded into two fundamentally different either α–helical or β-sheet conformations, carry out dissimilar biological functions. In living organisms α-helical secondary structure is adopted by different types of proteins such as myoglobin, keratin, collagen and more. Biological functions of β-sheet peptide/protein structures are different and associated with a wide range of human mental amyloid diseases such as Alzheimer, Parkinson, and more. The fundamental basis of these diseases is misfolding or refolding of natively soluble α-helical amyloid proteins into solid-state β-sheet fibrillary structures.

Bioinspired chemically synthesized biomolecules mimic their biological counterparts. Although these artificial and biological peptides/proteins molecules are completely dissimilar in origin and environment they demonstrate the common properties of folding and refolding into identical secondary architectures. In this work[1] we show that these two structural conformations, native (helix-like) and β-sheet, exhibit exclusive and different sets of fold-sensitive physical properties which are surprisingly similar in both biological and bioinspired materials. Native (helix-like) self-assembled fold having asymmetric structure, demonstrates ferroelectric-like pyroelectric, piezoelectric, nonlinear optical and electrooptical effects. β-sheet peptides/proteins structures acquire unique visible fluorescence (FL), and reveal a new property of lossless FL photonic transport followed by a long-range FL waveguiding in amyloidogenic fibers. Applied thermally-mediated refolding native-to-β-sheet allows to observe in details adoption, disappearance, and switching of the revealed physical properties in each fold and study dynamics of all critical stages of refolding from the metastable (native) helix-like conformation *via* intermediate disordered state to stable β-sheet fibrillary ordering. In the intermediate state appearance of the visible FL provides imaging, monitoring and direct observation of the early stages of seeding and nucleation of β-sheet fibrils. Found diverse fold-sensitive physical properties give a new insight in biological refolding processes and paves the way for development of advanced physical methods of folds' recognition, bioimaging, light diagnostics and therapy at nanoscale and peptide/protein nanophotonics from new visible FL bionanodots to bioinspired multifunctional peptide photonic chips.

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The Peptide Nucleic Acid leitmotif and its application in miRNA targeting

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Peptide nucleic acid (PNA) is an attractive and powerful nucleic acid mimic developed by Nielsen and co-workers in 1991.[1] PNA possesses a pseudo-peptide backbone of N-(2-aminoethyl)glycine motif (*aeg*) to replace the sugar phosphate one of DNA/RNA; whereas the nucleobases are linked to the main skeleton through a methylene carbonyl linker.[1] The peptide backbone, associated with the conserved ability of PNAs to form complementary Watson-Crick-Franklyn base pairs, is responsible for a number of desirable properties including greater affinity for complementary nucleic acids, enhanced specificity, and excellent resistance to chemical and enzymatic degradation.[2,3] Recently, miRNAs, a class of small non coding RNAs, [4] have emerged not only as novel cellular regulators but also as significant biomarkers being deregulated in various human diseases. [5] As a result, miRNAs represent an appealing class of molecules in both drug development and diagnostic field. In this respect, PNAs based molecules represent excellent candidates for miRNAs targeting. We will exemplify the ability of PNAs based molecules to target miRNAs in two case studies: a) PNAs as analogues of tumour suppressive miRNA-34a;[6] b) PNAs as smart receptor probes for multiple detection of miRNAs biomarkers.[7] In the first case, the design and synthesis of PNA-based analogues of tumour suppressive miRNA-34a were realized, and their interaction with two binding sites on the target MYCN mRNA was investigated by molecular dynamics simulation and spectroscopic techniques. In the second case PNA based smart probes (fluorescent and electrochemical), were synthesized and characterized by spectroscopic/electrochemical techniques. These smart probes were developed to address the lack of fast /cost effective diagnosis in several diseases.

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Pursuit of "Qualified" Antimicrobial Peptides to Challenge the Antibiotic Resistance

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Antimicrobial peptides (AMPs) represent valid tools to tackle the antibiotic resistance phenomenon as they act thorough alternative mechanisms of actions with respect to conventional antibiotics.[1] Among them, temporins, LL-37, aurein-1.2 and magainin have interesting properties for biological investigations due to *i)* short sequence length, *ii)* activity against a wide range of pathogens, *iii)* additional chemotactic activity and immunomodulatory effects.[2] The 13-mer temporin L (TL) peptide is one prominent example, showing elevated antimicrobial potency, strong affinity for Gram-negative such as *P. aeruginosa* and *E. coli*, although it also exhibits a significant hemolytic activity at microbicidal concentrations.[3] Our recent efforts have been aimed at improving the therapeutic index and drug-like features through the application of different chemical approaches. A library of macrocyclic peptide analogues of TL was obtained by embracing different intramolecular linking strategies (*e.g.,* lactam, 1,4-triazolic and hydrocarbon bridges),[4] while chemical tag motifs, including fatty acids, were introduced at key locations, to further control target cell specificity.[5] Antimicrobial peptidomimetics with greater broad-spectrum antimicrobial activity, including resistant strains, and less toxicity, were thus identified as encouraging candidates for the development of new antimicrobial "weapons" with further biomedical applications.

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Radiolabelled peptides in cancer diagnosis and therapy

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There has been steadily increasing interest in the clinical application of radiolabelled peptides in cancer diagnosis and treatment over recent years. The targeting of somatostatin receptors with radiolabelled peptides provides a paradigm where this type of research has gone full circle from bench to routine patient care. The somatostatin analogue octreotide was described in the late 1980s and utilised to treat carcinoid syndrome by activating somatostatin receptors (SSTRs) overexpressed in certain neuroendocrine tumours. It was soon realised that radiolabelled octreotide could be used to visualise tumours in vivo. ^{111}In-DTPA-Octreotide was approved by the FDA in the early 1990s for gamma camera imaging and single photon emission computed tomography (SPECT). It was immediately recognised as a sensitive and specific tool to image these types of cancers. In the 1990s somatostatin receptor targeting peptides were further developed improving binding affinity and allowing coupling to other radiometals. Positron emission tomography (PET) imaging with these analogues labelled with ^{68}Ga followed. Multiple case series demonstrated the superiority of ^{68}Ga-SSTR PET imaging compared to ^{111}In-SSTR scintigraphy in the 2000s. This resulted in the approval and registration of SSTR binding agents for PET imaging by both the FDA and the EMEA in the 2010s. The use of these same peptides to deliver lethal doses of radiation to tumours was also extensively investigated in the 2000s and 2010s. These preliminary efforts lead to the phase 3 NETTER-1 trial which showed significantly improved progression free survival of patients with advanced neuroendocrine tumours treated with ^{177}Lu-DOTATATE compared to controls. The last few years have seen approval and registration of this treatment around the world.

As the use of this therapeutic and diagnostic (theranostic) approach has been validated, there is great interest in exploring other peptide systems for this purpose. Focus has largely remained on G-protein coupled receptors given their biological relevance and high levels of expression in certain cancers. At the time of this writing there are ongoing registered trials (phase 1 to 3) addressing Gastrin Releasing Peptide Receptor (GRPR), Cholecystokinin-2/Gastrin (CCK2) receptors, chemokine receptor 4 (CXCR-4) receptors, neurotensin receptors and new indications for existing SSTR agents. Novel SSTR binding compounds are also being evaluated in clinical trials. These studies are mostly focused on therapeutic endpoints in specific diseases and utilise PET imaging for patient selection and treatment monitoring. The increasing recognition and availability of PET with ^{68}Ga labelled compounds as a sensitive and specific way to image and characterise cancer has been a major driver for development of these types of applications.

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Peptide based hydrogels and nanogels for biomedical applications

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Due to their low cost, biocompatibility and high tunability peptides have been proposed for many applications in biomedical and biotechnological field [1]. In the last years, short and ultrashort peptides have been also proposed as innovative building blocks for the fabrication of supramolecular biomaterials like nanotubes, nanospheres, fibers, hydrogels (HGs) and nanogels (NGs) [2]. HGs are self-supporting materials, structured as supramolecular hydrophilic networks associated with the construction of space-spanning structures characterized by a non-Newtonian behaviour. On the other hand, NGs are hydrogel particles with a size in the nano-range. NGs combine the same hydrated inner network of hydrogels with the size of injectable nanoparticles such as micelles and liposomes. They can be prepared by top-down methodologies for submicronization of HGs in presence of opportune stabilizing agents [3]. Here we describe a novel class of peptide based HGs and NGs as potential extracellular matrices for tissue engineering and/or delivery systems for the vehiculation of active pharmaceutical ingredients (drugs and MRI contrast agents) [4]. The functional and mechanical properties of these supramolecular architectures were modulated by punctual modification of the peptide primary sequences or by combining two or more sequences together.

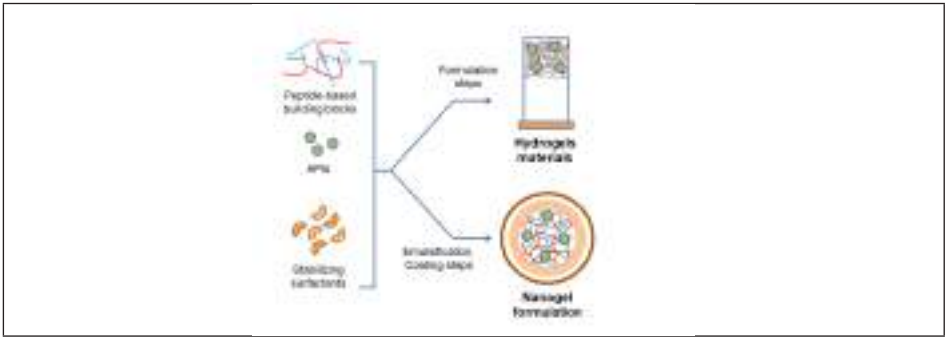

Figure 1. Schematic representation of peptide-based hydrogels and nanogels filled with active pharmaceutical ingredients

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A cognitive protection peptide for cannabis-based pain treatment

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Activation of cannabinoid CB1 receptors (CB1R) by Δ9-tetrahydrocannabinol (THC), the main psychoactive component of Cannabis sativa, produces useful analgesic effects. CB1R activation, however, has also undesirable outcomes (e.g., memory loss) that constitute important drawbacks for the use of cannabinoids as therapeutic agents.

We have explored the possibility of dissociating beneficial from detrimental THC effects through an approach based on splitting the cannabinoid CB1 and serotonin 5HT2A receptor heterodimer, *i.e.* CB_1R-$5HT_{2A}R$, whose co-activation by THC triggers cognitive impairment. We recently showed that peptides reproducing the CB_1R transmembrane (TM) helices 5 and 6 involved in CB_1R-$5HT_{2A}R$ dimerization, when fused to a cell-penetrating peptide (CPP), can access and disrupt the complex formation,[1] avoiding the adverse cognitive effects of THC but preserving analgesic response.

Full length TM5 and TM6 helices, however, are unwieldy drug leads due to limitations such as low water solubility, high protease susceptibility, poor membrane permeability and immunogenicity. We will report how, starting from those prototypes,[1] we developed a downsized, protease-resistant, orally available, non-immunogenic peptide that, fused to an enhanced blood-brain barrier-crossing CPP, restricts both *in vitro* and *in vivo* the CB_1R-$5HT_{2A}R$ heterodimer formation (Figure 1) responsible for unwanted cognitive impairment.[2] In addition, we show that our candidate (EP19382856.3, WO2021064165) does not alter THC analgesia in mice upon chronic oral administration. To our best knowledge this may become the first drug-like peptide able to protect medical cannabis users from cognitive deterioration while ensuring pain relief.

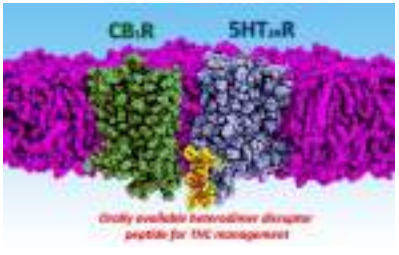

Figure 1. Heterodimer complex between CB_1R (green) and $5HT_{2A}R$ (blue) disrupted by an optimized CPP-coupled candidate (red backbone, yellow surface).

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Self-Assembled Peptides for Electronic and Piezoelectric Applications

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In recent years, a key direction in the field of electronics and electro-optics involves the transition from inorganic to organic components, thus paving the way towards flexible and wearable electronic devices. Bio-inspired organic materials may be the next-generation of organic optoelectronic devices, based on self-organization principles, which allow facile synthesis, eco-friendliness, resistance to oxidation and no need for heavy metal doping. Recent advances in bioorganic nanotechnology have established the notion that very simple building blocks, such as dipeptides, can form regular nanostructures with distinct mechanical, optical, piezoelectric and electronic properties. In particular, members of the diphenylalanine (FF) peptide archetypical family have been shown to form various morphologies and ordered nanostructures such as tubes, rods, fibrils, spheres, plates and macroscopic hydrogels with nano-scale order. These unique self-assembled peptide materials may be useful in the emerging field of implantable medical devices, which requires biocompatible power solutions to enable continuous and wireless operation.

The abundant mechanical energy stored in our body, ranging from body motion, walking, breathing to internal movement of organs, heartbeats and blood pressure, can be converted into electrical energy by the piezoelectric effect to serve as an autonomic electrical source. Currently, the field of piezoelectric materials mainly relies on lead-based ceramic materials such as lead zirconate titanate (PZT). However, as lead is toxic and entirely non-biocompatible, there is an emerging need for alternative materials with strong piezoelectric performance, especially in the field of bio-applications. Piezoelectric bio-inspired materials have attracted significant attention in recent years as promising alternatives for currently used poisonous piezoelectric materials owing to their strong piezoelectricity along with their biocompatibility. Specifically, several studies have explored the piezoelectric properties of the FF peptide. Although significant progress has been made toward the functionalization of piezoelectric biomaterials, challenges in the formation of well-ordered nanostructures limit their application. Controlling the organization of such assemblies is a crucial milestone in engineering applicable piezoelectric materials since the magnitude of the piezoelectric response is dictated by the molecular organization at the nanoscale. Here, we demonstrate the functionalization of piezoelectric FF derivatives by nano-structural alignment. We have developed a custom-made measurement system for piezoelectric performance evaluation, calibrated using a commercially available piezoelectric material. Utilizing this system, we show the realization of self-assembled peptides as a promising piezoelectric alternative for bio-compatible energy solutions.

Designing New Bioinspired 3D Hydrogels for Tissue Regeneration

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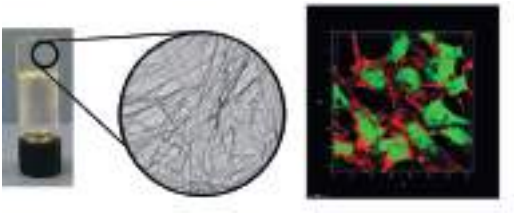
The emerging demand for tissue engineering scaffolds capable of inducing bone regeneration using minimally invasive techniques prompts the need for the development of new biomaterials. One promising route is molecular self-assembly, a key direction in current nanotechnology and material science. In this approach, the physical properties of the formed supramolecular assemblies are directed by the inherent characteristics of the specific building blocks. Molecular co-assembly at varied stoichiometry substantially increases the structural and functional diversity of the formed assemblies, thus allowing tuning of their architecture and physical properties.

Here, in line with polymer chemistry paradigms, we applied a co-assembly approach using hydrogel forming peptides, resulting in a synergistic modulation of their mechanical properties to form extraordinarily rigid hydrogels which supported osteogenic differentiation based on cells-mechnosensing. Furthermore, we designed a multi-component scaffold composed of polysaccharides, short self-assembling peptide and bone minerals. We demonstrate the formation of a rigid, yet injectable and printable hydrogel without the addition of cross-linking agents. The formed composite hydrogel displays a nanofibrous structure, which mimics the extracellular matrix and exhibits thixotropic behaviour and a high storage modulus. This composite scaffold can induce osteogenic differentiation and facilitate calcium mineralization.

This work provides a conceptual framework for the utilization of co-assembly strategies to push the limits of nanostructure physical properties obtained through self-assembly for the design of new biomaterials for tissue engineering and personalized medicine applications.



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Quorum sensing peptides as microbiota-derived mediators in different diseases

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Several diseases, including colorectal cancer, sarcopenia and psychiatric/mental disorders, are associated with alterations in the human microbiota composition. However, to date, the central mediators between the gut bacteria and these diseases are largely unknown. *In vitro* and *in vivo* studies from our group have now shown that quorum sensing peptides, *i.e.* metabolites that are constitutively produced by the microbiota and traditionally viewed as inter-bacterial communication molecules, are able to functionally influence host cells, and thus might be part of the central mediators in the microbiome-host crosstalk phenomenon.

In an orthotopic colorectal cancer mice model for example, a quorum sensing peptide from *E. faecium* (*i.e.* EntF*) was found to promote tumour metastasis, with metastatic lesions found in both liver and lung tissues (Figure 1). Moreover, colonization studies in gnotobiotic mice with EntF*-producing *E. faecium* strains, also indicated the presence of EntF* in the colon content, the feces and the serum of the conditioned mice, thereby indicating the possible role of the microbiota in this disease. In sarcopenia, different quorum sensing peptides showed effects on C2C12 myoblast cells, with effects observed on both viability and inflammation. In psychiatric disorders, a quorum sensing peptide from *Bacillus* species (*i.e.* PapRIV) demonstrated to have pro-inflammatory effects on BV-2 microglia cells, cells which are crucial in physiological brain function.

Altogether, these results strongly indicate a causal relationship between microbiota-derived quorum sensing peptides and different diseases.

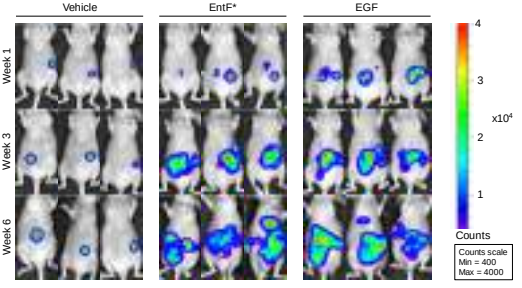

Figure 1: The *in vivo* metastatic potential of the colorectal cancer cells after treatment with vehicle, EntF* quorum sensing peptide (100 nmol/kg) and positive control (EGF, Epidermal Growth Factor, 0.1 mg/kg).

Chemoselective Modification of Cysteine and Selenocysteine in Peptides and Proteins

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Chemoselective modification of peptides and proteins has wide applications in chemical biology and pharmaceutical development. We report an efficient chemo- and stereoselective cysteine (Cys) functionalization protocol via rationally designed *β*-addition of ynamides. A substituent of the terminal ynamides offers a handle for functionality diversification. This Cys modification with ynamides proceeds efficiently in a slightly basic aqueous media (pH 8) to provide a series of *Z*-isomer of the corresponding conjugated products with excellent stereoselectivity (> 99%) and superior stability. All the reactive peptide side chain functional groups such as amino, carboxyl, primary amide, and hydroxyl groups, as well as the unprotected imidazole and indole rings are compatible. This method displays a broad substrates scope including linear and cyclic peptides and proteins. The potential application of this method in peptide and protein chemical biology was exemplified by Cys-bioconjugation with ynamides containing different functional molecules, including drug, fluorescent and affinity tags. In addition, this strategy is also compatible with click chemistry (performed in one pot), which remarkably extends the toolbox for further applications.

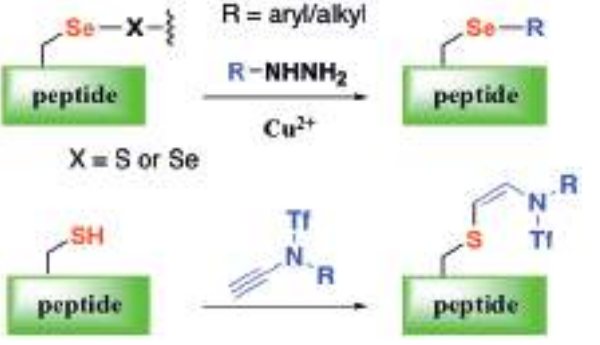

Figure 1. Chemoselective modifications for Cys and Sec in peptides and proteins.

The development of new efficient, chemoselective bioconjugation tools for reactive functional groups is highly desired, especially those that are chemoselective even in the presence of free Cys residues, or disulfides. Herein, we report the chemoselective modification of peptides and proteins via a reaction between selenocysteine residues and aryl/alkyl radicals. *In situ* radical generation from hydrazine substrates and copper ions proceeds rapidly in an aqueous buffer at near neutral pH (5−8), providing a variety of Se-modified linear and cyclic peptides and proteins conjugated to aryl and alkyl molecules, and to affinity label tag (biotin).

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Spontaneous assembly of functional membrane proteins from soluble membrane active peptides

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Experimental structural techniques have provided a wealth of molecular detail information on how proteins perform their functions in membranes. In contrast, little is currently known about functional structures and dynamics of pores that form only transiently in the membrane. This is chiefly due to a lack of technology that can capture transient structures in fluid membranes.

Here we demonstrate and experimentally validate a new methodology, based on unbiased long-timescale atomic detail equilibrium molecular dynamics simulations, that captures the entire assembly process of transient pores in lipid bilayers multiple times. This provides an unbiased and fully converged description of the assembly process.

Starting from unfolded peptides in water this approach predicts the insertion mechanisms and native state structures of membrane active peptides at atomic resolution,[1] accurately reproducing experimental ensemble averages and partitioning data determined via synchrotron radiation circular dichroism spectroscopy and in vitro translocon experiments.[2]

Once inserted peptides spontaneously assemble into functional channels in the bilayer that continuously form and disband.[3] Remarkably, a single peptide is able to generate a large ensemble of functional pores of different sizes, lifetimes, and conductivities. This explains why, despite millions of years of co-evolution pore-forming peptides are still highly toxic to bacteria. The largest pore size was validated indirectly using a an assay that measures peptide-induced leakage of dyes encapsulated in vesicles. Finally, the approach is applied to attempt the design of novel functional peptides.

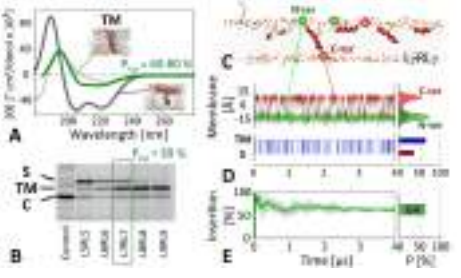

Figure 1. Oriented CD (A) and *in vitro* partitioning experiments (B) provide thermodynamic averages of the insertion propensity of a peptide (GL_7RL_7G). Unbiased MD simulations reveal that the native state is an ensemble of surface bound (S) and transmembrane configurations (TM), and provide additional information on the atomic detail structural ensemble, membrane insertion mechanism (C), kinetics (D), and thermodynamics (E), which are in quantitative agreement with the experiments (PTM = 70±10%).

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Metal-coupled folding as the driving force for zinc proteins function - insight into sequence-structure-stability relationships in zinc finger, hook and clasp domains

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Metal ions are essential elements required for many cellular processes including proliferation, signalling, DNA synthesis and repair. Extensive research over the past few years has demonstrated that Zn(II) ions play a unique role in protein folding and structure stabilization. Yet factors governing these metal-coupled events as well as zinc protein affinity remains unclear. Our study on zinc proteins underlines the importance of a deep understanding of relationship between sequence, structure and affinity.[1] Commonly known structural zinc sites display high Zn(II) affinity and compact architecture needed for sequence-specific binding.[2] They usually bind Zn(II) by Cys and His residues in structurally diverse tetrahedral cores. Our recent study shows that sequence composition of zinc cores found in zinc fingers influences the metal dependent folding and Zn(II) affinity.[3] Besides intraprotein zinc sites, interprotein Zn(II)-binding sites are getting acknowledged as the factors paramount for zinc-proteome biology. Rad50 zinc hook protein complex arose to be an unequalled example of Zn(II) interprotein site, conserved from Archaea to humans and even viruses. Our group's findings indicate that metal binding by the hook motif initiates a nucleation process resulting in rigid superhelical structure.[4] Moreover, our newest research suggests that Zn(II) can be replaced with Cd(II) in zinc hook causing a significant change of dimer's conformation and complex's stability.[5] Another example of Zn(II) binding at the protein interface is the zinc clasp - zinc-mediated interaction between the cytosolic C-terminal tail of the CD4 receptor and an N-terminal fragment of non-receptor protein tyrosine kinase (Lck). Our rationally optimized zinc clasp-based motif highlights the utilization of metal-driven interactions to develop reversible protein heterodimerization systems.[6] Overall, using a set of chemical strategies, spectroscopic and structural approaches we aim to study the relationship between structure-function-stability of structural zinc-binding sites.[1-6]

Acknowledgment
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Influence of xenobiotic metal ions on structure and folding of the prokaryotic zinc finger Ros87

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Metalloproteins must bind their metal cofactors with suitable affinity to carry out their biological function; even when the cofactor plays an exclusive structural role, it is essential to protein activity. For this reason, great attention has been devoted to the influence of xenobiotic metal ions on the structure, folding mechanism, and functions of metalloproteins. Xenobiotic ion can act in different ways: they can address the correct folding of a protein either maintaining or inhibiting its function. Moreover, in some cases, they do not address the correct folding or they form mixed complexes together with the native metal ion.

In the case of zinc finger domains, both eukaryotic and prokaryotic, the active fold is achieved by combining the structuring effect of the zinc cofactor and the formation of a hydrophobic core. In the case of a prokaryotic domain, an extensive hydrophobic core contributes to the folding mechanism of a larger domain. Within this context, we here report the study of the structural and functional effects of xenobiotic metal ion replacement in the coordination sphere of zinc fingers. The effects of the binding of Co(II), Ni(II), Hg(II) and Cd(II) on the structure and folding mechanism of Ros87, the DNA binding domain of the prokaryotic zinc finger protein Ros, will be examined and discussed.

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Designed peptides as potent inhibitors of amyloid self-assembly and cross-seeding of IAPP and Aβ42

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Amyloid self-assembly is linked to numerous devastating cell- and neurodegenerative diseases. Two prominent representatives are Alzheimer' disease (AD) and type 2 diabetes (T2D). Epidemiological evidence suggests that T2D patients have an increased risk of AD and vice versa. In addition, the two diseases appear to be linked at the molecular level as well. In fact, cross-seeding interactions between Aβ and IAPP, the key amyloid polypeptides of AD and T2D, respectively, dramatically accelerate amyloid self-assembly of both polypeptides as shown by both in vitro studies and in vivo studies with mouse models [1,2]. Also, the two polypeptides colocalize in AD or T2D amyloid deposits in humans and mouse models [3]. Therefore, molecules targeting amyloid self-assembly and reciprocal cross-seeding of Aβ and IAPP could be promising candidates for anti-amyloid drugs for both AD and T2D.
We have previously shown that designed IAPP-derived peptides are potent inhibitors of amyloid self-assembly of both IAPP and Aβ [4]. Here we will present a novel class of conformationally constrained peptides which are both nanomolar affinity cross-amyloid inhibitors of IAPP and Aβ42 and able to effectively suppress reciprocal cross-seeding of the two polypeptides. These peptides were designed to mimic Aβ/IAPP cross-interaction surfaces and were found to function via a novel and unexpected co-assembly mechanism. Their favorable properties make them to promising leads for the development of anti-amyloid treatments for both AD and T2D.

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PNA to encode and fold peptide

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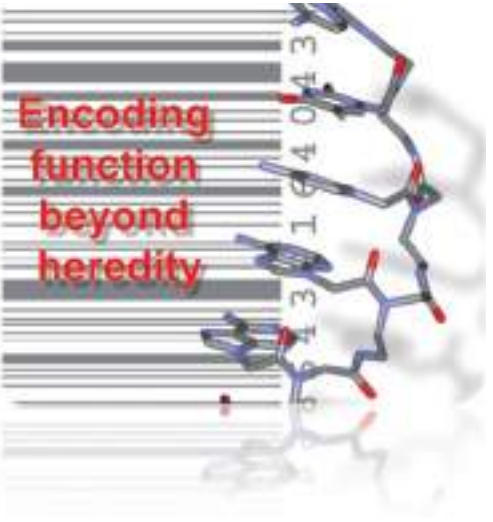
The programmable nature of nucleic acid hybridization has inspired a number of applications beyond their natural function in heredity. Peptide Nucleic Acids (PNA) are endowed with attractive properties for this endeavor as they are more robust and form more stable duplex than their natural counter parts. Several applications from our laboratory to encode libraries and program the folding of peptide will be presented including the use of PNA to assist in the ligation of peptides and folding into a helical conformation as well as an example of DNA display of peptides amenable to Darwinian evolution.



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Hybrid α/β Peptide Ligands Targeting $\alpha_4\beta_1$ Integrin: Therapeutic and Diagnostic Applications

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The $\alpha_4\beta_1$ integrin is a heterodimeric cell surface receptor expressed on most leukocytes, involved in the development and sustainment of inflammation, and in several inflammation-related diseases.
We developed a new class of hybrid α/β peptidomimetic ligands (Figure). Among the antagonists, the peptidomimetic **1** emerged as an effective anti-inflammatory agent in a guinea pig model of allergic conjunctivitis, and was found to inhibit leukocytes recruitment to the retina, potentially useful for the treatment of Age-related Macular Degeneration.
Cyclization of the structure yielded the peptides **2**, among the most potent $\alpha_4\beta_1$ integrin agonists reported to-date. Recently, such agonists rose much interest for their ability to steadily block leukocytes rolling onto the endothelial surface, preventing them to reach the sites of inflammation. Besides, the cyclopeptides **2** allowed to explore by QM computations the structural requirements at the basis of agonism or antagonism. The simulations suggested of a possible role of the agonists as small-molecule PPI stabilizer, capable to prearrange the receptor in a semi-activated conformation.

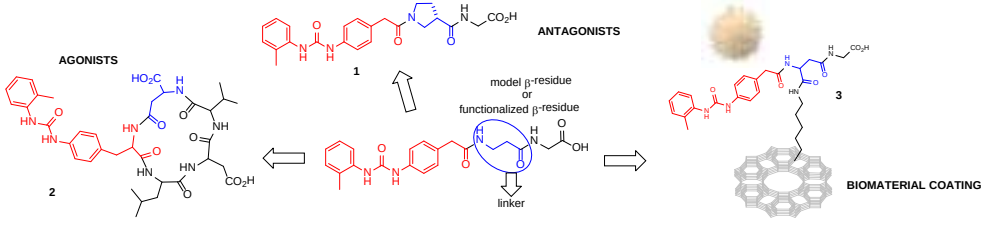

Figure. Diverse applications of hybrid α/β peptidomimetic $\alpha_4\beta_1$ integrin ligands

Finally, α/β peptidomimetics carrying linkable side chains can be grafted onto biomaterials, nano- and microparticles, for the design of functional cell-responsive materials. For instance, zeolite monolayers coated with peptide **3** were able to capture selectively α4β1 integrin-expressing cells.

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Multicomponent Supramolecular Polymers as a Platform for the Design of Glycoconjugate Vaccines

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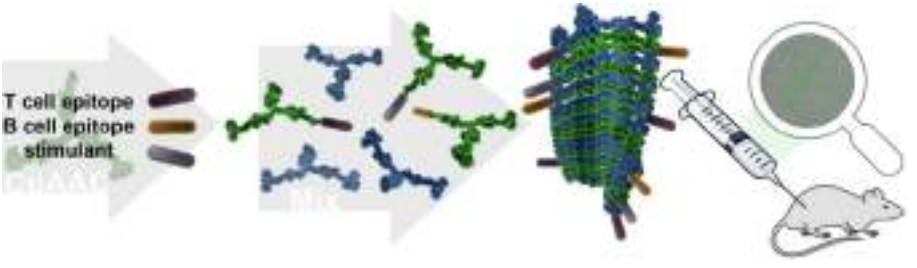
Peptide secondary structures can be harnessed to design monomers capable of self-assembling into nano-scaled supramolecular structures in aqueous media.[1,2] Decorating the surface with immunogenic molecular patterns results in pathogen-mimicking entities and potential vaccine candidates.[3] In the context of antitumor vaccines, the challenge is to overcome self-tolerance mechanisms to enforce an immune response against endogenous, tumor-associated glycopeptide motifs.[4] To this end, a co-stimulation of B cells with Th cells is mandatory, which we aim to achieve using a co-presentation of different epitopes and immunostimulating agents at the surface of multicomponent supramolecular polymers. Mucin 1 (MUC1) is well-known for undergoing alterations in O-glycosylation during tumorigenesis, and is thus an excellent tumor-associated target structure for immunotherapy. In this contribution I discuss the use a fully synthetic glycopeptide from the MUC1 tandem repeat sequence, which consists of 22 amino acids bearing the Tn and 2,3-Sialyl T tumor associated antigens. As T cell epitope we chose a small fragment from highly immunogenic tetanus toxin (p30). Additionally, an imidazoquinoline as potent TLR7/8 agonist, was synthesized. These epitopes were conjugated to supramolecular monomers and mixed in aqueous solution to yield a polymeric vaccine formulation. The vaccines were administered intraperitoneally to C57BL/6 mice and the antisera were collected after three further boosts. High antibody titers of the IgG type were observed in all mice. Furthermore, FACS analysis confirmed the high binding affinity of the generated antibodies to T47D tumor cells. These results support the potential of this modular supramolecular platform approach for the development of antitumor vaccines.



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Membrane-binding Peptides for Extracellular Vesicles On-chip Analysis

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Extracellular vesicles (EV) are membranous micro- and nano-sized biological particles released by cells that shuttle an impressive amount of molecular information, thus representing a phenomenal source of biomarkers. To date, the highest clinical relevance is thought to reside in small EV (sEV), including exosomes, which are arising unparalleled expectations as the next generation theranostic tools. Here we report on the use of membrane curvature sensing peptides (MSPs) as new, highly efficient ligands to directly integrate sEV capturing and analysis on a microarray platform[1,2]. Samples were analyzed by label-free, single particle counting and sizing, and by fluorescence co-localization immune staining with fluorescent anti-CD9/anti-CD63/anti-CD81antibodies. Peptides performed as selective yet general sEV baits and showed a binding capacity higher than conventional anti-tetraspanins antibodies. The role of surface chemistry for optimal peptide performances was found to be crucial, as capturing efficiency is strictly bound to probes surface orientation effects. This work represents the first proof-of-concept demonstration of the use of peptides as molecular baits for extracellular vesicles analysis. We anticipate that this new class of ligands, also due to the versatility and limited costs of synthetic peptides, may greatly enrich the molecular toolbox for EV analysis.

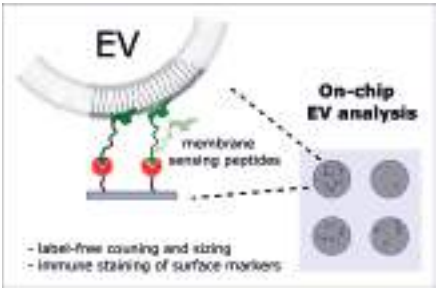

Figure 1. MSPs are used to capture sEV on microarrays, which are then characterized in terms of size, count and surface markers

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Semisynthetic approaches for studying post-translational modifications

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Glycoproteins are of special interest because of their huge therapeutic potential, but also because there is still limited understanding of the oligosaccharide's function. Moreover, glycoproteins present microheterogeneity in Nature, which makes it difficult to elucidate the correlation between glycan structures and functions. We developed a platform with which we can systematically test what correlation exists between the structure of an N-linked glycan and its effect on protein structure and stability. Moreover, we replaced the glycans by polyethylenglycol (PEG) to distinguish between sugar-specific or solubility-based effects.

By exploiting CuAAC and genetically incorporated non-natural amino acids we could generate isoform Erythropoietin (EPO) variants with defined synthetic azide-glycans at each individual natural occurring glycosylation sites. The conjugates displayed high *in vitro* bioactivity depending on the structure of the glycans and their position.[1]

To investigate if PEGylation can "replace" glycosylation, we employed the incorporation of the nnAA *p*-azidophenylalanine (pAzF) into bacterially expressed EPO at each single naturally occurring glycosylation site in combination with the chemoselective Staudinger-phosphite reaction to install branched PEG chains at defined sites. PEGylation with two short PEG chains at positions 24, 38, or 83 significantly decreased unspecific aggregation and proteolytic degradation while biological activity *in vitro* was preserved or even increased in comparison to full-glycosylated EPO.[2] The developed molecular tools are expected to be generally applicable to engineering of therapeutic semisynthetic proteins.

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Physical gels formed by Dopa-containing ultra-short peptides

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Physical gels are materials consisting primarily of a solvent (often water) and have solid-like properties, due to the presence of a fibres network which immobilises the liquid phase.
The self-aggregation of small gelator molecules leads to the formation of entangled Self-Assembled Fibrillar Networks (SAFINs) through a combination of non-covalent interactions like H-bonding, π–π stacking, donor–acceptor interactions, metal coordination, solvophobic forces (hydrophobic forces for gels in water) and van der Waals interactions. These fibres form a three-dimensional network that immobilises the water, thus behaving as a supramolecular cross-linked polymer in the hydrogel formation.

We present here ultra-short peptides containing Tyr (tyrosine), Dopa (3,4-dihydroxyphenylalanine) or halogenated aromatic ring, that are able to form additional H-bonds with other groups, thus improving their gelling ability. The possibility to form hydrogen bonds and the presence of the aromatic ring allow L-Dopa molecules to interact through a series of non-covalent interactions.
The additional presence of the catechol moiety really makes this compound unique: it has implications in the self-assembly process of Dopa itself and with other substrates and highly increases the number of applications of the final material.

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Halogenation as versatile tool for tuning structures and properties of amyloid peptides

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Besides pathological roles in many diseases, *e.g.*, Alzheimer's, Parkinson's, Creutzfeldt–Jakob, and Huntington's, amyloid peptide architectures have found many other non-biological applications such as forming highly ordered nanomaterials. Together with their biocompatibility and the ease of production, amyloidogenic peptides show a very polymorphic behavior yielding a broad range of hierarchical structures, such as tapes, ribbons, fibers, nanoparticles, and nanotubes.

Subtle variations in the experimental conditions, peptide sequence or its chemical functionalization may impact the self-assembly pathway and, consequently, the resulting nanostructures.

Here we report how halogenation can be used as versatile tool to tune structure and properties of amyloid peptides. Specifically, we show that depending on the number, position, and nature of the halogen atoms introduced into either one or both phenylalanine benzene rings of the amyloid β peptide-derived core-sequences such as DFNKF (H_2N-Asp-Phe-Asn-Lys-Phe-COOH), KLVFF (H_2N-Lys-Leu-Val-Phe-Phe-COOH) and DSGYEV (H_2N-Asp-Ser-Gly-Tyr-Glu-Val-COOH), different architectures and properties can be obtained in a controlled manner. [1,2,3]

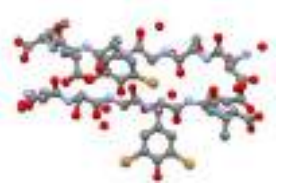

Figure 1. Crystal structure of DSGY(3,5-Br)EV

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Design of enzyme-inspired multivalent catalysts through functional patterning of nanosystems

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Enzymes are examples of the exquisite tools Nature evolved to perform a broad range of functions. Native enzymes often possess levels of chemo-, enantio-, and site-selectivity that are unparalleled by synthetic catalysts.[1] This is reflected in the growing interest of the scientific community in the development of artificial enzymes. The use of nanoparticles (NPs) provides new opportunities for the development of effective and safe ensembles of molecules for constructing enzyme-inspired assemblies. The self-assembly of peptides on the surface of metal nanoparticles has recently emerged as a powerful means towards creating arrays of functional groups in a defined geometry and space.[2,3] This strategy provides the opportunity to induce cooperativity between the functionalities in the assembly, a feature typical of enzymes. We present steps towards multivalent catalysts by a fine-tuning of the orientation, the local hydrophobicity, and the proximity of amino acids and short peptide ligands on the surface of nanoparticles or within functional polymers. Such systems should allow for creating hydrophobic regions decorated with catalytic moieties in the nanostructures reminiscent of the catalytic pockets within enzymes.

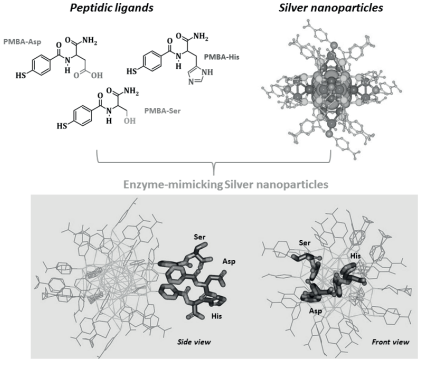

Figure 1. Design of enzyme-mimicking multivalent silver nanoparticles

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Tissue engineering and differentiation on neuronal stem cell targets through membrane active peptide

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Neurogenesis is an important biological process under intense investigation. Neurodegenerative diseases such as Alzheimer's and Parkinson's are devastating because neurons cannot be replaced in the diseased areas of the brain. The generation of new neurons from stem cells, however, has the potential to become a viable treatment option for patients suffering from neurodegenerative diseases In the field of tissue engineering, the design of a scaffold able to guide the process of tissue regeneration is one of the most challenging goals. The ideal scaffold should promote and control specific events at the cellular and tissue levels and should be made of a highly biocompatible material that does not have the potential to elicit an immunological or clinically detectable foreign body reaction [1, 2]. In the present work, three-dimensional biodegradable polymeric scaffolds (3D-PCL) with controlled morphology, macro, and micro and Nano-mechanical were functionalized with bioactive peptides with the final aim to promote the differentiation on neuronal stem cells. For bio-functionalization, we used C8 peptide [3], an octapeptide derived from the MPER region of glycoprotein gp36 of the feline immunodeficiency virus (FIV). C8 peptide, in addition to performing an antiviral activity blocking cell entry, [4] showed in confocal microscopy experiments, the ability to destabilize membrane vesicles, producing a complex network of membrane tubes. [5] On this basis, we functionalized biocompatible polymeric scaffold with C8 peptide and evaluated the biological activity of these scaffold using SH-SY5Y neuroblastoma cells. Accordingly, we studied the effect of the surface modification on the mechanical and functional performances of the scaffold and expression of differentiation on neuronal stem cells, also showing a morphological and analytical approach to study the functionalization/bioactivation treatment, the immobilized ligands, and the biological features.

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A helical antimicrobial peptide selectively detects sulfate anions

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TOPIC: Folding and Aggregation of Peptides *(or Peptides in Diagnostic Applications)*

Selective identification of environmentally relevant anions, such as sulfate, can be achieved by chemical sensors, for instance by using dye-containing compounds or electrochemical probes. In this connection, peptide-based chemosensors are an environmentally friendly, intriguing option. Nonetheless, so far only a few examples of peptide-based sensors for sulfate, and in general anions, have been reported.[1] They are almost always based on cyclopeptides, and/or contain fluorescent dyes.[2,3] We herein describe a linear cationic peptide, endowed with antimicrobial activity, able to change its helical structure in response to the presence of sulfate anions in water. We demonstrate that the apolar C-terminal capping moiety is the main responsible of peptide selectivity. The selective chiroptical response to sulfate is mantained once the peptide is linked to gold nanoparticles (NPs), and the interaction with sulfate results in precipitation of the peptide-decorated NPs. Transmission electron microscopy shows that sulfate causes disruption of the peptide fibers formed in water (Fig. 1). Our results support the view that sulfate selective recognition stems from a combination of polar and apolar features in the peptide sequence, with its well-developed helical structure playing a key role.

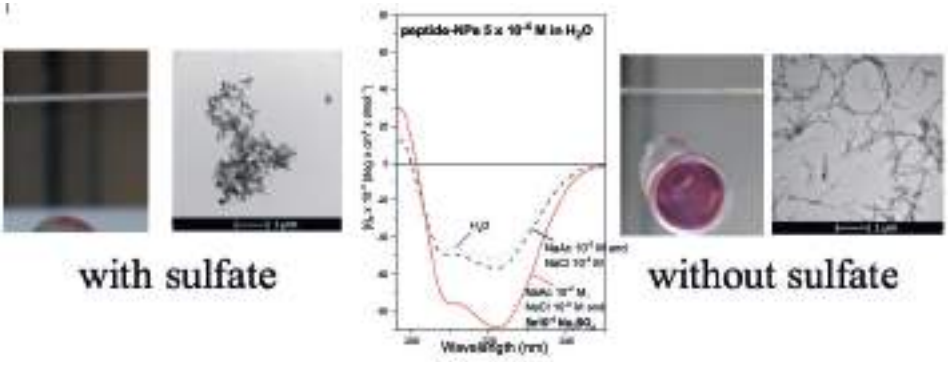

Figure 1. Left: peptide-decorated gold nanoparticles in an acqueous solution containing sulfate (Na_2SO_4) in the presence of a tenfold excess of NaCl, NaBr, NaI, $NaNO_3$, and NaAc. Sulfate concentration: 10^{-3} M; Each of the other salts: 10^{-2} M concentration. Right: the same solution, but without sulfate anions. Central inset: CD spectra of peptide-NPs (peptide concentration: 5 x 10^{-4} M) in water (dotted line), after the addition of sodium acetate (NaAc) and sodium chloride (final concentration: 10^{-2} M each, dashed line), and after the addition of Na_2SO_4 (final concentration: 5 x 10^{-4} M, solid line).

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Lost in translation: development of single and novel therapeutic peptidomimetic against multiple pathologies

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Aberrant control of RNA translation has emerged as a common feature across multiple pathologies, such as cancer and neurodevelopmental disorders, like Fragile X Syndrome (FXS). A loosened control of the translation machinery results in increased synthesis of specific proteins involved in cell morphology and proliferation, leading to pleiotropic effects, possibly establishing a link between different diseases [1]. The translation of these mRNAs is influenced by the level of activation of the eukaryotic translation initiation factor 4E (eIF4E) by the physical interaction with the eukaryotic translation initiation factor 4G (eIF4G), or by its inhibition by 4E-binding proteins (4E-BPs), a well-characterized group of proteins that repress protein synthesis [2]. Indeed, 4E-BPs and eIF4G compete for the same binding site with eIF4E. Particularly interesting among the different 4E-BPs is the Cytoplasmic FMRP Interacting Protein 1 (CYFIP1) [3], whose involvement in cancer and a range of neurological disorders has been recently established [4]. CYFIP1 is the binding partner of FMRP (fragile X mental retardation protein), a mRNA binding protein that acts as a translational repressor of transcripts that encode for proteins implied in synaptic architecture and maturation, whose absence being the main cause of Fragile X Syndrome (FXS) [5]. Like all other 4E-BPs, CYFIP1 interacts with eIF4E through a α-short helix, which, however, displays a low degree of sequence conservation compared to other 4E-BPs, therefore assuming a specific binding mode [6,7,8]. Here we designed and synthesized a novel CYFIP1-derived peptidomimetic, named Cy-9B, able to inhibit the eIF4E activity. The Cy-9B design and the characterization of the eIF4E/Cy-9B binding was carried out using advanced computational approaches and the *in silico* results were validated with *in vitro* experiments. The ability of the Cy-9B to enter into the cells and the impact on the translation process was analysed on a lung cancer cell line, after the peptidomimetic treatment. Results show that Cy-9B is able to inhibit the eIF4E/eIF4G interaction, reducing the global translation rates and cellular growth. Lastly, Cy-9B was also tested on *Fmr1* KO hippocampal neurons, that represent a valid model for FXS. The treatment of neurons with Cy-9B restores the normal translation levels of PSD95, that is an important synaptic marker of synaptic plasticity. Taken together these results, obtained through multidisciplinary approaches, are very promising for the development of a single and novel molecule that could be used as a therapeutic strategy against multiple pathologies.

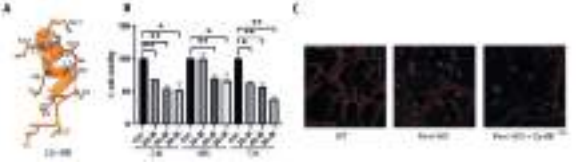

Figure 1. (A) Ribbon representation of Cy-9B peptide; (B) MTT analysis of A549 lung cancer cells treated with different concentration of Cy-9B peptide; (C) Immunofluorescence on primary hippocampal neuron: representative images showing DIV14 neurons stained for DAPI (blue), tubulin (red) and PSD95. Arrowheads point to postsynaptic PSD95 clusters. Images are acquired with 63x objective zoom 1x. The scale bar=10 µm.

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β-Amyloid fibrils catalyze neurotransmitter degradation

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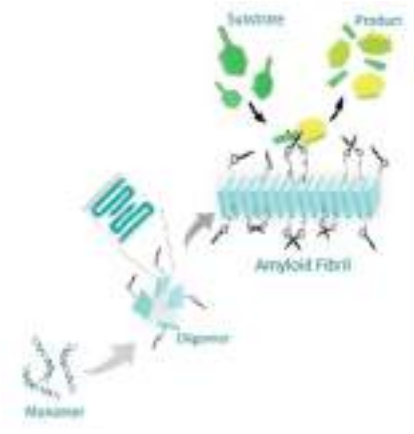

Figure 1. β-amyloid (Aβ42) assembles β-sheet fibrillar structure with biocatalytic activity.

Amyloid fibrils are one of the hallmarks of Alzheimer's disease (AD), although a causative link between plaque-forming amyloid fibrils and AD pathology remains to be clarified. This study demonstrates, for the first time for a naturally occurring amyloid, that fibrils comprising the 42-residue Amyloid-β peptides (Aβ42) exhibit significant catalytic properties. Aβ42 fibrils catalyzed the hydrolysis of the model ester, para-nitrophenyl acetate (pNPA), and of acetylthiocholine, a surrogate for the neurotransmitter acetylcholine. Aβ42 fibrils also catalyzed the oxidation of the prominent neurotransmitters, dopamine and adrenaline. Importantly, the catalytic activity was specifically exhibited by mature Aβ42 fibrils, as opposed to the peptide monomers, or oligomeric Aβ42, the putative neurotoxic species. Importantly, maximal catalytic activity was exerted by the full-length Aβ42 fibrils, whereas fibrillar assemblies comprising Aβ42 subdomains exhibited significantly lower catalytic activity. The catalytic activity of Aβ fibrils may be implicated in pathophysiology pathways associated with the generation of AD amyloid plaques.

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PEPTIDE SHOWCASE

Therapeutic peptide industrial chemistry and analytical consideration

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Therapeutic peptides market is keeping the high interest observed in the recent years. In fact, three peptide APIs were approved by FDA in 2019, together with other peptide drug conjugates[1], while the overall market for the already commercialized peptides is growing at multi-billion sales level. Parallelly to this, the complexity of the produced peptides is growing at similar pace, through the choice of longer sequences and the use of non-proteinogenic/non-natural aminoacid, with the target to increase their stability and bioavailability, reaching even the oral formulation in some cases. This target poses challenges to the manufacturing processes of Peptide API, both in the Upstream (synthesis of the crude peptide API) and Downstream (purification and isolation the pure peptide API), that need to reach high quality at an effective cost and be also innovative, as to avoid any patent infringement. Even more, this increased complexity requires the refinement of the existing analytical tools to clearly establish the purity of the products that reach the market. Furthermore, sophisticated characterization tools must be applied to fully establish the physio-chemical characterization of the manufactured peptides and satisfy the recent tight request of the regulatory agencies[2]. All these aspects will be presented and discussed, starting from the case studies occurred in our laboratory[3].

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Development of Analogues of the Venom Toxin ProTx-II as Selective Blockers of the Ion Channel $Na_v1.7$ for the Treatment of Pain

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Peptide toxins based on the cystine knot motif have evolved to block ion channels but might display toxicity if dosed in a pharmacological setting[1]. Protx-II is a 30 amino acids peptide derived from the venom of the tarantula that displays blockage of $Na_v1.7$, a sodium ion channel that has been associated with pain signaling. Although ProTx-II displays selectivity over other Na_v1 subtypes, here we report a structure activity relationship study to provide analogues with a better selectivity towards $Na_v1.7$ Protx-II peptide toxin comprises many cationic and hydrophobic residues in the sequence (Fig 1).

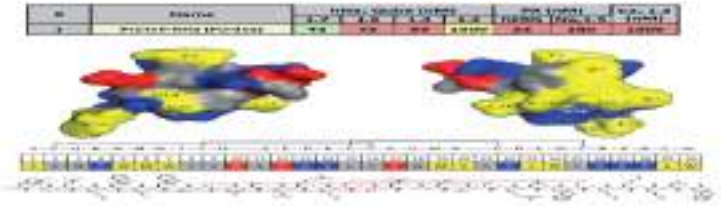

Figure 1. Sequence of inhibitor cystine knot ProTx-II: cationic residues are highlighted in blue and hydrophobic residues in yellow

As a strategy for optimization, substitutions with natural and non-natural amino acids were considered to minimize the positively charged hydrophobic surface and avoid channel-blocking potency via non-specific interactions with lipid membranes. In this process new mutations were identified having a large positive impact on the refolding yields, the selectivity profile and that limited the ability of the peptides to cause pseudo-allergic reactions mediated by activation of mast cells and histamine release.

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Peptide Optimization by Novel "Fatty" Amino Acids

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Besides many other association partners, fatty acids can bind to mammalian plasma albumin. Although the physiological role of this binding is the transport of free fatty acids, it soon turned out that albumin is a perfect blood shuttle for many other molecules attached to or modified with fatty acids. Binding to albumin can protect from enzymatic degradation and renal clearance and thus significantly increase the plasma half-life of fatty acylated compounds.

The economic breakthrough of this effect started with the approval of Novo Nordisk's "Liraglutide", a palmitoylated derivative of the peptide hormone GLP-1. The fatty acylation enables a 24 h/day plasma presence without lowering its receptor binding affinity, both necessary for the desired pharmacological effect of glucose lowering. [1] Liraglutide is the best-selling peptide drug with a turnover close to $ 5 billion. Further optimization of albumin binding by changing the fatty acid moiety and the linking chemistry to GLP-1 led to the API "Semaglutide". It is now fully stable against metabolic degradation, which could change the API's administration period from once-daily to once-weekly. [2]

This tremendous effect, mainly caused by changing the linker-fatty acid composition, demonstrates the importance of identifying the best amino acid - linker - fatty acid combination for each peptide to be modified. By today, eleven different fatty acid binding sites are known on the albumin surface, which differ in affinity for various fatty acids. Of course, besides Albumin, numerous other binding partners are also targetable for hydrophobic interactions with "fatty" peptide modifications.

We present here our activities on developing dozens of novel hydrophobically modified amino acids to provide the researcher with the suitable toolbox of building blocks for the fine-tuning of a peptide's hydrophobic binding properties. This does not only comprise acylations, but also alkylations, bulky three-dimensional modifications, introduction of further functional terminal groups or multiple fatty acylation on one single amino acid.

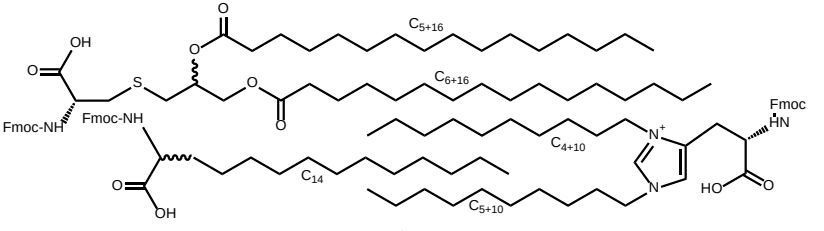

Figure 1. Examples of "Fatty" Amino Acids.

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Optimized Synthesis and Purification Strategies for SPPS

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In the last fifteen year, microwave-based SPPS processes have provided significant benefits for the production of synthetic peptides. Microwave (heating via dipolar polarization and ionic conduction) peptide synthesis provides high purities, (because drive reactins to completion while minimizing side-reactions), accelerated cycles times (as low as two min/residue for R&D scales), high efficiency (low eccess of reagents and solvent) and full cGMP compatibility (21CFR part11 conformance).

Initially these methodologies has been used almost exclusively in the research field. In the last few years, these processes has expanded beyond its traditional R&D role into GMP processes for both personalized peptide vaccines and larger-scale kilogram quantities of peptide API's. The benefits realized using special chemistries and specific usage of microwave irradiation have been higher purity, reduced reagent and solvent consumption and an easier ability to incorporate more viscous green solvents. Peptide new chemical entities can be generated more efficiently, faster and compliant with FDA regulations. Most recently, an improved purification prep process at elevated temperature was developed that compliments the benefits of microwave SPPS and helps in the purification of hydrophobic peptide, in reducing peak tailing and reducing system backpressure. This optimized synthesis and purification process will be presented.

International Industrial Collaborative Opportunities with KelAda

Malachi Gillick-Healy, Brian Kelly, and Mauro Adamo

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make use of previously neglected, cheap and abundant renewable sources;

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are easily "platformed", enabling portfolios of novel compounds for bioactivity to be made.

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3) Small Molecule Therapeutics KelAda has a on-going program of research in drug/RNA interaction with a view to identifying small molecule motifs—e.g. peptides—which exhibit bioactivity by affecting RNA metabolism, binding and function.

Contact us to learn more about how we can collaborate to target better processes.

POSTER

Semax, a synthetic regulatory peptide, affects copper-induced Aβ aggregation, amyloid formation and ROS production

M.F.M. Sciacca, M.F. Tomasello, M.L. Giuffrida, I. Naletova and F. Attanasio

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Alzheimer's disease (AD), the most common form of dementia, is characterized by the aggregation of amyloid beta protein (Aβ). The aggregation and toxicity of Aβ are strongly modulated by metal ions (Cu^{2+}) and, also, by phospholipidic membranes. Moreover, it is postulated that the generation of reactive oxygen species (ROS) in Fenton-like reaction connected with Cu^{2+}/ Cu^{+} redox cycling of the Cu^{2+}-Aβ complex can play a key role in the molecular mechanism of neurotoxicity in AD. Multifunctional compounds able to both inhibit fibrillogenesis, in particular the formation of oligomeric species, and prevent the formation of the Aβ:Cu^{2+} complex are of particular interest and have been evaluated as candidate drugs, but, to date, no treatments are available for the pathology. Here we tested the anti-aggregating and ROS generation quenching properties of a heptapeptide, Semax (Met-Glu-His-Phe-Pro-Gly-Pro), an ACTH-like peptide able to form a stable complex with Cu^{2+} ions[1, 2] and having neuroprotective and nootropic effects.[3] We demonstrated through a combination of spectrofluorometric, calorimetric, cytofluorimetric and MTT assays that Semax is able to prevent the formation of Aβ:Cu^{2+} complexes, has anti-aggregating and protective properties especially in the presence of Cu^{2+} as well as inhibits the copper catalyzed oxidation of Aβ. We find that these properties of Semax confer cytoprotection to RA-differentiated SH-SY 5Y cells against Aβ aggregation and oxidative stress induced by copper catalyzed oxidation of Aβ peptide. The results suggest that Semax inhibits fiber formation by interfering with the fibrillogenesis of Aβ also in the presence of Cu^{2+} as well as influences the redox cycling of the Cu^{2+}-Aβ complex and decreases the level of associated ROS production by trapping Cu^{2+}. This study provides further insights in the potential role of Semax in neurodegenerative disorders and into the design of new compounds with therapeutic potential for AD

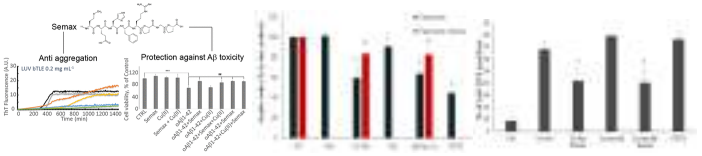

Figure 1. Semax properties

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The syntetic eptapeptide Semax, a fragment of the ACTH hormone, sustains differentiated neuroblastoma, by stimulating the mitochondrial function and improving bioenergetic

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Semax is the active component of drugs originally used as treatments for brain hypoxia, ischemia, brain traumas, and to facilitate adaptive processes to extreme situations. Semax is now used as a nootropic for mental enhancement in healthy people and for treating many cognitive disorders. Semax is a syntetic eptapeptide consisting of the Met-Glu-His-Phe fragment of ACTH and the C-terminal tripeptide Pro-GlyPro. These fragments are well known for their potent neuro-regenerative and cognitive activities,[1] and Semax is able to stimulate learning and memory formation in rodents and humans.[2] However, the molecular mechanisms underlying the action of Semax, are still unknown. At the cellular level, Semax was shown to prevent the death of cultured neurons, and to increase the expression of neurotrophine and their receptors[3], thus implying that Semax might modulate brain functions by influencing neurotrophins functions. Here we investigated the mechanism by which Semax acts and describe the effects at the cellular level on RA-differentiated SHSY5SY cells. Interestingly, when differentiated in the presence of Semax, cells present improved mitochondrial functions and mass, increased ATP levels and improved resistance to stressors. Furthermore, we found that Semax increase the BDNF expression and release, trough the activation of melanocortin receptor 4 (MC4R) and the p-CREB signaling pathway. We suggest that Semax promotes cognitive brain functions by activatining MC4 receptor and modulating the expression of the BDNF/trkB system which in turn stimulate the mitochondrial function. Thus, Semax provide neurons with the ability to better exploit fuels, either under basal or stressful conditions, which results in the improvement of neurons viability as demonstrated by the means of several experimental approaches. The finding that Semax, by modulating neurotrophins levels, improve the mitochondrial functions, has important implications for neurodegenerative and psychiatric diseases.

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Peptide Nucleic Acid based "light up" probes for the early diagnosis of Celiac Disease

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Celiac disease (CD) is an autoimmune disorder triggered by the ingestion of proteins contained in wheat (gluten), barley (hordein), and rye (secalin) in genetically predisposed individuals [1]. The onset of the disease is often characterized by generic symptoms (e.g. diarrhea, steatorrhea, weight loss, bloating, flatulence, abdominal pain), also associated with other diseases or many individuals may have no symptoms at all. Celiac disease is generally diagnosed by serologic testing of specific antibodies and must always be confirmed by duodenal mucosal biopsies, an invasive technique. It is therefore an urgent matter to identify new non invasive biomarkers to avoid biopsies and develop an early and accurate diagnostic assay. This goal can be obtained employing microRNAs, small non coding RNA, that are deregulated in numerous disease including the celiac ones. Interestingly miRNAs show different expression profile in healthy or at-risk subjects [2] and can be employed as biomarkers. In this work a group of circulating miRNAs deregulated in celiac disease have been identified (miRNA-449a, miRNA-492, miRNA-21, miR-486) and smart probes based on peptide nucleic acids (PNAs) [3] were designed in order to develop an innovative, non-invasive kit for the early diagnosis of this disease. AntimiRNA PNA "light up" probes, fully complementary to miRNA sequences, were realized and tagged with the intercalating fluorophore thiazole orange (TO) , which lights-up upon hybridization in presence of the target [4]. Circular dichroism and fluorescence experiments were carried out to demonstrate the formation of the PNA-RNA complexes and validate the feasibility of this new approach.

Figure 1. PNA light-up probe interaction with single-stranded miRNA target.

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Structure based development of HtpG-derived antigens against *M. tuberculosis*

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Tuberculosis is a highly contagious disease caused by *Mycobacterium tuberculosis* (Mtb), which infects 10 million people and causes 2 million casualties annually. Although the disease can be treated with first-line antibiotics as well as last resort ones, resistances are being observed even to the last resort antibiotics [1-3]. A vaccine against tuberculosis is commercialized, the BCG, but estimates point to an efficiency that varies between 0% and 80% in adult subjects and of around 50% in children. All these data highlight the need for new therapies and vaccines.

Among newly identified antigens, we have recently demonstrated that $HtpG_{Mtb}$, a homolog of the bacterial chaperone Hsp90 of *E. coli*, is able to effectively induced dendritic cells activation. Most important, when $HtpG_{Mtb}$ is fused to ESAT6, the overall molecule is highly effective in boosting BCG immunization and lowering ESAT6 cytotoxicity [4]. In addition, inclusion of $HtpG_{Mtb}$ to the Ag85B-ESAT6 fusion protein leads to an increase in the immune reactivities and protective efficacy of Ag85B-ESAT6 [5].

Using a structural vaccinology approach, we used several bioinformatic tools to predict immunogenic epitopes and dissect the contribution of individual domains to folding and antigenicity. Therefore, we performed experimental and computational analyses of $HtpG_{Mtb}$ and smaller truncated variants characterized them using a plethora of biophysical assays, as light-scattering (SEC-LS), Circular Dichroism (CD) and Isothermal Titration Calorimetry (ITC) [6]. This information resulted in the production of new antigens, which were experimentally validated at the University of Daejeon, South Korea, by identifying the most relevant regions of the molecule for vaccine development.

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Self-assembled peptide-based nanofiber: smart delivery system in triple-negative breast cancer

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Triple-negative breast cancer (TNBC), defined by the lack of both estrogen and progesterone receptor as well as human epidermal growth factor receptor 2, is the most complex and aggressive subtype of breast cancer. Although chemotherapy poses the cornerstone in TBNC treatment, its use is seriously dissatisfactory due to issues related to formulation and pharmacokinetics of drugs. To overcome these limitations, herein, we present a self-assembled peptide-based nanofiber as smart platform to achieve a selective on-demand drug release into breast cancer cells. The nanofiber consists of two structured self-assembled peptides featured by the presence of an aliphatic sequence of six alanine residues and a lipid tail (nonadecanoic acid) attached to the amino group of lysine in C-terminal, and by positively and negatively charged amino acid residues.[1] On the external surface, the nanofiber is decorated by doxorubicin (Doxo) as chemotherapeutic, the cell-penetrating peptide, named gH625, that increases cellular uptake and promotes endosomal escape of nanofiber into cancer cells,[2] and the peptide targeting the epidermal growth factor receptor (EGFR) overexpressed on cancer cells.[3] Doxo is covalently bound to the fiber surface through an on-demand strategy which involves the over-expression of matrix metalloproteinase 9 (MMP-9) in cancer cells, by the introduction of the specific MMP-9 cleavage sequence between Doxo and the fiber. Biophysical assays, including dynamic light scattering, zetametry, and fluorescence assays, were performed for determining the size, charge and critical micelle concentration of fiber, and its formation was confirmed by transmission electron microscopy. The proteolytic cut for the on-demand drug release was performed in presence of recombinant MMP-9 and *in vitro* using cells overexpressing MMP-9. The cytotoxicity profile of the nanofiber carrying Doxo was established on the triple negative cancer cell lines and the internalization pathway of the nanofiber was monitored by drug fluorescence.

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Antibacterial activity and serum stability of panusin, a lobster β-defensin, is conserved in the carboxy-terminal region

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The β-defensins are one of the most abundant and studied families of antimicrobial peptides (AMPs). Because of their selective toxicity on bacterial membranes and broad spectrum of microbicidal action, these AMPs are potential therapeutic agents (1). Panusin (PaD) is a β-defensin-like AMP from the spiny lobster *Panulirus argus*, structurally related to mammalian defensins by the presence of an αβ domain stabilized by disulfide bonds (2). Previous studies of PaD suggest that its C-terminal (Ct-PaD) contains the main structural determinants of antibacterial activity (2). To test this hypothesis, we aimed to obtain PaD and Ct-PaD, to determine the influence of the C-terminus on antimicrobial activity and proteolytic stability. After successful solid phase synthesis and disulfide folding, we evaluated the antibacterial potency of both peptides and their cytotoxic activity in human cells. Moreover, proteolytic stability in human serum was also determined, and circular dichroism (CD) experiments were performed to investigate their conformation in both aqueous solution and in SDS micelles. Results showed that truncated Ct-PaD is more active than native PaD, confirming the importance of the C-terminal domain in biological activity. Differences in antibacterial activity also suggest a role of positively charged residues at the C-terminus in providing selectivity and an increased ability to bind negative bacterial membranes. In addition, since Ct-PaD, same as PaD, lacks hemolytic and cytotoxic activity on human cells, it appears that the build-up of charges at the C-terminal does not influence the cytotoxic effects of the truncated peptide. LC-MS stability studies in human serum showed high $t_{1/2}$ values for PaD and slightly lower for Ct-PaD. Apparently, the absence of a native disulfide bond in Ct-PaD alters, though not decisively, proteolytic stability. Secondary structure analysis showed both peptides adopting an increasingly ordered structure in hydrophobic environment, in tune with their ability to perturb bacterial membrane systems. Altogether, we conclude that both panusin and its C-terminal region retain essential structural features for the development of novel antimicrobials.

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Analogues of PMAP-36: synthesis, conformational analysis and bioactivity

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Cathelicidins constitute a family of host defence peptides (HDPs) and play an important role in the innate immune response. Although the sequences of cathelicidins are highly variable, almost all cathelicidins show direct antimicrobial activity against many different bacteria, viruses, fungi, and parasites. In this contribution, we focused on porcine cathelicidin PMAP-36 designing and synthetizing a series of truncated analogues with the aim at identifying the minimal length necessary to maintain the biological activities, to favour the interactions with the bacterial membranes and to prevent the enzymatic degradation.

Peptide Sequences
Ac-KRLKKIGKVLKWIPPIVGSI-NH_2
Ac-KRLKKIGKVLKWIAKIVGSI-NH_2
Ac-KRUKKIGKVLKWIAKIVGSI-NH_2
Oct-KRLKKIGKVLKWIAKIVGSI-NH_2
Laur-KRLKKIGKVLKWIAKIVGSI-NH_2
Palm-KRLKKIGKVLKWIAKIVGSI-NH_2
Ac-KRLKKIGKVLKWIAKIVGSI-Aun-NH_2
Ac-K(Pal)RLKKIGKVLKWIAKIVGSI-NH_2

Figure 1. Peptide Sequences

A thorough conformational investigation (CD, NMR) confirmed an amphiphilic α-helical structure for all the peptides in membrane mimicking environment.
Bioactivity tests revealed the maintenance of the antibacterial activity even for the truncated analogues.

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Stability to proteolysis of GE11 analogues on gold nanostructures

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Among many targeting ligands, peptides have emerged for cell targeting of nanomaterials in diagnostic and therapeutic applications. This is due to their scarce immunogenicity as well as for their affinity for different cellular targets and the possibility of being introduced in multiple copies on nanosystems thus providing high affinity for the target. Unfortunately, their sensitivity to proteases strongly hampers their application *in vivo*. In the present work we show that the stability to proteolysis is strongly improved without reducing the targeting activity when peptides are presented on properly functionalized nanostructures.

Gold nanostructures were functionalized with GE11, a dodecapeptide already known in literature as ligand of the EGF Receptor, which is overexpressed in many tumours of epithelial origin. Two types of nanosystems were prepared by linking the targeting unit directly to the gold surface or through a PEG chain, thus obtaining nanostructures presenting different peptide density and exposure [1]. In the presence of 20% human serum or isolated serine proteases, we observed that while the isolated peptide was rapidly degradated, no proteolytic fragments were detected during incubation of the nanosystems and after 24 h digestion. Moreover, the nanostructures preserved their targeting activity and selectivity on cancer cells. Molecular dynamic calculations of the interaction between a serine protease, the chymotrypsin, and the nanosystems helped us to understand the reason of the targeting unit protection against the enzymatic degradation. Namely, we found that the constraint imposed by the peptide anchoring to the gold surface inhibits the formation of the enzyme-peptide complex, the first step in the mechanism of peptide digestion. These results support the employment of peptides as active targeting unit in nanomedicine [2].

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Peptides with angiotensin I-converting enzyme inhibitory and antioxidant activity from raw and cooked trout meat protein hydrolysate

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According to published data, fish proteins are a rich source of peptides with biological activity, including angiotensin converting enzyme (ACE) inhibitors and antioxidant peptides [1]. Peptides with such activity can play a role in the prevention of cardiovascular diseases. Trout (*Oncorhynchus mykiss*) is one of the most popular fresh water fish in Poland, with high digestibility of proteins exceeds 98%, so it may be the source of biopeptides in the human diet. Raw and high temperature treated trout myofibrillar and sarcoplasmic proteins were examined as a bioactive peptides precursors after *in vitro* digestion.

The first, *in silico*, part of the study used computer tools available in UniProt and BIOPEP-UWM databases (http://www.uwm.edu.pl/biochemia/), Fragment Ion Calculator and SSRCalc applications [2]. Samples were *in vitro* digested according to INFOGEST method [3]. Hydrolysates were analysed for their ACE inhibitory and antioxidant activities and were used in a screening for ACE inhibitory and antioxidant peptides as well. First, samples were separated using RP-HPLC method, then amino acid sequences were identified using LC-MS/MS method [2].

Hydrolysates of trout myofibrillar and sarcoplasmic proteins obtained from raw or cooked trout tissue showed ACE inhibitory and antioxidant activity. The difference between raw and high temperature treated samples were observed. The ACE inhibitory and antioxidant fragments selected based on the results of *in silico* studies were identified *via* RP-HPLC-ESI-MS/MS method.

It was concluded that trout proteins can be the source of ACE inhibitory and antioxidant peptides.

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Barley (*Hordeum vulgare L.*) flakes as a source of antioxidant peptides

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Barley (*Hordeum vulgare L.*) is one of the ancient cereals cultivated until nowadays. A diet composed of cereals, including barley flakes, could have a beneficial effect on human health e.g. reduce the risk of many chronic diseases. It is resulted from the presence of biologically active (bioactive) peptides. Bioactive peptides are known as the factors contributing to prevention of diet-related diseases [1]. Peptides released after enzymatic hydrolysis may have a wide spectrum of biological functions, e.g. antioxidant activity.

The aim of the study was the characteristics of antioxidant peptides derived from proteins originating from barley flakes.

The experiment included *in silico* and *in vitro* analyses. *In silico* (bioinformatic) analysis included the calculation of the profile of potential biological activity of barley proteins, computation of A parameter, simulation of proteolysis using human digestive enzymes. All definitions of the parameters applied are available in BIOPEP-UWM database [2]. UniProt [3] database was the source of 102 barley protein sequences taken for the analyses. Then, *in silico* results were verified using experimental analysis. The *in vitro* methods included the extraction of barley flakes their proteins, enzymatic hydrolysis using the digestive protocol of Infogest [4] and assessment of antioxidant activity of extracts and hydrolysates (tests involving ABTS+ and DPPH scavenging activity).

In silico and *in vitro* results showed that barley protein hydrolysates had the antioxidant potential. It may be related to the presence of peptides with the above-mentioned biological activity. The research also confirms the usefulness of bioinformatic methods in analysis of food proteins.

These promising results were the prerequisite to undertake the next step of our studies relying on the identification of antioxidant peptides in both barley flake extract and hydrolysate samples. The result are in progress now.

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The C-terminus of the GKY20 antimicrobial peptide, derived from human thrombin, plays a key role in its membrane perturbation capability

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In a previous biophysical study, we characterized in detail the mechanism of action of the antimicrobial peptide GKY20 showing that it selectively perturbs the bacterial-like membrane employing peptide conformational changes, lipid segregation and domain formation as key steps in promoting membrane disruption [1]. Here, we used a combination of biophysical techniques to similarly characterize the antimicrobial activity as well as the membrane-perturbing capability of GKY10, a much shorter version of the GKY20 peptide. GKY10 is only half of the parent peptide and consists of the last 10 amino acids (starting from the C-terminus) of the full-length peptide.

Figure 1. Human thrombin structure (pdb:3U69). The portion corresponding to GKY10 is highlighted in red. GKY20 N-terminal portion is highlighted in blue. The remaining sequence of human thrombin is reported in yellow.

Despite the large difference in length, we found that GKY10, like the parent peptide, retains the ability to adopt a helical structure and to induce lipid segregation upon membrane-binding. Overall, our results suggests that the amino acidic sequence of GKY10 is responsible for most of the observed behaviors of GKY20. Our results shed further light on the mechanism of action of the full-length peptide and provide useful information for the design and the development of new peptides that serve as antimicrobial agents.

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Peptide encapsulation for structural and functional studies

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Nature's idea of self-assembly has been exploited in several nanobiotechnological applications, particularly in targeted drug delivery and cellular imaging, using viral capsids, lipid membranes and proteins[1]. Among the latter, ferritin nanocages (Fts) are of great interest. In fact, Fts possess the capability to disassemble and reassemble in extreme experimental conditions and, together with the hollow structure and the ease of production, they represent suitable nanosystems for small molecules encapsulation.

Since it has a low immunogenicity, human Ft (hFt) seems to be of particular interest. Native hFt is made up of 24 subunits that naturally self-assemble to form a nanocage structure made-up identical amounts of 2 types of subunits, L chains (light) and H chains (heavy)[2]. H chains have a high affinity for the TfR1 transferrin receptor, over-expressed on the cell surface of different cancer cell lines[3]. For these reasons, we focused our attention on nanocages composed entirely by the human H-chain (hHFt), that can be exploited as cargos of antitumor drugs. As additional advantage, drug encapsulation in hHFt can also increase the solubility in aqueous media and/or the thermal stability, and decrease the immunogenicity and other side-effects. For this purpose, an efficient and versatile protocol has been developed for the encapsulation and release of different molecules including metallodrugs, polypeptides and bioactive molecules. In the present work, we reported the production and the characterization of a hHFt nanocage loaded with a small antimicrobial peptide (AMP), which is amenable for structural and functional studies. The obtained data open interesting perspectives to develop new nanoparticles which combine the properties of the hFt with those of the cargo molecule, with synergistic effects.

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A Convenient Synthetic Route to (2S, 4S)-4-Methylproline

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Proline is able to influence the activity and reactivity of peptides and proteins because of its conformational properties. Proline and its substituted analogues can induce turns in peptides in close proximity to pharmacophores to play an essential role in biological recognition processes. As a result, the incorporation of proline derivates into peptides and peptidomimetics represent a powerful tool in the study of structure-activity relationship.[1] Many proline derivatives with different substituents at the Cγ atom are commonly synthesized from 4-hydroxyproline through functionalization or derivatization. [2] Since Koskinen's synthesis in 1989, [3] several approaches have been reported for the synthesis of the non-natural amino acid (2S, 4S)-4-methylproline, involving rare and expensive reagents, in order to achieve high enantioselectivity. At the same time, several reported protocols present challenges in the separation of the two diastereomers of 4-methylproline. Here, we present a cheap, convenient, and versatile synthetic route for the preparation of (2S, 4S)-4-methylproline starting from pyroglutamic acid (Figure 1).

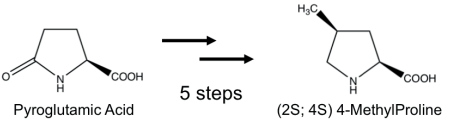

Figure 1.

Moreover, incorporation studies with both (4R)- and (4S)-methylproline into the model protein *E. coli* Thioredoxin show that (4R)-methylproline is a viable substrate for ribosomal protein synthesis, but not (4S)-methylproline.

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Lactoferrin-derived KDEON peptide and its biological properties

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Antibiotic resistance is expected to be one of the greatest health threats of the 21st century. If no action against post-antibiotic era is taken, antibiotic resistance will provoke more deaths than cancer by 2050, drastically increasing the global economic burden. [1] Innovative strategies are therefore urgently needed to develop alternative therapies to conventional antibiotics, able to tackle multidrug resistant bacterial pathogens with different mechanisms of action. [2] Cationic peptides (AMPs), small polypeptide molecules found in all living organisms as component of innate immunity, could constitute one of the most promising alternatives to existing antibiotics with limited resistance development. [3] More precisely, cationic AMPs interact with the negatively charged components of the bacterial cell wall (lipoteichoic acid in Gram+ and lipopolysaccharide, LPS, in Gram– bacteria), and then destabilize the integrity of cytoplasmic membrane. [2,3] Since LPS induces the production of inflammatory cytokines in the host, leading to septic shock syndrome, scientific research turned its attention to AMPs able to kill pathogenic Gram– bacteria and to neutralize LPS. Lactoferrin, an abundant iron-chelating protein found in most of the exocrine secretions, and its derived AMPs neutralize the toxic effect of LPS by binding to LPS molecules causing disorganization and destabilization of the bacterial surface. [4] In order to maintain these properties, we designed a short AMP named KDEON containing two of the three typical residues of lactoferrin-derived AMPs (Tryptophan, Phenylalanine and Lysine), consistently repeated in its primary structure (WWKKWWKKWWK). The peptide was characterized for its capability to bind lipid A, a portion of LPS that anchors it to the outer membrane, for its spectrum of activity, its mechanism of action, antibiofilm potency and cytotoxicity.

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Topoisomers of a snake venom-derived peptide with anti-infective and antitumoral activity

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In order to fight antimicrobial resistance (AMR), it is essential to have a good understanding of the mechanisms underlying infection, in particular those related to the interaction between anti-infective agents and pathogen targets. In this regard, antimicrobial peptides (AMPs) are emerging as promising therapeutic alternatives in the worrying context of global AMR, for reasons that include their simple but effective mechanisms of action, broad spectrum, amenability to structural modification by versatile peptide engineering techniques, and affordable production costs. Ctn[15-34][1,2], the C-terminal section of crotalicidin (Ctn), a cathelicidin from a South American pit viper, is an antimicrobial and antitumoral peptide candidate with remarkably longer stability in human serum than the parent Ctn. In this work, a set of topoisomers of both Ctn and Ctn[15-34], including the retro, enantio and retroenantio versions, have been synthesized and tested to investigate the structural requirements for activity. All topoisomers were as active as the cognate sequences against gram negative bacteria and tumor cells, while slightly more toxic towards normal cells. More importantly, the enhanced serum stability of the D-amino acid containing versions suggests that such topoisomers must be preferentially considered as future antimicrobial and anticancer peptide leads.

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Antibiofilm and antiviral properties of the antimicrobial peptide Temporin G

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The recent pandemic has made clear the urgency to find new classes of drugs capable of having new biological properties and different mechanisms of action compared to the classic drugs used today. In this context, antimicrobial peptides (AMPs) of the innate immunity hold great promise [1]. Recently, a 13-residues-long AMP derived from frog skin, named Temporin G [TG: FFPVIGRILNGIL-NH_2] has been characterized for its antibacterial and antiviral properties. TG was found to provoke ~50 to 100% reduction of biofilm viability in the concentration range from 12.5 to 100 μM versus different *Staphylococcus aureus* strains and to be active against persister cells (about 70-80% killing at 50-100 μM), without showing any critical cytotoxicity in vitro up to 100 μM against human keratinocytes [2]. Furthermore, when used at sub-inhibitory concentrations, TG potentiated the antimicrobial activity of tobramycin in reducing bacterial growth. Regarding its antiviral activity, TG significantly inhibited the early life-cycle phases of influenza virus as proved by the in vitro hemagglutinating test that revealed the existence of TG interaction with the viral hemagglutinin (HA) protein. The TG/HA complex formation was also confirmed by the hemolysis inhibition assay and molecular docking studies. These latter showed the capability of TG to block the conformational rearrangements of HA2 subunit, neutralizing the virus entry into the host cell by interfering with the viral envelope fusion with intracellular endocytic vesicles [3]. These results have highlighted the high potential of this peptide for the development of new drugs with both antibacterial and antiviral action.

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The peptide Hylin-a1 is able to inhibit Gram-positive bacterial infections

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In recent years, the resistance of pathogenic microorganisms to common antimicrobial agents is representing a severe public health problem. Moderate and wise use of antimicrobials and prevention of infections are the most effective methods for decreasing the spread and development of resistance. Therefore, the World Health Organization (WHO) intensified searching for new agents capable of fighting emerging bacteria [1]. Antimicrobial peptides (AMPs), also known as host defence peptides (HDPs), play a crucial role in the innate immunity, representing one of the first barriers against external attack [2]. In the present study, we evaluated the antibacterial activity of the Hylin-a1 peptide, derived from the frog skin of *Heleioporus albopunctatus*, against several strains of Gram-positive bacteria, e.g., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Streptococcus mutans*, and clinical isolates). The peptide toxicity was evaluated on human keratinocytes (HaCaT cells) by the metabolic 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT), identifying a 50% cytotoxic concentration (CC_{50}) at 50 μM. In addition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and time-killing assays were performed to deepen how the peptide exerted its action on bacterial cells. Hylin-a1 showed a very strong antibacterial effect against *Staphylococcus aureus* and its clinical isolates. In detail, the peptide inhibited the bacterial infectivity at the MIC concentration of 6.25 μM. On the other hand, Hylin-a1 also interfered with the activity of other Gram-positive bacteria with MIC values ranged from 25 to 6.25 μM. Altogether, the results indicated Hylin-a1 as a peptide with potential therapeutic effects against a wide variety of human pathogenic bacteria.

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Carnosine-derived dipeptides as antineoplastic entites

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Peptides represent a unique class of therapeutics with high selectivity and efficacy, yet short half-life. Small peptides could change the fate of different drugs, for example carnosine, composed only of β-Ala and His, is able to protect healthy cells from the cytotoxicity of chemotherapeutics[1,2,3]. It is also worth to mention histidine itself can act selectively on cancerous cells and so its usage in cancer research is justified.

Unfortunately, as mentioned, peptides face a short life so their analysis *in vivo* might be hard, yet computational chemistry can help us understand more the fate and activities peptides could possess when isolated from hydrolyzing enzymes.

Therefore we present a computational study of dipeptides based on carnosine to analyze their possible efficacy and targets. Later such entities can be conjugated with other chemicals to prolong their half-life and might be of use against cancer, like carnosine itself.

A short *in vitro* study is also presented, to understand how theory binds with practice and how *in silico* can explain the *in vitro*.

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Molecular mechanism of inhibitory effects of designed conformationally constrained peptides on amyloid self- and cross-assembly of IAPP and Aβ42

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Amyloid self-assembly is linked to the pathogenesis of Alzheimer's disease (AD) and type 2 diabetes (T2D). Epidemiological, pathophysiological, and biochemical evidence suggest that onset and pathogenesis of the two diseases are linked to each other. In this context, T2D patients have an increased risk of AD and *vice versa*. Cross-seeding interactions between the key amyloid polypeptides of AD and T2D Aβ and IAPP, respectively, dramatically accelerate amyloid self-assembly and may act as molecular links between the two diseases.[1,2] However, previous work by our group and others has also shown that cross-amyloid interactions can be used to design cross-amyloid inhibitors.[3,4]
Here we will present structural and functional studies on a novel class of conformationally constrained peptides which are both nanomolar inhibitors of amyloid self-assembly of IAPP and Aβ42 and able to effectively suppress their reciprocal cross-seeding. These peptides were designed to mimic Aβ/IAPP interaction surfaces and found to function *via* a novel co-assembly mechanism. Several biophysical, biochemical, and advanced microscopy methods were applied to characterize their structures, interactions, co-assemblies, and functions. The results suggest a possible mechanistic scenario for the potent self- and cross-amyloid inhibitor function of this novel class of peptides and should assist in the development of drugs targeting amyloid formation in both AD and 2TD.

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A straightforward chemical strategy for the triple functionalization of a peptide-based multimodal bioimaging probe

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The application of peptides in bioimaging is attracting increasing attention, especially in oncology where peptides able to selectively target tumor antigens and conjugated to imaging molecular probes can be exploited as homing units for the targeted delivery of the imaging agents to the tumor site.[1] Peptide-based imaging probes harbouring a set of reporters, such as fluorophores and radionuclides, appear of great interest in the diagnostic field for frontier applications in multimodality bioimaging, a modern methodology that crosses imaging data obtained through different and complementary techniques.[2] However, preparation of peptide based multimodal probes is not trivial due to challenging synthetic procedures, often performed on solid phase. The development of cost-effective and green chemical procedures enabling the site-specific multiple functionalization of peptide-based imaging probes is strongly desired to further the effective wide-spreading of multivalent peptide probes in bioimaging.[3] Herein, we present a chemical strategy that enables the site-specific triple-functionalization of a peptide-based imaging probe completely performed in solution and requiring mild conditions. The approach exploits native chemical ligation (NCL) and thiol-maleimide addition reactions. The functionalization strategy is straightforward, economically and environmentally more sustainable than classical peptide labelling approaches performed on solid-phase, and appears of general applicability for the development of multivalent peptide probes with applications in multimodality imaging.

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Metabolism and metabolites identification of selective αvβ3 [^{99m}Tc][Tc(N)PNP]-tagged RGDechi peptides

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RGDechi peptide has been characterized as potent and selective antagonist of $\alpha_v\beta_3$ receptors.[1,2] Studies on animal models of ^{18}F- and [^{99m}TcN(PNP)]-tagged peptide confirmed the ability of the radiolabeled derivative to discriminate between $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins. Unfortunately, the absolute uptake of the tracers in $\alpha_v\beta_3$ positive tumors resulted low[3], likely because of the *in-vivo* degradation as demonstrated by in-vitro studies. For enhancing the bioavailability and accumulation at tumor site, the two derivatives RGDechi[1-17] and ΨRGDechi[4] were tagged with the [^{99m}TcN(PNP)]-moiety, *in-vitro*/*in-vivo* stabilities were assessed in mouse, human sera, human whole blood and murine tissue homogenates, and compared with the degradation profile of the native RGDechi. Data obtained from RP-HPLC-UV/Radio and mass spectrometry analysis support the expected formulation consisting of the [Tc(N)PNP]-scaffold bound to one cysteine-N,S chelator carrying the peptide, in a syn/anti isomeric arrangement. Compounds resulted stable in whole blood and sera, but not in mouse kidney homogenate and urine. Findings collected allow the full identification of the degradation products for the radiolabeled peptides that resides in a common cleavage site identified between the Asp7↓Asp8. Our study demonstrated a different metabolic fate of the radiolabeled RGDechi compared to the unlabeled peptide probably due to the conjugation of the [Tc(N)PNP]-moiety that might limit the recognition of the peptides by the serum enzymes. These achievements provide important information to improve the enzymatic stability of the RGDechi-based peptides, which can result in the overall magnification of the pharmacokinetic profile of corresponding radiolabeled compounds.

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Des3PI: A Computational Fragment-based Approach to Design Peptides Targeting Protein-Protein Interactions

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Protein-protein interactions (PPI) play crucial roles in many cellular processes and their deregulation often leads to cellular dysfunctions[1]. One promising way to modulate PPI is to use peptide derivatives that bind their protein target with high affinity and high specificity[2].

Peptide modulators are often designed using secondary structure mimics. However fragment-based design is an alternative emergent approach in the PPI field. Most of the reported computational fragment-based libraries targeting PPI are composed of small molecules or already approved drugs[3,4], but according to our knowledge, no amino acid based library has been reported yet.

In this context, we developed a novel fragment-based approach called Des3PI (Design of Peptides targeting Protein-Protein Interactions) with a library composed of single amino acids. Our goal is to find the optimal sequence of cyclic peptides that will bind a given protein surface with high affinity. Each amino acid of the library is docked into the target surface using Autodock Vina. The resulting binding modes are geometrically clustered, and in each cluster, the most populated amino acids are determined and form the hotspots that will compose the optimal cyclic peptide.

This approach has been applied on five proteins: Mcl1, Ras, Abeta, PDZ and SH3 domains. For each target, the best peptides determined by Des3PI have been tested *in silico*. First, the peptides were blindly redocked on their target. Secondly, the stability of the correctly redocked complexes has been verified using 200 ns MD simulations. DES3PI shows encouraging results with at least three peptides for each protein target that succeeds in passing the two in silico validation steps.

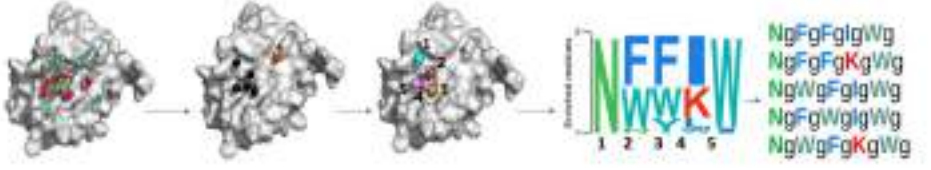

Figure 1. Overview of Des3PI's method

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Characterisation of the novel antimicrobial peptide B7-005: *in vitro* antimicrobial activity against ESKAPE pathogens and biocompatibility with human cells

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Antibiotic resistance is spreading through bacteria worldwide, leading to an increasing number of multidrug-resistant pathogens that are insensitive to most commonly used antibiotics. The World Health Organization has compiled a list of multi-drug resistant pathogens responsible for the majority of nosocomial infections. Among them, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp* (the E.S.K.A.P.E.) are of greatest concern [1multidrug-resistant tuberculosis has been long regarded by WHO as a global priority for investment in new drugs. In 2016, WHO was requested by member states to create a priority list of other antibiotic-resistant bacteria to support research and development of effective drugs.","container-title":"The Lancet Infectious Diseases","DOI":"10.1016/S1473-3099(17[]. New classes of drugs with different mechanisms of action are urgently needed to overcome this problem. Great hopes are focused on proline-rich antimicrobial peptides (PrAMPs). PrAMPs generally have low cytotoxicity due to an intracellular mode of action which is based on inhibition of bacterial protein synthesis, but also a relatively narrow spectrum of activity. To further potentiate these compounds, the mammalian PrAMP Bac7(1-16) was optimized to obtain the peptide B7-005 [2]. This peptide displayed improved antimicrobial potency and a broader spectrum of activity than the original peptide. We are currently investigating the potential of B7-005 to target ESKAPE pathogens. To this aim i) we are evaluating the *in vitro* antimicrobial activity of B7-005 against *E. coli* and representative strains of all ESKAPE pathogens and determining the minimum inhibitory and bactericidal concentrations; ii) we are testing the propensity of B7-005 to allow the development of resistance mechanisms in *E. coli*; iii) we are describing the biocompatibility of the peptide with human cells using a keratinocyte cell line. B7-005 may be a promising agent to develop a safe drug that does not cause the development of bacterial resistance while effectively killing ESKAPE pathogens.

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α-helix conformation and stability analysis of a VEGF mimetic peptide switched in the N-capping region from L- to D- amino acids: more than a preliminary view

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The correct helical fold of the VEGF mimetic peptide QK [1, 2], as in the most helical peptides, starts from the N-capping region considered the key factor driving the folding process [3, 4]. We modified this region introducing the first three amino acids in their D stereo isoform to evaluate the effect of such a modification on peptide folding and stability. The effect of the chiral inversion at the N-terminal region of QK was investigated through conformational studies in solution by circular dichroism and NMR spectroscopy. In serum stability and in vitro proliferative effect of QK after this modification was verified, reporting the first example of a N-capping chiral inversion on folding and biological behavior of an alpha-helix bioactive peptide.

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Cysteine-containing peptides for generation of modulable self-supporting hydrogels matrices

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Peptides cover important biological roles, being both functional and structural elements. They have been widely used as constitutive building blocks for the generation of supramolecular architectures, including nanofibers, nanodots, and hydrogels (HGs).[1] Specifically, peptide-based HGs drew increasing interest due to their large range of applications. Originated by a multiscale self-assembling phenomenon, structural studies and molecular dynamics simulations conducted by us pointed out the capability of some completely aromatic peptides to gelificate.[2] We enlarge our gelling peptide library, reporting about the effect of the introduction of a Cys residue in the heteroaromatic sequence of (FY)3 and in its PEGylated variant. Both FYFCFYF and PEG8-FYFCFYF are able to self-assemble in cross-β based supramolecular nanostructures, but gelification occurs only for FYFCFYF.[3] The resulting HG can undergo an additional cross-linking step *via* Cys-Cys oxidation with a consequent rigidity improvement (value of storage modulus G' from 970 to 3360 Pa). The Cys insertion made the hydrogel responsible to the external stimulus of oxidation and could additionally allow the hydrogel derivatization with targeting moieties or with biologically relevant molecules.

Figure 1. Structure of Cys-containing peptides and schematic representation of the double interfaces supposed organization.

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Solid-state optical properties of self-assembling amyloid-like peptides with different charged states at the terminal ends

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The self-assembling of small peptides not only leads to the formation of intriguing nanoarchitectures, but also generates materials with unexpected functional properties [1]. Oligopeptides can form amyloid-like cross-β assemblies that are able to emit intrinsic photoluminescence (PL), over the whole near-UV/visible range, whose origin is still largely debated [2]. As proton transfer between the peptide chain termini within the assembly is one of the invoked interpretations of this phenomenon [3], we here evaluated the solid-state PL properties of a series of self-assembled hexaphenylalanine peptides characterized by a different terminal charge state. Overall, our data indicate that the charge state of these peptides has a marginal role in the PL emission as all systems exhibit very similar multicolour PL associated with a violation of the Kasha's rule [4]. On the other hand, charged/uncharged ends occasionally produce differences in the quantum yields. The generality of these observations has been proven by extending these analyses to the $A\beta_{16\text{-}21}$ peptide. Collectively, the present findings provide useful information for deciphering the code that links the spectroscopic properties of these assemblies to their structural/electronic features.

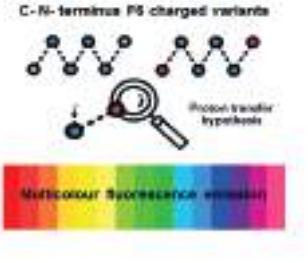

Figure 1. Schematic representation of multicolour fluorescence emission due to peptide nanofibers

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All aromatic hydrogelators from a dopamine and 2-naphytlalanine hexapeptide library

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Peptides have been proposed as versatile candidates for the development of hydrogels. Recently, we developed a novel hybrid polymer–peptide conjugate, PEG8-(FY)3, which can self-assemble into a self-supporting soft hydrogel over dry and wet surfaces.[1] This aggregative model was demonstrated by molecular dynamics simulations. Here, we describe the synthesis and supramolecular organization of six novel hexapeptides rationally designed by punctual chemical modification of the primary peptide sequence of the ancestor peptide (FY)3. Non-coded amino acids were incorporated by replacing the phenylalanine residue with naphthylalanine (Nal) and tyrosine with dopamine (Dopa). We also studied the effect of the modification of the side chain and the corresponding PEGylated peptide analogues, on the structural and mechanical properties of the hydrogel. Secondary structure, morphology and rheological properties of all the peptide-based materials were assessed by various biophysical tools. The *in vitro* biocompatibility of the supramolecular nanostructures was also evaluated on fibroblast cell lines. We conclude that the PEG8-(Nal-Dopa)3 hydrogel possesses the right properties to serve as a scaffold and support cell growth.

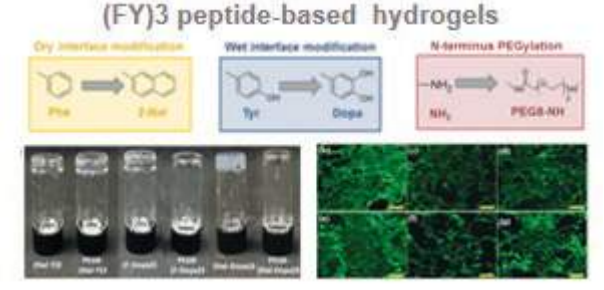

Figure 1. Chemical modification of (FY)3 ancestor peptide, macroscopical hydrogels and *in vitro* biocompatibility test.

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Applications of NMR spectroscopy for protein-peptide interaction studies on living cells

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The interaction of peptides with biological membranes is central to a number of biological processes, such as the insertion and folding of peptides in membranes, the rupturing of membranes by toxins and the membrane-mediated mechanism of peptide-protein interaction. To target membrane-bound receptors, several homonuclear NMR spectroscopic experiments have been extensively used in their physiological environment [1,2], detecting binding events and providing information on the bound conformation of the ligands. Herein, we show how NMR methodologies has been used to investigate binding events at the cell surface between designed VEGF mimetic peptides and membrane-bound proteins [3,4].

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Amyloid-like Prep1 peptides exhibit an intrinsic fluorescence signature in vitro and in living cells

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Extensive characterizations of amyloid-like aggregates carried out in the last decade have unravelled that they are endowed with unexpected and interesting properties that go well beyond their peculiar structural organization. Indeed, these assemblies present unusual spectroscopic properties whose underlying physicochemical determinants remain still quite elusive. In particular, they can emit a blue fluorescence upon excitation with near-UV radiation (~370 nm). Moreover, they are also able to emit at much higher wavelengths extending to the red and near-infrared region when excited with radiation with wavelengths in the range 600–670 nm.[1] Interestingly, we here show that the peptides PREP1[117-132] and R8PREP1[297-311], extracted from the sequence of the homeobox protein PREP1, are able to form β-rich amyloid-like at physiological conditions that, in addition to these emissions, also present a strong fluorescence emission in the green region (maximum at 520 nm upon excitation at 440–480 nm) in very different contexts as solution, solid-state, and living cells.[2,3]

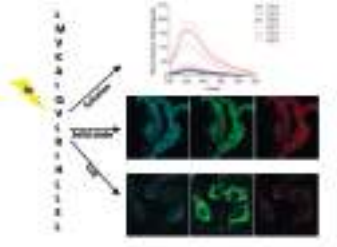

Figure 1. The intrinsic fluorescence of PREP1[117-132] β-sheet rich aggregates in solution, solid state and cells.

These peptides also present a remarkable structural versatility adopting a β-sheet or alpha-helical organization in dependence of the physico-chemical environment, a feature paralleled by their ability to emit fluorescence.[2,3] In particular, we show that the reversible structural transition exhibited by PREP1[117-132] passing from neutral to acidic pH is an effective molecular switch to turn on and off its fluorescence emission. This finding is particularly intriguing considering the recent interest in reversible and non-reversible amyloids and their roles in functional or pathological processes.

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The interplay between carbonyl carbon pyramidalizations and n→π* interactions in biomolecules

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Biomolecules generally operate in the intricate environment of living organisms by adopting well-defined structural states that are attained through the combination of multiple electronic and steric effects. Despite the huge amount of data collected over the years, the identification and the estimation of the role that non-covalent interactions play in dictating or modulating the spatial organization of this class of molecules remains a challenging task, even for biomolecules with limited complexity. In recent years, it has been suggested that, in addition to well-known forces as hydrogen bonding and electrostatic/van der Waals interactions, the structure of biomolecules is stabilized by minor, yet crucial, effects denoted as secondary forces. [1] Among these, particularly interesting is the interaction between carbonyl groups and nucleophiles (including other carbonyl groups) through contacts in which the nucleophile donates lone-pair (n) electron density into the empty π* orbital of the carbonyl group (n→π* interaction). [2,3] It has also been suggested that n→π* interactions favour local distortion of the carbonyl group planarity and that the observation of the carbonyl carbon pyramidalization may be a signature of this interaction. [4,5] Since we previously showed that distortions, including pyramidalization, of the carbonyl carbon group in proteins and peptides are mainly dictated by local conformational effects, [6-8], we interrogated the Cambridge Structure Database to gain further insights into this puzzling issue. In particular, we preliminary searched the database for structures containing carbonyl derivatives as amides, esters, and thioesters. Independently of the presence of a nearby nucleophile, we could detect a significant conformation-dependent pyramidalization for these classes of compounds, thus indicating the generality of the conformation-induced pyramidalization previously reported only for peptides and proteins. In other to evaluate a possible interplay between the n→π* interaction and the pyramidalization, we evaluated the deformation of the carbonyl group as function of the presence/absence of a nearby nucleophile. Interestingly, this comparative analysis demonstrated that the n→π* donation was indeed able to increase the conformation-dependent pyramidalization. These findings indicate that the pyramidalization-inducing conformations represent a prerequisite for the donation.

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Nanosystem for antiviral delivery

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For medical and health care, nanotechnology is a science that increasingly stimulates the development of therapies through the preparation of nanometric materials, which have unique physico-chemical properties, which can be exploited in different biomedical applications. Biomedical research in the field of infectious diseases is focused on the development of targeted delivery systems of sustainable and biocompatible drugs, with low toxicity, high stability and therapeutic efficacy [1]. The infection process involves one or more glycoproteins present on the external surface of the enveloped viruses, their conformational change is a crucial step that governs the fusion of viral and cellular membranes in the entry of enveloped viruses [2,3]. Antiviral peptides are able to interfere with the conformational modifications of surface glycoproteins that are required for viral fusion, inhibiting viral attachment and penetration, competing for receptor sites. Peptides mimicking domains of viral glycoproteins are apt to interfere with the fusion event, we have developed a peptide sequence with a high potential to inhibit the entry of Herpes simplex virus type 1 and we developed a strategy, similarly to other viruses, to deliver these sequences directly to the membrane through cholesterol conjugation in order to potently block fusion [4]. The peptide conjugated to polyethylenglycol and cholesterol interacts with viral and cell membranes thanks to the presence of cholesterol and blocks the conformational rearrangements of the glycoprotein B [5,6]. Our results show that the most efficient nanosystem is able to self-assemble into looser aggregates and bind extensively to membranes where fusion takes place increasing the local peptide concentration.

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New hope in the war of antibiotic resistance: Antimicrobial Peptide

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Bacteria are ubiquitous. A large percentage of the world's bacteria cause infection and disease determining a dramatic impact on public health. With the first-line antibiotics becoming less effective, patient care is becoming more expensive for the need of more costly medications. Besides, these have only limited action against resistant strains. Conventional antibiotics are inefficient against biofilms, due to enhanced persistence of bacteria grown in the sessile mode respect to bacteria grown planktonically. The production of an exopolysaccharide matrix and phenotypic changes contribute to biofilm resistance to antibiotics.

Antimicrobial peptides (AMPs) have rapidly captured attention as novel drug candidates. These are relatively short, commonly consisting of 10–50 amino acids, display an overall positive charge ranging from +2 to +11, and contain a large segment (typically 50%) of hydrophobic residues. Many AMPs exhibit a broad range of antimicrobial activity against Gram-positive and Gram-negative bacteria but also fungi, viruses, and unicellular protozoa. Generally, positively charged peptides target the negatively charged cellular membranes of bacterial cells resulting in cellular membrane destabilizations that leads to rapid cell death. [1] However, available AMPs may exploit also different mechanisms. [2]

WMR-K is a 14 amino-acid peptide derived from the marine AMP mixinidin, which is one of the shortest AMPs discovered so far. It showed potent antibacterial activity against a broad range of bacteria and yeast pathogens at minimum bactericidal concentrations (MBC) between 1 and 10 μM. In particular, the microbicidal activity was evaluated against *E. coli*, *P. aeruginosa*, *S. typhimurium*, *K. pneumoniae* and *S. aureus* and the minimal inhibitor concentration calculated was 2, 2, 1.2, 3, 2 μM respectively. Moreover, in vitro experiments on J774 murine macrophages demonstrated that WMR-K has an anti-inflammatory activity at 30 μM and it's no toxic up to 100 μM.

WMR-K will be used to obtain a versatile nanosystem useful on different type of bacterial infections and antibiotic resistance pattern.

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New structural determinants for antimicrobial peptides?

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Antimicrobial peptides (AMPs) AMPs are a skilled class of new antibiotics that present increasing applications in the public health as well as is in the food industry as bactericidal, bio-preservatives and anti-biofilms. [1,2] Numerous studies are devoted to discovering AMPs with improved performances, such as high antimicrobial activity, low cytotoxicity against human cells, stability against proteolytic degradation, and low costs of production. When designing new AMPs, several physicochemical features, as hydrophobicity, net positive charge, propensity to assume amphipathic conformation and self-assembling properties, must be considered. Starting from the sequence of the dodecapeptide 1018-K6, we designed a 10-aminocids peptide, namely RiLK1, which exhibited a stronger killing efficiency than the 1018-K6 peptide against *Listeria monocytogenes* and *Salmonella typhimurium*, as well as against fungal pathogens. [3, 4] The structural reasons that make RiLK1 a more efficient antimicrobial than 1018-K6 have been explored using a combination of CD, NMR, and fluorescence spectroscopies, together with optical and atomic force microscopies, and by comparing the two peptides both with respect to their ability to self-aggregate, and for their conformational and interaction profiles with a bacterial membrane mimic (SDS micelles). [5]

Our results show that the spectroscopic and microscopic profiles are clearly different for RiLK1 and 1018-K6.

If in pure water, both peptides adopt random conformations, the presence of SDS stabilizes the two peptides in ordered structures. In particular, the effect of SDS on 1018-K6 is to tighten its conformational distribution to the 100% of the beta form, on RiLK1 is to widen the conformational distribution with the possibility to adopt alpha structures, too. This suggests that RiLK1 may have a wider spectrum of mechanisms of action, and for all these features, RiLK1 represents a promising candidate for a new class of peptide-based antibiotics.

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Relationship excluded volume interactions/biological activity in D-leucine containing sequences

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Conus snail are a group of predatory living in tropical oceans all over the word. They can produce highly diversified conotoxins for defense and predation. Conotoxins are believed to number about 50.000, and could serve as a rich source of active compounds. Conotoxins are mainly disulfide bond-rich peptides of 10-40 residues. A small number of conotoxins have zero or only one disulfide bond; they are classified in different families of D-residue containing conotoxins: contryphans, conophan and conomap. Apart contryphans (eight amino acids long with highly conserved sequence motifs) other gene products that have been characterized with D-amino acids are quite a heterogeneous assemblage. Despite this it has been noted that there are preferential loci for modification, i.e. either for the second amino acid from the N-terminus, or on the third amino acid from the C-terminus. Another striking feature of conotoxins is the high content of different post-translational modifications. Here we are interested to epimerization of different residues. DNA cloning has shown that at those positions where a D-amino acid is found in the end product, a normal codon for the corresponding L-amino acid is present. This implies that the D-residue are formed from L-amino acid by a post-translational reaction. Here we report about homologues sequences of the native peptide conotoxins having L-leucine on the third amino acid from the C-terminus and the isomer with D-leucine in the same position. Our work highlight the importance of structural factors, beyond the disulfide pattern and electrostatic interactions, in the understanding of the functional properties of bioactive peptides. The latter needs to be considered when designing analogues for further applications.

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Combining biological structures and molecular self-assembly lead to ordered nanostructures

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The bioactivities of peptides encrypted in major food proteins are latent until released and activated by enzymatic proteolysis or food processing. The study of conformational properties of synthetic homologues of these bioactive peptides allows us to identify the biological structure. Peptide assembly structures have been widely exploited in fabricating materials promising for bioapplication. Peptides can self-organize into various highly ordered supramolecular architectures. Detailed studies of the molecular mechanism by which these versatile building blocks assemble can guide the design of peptide architectures with desired structure and functionality. It has been revealed that peptide assembly structures are highly sequence-dependent and sensitive to amino acid composition, the chirality of peptide and amino acid residues, and external factors, such as solvent, pH, and temperature. Combining biological structures and self-assembling in synthetic heterochiral peptides provides a powerful means to direct biological supramolecular materials formation. The creation of synthetic molecules that enable precise control over spacing and functionalization provides opportunities across diverse disciplines. Key requirements of functionalizable oligomeric scaffolds include the specific control of their molecular properties where the correct balance of flexibility and rigidity must be maintained in addition to the prerequisite of defined length. In this report, we describe our use of peptide model systems that fold cooperatively yet are small enough to be chemically synthesized to measure such quantities.

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Self-assembly of short peptides into hydrogels

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Bioactive peptides, deriving from natural protein fragments, capable of self-assembly into ordered supramolecular structures are ideal seeds to generate hydrogels. Indeed, self-assembling peptide (SAP)-based hydrogels show unique characteristics: high water content, tuneable mechanical stability, great biocompatibility, and low production costs [1-2].
To modulate SAPs' mechanical properties, the insertion of chemical modifications as the change of the chirality of single amino acids is a successful tool [3]. A short stretch spanning residues 268-273, ^{268}FINYVK^{273}, of the C-terminal domain of Nucleophosmin 1 showed amyloid-aggregation features [4]. Recently, we analyzed the structural properties of sequences deriving from a D-scan of this sequence. The kinetic and conformational features of the derived hexapeptides and the morphologies of microstructures were investigated by different biophysical techniques, and the biocompatibility of the hydrogels was examined in Hela cells. Results demonstrated that all sequences were able to self-assemble providing hydrogels endowed with different conformational intermediates in dependence of introduced chirality [5]. Since hydrogel formation can be strongly tuned by net charges at extremities of sequences, ongoing studies are focused on this effect of ultrashort [6] self-assembling sequence ^{268}INYVK^{273}, in four differently protected variants.

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Synthesis, spectroscopic and electrochemical studies of Dap homo-peptides, conjugated to ferrocene moieties

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TOPIC: Folding and Aggregation of Peptides

Electron transfer processes in proteins depend primarily on the distance between the centres involved, but are heavily mediated by the nature of the amino acid side chains, by backbone conformations and hydrogen bonds.[1-3] In particular, the macrodipole moment generated by ordered structures (*e.g.*, helices) appears to play a relevant role. In this contribution we report synthesis, conformation, and electrochemical behavior of new ferrocenyl-peptide systems (Figure 1). They are characterized by helical homo-peptide spacers, based on 2,3-diaminopropionic acid (Dap), with pendant ferrocenyl (Fc) moieties.[4]

Figure 1. Chemical structures of the Fc-peptide conjugates synthesized and studied in this work.

Thanks to the appropriate placement of the Fc redox probes along the peptide chains, we were able to modulate the electronic properties as a function of the applied potential.
Conformational studies in solution (^{1}H NMR, IR, and CD) and in the crystal state (X-ray diffraction) revealed the tendency of side-chain protected, Dap homo-peptides to adopt β-turn or helical conformations. An in-depth electrochemical (cyclic voltammetry) and spectroscopic (UV-Vis variations following oxidation) analysis, performed to map the charge transfer mediated by the peptide spacers, highlighted the influence of the molecular skeleton on the redox and optical properties of the molecules.

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Fluorescent nanospheres obtained by PEGylated tetratyrosine nanofibers heating process

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Due to their interesting mechanical, electrical and optical properties, nanostructures obtained by aggregation of aromatic peptides have been proposed as innovative tools for biomedical, biotechnological and industrial application.[1] Recently, several efforts have been employed in the development innovative materials as nanoscale fluorescence (FL) imaging probes.[2] In this context, we describe the synthesis and the functional properties of fluorescent tyrosine (Y)-based nanospheres, obtained by heating at 200 °C a solution of the PEGylated tetra-peptide, called PEG6-Y4.[3] At room temperature, this peptide self-organizes into not fluorescent water-soluble fibrillary aggregates. After heating, the aggregation phenomenon produces Y4-based nanospheres. These nanoarchitecture were found able to emit FL into blue, green and red spectral regions. After a deep investigation of their optoelectronic properties, these nanospheres could be exploited as promising tools for precise biomedicine in advanced nanomedical technologies (local bioimaging, light diagnostics, therapy, optogenetics and health monitoring).

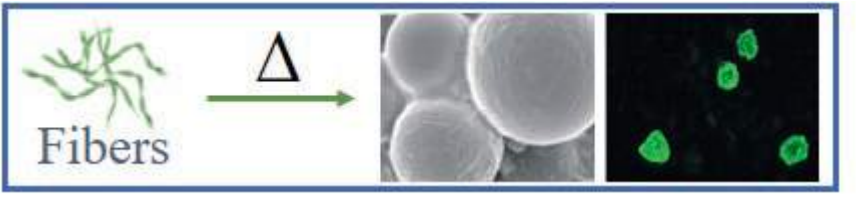

Figure 1. Fibers of PEG6-Y4 are converted in photoactive nanospheres *via* heating step.

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Insulin conjugates for receptor studies and therapeutic applications

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Insulin and insulin-like growth factors 1 and 2 (IGF-1/2) are important hormones that share similar 3D structures and cell membrane receptors. Insulin and IGFs cross-bind to these receptors with different affinities and trigger distinct but overlapping physiological effects; predominantly metabolic for insulin and predominantly mitogenic for IGFs. This complex system plays a major role in the regulation of metabolism, growth, development and lifespan as well as in the development of cancer, diabetes, growth-related and neurological diseases. [1]

In general, the development of any receptor-selective agonist/antagonist for both isoforms of insulin receptor (IR-A and IR-B) and for IGF-1R would be of great interest; even beyond if we talk about the discovery of an IGF-1R specific antagonist with potential anticancer clinical application. With this objective in mind, we designed the synthesis of different conjugates formed by insulin connected with different linkers to peptides derived from IR or IGF-1R sequences. To achieve these conjugates, we selectively modified the hormone[2] and peptides by introducing a terminal alkyne to perform an azide-alkyne cycloaddition reaction[3] with bis-azide linkers. The conjugates will be tested in binding and functional assays with IR and IGF-1R to investigate their binding affinities with the receptors and the possibly modulation of their physiological properties.

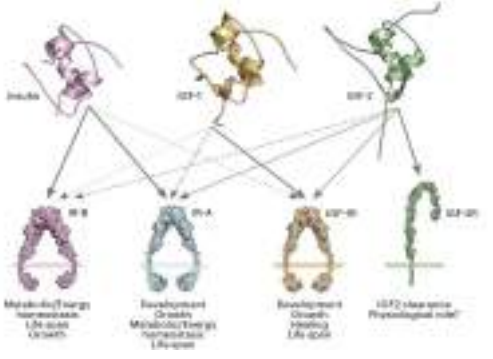

Figure 1. Insulin, insulin-like growth factor 1 and 2 and their receptors.

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Self-assembled peptide gels for the release of active compounds

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Low-Molecular-Weight Gelators (LMWGs) are a versatile class of compounds able to self-assemble into supramolecular architectures thanks to non-covalent interactions.[1] Peptide-based gelators are ideal candidates for the preparation of such materials because they are usually biocompatible and can be effectively synthesised and functionalised. The gelation process usually starts with a stimulus (pH change, ultrasound sonication, salt addition), that induces the formation of entangled fibres, which over time form a network strong enough to entrap the solvent.

The opportunity to form materials with unusual and interesting properties can be exploited when two gelators are mixed together to form a multicomponent system. In this work we prepared a library of multicomponent gel using two gelators, Boc-L-Dopa(Bn)$_2$-OH (**1**)[2] and Boc-L-Ala-Aib-L-Val-OH (**2**) [3]. Compound **2** is not able to form gels in water alone but can induce the gelation of **1** in phosphate buffer solution acting as both co-gelator and trigger. We obtained gels with increasing quantities of **2** which influence the final pH of the gels, in a range useful for both biomedical and cosmetic applications (7.4 - 6.5). Two bioactive peptides TFA-L-Val-L-Tyr-L-Val-OH (**A**) and Pal-L-Lys-L-Val-L-Lys-OH (**B**) with antiaging activity were synthesised and introduced in the formulation. The release profile of the two active ingredients and their permeation through pig skin were evaluated using Franz cells. The multicomponent peptide gels obtained in this work constitute a valid and biocompatible formulation for both drug delivery and cosmetic applications.

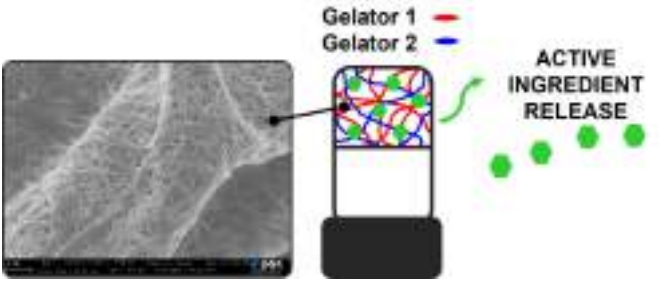

Figure 1. Multicomponent peptide gel for the release of active compounds.

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Development of Bombesin Based Peptide-Drug Conjugates and Combination with Conjugates Targeting Gonadotropin Releasing Hormone (GnRH), CD13 and Integrin Receptors

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After developing Peptide-Drug Conjugates (PDC) targeting GnRH-R, Aminopeptidase N (CD13) and integrin receptors, our research group identified the Gastrin Releasing Peptide Receptor (GRP-R) as suitable for targeted tumour therapy due to its overexpression in cancerous tissues such as breast, prostate, pancreatic and small-cell lung cancer[1]. Herein, we report on the selective delivery of the cytotoxic drug daunorubicin to prostate and breast cancer by targeting the mentioned receptors. We produced daunorubicin containing PDCs acting as drug delivery systems to safely reach the tumour environment, using many bombesin analogues as homing peptides. Here, after a receptor mediated internalisation by the malignant cells, the presence of a Cathepsin B cleavable spacer between the peptides and the drug ensures the release of the cytotoxic payload. *In vitro* experiments included the assessment of internalisation efficacy and cytotoxicity on the PC-3 human prostate cancer cell line and MDA-MB-231 and MDA-MB-453 human breast cancer cell lines. The stability of the compounds was ensured in mouse plasma and the Cathepsin B mediated release of the daunorubicin in rat liver lysosomal homogenate. The investigation of such properties led us to develop new peptide sequences to be studied in the same manner. Two bioconjugates revealed IC_{50} values in the low micromolar range, an efficient uptake by all the tested cell lines, high stability in plasma and a prompt release of the drug containing metabolite. The expression of GRP-R, GnRH-R and integrin receptors by common tumours induced us to question whether we could observe a synergistic effect by using mixtures of conjugates targeting these receptors. Therefore, we tested the cytotoxicity of the combinations between the lead bombesin based bioconjugates and those previously developed by our group, based on GnRH-III[2] and NGR[3], respectively. In conclusion, we highlight the importance of PDCs binding GRP-R, GnRH-R, CD13 and integrin receptors in targeted cancer therapy, with the possibility of further tailoring and optimisation.

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Constrained α-helical macrocyclic peptides for enhancing the Antibiofilm activity on native Temporin L peptide

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Antimicrobial peptides (AMPs) with broad-spectrum activity represent excellent candidates for inhibiting fungal biofilm formation and eradicating preformed biofilms. Among the frog skin AMPs, temporins represent encouraging candidates for the development of novel antibiofilm agents.[1]
Solution-state NMR studies revealed that Temporin L and some synthetic analogues exhibit a high helical content (>70%) responsible for their strong haemolytic activity, related to a "barrel stave" mechanism.[2]
Here, we presented cyclic temporin analogues that can disrupt established surface biofilms of Candida species at sub-MIC concentrations without being cytotoxic to mammalian cells.
The antibiofilm activities of all compounds were evaluated on representative Gram-positive and Gram-negative bacteria, *Candida* strains (*C. albicans, C. glabrata, C. auris, C. parapsilosis and C. tropicalis*) and the haemolytic activity was evaluated.[3] The helical content of the most promising peptides was predicted using CD spectra, and NMR spectroscopy was used to study their conformational changes. The mechanism of action was studied by performing fluorescence assays, and the improved stability compared to proteases, due to structural constraints, was assessed by the human serum biostability assay.

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Structure-based design of EGF-like bicyclic peptide inhibitors of protein Nodal activity

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Bicyclic peptides assembled around small organic scaffolds are gaining an increasing interest as new potent, stable and highly selective therapeutics due to their uncommon ability to specifically recognize protein targets, to the small size that favour tissue penetration and to the versatility and easiness of synthesis. Here, we report the design and *in vitro* testing of a bicyclic peptide targeting the onco-fetal protein Nodal. Nodal expression is normally restricted to embryogenesis, while after development its expression is associated with increased tumourigenesis, invasion of tumour cells and metastasis. It has long been investigated as a target for treating several types of cancers and to modulate stem cells fate. Nodal mostly works by binding to and activating the cell surface receptors ALK4 and ALK7 in cooperation with the co-receptor Cripto-1 to form a ligand–receptor complex that leads to the phosphorylation of Smad2/3 and the transcription of target genes, including Nodal itself [1].

Aiming to identify Nodal binders able to inhibit its signalling, we have rationally designed a bicyclic peptide by taking the loops of the EGF-like domain of Cripto-1 that binds Nodal and assembling them around a tribromomethyl-benzene (TBMB) scaffold [2,3]. Binding studies have demonstrated that the EGF-like bicyclic peptide binds Nodal and *in vitro* is able to interfere with Nodal-induced signalling and to suppress the Nodal-promoted proliferation of cancer cells.

This molecule is being further investigated as a potential therapeutic and as carrier for the targeted delivery of cytotoxic drugs in Nodal-1-positive tumours.

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Studying the structural determinants of aggregation in peptides of the Gadd45 protein family members

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The Growth Arrest and DNA damage-inducible 45 (GADD45) gene family encodes three related highly similar proteins, GADD45α, GADD45β, and GADD45γ [1], involved in fundamental physio-pathological processes which include growth arrest, cell cycle control, DNA repair and apoptosis. The antiapoptotic factor GADD45β together with the JNK kinase MKK7 represents an interesting therapeutic target in multiple myeloma [2]. We have found that GADD45β undergoes denaturation by forming β-rich amyloid-like aggregates that are cytotoxic in physiological conditions [3]. The characterization of the unfolding process of the two other members of the family (GADD45α and GADD45γ) highlighted analogies and differences. While GADD45α displays a behaviour somehow similar to that exhibited by GADD45β, GADD45γ exhibits a partial and reversible unfolding without forming any aggregate. Further characterizations of these systems have provided insights into the determinants that favour/disfavour the amyloid-like aggregation in GADD45 proteins and suggested that GADD45β amyloid–like aggregates are able to form non-toxic hydrogels and nanogels (Smaldone, Caruso et al. in preparation). Using structural/biophysical techniques, limited proteolysis experiments, mass spectrometry and peptide synthesis, we identified the protein region(s) contributing to the self-aggregation characteristics of these proteins and studied the shared and divergent residues that could impart the different properties to the three proteins. Interestingly, the characterization of the GADD45β-derived peptides indicates that they retain the ability of the parent protein to self-assemble and to form hydrogels.

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Peptide-capped Gold Nanoparticles: a biomimicking approach towards organ cryopreservation

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Transplant practise has improved the life duration and quality of many patients. However, in 2017 only 10% of the worldwide need was met due to the limited number of donor organs and the inefficient preservation methods, that hinder their long-term preservation. Strategies that extend the donor organ lifetime are thus an urgent global goal.[1] In nature, organisms living in extreme environment produce antifreeze proteins (AFPs), that can prevent the growth of the ice crystals and depress the freezing point of body fluids.[2] Among the different classes of AFPs, Type I AFPs are particularly interesting because of their moderate size, the simple primary structure, and the helical secondary structure.[2] Four different peptides derived from the N-*terminus* sequence of Type I AFPs HPLC6 were conjugated to gold nanoparticles (*Au*NPs), by exploiting a multimeric approach. The goal of decorating the nanoparticles with multiple copies of the peptides is combining the ice-binding capability of peptides with the size of a nanoparticle, mimicking the globular protein bulkiness. Although the conjugation of peptides presenting ice-binding moieties to *Au*NPs has already been successfully employed [3], this is the first time that a native truncated sequence derived from an AFP and its derivatives were conjugated to *Au*NPs in order to enhance their antifreeze activity. Three peptide sequences [4] were synthesized by MW-assisted SPPS using the Fmoc/t-Bu strategy and their N-*terminus* were on-resin coupled to 3-(Tritylthio)propionic acid, allowing in-solution conjugation to *Au*NPs. Moreover, a thiol-derived stapled peptide was designed and synthesized exploiting MW-irradation for the on-resin CuAAC.[5] The peptides and the conjugated AuNPs were fully characterized, and their ice recrystallization inhibition (IRI) activity was tested by sucrose sandwich assay using optical microscopy.

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Design of short catalytic peptides inspired by hydrolases

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Short catalytic peptides are tunable, low-cost biomolecules able to catalyze chemical reactions such as ester hydrolysis [1]. The main drawback is their low catalytic efficiency attributed to the lack of well-defined tridimensional structures characteristic for enzymes. Peptide self-assembly offers the possibility to obtain nanostructures with a higher degree of order leading to improved catalytic efficiency. Alternatively, the cyclization of linear peptides restrains their conformational freedom, which could stabilize the active site. However, better understanding of the principles that govern the catalytic activity of short peptides at the sequence level is needed. Our aim is to design short catalytic linear and cyclic peptides to assess whether the enhanced rigidity obtained by cyclization improves their catalytic efficiency. The design of the sequences was inspired by active sites found in hydrolases acting on ester bonds (EC 3.1.) and it was based on the selection of a fragment from the primary sequence containing the triad retaining its chemical environment. Three sequences (SGNYDYLHGE, CTLGLGSHCGG, and GGESTGHTGAGNDK) obtained from the 1-alkyl-2-acetylglycerophosphocholine esterase and protein-glutamate methylesterases (CheD and CheB), respectively, were synthesized using solid-phase peptide synthesis (SPPS). The cyclization was achieved by three different methods (head-to-side chain, disulfide bridge formation and side chain-to-side chain) depending on the sequence composition. The peptides were purified using semipreparative-HPLC and characterized by LC-MS and analytical HPLC. The catalytic activities of the linear and cyclic peptides were evaluated using the 4-nitrophenyl acetate (*p*-NPA) colorimetric test. A successful outcome of this project will contribute to a better understanding of the relationship between short peptide sequences and their catalytic activity applicable to peptides with esterase activity.

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The Proline hinge motif controls the immunomodulatory fucntion of bohevine herpesvirus 1-encoded inhibitor of TAP

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Varicellovirus-encoded UL49.5 protein is a potent inhibitor of transporter associated with antigen processing (TAP), allowing viruses to avoid recognition by cytotoxic T cells. The BHV-1 UL49.5 encodes a small, type I transmembrane protein acting upon TAP in two ways: causing conformational arrest within TAP and inducing its degradation. The exact mechanism of UL49.5 activity remains elusive. To determine the active sites of UL49.5, we established its 3D structure using two-dimensional nuclear magnetic resonance, circular dichroism and molecular dynamics in membrane-mimicking phospholipids.

The formation of N-terminal structural motifs was highly dependent on the membrane environment. Based on the revealed structure of UL49.5 we investigated its potential active sites, such as the ER-lumenal alpha-helix, stabilizing salt bridges or the “proline hinge” formed by the type II β-turn.

We designed mutations disturbing the structural motifs as confirmed by NMR/MD. UL49.5 variants were expressed in human melanoma MJS cells transduced with retroviral vectors. The results correlate the (in)stability of UL49.5 with TAP degradation. On the other hand, mutants failing to sensitize TAP to ERAD could efficiently inhibit the transporter, questioning the importance of degradation for UL49.5 activity. Finally, while the helical motif in the UL49.5 N-terminal domain seemed redundant for MHC class I downregulation, lack of the “proline hinge” resulted in a massive loss in protein function.

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Structural vaccinology for the design of vaccine antigens against difficult infectious pathogens

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Structural vaccinology is a rational-based approach to design immunogenic antigens aimed at generating an effective vaccine. It combines experimental methods like X-ray crystallography, molecular biology, electron microscopy and mass spectrometry, with computational methods like molecular modelling and epitope prediction [1-4].

The first step of this approach is the three-dimensional structure determination of the antigen using structural biology tools such as X-ray crystallography, cryo-electron microscopy, and nuclear magnetic resonance. Key to vaccine development is the knowledge of the exact regions of an antigen that are recognized and bound by antibodies. This knowledge may be acquired using experimental methods named as "epitope mapping", which makes use of x-ray crystallography, NMR and more recently cryo-EM. These methods provide the whole set of information needed to engineer new constructs with better properties in terms of elicitation of the immune response, stability in solution and ease of production [5]. Using this approach, we have currently identified and developed several vaccine antigens against difficult nosocomial pathogens, belonging to the so-called ESKAPE family. These studies are being implemented through an integrative effort of Partners of the Marie Skłodowska-Curie Action BactiVax - Anti-Bacterial Innovative Vaccines. This poster will report an update of our structural studies.

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Relation between fluctuations of free Zn(II) concentrations and formation of Zn(II)-binding sites in cysteine-rich motif of MTF-1

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An MTF-1 transcription factor is a key player that participates in the cellular Zn(II) homeostasis via activation of several genes expression. However, the exact molecular mechanism of MTF-1 activation has not been clarified. At the C-terminus, MTF-1 protein contains a unique cysteine-rich (Cys-rich) motif (CXCXCXCX), which is highly conserved among higher eukaryotes. It has been shown that the Cys-rich motif mediates homodimerization of MTF-1 [1], while substitution within Cys residues decreases the transcriptional response [2]. Here, we aimed to examine whether or not the Cys-rich motif may be involved in the metal-sensing. To conduct its physicochemical characterization, a set of shorter and longer peptides were synthesized. Their CD spectral changes upon Zn(II) bidding indicate a similar mode of structurization. The observed changes associated with S → Zn(II) charge transfer are weak, suggesting the subsequent formation of a random coil. Furthermore, to determine the metal-binding affinity of the Cys-rich motif, we performed the competition with chromophoric chelators and complexones. Interestingly, dissociation constant values obtained for the peptides forming ZnL complexes fall within the range of cellular free Zn(II) fluctuations. It confirms the possible role of the Cys-rich motif as a Zn(II) dependent switch under specific free Zn(II) changes. Moreover, the absorption envelope, obtained during UV-Vis Co(II) titration, contains three significant d-d bands typical for a pseudo tetrahedral coordination geometry and {SSSS} binding mode. Their hypsochromic shift indicates the presence of two binding sites. ESI-MS investigation reveals the formation of a Zn_2L_2 complex. To prove its formation in the solution, we performed an SEC analysis showing that the peptides form the dimeric binuclear complex under increasingly free Zn(II) concentration. Additionally, Zn(II) complexation performed in the cellular buffering system - metallothionein (MT) with thionein (T) showed that the motif is saturated with Zn(II) ions in the presence of MT only. The increasing amount of T caused Zn(II) ejection, which correlates well with the relation between thionein induction/inhibition and free Zn(II) fluctuations in HT-29 cells [3].

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Proregenerative peptides as part of a composites for bone reconstruction

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Bone tissue is continuously remodeled through the concerted actions of bone cells, which include bone resorption, formation and remodeling process. This process is under the control of local (e.g., growth factors and cytokines) and systemic (e.g., calcitonin and estrogens) factors that all together contribute for bone homeostasis [1]. However, in pathological fractures or in the case of large defects, the bone does not heal and requires reconstruction. Insufficient blood supply, infection of bones or surrounding tissues and systemic diseases (e.g. osteoporosis, cancer, diabetes) hinder or prevent regeneration [2]. Despite the multitude of available biomaterials to treat bone defects only a small number have achieved clinical use. Biomaterials are of a great significance to regenerative medicine, in bone reconstruction for example, and are undergoing constant improvement, especially when it comes to integration with body tissues and their ability to regenerate tissue. The scaffolds applied in bone regeneration should provide a solid structure support that responds to mechanical stresses and mimic the microenvironment for cell survival and tissue development. [3].

The project aims to devise and produce biologically active material to replace bone loss and act as a cell matrix, at the same time assisting in the regeneration of damaged tissue. The composition of the composite is chitosan/bioglass, containing pro-regenerative peptides slowly released into the bone environment. The obtained material has been fully characterized in terms of its physicochemical properties and biological activity.

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Ability of glucosyl platinum(II) complexes to modulate the amyloid aggregation of the C-terminal region of the Aβ peptide

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The self-aggregation of amyloid β (1-40 or 1-42, Aβ) peptide is implicated in the development of Alzheimer's disease (AD), the most common type of dementia [1]. To contrast this pathology many drug discovery processes are focused on identifying new compounds that can modulate self-recognition mechanisms. Among them, transition metal complexes are receiving great attention [2-3]. Indeed, thanks to their unique characteristics (e.g., oxidation, spin states and coordination fields of metal ions), transition metal complexes are able to interact with proteins and regulate their conformational and morphological features [4].

In this work, two glycosyl pentacoordinate Pt(II) complexes, named $1Pt_{dep}$ and 1Pt, were tested to prove their capability to affect the aggregation of two amyloidogenic fragments, $A\beta_{21-40}$ and $A\beta_{25-35}$, of the C-terminal region of the Aβ peptide [5]. In detail, by employing spectroscopic techniques (e.g., fluorescence assays, UV-vis absorption, and electrospray ionization mass spectrometry), we observed that $1Pt_{dep}$ was able to bind both Aβ fragments through a coordinative bond of the metal centre to the peptides and to deeply inhibit the formation of amyloid aggregates as confirmed by the morphological analysis of amyloid fibers. In conclusion, reported investigations can open new ways for the application of metallodrugs as potential new therapeutic agents in neurodegeneration.

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Design, Synthesis, and Biological Investigations of New TSP-1-deriving Peptides as TGF-β Activators

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Considering the limited possible toxic side-effects, relatively easy synthesis, and several opportunities of further structural modifications, peptides give a fundamental contribution to the cosmeceutical field.[1,2] The collagen turnover regulation is one of the main targets for the design of new compounds relevant in cosmeceutical area, with particular attention on peptide-based formulas.

The group of Transforming Growth Factors-β (TGFs-β) consists of five isoforms, namely TGF-$\beta_{1\text{-}5}$.[3] Latent form of TGFs-β, that is LAP-TGF-β, is biologically inert and requires a prior activation. Glycoprotein Thrombospondin-1 (TSP-1) was found to be the major TGF-β1 activator *in vivo*.[3] The *N*-terminal part of LAP-TGF-β, called the Latency Associated Peptide (LAP), is cleaved throughout the activation and therefore the concentration of free LAP may indicate the efficacy of LAP-TGF-β activators.

Among various TSP-1-deriving peptide sequences, KRFK and KVK peptides were found to be promising LAP-TGF-β_1 activating agents.[4] In this research, we address still unmet aspect of these peptides through the design of modified analogs, aiming at increasing the activity, stability, and bioavailability. Applying various modifications, such as including D-amino acids in the structure, retro-inverso strategy, or incorporation of unnatural amino acids, we expect that the final KRFK and KVK analogs may feature enhanced biological properties *in vitro*, increasing bioactivity and reducing susceptibility to enzymatic degradation when applied topically. The ability in inducing the release of active TGF-β of *in silico* designed and then synthesized compounds was evaluated by performing the assay on a *Biacore*™ X100 SPR biosensor. For the most effective activator(s) of LAP-TGF-β, conformational studies including NMR and CD will be performed.

Acknowledgements

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In depth analysis of the biological properties of the antimicrobial peptide Esc(1-21) and its one-residue-substituted analogs

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Esc(1-21) is a derivative of the frog-skin antimicrobial peptide Esculentin-1a, with a large spectrum of activity especially against Gram-negative strains, without being cytotoxic. It was previously characterized for its *in vitro* and *in vivo* anti-Pseudomonal activity [1,2]. Here, we deepened the study of the biological properties of this peptide by evaluating (i) its capability to inhibit biofilm formation of the enterohemorrhagic *Escherichia coli* O157:H7; and (ii) the plausible synergistic effect with colistin against multidrug-resistant *A. baumannii* clinical isolates. The results showed that Esc(1-21) inhibits *E. coli* O157:H7 biofilm formation at sub-MIC doses by inducing the expression of several genes involved in the regulation of biofilm, as well as in the stress response. It also showed a rapid kinetic of killing in line with its membrane-perturbing activity. Furthermore, when the peptide was combined with colistin at sub-MIC, the microbial growth of different multidrug-resistant *A. baumannii* clinical isolates was significantly slowed down while a potentiation of the membrane-perturbing effect was detected [3]. In parallel, to explore the effect of amino acid substitutions in the primary structure of the peptide, two analogs carrying a Pro- or Aib- residue in position 8 (instead of glycine) were synthesized. The Pro-containing analog showed a weaker activity or comparable to that of the parent peptide against a panel of Gram-positive and Gram-negative bacteria. Interestingly, the Aib-containing analog displayed an activity similar to Esc(1-21) against Gram-negative bacteria but higher against Gram-positive strains. In conclusion, both derivatives of Esc(1-21) turned out to be a promising agents against Gram-negative but also Gram-positive bacteria.

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Different loading and release of an antimicrobial peptide from alginate- and agarose porous scaffolds for bone regeneration

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Alginate/hydroxyapatite porous scaffolds have been widely studied to promote bone regeneration[1]. However, in the age of the microbial antibiotic-resistance, new attention must be paid to provide antimicrobial activity to these structures, to shield them from the colonization by antibiotic resistant pathogens. To this aim, Alginate/hydroxyapatite porous scaffolds were loaded with a non-cytotoxic proline-rich antimicrobial peptide, the B7-005[2]. The strong electrostatic interactions between the positively charged B7-005 and the negatively charged alginate allowed efficient peptide loading but totally hindered its release, even in the presence of a positive lactose-modified chitosan coating, which partially shielded alginate negative charges. Hence, alginate was substituted with the electrostatically neutral agarose for the preparation of scaffolds, obtaining as result similar structure, porosity, and mechanical performance in the dry state than the alginate counterpart, while providing a rapid B7-005 release within the first 24 h. The presence of the peptide did not impaired MG-63 adhesion and proliferation on the scaffold. Cells displayed a growing proliferation trend in 2 weeks after an initial stabilization phase. Likewise, the B7-005 loaded on- and released from the scaffolds revealed its efficacy against *E. coli, K. pneumoniae, and A. baumanni*, but not against *S. aureus* and *P. aeruginosa*.
The use of agarose instead of alginate for the preparation of porous scaffolds is therefore desirable to make them compatible with the loading with antimicrobial peptides. The combined use of agarose and antimicrobial peptides may provide scaffolds to promote bone and tissue regeneration despite the diffusion of antibiotic-resistant pathogens.

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Metabolic syndrome preventing peptides derived from caseins (bovine, caprine and ovine) after in silico proteolysis

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This work presents an in silico approach to compare the release of biological active peptides present in bovine, goat and sheep caseins. The casein sequences have been acquired from the universal protein knowledgebase UniProt: (P02662, P18626, P04653, P02663, P33049, P04654, P02666, P33048, P11839, P02668, P02670, P02669), available at http://www.uniprot.org/uniprot [2]. The sequences were analyzed with the BIOPEP-UWM database available at https://biochemia.uwm.edu.pl/en/biopep-uwm-2 [1]. The aim of the study was to detect the differences in the profiles of biological active peptides of caseins derived from the above-mentioned three animal species after in silico digestion with trypsin, chymotrypsin and pepsin in the BIOPEP-UWM database.

Table 1, Number of released peptides after in silico digestion

Protein / peptid activity	casein α_{S1}			casein α_{S2}			casein β			casein ϰ		
	B*	C	O	B	C	O	B	C	O	B	C	O
ACE inhibitor	7	7	6	4	6	6	5	4	5	6	6	6
DPP4 inhibitor	11	10	9	10	12	11	11	10	11	12	13	13
DPP3 inhibitor	1	1	1	-	1	1	3	3	3	1	1	1
antioxidative	4	3	3	1	2	2	2	1	1	-	-	-
antiamnestic	1	-	-	-	-	-	-	-	-	-	-	-
stimulating	2	1	1	-	-	-	2	2	2	1	1	1
renin inhibitor	1	1	1	-	-	-	1	1	1	1	1	1
anti inflammatory	-	-	-	-	1	-	-	-	-	1	1	1
immunomodulating	-	-	-	-	-	-	1	-	-	-	-	-
anticancer	-	-	-	-	-	-	1	-	-	-	-	-
antibacterial	-	-	-	-	-	-	-	-	-	1	1	1
hypotensive	-	-	-	-	-	-	-	-	-	1	1	1

*B-bovine, C-caprine, O - ovine

The four casein fractions namely α_{S1}, α_{S2}, β and ϰ after proteolysis contained peptides with 12 different bioactivities. The amount of released peptides indicated that all caseins can act as anti-MetS agents, especially lowering blood glucose levels and lowering blood pressure. One peptide derived from bovine β-casein has an anticancer and immunomodulating activities. Further research is needed if those peptides can pass into the bloodstream and be useful in the prevention of the Metabolic syndrome.

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Generation of a new AIF peptide targeting Human Cyclophilin A to inhibit AIF-mediated cell death

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The interaction between Apoptosis Inducing Factor (AIF) and Cyclophilin A (CypA) plays a key role in neuronal cell death upon apoptotic stimuli.[1] The blocking of this interaction through a synthetic fragment mimicking the amino acid region of AIF, spanning residues 370-394 (AIF(370-394)), induces neuroprotection in neuronal cell models of glutamate-induced neurotoxicity.[2] Here, we report the generation of the disulphide-bridged and shorter variant AIF(381-389) of this peptide and its biochemical and structural characterization by NMR in the free and CypA-bound state.[3] AIF(381-389), despite its small dimensions, less than half the size of the precursor, shows an affinity *in vitro* comparable to that of AIF(370-394). NMR-based 3D models of the isolated AIF(381-389) and in complex with CypA demonstrate that the cyclization significantly restricts the conformational space explored by AIF(381-389), resulting in the formation of intramolecular hydrophobic interactions and an extensive network of hydrogen bonds involving the backbone atoms which significantly stabilize a β-hairpin structure very similar to that adopted in the isolated protein and in the complex with CypA, in contrast to the still very flexible structures adopted by the linear AIF(370-394) peptide and its stapled analogues.[4,5] Moreover, the new peptide shows a remarkably higher resistance to degradation in human serum and an improved ability to prevent cell death in a cellular model of glutamate-induced neurotoxicity compared to the AIF(370-394) parent peptide.[3]
The structural data obtained are very useful for drug development programs based on structure-activity-relationship studies and on computational approaches for improving the druggability of the peptide and to predict new highly effective, target selective, stable and cell-permeable inhibitors.

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The Thorpe-Ingold effect is highly efficient in promoting the β-turn conformation in an N$^{\delta}$-acylated, (E) β,γ-olefin dipeptide amide isostere

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A promising conformationally constrained δ-amino acid residue, a dipeptide mimic, is obtained by replacing its central amide group with an (E) $C^{\beta}=C^{\gamma}$ alkene unit (olefin isostere), as shown in the Figure.

In the present work, we have synthesized the compound with both α- and δ -CH_2- atoms characterized by the highest possible level of methylation. The 3D-structural properties of this hitherto unexplored tetramethylated analog have been compared to those of the known unsubstituted prototypical compound. Specifically, we have investigated by DFT calculations, crystal-state X-ray diffraction, and FT-IR absorption / NMR spectroscopies in solution the extended *vs* folded preferences of the two compounds.

In particular, a relevant piece of information was extracted from the X-ray diffraction analyses. All three crystallographically independent molecules in the asymmetric unit of the N$^{\delta}$-Boc, -NH*i*Pr blocked, unsubstituted -CH_2- compound are essentially extended (or slightly kinked) and devoid of any intramolecular H-bond. In contrast, the N$^{\delta}$-Boc, -NH*i*Pr blocked, tetramethylated -CH_2- analog is folded in a conformation close to that of a type-I(I') β-turn, stabilized by an intramolecular (urethane) C=O···H-N (amide), C_{10}-ring forming, H-bond. Our FT-IR absorption / NMR data and the results of the DFT calculations strongly support these conclusions, highlighting the extremely effective role of this type of tetramethylation primarily promoted by the Thorpe-Ingold effect. In summary, these finding open interesting perspectives in the design of conformationally constrained peptidomimetics.

An improved application of the Carpino's acyl fluoride C-activation methodology to the SPPS of C^{α}-tetrasubstituted α-amino acid sequences

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We developed a novel, improved dyad of α-amino protection/α-carboxyl activation procedures in SPPS. This combination demonstrated to be quite appealing, in particular with the sterically hindered, C^{α}-tetrasubstituted α-amino acids. The *ortho*-nitrophenylsulfenyl (*o*-NPS) α-amino protection, which can *not* generate the chirally dangerous and poorly reactive 5(4*H*)-oxazolone intermediate, was combined with the Carpino's highly efficient, aminoacyl fluoride C-activation.

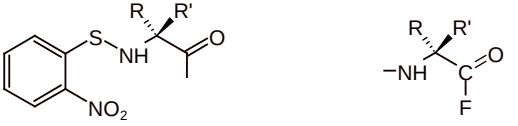

Figure 1. An *o*-NPS-α-amino acyl (left) and an α-aminoacyl fluoride (right).

1. We first SPPS synthesized Fmoc-$(Aib)_4$-L-Ala-OH using the Carpino's procedure, in particular with Fmoc-L-Ala-SASRIN and Fmoc-Aib-F. This method is confirmed to be *highly efficient*.
2. Then, using the same SPPS procedure as above, we made an attempt to prepare the *much more hindered*, tetrapeptide sequence -$[D\text{-}(\alpha Me)Val]_4$-, using five equivalents of Fmoc-D-(αMe)Val-F. In this case, the synthesis essentially *failed* and the desired tetrapeptide was *not* found.
3. In the third step, our synthetic target was the tripeptide *o*-NPS-L-(αMe)Phg-Aib-D-(αMe)Phg-O*t*Bu by *solution* methods. We employed *o*-NPS-Aib-F and (separately) the two enantiomeric *o*-NPS-(αMe)Phg-F. The α-amino group of the nucleophile was preactivated with BTSA. The final product was obtained in *excellent* (92 %) yield, after chromatographic purification.
4. We initially applied the *o*-NPS protection to SPPS for the production of the hexapeptide -L-Ala-$(Aib)_4$-L-Ala- using the *milder* Carpino's C-activation *HATU/HOAT* method, Fmoc-L-Ala-SASRIN, and *o*-NPS-Aib-OH. The desired product was obtained in a *very limited* yield.
5. Our next step was the *first* application of the *o*-NPS-/-CO-F dyad to *SPPS* [synthesis of the sequence -L-Ala-$(Aib)_3$-L-Ala-]. Here, we took advantage of Fmoc-L-Ala-SASRIN and *o*-NPS-Aib-F. Satisfactorily, the *largely major* peak in HPLC is that corresponding to the target pentapeptide.
6. Finally, we synthesized the *highest sterically congested* dipeptide sequence -$[L\text{-}(\alpha Me)Phg]_2$-. To this aim, we exploited *o*-NPS-L-(αMe)Phg-F and BTSA. The HPLC results are *extremely promising* (88 % overall yield).

Transforming a crude peptide sequence into valuable antimycobacterial products

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The incidence of nontuberculous mycobacteria (NTM) infections has been increasing over the last decades with the most prominent NTM infections being caused by the *Mycobacterium avium* complex and *M. abscessus*. Treating these infections requires a multi-drug regimen, lasting until the patient is culture-negative for one year. This is, however, highly toxic to the patient, making it imperative to find new antimicrobial agents[1].

Found in almost every living organism, antimicrobial peptides not only display antimicrobial activity but also display anti-inflammatory, anti-cancer, and wound healing properties. They have taken the role of being the most promising substitute for antibiotics both from a monotherapy point of view and in conjugation with other pre-existing molecules[2].

Therefore, this work is aimed at testing a positional scanning library with 114 peptides, generated using the bovine lactoferricin peptide (LFcin17-30), which is one of the peptides with highest reported activities against *M. avium*[3]. The activities of these peptides against NTM growing in planktonic cultures, biofilms, and inside macrophages, will be used to derive quantitative structure-activity relationship (QSAR) models, relating the activities and sequences of the peptides. After validation, the derived QSAR models will then be used to select an experimental set of 50 peptides. The most promising peptides will next be tested in *in vitro* 3D infection models, that more closely relate to *in vivo* conditions. The results obtained will allow the identification of LFcin peptides with promising activities against animal models of mycobacterial infections. We thus expect to find new antimicrobial compounds that display high activity against NTM and can be used as new therapeutics to treat these infections.

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AlphaFold predictions of peptide-MHC complexes

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The identification of Tumor Associated Antigens (TAAs) has important implication for innovative immunotherapy approaches. [1] However, TAAs may suffer from the immunological tolerance due to their expression in normal cells. To potentiate their immunogenicity, we are currently developing heteroclitic peptides by introducing *ad hoc* modifications in the TAAs to improve their affinity for the Major Histocompatibility Complex (MHC).[2,3] This strategy would greatly benefit from tools providing reliable *a priori* predictions of the affinity of the designed peptide for MHC. Although some machine-learning servers can provide rough estimations of the strength of these bindings, the difficulties in generating solid three-dimensional models of these complexes represent a major limitation in the fast development of effective heteroclitic peptides. We here evaluate the ability of AlphaFold algorithm, whose development are revolutionizing structure biology, the three-dimensional structure of MHC-peptides. As a validation, we conducted an *ab initio* prediction of the structure of the GP33 epitope complexed with the murine MHC H-2D^b. The impressive agreement between the experimental and the predicted model is shown in the Figure.

Figure 1. Fitting of the experimental (green) and the AlphaFold predicted (cyan) structure for the GP33-MHC H-2D^b complex

On the basis of these results, we generated the structure of other complexes including the one that MHC H-2D^b forms with the widely studied epitope HPV E7 epitope. Collectively, these analyses provided interesting clues on the peptide recognition by MHC molecules.

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Small peptide conjugates for neuroprotection and Aβ detection

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Peptides may represent an opportunity for therapeutic intervention that closely mimics natural pathways. [1] Bio-conjugated peptides play an important role in several fields of biomolecular and medicinal chemistry.[2]. Peptide-based epitopes with covalently attached other moieties able to explicate additional or complementary functions, including BBB permeation, metal chelation or aggregates disassembling, targeted imaging, and treatment, hold a promising potential for applications in Alzheimer's disease (AD). In our laboratory, we have been synthesizing a variety of small peptides bio-conjugates differing by the peptide epitope or the conjugated scaffold.[3,4]

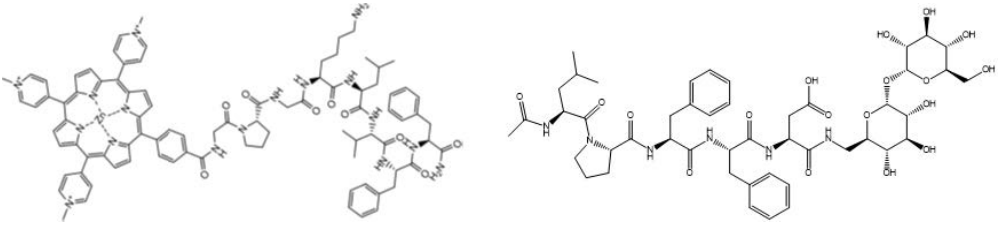

Figure 1. Representative Structures of some Peptide Conjugates

A range of molecular details, together with measured biological effects, have been listed with these systems, all of them accounting for the observed neuroprotection against the toxic insult induced by Aβ oligomers in cultured neurons. In this communication an overview of the design principles of the peptide conjugates, their neuroprotective activity, and their capability in detecting Aβ peptide in solution is described in terms of potential use of these compounds as theragnostic agents and for the targeted drug delivery.

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Potent and selective C-terminal carboxamide peptide inhibitors of Zika virus NS2B -NS3 protease

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Zika virus (ZIKV) is a member of the *Flaviviridae* family that can cause neurological disorders and congenital malformations [1]. The ZIKV NS2B-NS3 serine protease is an attractive target for the development of new antiviral agents [2]. We will report a SAR study on a series of peptide inhibitors of the NS2B-NS3 protease [3]. Substrate-like linear tripeptides bearing a primary carboxamide functionality were first identified to inhibit the NS2B-NS3 protease of ZIKV in a non-covalent and competitive manner. A systematic exploration of the residues at the P1, P2 and P3 positions, as well as the investigation of the N-terminal and C-terminal ends of the linear tripeptide (Figure 1) allowed the identification of peptides with sub micromolar potency bearing phenylglycine as arginine-mimicking group and a benzylamide as C-terminal moiety. The application of these findings to peptides bearing 4-substituted phenylglycine [4] at the P1 position to afford low nanomolar ZIKV protease inhibitors with good selectivity against proteases of related Flaviviruses will also be discussed.

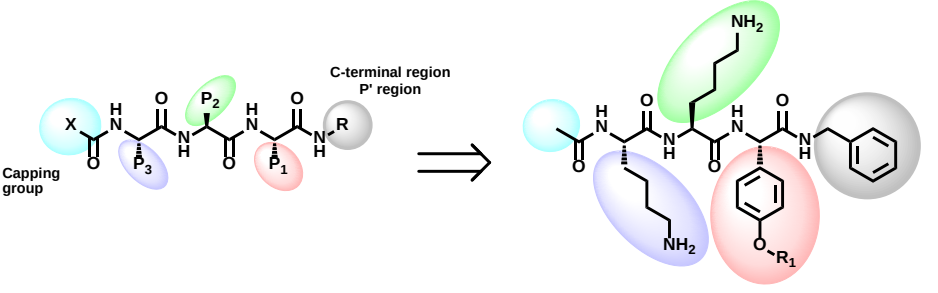

Figure 1. SAR approach on ZIKV NS2B-NS3 protease inhibitors

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PMAP36 peptide derivatives linked to polysaccharide scaffolds for new antimicrobial materials

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The development of antimicrobial materials recently have gained increased interest for a variety of applications, like medical devices able to prevent bacterial infections. In this contribution, we present the development of bio-functional smart materials based short analogues of the antimicrobial cathelicine PMAP36, covalently linked to hyaluronic acid, cellulose and starch.
In our contribution we present the synthesis of selected analogues of PMAP36 and the study of the structure-activity relationship. The chemo-enzymatic oxidation of the primary alcoholic functions to obtain aldehyde groups on the polysaccharide matrices (hyaluronic acid, cellulose and starch). The subsequent coupling of the peptide was obtained through a chemoselective ligation to obtain a stable thiazolidine ring.

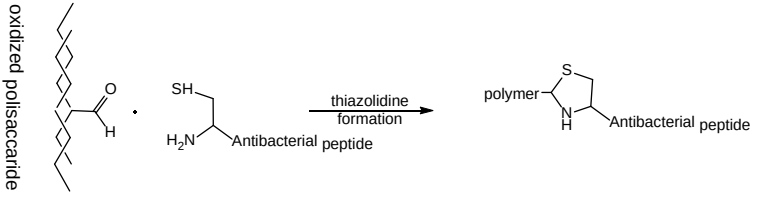

Figure 1.Thiazolidine formation

The physico-chemical properties of the final materials will be analysed and discussed. The peptides and the peptide-functionalized materials were tested on bacterial strains, showing that peptides are able to inhibit bacterial proliferation even when covalently linked to the polisaccaridic matrix. The activity is maintained even after UV-sterilization treatment.

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Cyclic peptoids as a playground for studying weak interactions

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Cyclic peptoids (cyclic oligomers of *N*-substituted glycine) belong to the realm of peptidomimetic compounds and are particularly appealing because of their peculiar features like exceptional biostability, easy synthesis and conspicuous diversity.[1] In cyclic peptoids, the lack of the amide protons prevents the formation of the typical NH ··· OC hydrogen bonds, which are substituted by CH_2 ··· OC hydrogen bonds. Cyclization and incorporation of specific side chains allow to overcome the backbone flexibility caused by the isoenergetic *cis*/*trans* amide bond conformations.[2] Thanks to the appropriate combination of functional side chains and ring size, it is possible to obtain a variety of solid state supramolecular architectures like columns,[3a] tubes[3b] and layers.[3c] Recently, we have reported for the first time the crystal structures of four cyclic dodecapeptoids (Figure 1).[4] Interestingly, all the compounds show an unprecedented *ccccctccccct* (*c = cis*, *t = trans*) sequence of the amide bond configuration, resulting in enantiomorphic right- and left-handed polyproline type I helices bridged by *trans* residues. This peculiar backbone conformation is sustained by both one-sided and reciprocal CO ··· CO interactions (distances below 3.22 Å), intramolecular backbone-to-backbone CH ··· OC contacts and backbone-to-side chains C5 CH ··· OC hydrogen bonds. These contacts were also investigated through QTAIM, searching for bond critical points (BCPs).

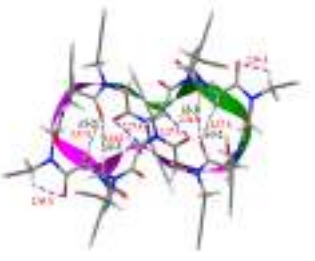

Figure 1. Crystal structure of cyclo-$(Npa)_{12}$. Polyproline type I helices and intramolecular contacts are highlighted.

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Design of helical foldamers for chemical catalysis

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Peptide-based catalysts have been demonstrated to be effective in an increasingly variety of reactions. Their ease of preparation, modular and tunable structures as well the possibility of multifunctional substrate activation modes make peptides well-suited as catalysts.
Foldamers are synthetic, conformationally defined mimics of proteins and other biopolymers. In recent years, these non-natural oligomers adopting highly stable and predictable architectures demonstrated their attractiveness in providing original solutions to the development of artificial enzymes.[1] The modular nature of foldamers and the tight control of the folding propensity enables the exploration of new moieties and new geometries for sets of reactive side chains, and the encoding of functionally important dynamics.
Here we report our effort in the design of foldamers to achieve controllable chemical consequences in virtue of their primary and secondary structures. We made use of helical foldamers to run the hydrolysis of esters, [2] and to efficiently template a C-C bond macrocyclization.

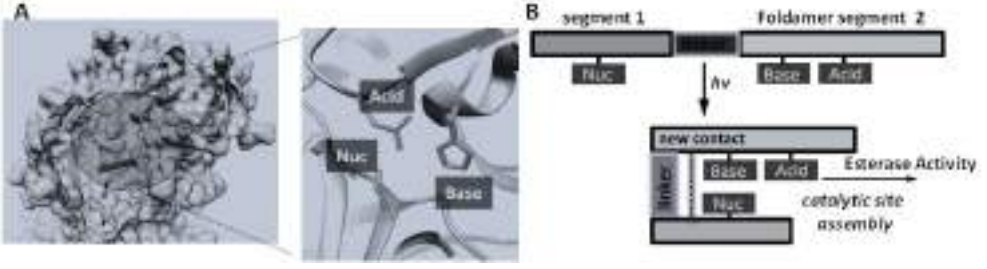

Figure 1. The catalytic action of serine peptidases depends on the interplay of a nucleophile, a general base and an acid (A). Our approach to a photo-responsive helical foldamer with esterase activity (B). Two peptide segments are connected by a switchable linker which allows a conformational change upon irradiation with UV-light and the assembly of a catalytic site.

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A new class of cell penetrating peptides and their binding affinity with nucleic acids

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Cell penetrating peptides (CPPs) are short peptides able to cross plasma membranes simultaneously being harmless to the rest of cellular structures. The most common group of CPP are arginine-rich peptides. Due to their positive charge, they have strong affinities for multiple negatively charged compounds and structures of the cell. One of them is binding between CPP and lipid bilayer resulting transfer peptides inside the cell even at low micromolecular concentrations in vivo and in vitro. Effectiveness membrane penetration depends on number of guanidine moiety, 6 – 8 are the most recommended [1]. This type of compounds are capable of delivering a wide range of molecules into target cell. Gene therapy can be promising to treat many diseases, but molecules for this treatment such as nucleic acids are not able to cross the cell membrane and delivery vectors like CPP can be solution for this issue [2].
Our group designed and synthesized of analogs of the fluorescein labelled DAPEG peptidomimetics able to cross the cell membrane and may have a potential of interacting with nucleus structures, particularly DNA. This compounds are homopolymers built of L-2,3-diaminopropionic acid (Dap). Each of beta amino groups of the Dap residues were modified by functionalized oxa-acids containing a guanidine moiety. *N*-terminus of the DAPEG peptidomimetics were labelled with 5/6-carboxyfluorescein succinimidyl ester. After syntheses binding affinity between compounds and nucleic acids were investigated, results of this studies will be presented on the poster.

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Soft materials as a novel diagnostic tool for MRI applications

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Magnetic Resonance Imaging (MRI) is a diagnostic technique that allows obtaining high-definition images of the interior of the human body. It represents a non-invasive method since it uses magnetic fields, overcoming the need of ionizing radiations. MRI is a very accurate but not a very sensitive technique. The use of positive (T1) or negative (T2) contrast agents (CAs) allows increasing the contrast between normal and pathological regions. Among T1-CAs, the most effective ones are based on kinetically and thermodynamically stable gadolinium (Gd) complexes. In the last two decades, interest has focused on the possibility of delivering these complexes through supramolecular structures, with the aim of improving the relaxation performance, the distribution profile and clearance of CAs [1]. In this scenario, we have recently proposed peptide-based hydrogels [2] and hydrogel nanoparticles (namely nanogels) derivatized or loaded with Gd-complexes as novel diagnostic tools [3]. We have evaluated the structural and relaxometric behaviour of this class of supramolecular CAs. An increase of performance could allow to reduce the administered CA dose and in turn to overcome the problematic issues related to the toxicity of free gadolinium ions.

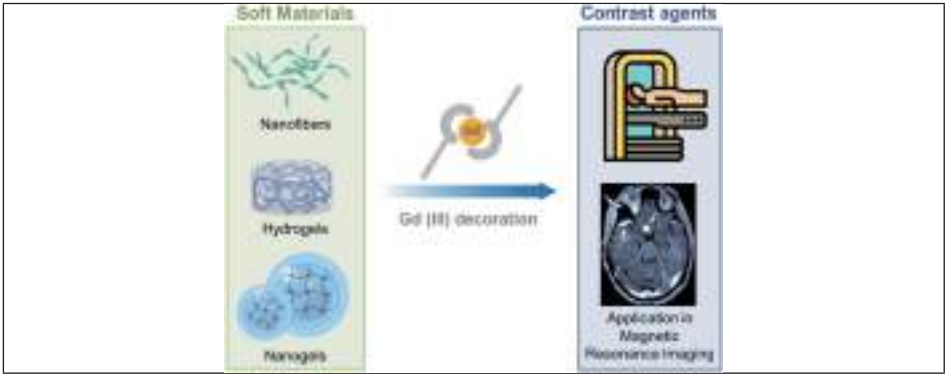

Figure 1. Schematic representation of multicolour fluorescence emission due to peptide nanofibers

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The high-resolution structure of the human Annexin IV: insight into calcium-binding sites

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The molecular mechanisms of how cancer cells promote their own survival and avoid apoptosis in response to commonly used chemotherapeutics are multiple and consist of a set of signalling pathways, which can be activated by a large amount of stimuli to promote chemoresistance [1]. Chemoresistance is thus becoming a serious problem that cancer research seeks to understand and overcome.
In this context, Annexin IV (ANXA4) is an interesting oncoprotein being its overexpression ~~is~~ involved in chemoresistance [2]. ANXA4 belongs to the family of annexins that are proteins able to bind calcium ions and phospholipids and contribute to biological processes such as endocytosis, exocytosis, cell division, apoptosis, and growth regulation [3,4]
Recently, a chemoresistance model after paclitaxel treatment has been reported. In particular, in this model FHIT protein can bind and delocalize ANXA4 from plasma membrane to cytosol in paclitaxel-resistant lung cancer cells, thus restoring their chemosensitivity to the drug [5-7].
With the aim of gaining structural insights into this process and of developing bioactive molecules able to modulate it, we have undertaken crystallographic studies on human ANXA4. The absence of a literature reports on high resolution structure of human ANXA4 and some controversial indication on its calcium binding compared to the bovine counterpart prompted us to collect diffraction data on crystal of this protein by using the Grenoble ESRF synchrotron radiation. The refinement of the structure provides a clear picture of the binding of calcium ions to the protein and of the role that flexible regions plays in the recognition. Moreover, the analysis of the crystalline contacts unravels a network of contacts among charged arginine side chains, in line with the recently reported propensity of the guanidinium group to self-interact [8].

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Antimicrobial peptides can generate tolerance by lag and affect antimicrobial treatments

Daniel Sandín[1], Javier Valle[2], David Andreu[2,*] and Marc Torrent[1,*]

The unrelenting emergence of multi-drug resistant bacteria spurs efforts toward discovery of new antibiotic molecules, as well as a better understanding of their mechanism of action, to successfully fight infections. Antimicrobial peptides (AMPs), extensively studied for their efficacy and broad spectrum of action, have yet in only a few cases reached clinical stages. Bacterial tolerance is the first adaptative step acquired to prevail against the stress produced by antimicrobial agents. Prior studies with conventional antibiotics have shown that bacteria easily generate tolerance and that their survival mechanisms appear to be effective when treated with different antibiotics. To extend these studies to AMPs, one must first elucidate whether they can generate tolerance and how broad toward different agents are the mechanisms involved.

In this study, we first generated tolerant and/or resistant *E. coli* populations by consecutive incubations with several well-known AMPs (polymyxin B, pleurocidin and LL-37). Throughout the kinetic cycles, we also measured differences in colony appearance time and shape, with different bacterial behaviors observed depending on the AMP used. Only the strain cultured with polymyxin B was able to generate resistance, but all three AMP treatments led to tolerant populations. Cross-tolerance, however, only appeared in pleurocidin-challenged bacteria. Evolved strains treated with conventional antibiotics also showed cross-tolerance and collateral sensitivity that seemed dependent on the AMP target. Genomic analysis of the evolved populations showed some interesting mutations that could be related with tolerance development and will be studied in order to find new antimicrobial approaches.

In conclusion, our results have shown that bacteria develop tolerance –though rarely resistance– when under AMP stress. Moreover, this tolerance can trigger cross-tolerance when treated with other agents. However, collateral sensitivity events also occur on treatments with different mechanism of action. Our work adds value to AMP research and the possibility of replacing or synergizing classic antibiotics with AMPs to deal with bacterial resistance.

Site-Specific labelling of recombinant humanized antibody fragments

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Targeted therapies using monoclonal antibodies and their combination regimens are at the forefront in cancer theranostics. Advanced understanding of the protein engineering technology has paved the way for the design and evolution of antibody-derived functional fragments into humanized and therapy-optimized versions [1]. Antibody's fragments obtained from mAbs against the two onco-fetal proteins Nodal and Cripto-1, selectively expressed in many solid tumours and actively investigated as theranostic biomarkers [2], have been prepared as recombinant molecules bearing sites for the selective stapling of diagnostic tracers or cytotoxic payloads. The humanized recombinant Fabs of the anti-Nodal (3D1) [3,4] and anti-Cripto-1 (1B4 and 10D1) [5] murine monoclonal antibodies have been here used to implement the technology based on a one-step site-specific labelling of monomeric Fabs [6] using the short linker peptide LQSP [7] opportunely functionalized with tracers like FITC and Biotin. These molecules retain the binding affinity for the antigens suggesting their potential use for imaging applications on Nodal and Cripto-1 positive cancers cells. In general, we demonstrate that our strategy is an effective and versatile tool to develop site-specific labelled biomolecules for theranostic purposes.

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From cyclic peptoids to azamacrocycles

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Cyclopeptoids, cyclic oligomers of *N*-alkyl glycines, are an intriguing family of peptidomimetics used for a wide range of applications. [1] There is a continuous effort towards the design, synthesis and characterization of novel cyclic peptoids involved in various fields of research: catalysis [2], synthetic ionophores [3], crystal engineering. [4] Recently, we have developed a new synthetic methodology based on the intra-annular amide reduction of cyclic peptoids in order to obtain the polyaza-macrocycles. [5]

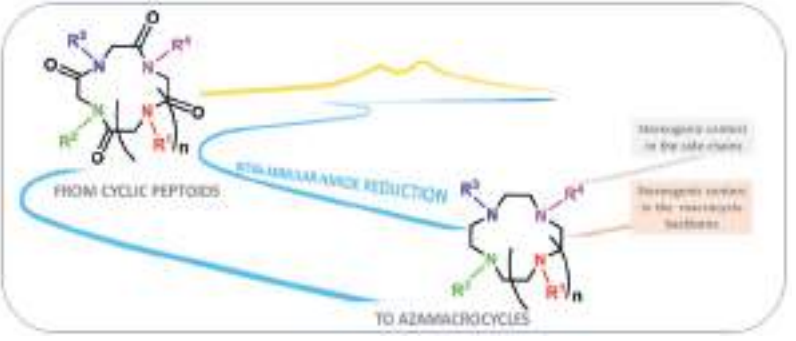

Figure 1. Synthetic route: from cyclic peptoids to peraza-macrocycles

The advantages of this ingenious approach are: the use of a general synthetic procedure, the access to macrocycles with different cavity size, the unlimited introduction of different functional groups.
More recently, we have applied the same methodology for the synthesis of chiral peraza-macrocycles to explore the effect of the incorporation of stereogenic centers in the side chains or in the macrocycle backbone. [6] This presentation will highlight how the development of this synthetic methodology paved the way to the exploration of novel *N*-substituted peraza-macrocycles as MRI contrast agents, asymmetric catalysts and chiral receptors.

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Viral weapons against bacteria: structural clues to fight antimicrobial resistance

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Antimicrobial resistance (AMR) poses a serious threat to global human and animal public health. The excess and improper use of antibiotics is increasing the number of reported resistant microbial strains, compromising the conventional clinical treatments. The World Health Organization (WHO) has declared AMR a health emergency and has announced that the deaths attributable to AMR every year will be of 10 million in 2050, exceeding all the other major causes of death. To respond to AMR threat, an immediate action is required. Structural insights of key molecular players of AMR are fundamental for a deeper understanding of antimicrobial resistance and for the exploration of alternative therapeutic strategies.

A group of six scary nosocomial pathogens is named with the acronym 'ESKAPE' because capable of 'escaping' the biocidal action of antibiotics classified as highly important for human medicine. ESKAPE bacteria are a serious health concern, as they increase frequency of treatment failures and severity of human infections by adapting to altered environmental conditions and by acquiring resistance determinants. Among ESKAPE bacteria, three are most problematic, the highly resistant Gram-negative (Gram-) *K. pneumoniae, P. aeruginosa and A. baumannii*, typically associated with infections in severely ill hospitalized patients. As in other bacteria (1,2), their cell envelope shield is the first line of defence against stress conditions and is crucial to resistance to antibiotics (3-5). This complex structure exposes two important barriers, capsule polysaccharides (CPS) and lipopolysaccharides (LPS). CPS is an important virulence factor of all Gram- ESKAPE pathogens as it protects bacteria from harsh environmental conditions, immune system response and phage infection. A strong correlation exists between CPS overproduction and hypervirulence in *K. pneumoniae*, as poorly capsulated strains or capsule-deficient mutants are more efficiently phagocytized by macrophages and neutrophils. In a previous work, we determined the first crystal structure of a CPS-degrading enzyme encoded in a phage acting against *K. pneumoniae* (5). Those enzymes, especially when directed to hypervirulent strains, have strong applications in making highly resistant bacteria susceptible to antimicrobials and innate immunity. Structural and functional data on depolymerases with different serotype specificity will provide a structural framework for enzyme-engineering to produce serotype-broad-active enzyme complexes against *K. pneumoniae*.

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Delivery of inhibitors of the phosphatase SHP2 protein-protein interactions

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SHP2 is a tyrosine phosphatase involved in several cellular processes, as a cell signal transducer. It is characterized by two Src homology 2 (SH2) domains towards the N-terminal part of the sequence, indicated as N-SH2 and C-SH2, a PTP catalytic domain, and a hydrophilic tail. Under basal conditions, it occurs in a self-inhibited form, in which the catalytic site is blocked by the N-SH2 domain. Many mutations destabilize the N-SH2 / PTP interface, causing the abnormal activation of SHP2 and leading to developmental disorders, juvenile leukemia, and other malignancies[1-3].
Peptide sequences have been developed and optimized, in order to selectively bind SHP-2 on the SH-2 domains to inhibit their interactions[3]. To optimize the delivery of these peptides within cells, we investigate the conjugation with Cell-Penetrating Peptides (CPPs) as carriers. We focus our attention on different CPPs (Penetratin, Tat, ecc.) and we explore diverse synthetic pathways for an efficient and modular conjugation to link carrier and cargo peptides.
To investigate the effective penetration of the carrier-cargo group and its selectivity for different cell environments (e.g. release from endosomes to the cytosol) we label these carrier-cargo structures with pH-sensitive fluorescent probes such as Naphthofluorescein[4].

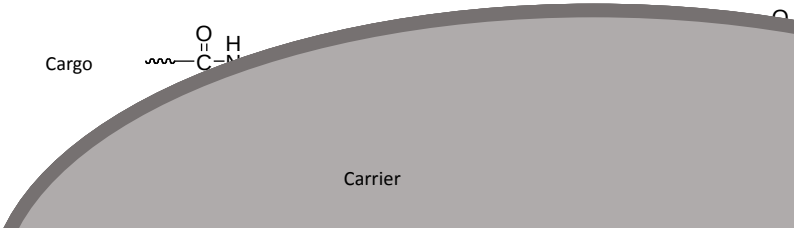

Figure 1. Cargo-Carrier conjugates (left); a pH-sensitive fluorescent probe (right).

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Plant peptides which modulate the immune system

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Among the bioactive compounds which affect the immune system and demonstrate other biological activities there are biopeptides (biologically active peptides). Some of these biopeptides activate macrophages, stimulate natural killer (NK) cells, phagocytosis, increase the production of leukocytes and affect the immunity modulators (e.g. cytokines, nitric oxide and immunoglobulins)[1].
The work uses over 500 sequences of plant food proteins (including their fragments) deposited in the BIOPEP-UWM database, as well as programs available in the database, in order to search for bioactive fragments of sequences (immunomodulatory, immunostimulatory, antibacterial, antivirus, antifungal, anticancer) in the analysed proteins which may affect the human immune system. The profiles of potential protein bioactivity were determined, the frequency of occurrence of bioactive fragments in the studied proteins (A parameters)[2] was calculated, and the monocatalytic simulations of protein hydrolysis with the use of three enzymes (ficin or stem bromelain or pepsin pH >2) were carried out in order to release biopeptides with selected activities from protein structures[2, 3].
In the analysed protein sequences, the most frequently occurring biosequences with immunomodulatory activity were YG, GLF, YGG, IKPR (sequences recorded in the form of a single letter code of amino acids) and GVM, EAE, LGY, LLY, GFL with immunostimulatory activity. In the studied proteins, there were also fragments of IQL, YVL, EIPT sequences – sequence fragments with antibacterial activity, and VVV with anticancer activity. However, during the *in silico* hydrolysis of proteins, only bromelain released biopeptides with YG, IKPR sequences, and most of them from rice proteins. Immunomodulatory peptides (YG) were also released from the structures of wheat, rye, barley, pea and peanut proteins. These biopeptides may be potential nutraceuticals/ingredients in foods designed to meet the specific needs of the organism.

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Peptide interfaces for CRP molecular recognition – development, characterization and application

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Herein, we have presented the peptides-based materials development and characterization for molecular recognition of C-reactive protein (CRP). The three CRP-binding peptides (P2, P3, P9) have been recognized using phage-display technology. The binding efficiency of the peptides exposed on phages towards the CRP protein was demonstrated via biological methods (plaque and ELISA tests). Fibres of the selected phages interact differently with each other, which was confirmed by transition electron microscopy. In addition, computer modelling has shown that although peptides bind in the same places to CRP, they have a different binding energy. These studies have also revealed that one of the investigated peptides (P3 peptide) exhibits the highest binding energy towards CRP measured yet. Then PM-IRRAS analysis has demonstrated and confirmed the result of the computational modelling that the strongest interaction occurs between the CRP and P3 peptides. Finally, the cysteine labelled P3 peptide-modified electrodes were fabricated and applied for biomolecular recognition of CRP.

Acknowledgements
This work was funded by the Polish National Science Centre under the SONATA 13 grant UMO 2017/26/D/ST5/00980 to Dr Katarzyna Szot-Karpińska. Financial support from project 3/DOT/2016 funded by the City of Gdynia, Poland, is also acknowledged.

Reactivity of vasopressin and its selenium analogues with mercury: LC-MS/MS investigation

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Selenium is principally present in biological systems in the form of the naturally occurring amino acid selenocysteine (Sec), which has been identified in 25 human proteins[1]. This residue, as soft Lewis base, shows strong affinity to soft Lewis acids, such as heavy metals, with a powerful environmental toxicity. Previous studies on mercury demonstrated its ca. 10^6 times higher affinity to Se than to sulfur, which is mostly compensated by the 10^5 higher cellular abundance of thiols[2]. In this work, the reactivity of Hg(I) and Hg(II) compounds towards vasopressin (AVP) analogs containing Cys and Sec groups (Figure 1) has been investigated by reversed-phase HPLC coupled with electrospray MS/MS detection (LC-MS/MS). The selected peptide is an hormone with antidiuretic and vasopressor actions containing a disulphide bridge, and its diselenide and selenylsulfide analogues (Figure 1). Taking into consideration the stability of Se-Hg bonds, our results support the hypothesis of a binding preference of Hg to Sec residues in selenoproteins.

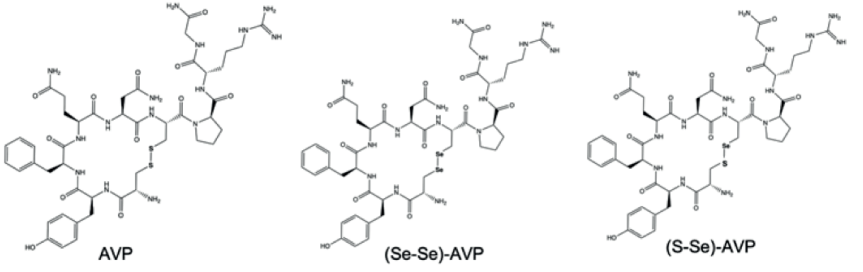

Figure 1. Vasopressin (AVP), diselenide (Se-Se)-AVP and selenylsulfide analogues (S-Se)-AVP structures

Acknowledgements
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A tribute to Prof. Louis A. Carpino: A statistical survey of geometry and conformation of the Fmoc-amino group in peptides from X-ray diffraction structures

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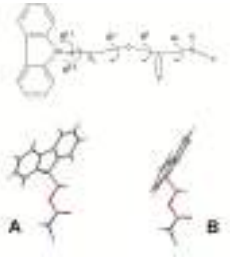
We investigated a significant aspect of peptide chemistry strictly related to the production of Prof. Louis A. Carpino, a giant in our science, who passed away in early 2019. We performed a statistical survey on the relevant geometrical (bond lengths and bond angles) and conformational (torsion angles) properties of the currently most extensively utilized 9-fluorenylmethyloxycarbonyl (Fmoc) α-amino protecting group introduced by Carpino in peptide synthesis fifty years ago. In this (large) statistical compilation we considered all X-ray diffraction structures published to date on amino acid / linear peptide derivatives and Fmoc-labeled amines. Both Fmoc secondary and tertiary urethane functions (131 and 22 examples, respectively) were separately analyzed.



Interestingly, in particular, for the conformational properties: (a) Only a few occurrences of the ω torsion angle (see top part of the Figure) in the Fmoc secondary urethane groups are found in the *cis* (ω ≅ 0°) disposition (intriguingly, in all of them the Fmoc group is linked to a C^{α}-tetrasubstituted α-amino acid). Regarding the Fmoc tertiary urethane group, the ω torsion angle (defined with the C^{α}-atom of the α-amino acid labelled C in the top part of the Figure) is *cis* in Fmoc-L-Pro-OH and Fmoc-L-Hyp(*p*IPh)-OH; (b) The Fmoc θ^1 torsion angle is exclusively *trans*; (c) The values of θ^2 show a much wider distribution, but the region $\theta^2 = 0° \pm 70°$ is completely disallowed; (d) The $\theta^{3,1}$ and $\theta^{3,2}$ torsion angles are restricted to values close to the *trans*, *gauche*$^-$ and *gauche*$^+$ dispositions. The conformations in which one of them is *trans* largely prevail.

The two most common conformational combinations for the Fmoc secondary urethane group are illustrated in the bottom part of the Figure (A and B). In both of them, the fluorene moiety, although differently oriented, is tilted by 60° with respect to the urethane plane.

Native chemical ligation as a way for obtaining fully synthetic metallothioneins

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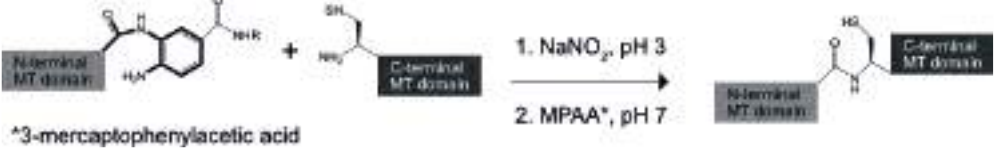
Metallothioneins (MTs) are a group of small, heterogeneous proteins that share a common feature, namely high cysteine content (up to 30% of all residues), which grants them the capability to coordinate Zn(II) and Cu(I) in mammals. MTs are involved in hazardous toxic metal ions metabolism and cell cycle regulation. Mammalians MTs bind up to seven Zn(II) ions with various affinities, which means that MTs are present under cellular conditions in variously metallated states ($Zn_{4-7}MT$). Unfortunately, a complete understanding of human MTs is still beyond scientific knowledge. An additional variable that complicates the understanding of MTs' function. is the fact that MTs can undergo posttranslational modifications, namely N-terminal acetylation or phosphorylation [1].



*3-mercaptophenylacetic acid

The role of these modifications in MTs case is unknown, as obtaining such modified MTs is not trivial. To enable further research on posttranslationally modified MTs, we have investigated the possibility of obtaining MTs via native chemical ligation (NCL), as classical microwave-assisted solid-phase peptide synthesis. We have used native chemical ligation to get complete, synthetic protein to tackle this obstacle. The NCL was performed by activating entirely unprotected peptide o-aminoanilide with $NaNO_2$ to a thioester surrogate, followed by a reaction catalyzed by 3-mercaptophenylacetic acid [2]. The biophysical properties of such synthetic MTs were compared with MTs obtained by heterologous expression in *E. coli* using the IMPACT expression system. The presented research opens up new pathways to receive and characterize posttranslationally modified MTs, which may exhibit different properties than their unmodified counterparts.

The research was supported by the National Science Centre of Poland under Opus grant 2018/31/B/NZ1/00567 to A. K.

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Getting insights on structural and energetic properties of reciprocal peptide-protein interactions

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Peptide-protein interactions play a pivotal role for many metabolic and cellular processes and are strictly associated to the onset of largely spread human diseases such as cancer and neurodegenerative pathologies. Despite the progress in the structural and energetic characterization of peptide-protein interfaces, an in-depth knowledge of the molecular details of key binding interactions occurring between the peptide and its target counterpart is still missing.

In the present study, we proposed an exhaustive and comprehensive *in silico* morphological and energetic analysis of putative peptide binding sites by focusing on both peptide and protein standpoints. Starting from the PixelDB database[1], a non-redundant collection of high-quality 3D crystallographic structures of peptide-protein complexes, several interpretable geometrical and energetic descriptors have been reciprocally computed in order to explore the physicochemical property space and highlight the differences of the secondary structure contents. Next, we investigated the most frequent peptide-protein residue pairs at the binding interface and made extensive 3D-GRID energetic distribution analyses. To better assess the difference between peptide-protein and protein-protein interacting residue pairs, an additional analyses by using a non-redundant database tailored for protein-protein interactions was carried out[2].

The ultimate aim is to investigate the peptide affinity-enhancing interactions with the partner protein to be further exploited in rational drug design strategies.

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Microfluidic impedance cytometry: a new tool to study antimicrobial peptides at the single-cell level

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Antimicrobial peptides (AMPs) represent a promising class of compounds to fight resistant infections. In most cases, they kill bacteria by making their membrane permeable.[1, 2]

With the aim of understanding the activity and selectivity of AMPs, we are currently developing microfluidic impedance cytometry as a new tool to study the effects of AMPs at the single cell level. This technique involves the detection of electric field screening caused by individual cells flowing over patterned electrodes integrated in a microchannel, measured through variations in electric current under an applied AC potential. The measured frequency-dependent impedance depends on cell features, i.e., volume and dielectric properties[5] and can be analyzed with appropriate signal processing for the characterization of cellular electrophysiology.[6]

In our experiments, we studied the effect of the antimicrobial peptide DNS-PMAP23 on both bacteria and human cells, using a coplanar-electrode microfluidic impedance chip.[6] For these studies, we selected human erythrocytes (obtained from healthy donors) and *Bacillus megaterium* Bm11 cells, which are commonly used as Grampositive bacterial model organism. Comparison of these data with traditional approaches indicate that impedance spectroscopy is a very promising approach for the development of next-generation fast and sensitive antimicrobial activity assays.

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Identification of peptide markers from allergenic seafood tropomyosins

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Crustaceans and molluscs are mentioned on a list of 14 allergen sources whose presence must be declared on the product label [1]. The diversity of seafood species makes it difficult for food producers to include accurate information on the origin of allergen on a food product label. Tropomyosins are the main seafood allergens. The aim of this study was to find the fragments peptides markers common to many sequences of tropomyosins which can be identified in processed seafood products with selected proteomic tools, following enzymatic hydrolysis.

Proteins were extracted from 4 products that had been defrosted, cooked or fried without fat, as well as from two ready-to-eat products. Proteins were hydrolyzed with trypsin, lyophilized and the hydrolysates were analyzed by RP-LC-MS/MS. 40 peptides present in at least two samples were selected. MQQLENDLDQVQESLLK peptide was present in samples obtained from crustaceans products, and VAECESEIQGLNR peptide - in products containing cephalopod molluscs, subjected to cooking and frying, as well as in defrosted raw material. Peptides such as LAEASQAADESER, LAMVEADLER and IVELEEELR have been identified in defrosted, cooked as well as ready-to-eat products.

In the future, these studies may be included in a procedure comprising identification of food allergens.

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Aggregation properties of a therapeutic peptide for rheumatoid arthritis: a spectroscopic and molecular dynamics study

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The biological properties of therapeutic peptides, such as their pharmacokinetics and pharmacodynamics, are correlated with their structure and aggregation properties. In this contribution, we report on the aggregation properties of a therapeutic peptide (CIGB-814), currently in phase 2 clinical trial, for the treatment of rheumatoid arthritis over a wide range of concentrations (μM–mM). We applied spectroscopic techniques (fluorescence, electronic circular dichroism, resonance, and dynamic light scattering), atomic force microscopy, and molecular dynamics simulations to determine the aggregation mechanism of CIGB-814. We found that the hierarchical aggregation of CIGB-814 at micromolar concentrations was initiated by the formation of peptide oligomers.[1] Subsequently, the peptide oligomers trigger the nucleation and growth of peptide nanostructures (cac = 123 μM), ultimately leading to the fibrillization of CIGB-814 (cac' = 508 μM). These results pave the way for a deeper understanding of the CIGB-814 therapeutic activity and may give important insights on its pharmacokinetics.

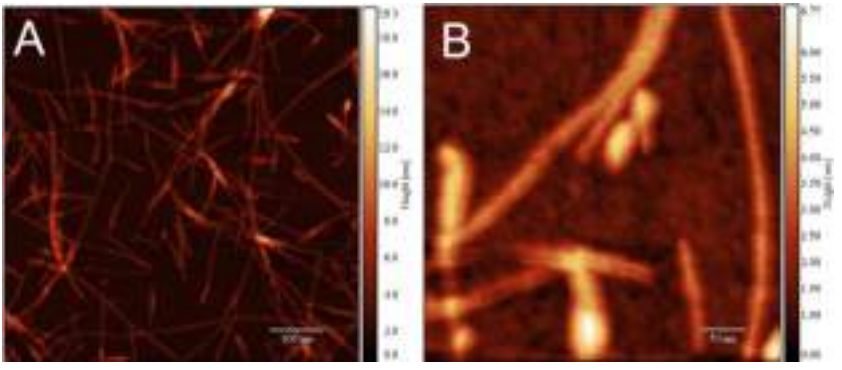

Figure 1. AFM imaging of CIGB-814 on mica. Deposition solution concentration: 1.8 mg/mL (≈600 μM). Scale bar: 500 nm (left); 100 nm (right).

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Zeolites employed as basic catalyst for nucleophilic substitution reactions: An analysis of the adopted approach and hypothesized new perspectives

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An excursus of substitution reactions performed on different halo-compounds and several cyclic sulfamidates that are readily accessible to nucleophilic attack is reported. [1-5] Essentially, a cysteine sulfhydryl group is employed as nucleophile and the process is promoted by the basic sites of activated Zeolites A (4 Å molecular sieves). [6] The results obtained by performing *in situ* FT-IR analysis, executed on molecules adsorbed on the catalyst, suggest that the basic sites of zeolites, corresponding to framework oxygens, are located on the external surface of the crystals. It is also worth mentioning that the zeolites are easy to handle and easy to be separated by the reacting mixture, in order to be recycled and reused for other chemical processes. This issue is actually under our consideration and will be the object of our future investigation. The moderate basicity of the zeolite lattice surface was the prerequisite for conducting chemoselective and in some case stereoselective peptide modifications. A wide range of chemical modifications was carried out on peptide molecules and the developed methodology allowed an efficient one-pot introduction of exogenous moieties into peptides, useful for developing peptidomimetic and/or peptide-based probes. (figure 1)

Figure 1. Thio-Alkylation of peptide sequences performed with different compounds catalysed by 4 Å MS.

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Design and evaluation of peptides targeting Ship2-Sam

M. Vincenzi[1], F. A. Mercurio[1], C. Di Natale[2,3], R. Palumbo[1], L. Pirone[1,5], S. La Manna[4,5], D. Marasco[4,5], E. M. Pedone[1,5] and M. Leone[1,5]

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Ship2 is a lipid phosphatase that interacts with the EphA2 receptor through a heterotypic Sam (Sterile alpha motif domain)-Sam association.[1] EphA2 is upregulated in many types of tumours and the process of receptor endocytosis and degradation has been analysed as potential route to decrease tumour malignancy. Binding of Ship2-Sam to EphA2-Sam is important to modulate receptor endocytosis/degradation thus, peptide inhibitors of this Sam-Sam complex hold a certain interest as potential anticancer agents. We have previously designed the positively charged "KRI3" peptide, that binds Shp2-Sam and represents a weak antagonist of the Ship2-Sam/EphA2-Sam complex.[2] Herein we report on different linear and cyclic KRI3 analogues. A multidisciplinary approach based on computational studies and conformational analyses by CD and NMR was conducted to study the novel KRI3 analogues. In addition, different techniques (NMR, MST and SPR) were employed to evaluate peptides binding to Ship2-Sam. Our studies revealed the important role played by tyrosine residues in the interaction with Ship2-Sam. On the contrary, peptide cyclization through a disulphide bridge or an increase of the positive charge of the sequence improves unspecific interactions with a negligible effect on the binding affinity to Ship2-Sam. *In vitro* cell-based assays were conducted as well and highlighted the capacity of KRI3 peptide to be internalized into PC3 cells even without conjugation to a cell penetrating peptide sequence.[3]

Acknowledgement
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Larazotide tripeptide derivatives as potential Main protease inhibitors

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The outbreak of novel coronavirus disease caused by the pathogen SARS-CoV-2 has resulted in over 61.8 million infections and over 1.4 million deaths worldwide.[1] These reported estimations highlight the need for drug discovery and development of antiviral treatments to combat this deadly virus. While vaccines are a central pillar of our grater efforts to fight the ongoing of COVID-19 pandemic,[2] therapeutics should provide many distinct advantages maintaining a complementary approach. Most of anti-COVID therapeutics resulting from several repurposing campaigns are penalized by a specific administration intended exclusively for hospital practices coupled with most severe cases of infections. In this field, several progresses have been reached with monoclonal antibodies (mABs), but their use is strongly limited by the economic costs, the problematic production practice, and the global shortage in supply.[3]

For these reasons small molecules discovery could represent a valid alternative approach to expand anti-sars-CoV-2 therapeutics arsenal. By observing similar structural motifs of peptidomimetic M^{pro} inhibitors N3 and 13b with AT1001 (Larazotite acetate), currently in phase 3 trials in celiac disease [4] as zonulin inhibitor, we developed a new rational and ambitious research program aimed to investigate new antivirals. We experimentally confirmed [5] the AT1001 binding towards of M^{pro} enzyme, providing us a structural rational to design and synthesize five AT1001 derivatives.

In the present work, we discuss the process leading to the development of a new series of Larazotide tripeptide derivatives. Considering the previous results concerning AT1001 analogues, we collected pivotal clues to design a new series of more potent M^{pro} inhibitors.

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Functional stapled fragments of human preptin of minimised length

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Preptin is a 34-amino-acid-long peptide derived from the E-domain of a precursor of insulin-like growth factor 2 (pro-IGF2) with bone-anabolic and insulin secretion amplifying properties. Here, we describe the synthesis, structures, and biological activities of six shortened analogues of human preptin. Eight- and nine-amino-acid-long peptide amides corresponding to the C-terminal part of human preptin were stabilised by two types of staples to induce a higher proportion of helicity in their secondary structure. We monitored the secondary structure of the stapled peptides using circular dichroism. The biological effect of the structural changes was determined afterwards by the ability of peptides to stimulate the release of intracellular calcium ions. We confirmed the previous observation that the stabilisation of the disordered conformation of human preptin has a deleterious effect on biological potency. However, surprisingly, one of our preptin analogues, a nonapeptide stabilised by olefin metathesis between positions 3 and 7 of the amino acid chain, had a similar ability to stimulate calcium ions' release to the full-length human preptin. Our findings could open up new ways to design new preptin analogues, which may have potential as drugs for the treatment of diabetes and osteoporosis (1).

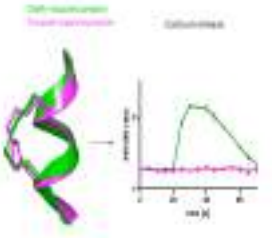

Figure 1. Analysis of calcium mobilisation in human U2OS cells by preptin fragments.

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Synthesis and nucleic acid binding evaluation of a thyminyl L-diaminobutanoic acid-based nucleopeptide

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Synthetic conjugates composed of nucleobases inserted into a peptide backbone, also referred to as nucleopeptides, may show many interesting properties including nucleic acid-binding ability or formation of supramolecular networks useful for various biomedical applications.[1,2]
We have already explored several nucleopeptide structures composed of different nucleoamino acid monomers and found interesting biomolecular binding properties in case of diamino acid-based cationic nucleopeptides realized by sequential oligomerization of nucleobase-bearing and base-free units.[1,3]

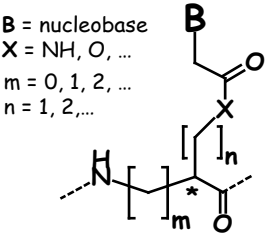

Figure 1. Generic nucleopeptide composed of a peptide backbone on which nucleobases are anchored through a suitable linker

Here, the synthesis of a nucleopeptide based on L-diaminobutanoic acid (DABA) realized alternating in the sequence thymine-bearing DABA moieties with free ones, and its characterization, will be presented. In addition, the ability of this nucleopeptide to interact with nucleic acid targets in both single and double stranded forms, explored by CD analysis, will be also reported.

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Identification of a novel vasoactive pentapeptide through a bioassay-guided fractionation approach

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Milk and milk-derived products represent an important source of amino acids, proteins, fat, vitamins, calcium, fatty acids, and several other bioactive compounds needed for the biochemical and physiological functions of our cells. During the last decade, numerous casein-derived peptides with cardiovascular properties have been identified and tested for their properties as anti-hypertensive compounds. However, most of these have failed during the translation in-vivo, probably because they have been identified by protein hydrolysates of cheese sources, thus giving rise to a series of chemically unstable peptides to gastrointestinal digestion.

Herein, in order to overcome this problem, we applied *in-vitro* simulated human gastro-intestinal digestions (GID) using endogenous enzymes to mimic a physiological process on buffalo ice-cream (BIC) matrix, which until now had never been exploited to obtain new possible bioactive peptides. Although many peptides have come out through this experimental approach, we decided to investigate the peptide that emerged in more abundant quantities, and therefore, *in vivo*, would have been more likely to exert a biological action. We proceeded to investigate the possible direct vascular effects of the new pentapeptide namely PG1 (QKEPM), which originates from the GID of α_{S1}-casein (f146-150). The main purpose of this work was to investigate its possible vascular action both *ex-vivo*, on the modulation of vascular tone, and *in-vivo* testing its possible hemodynamic effects in a mice model of arterial hypertension induced by Ang II. [1,2]

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Discovery of a Novel Tetrapeptide against Influenza A Virus: Rational Design, Synthesis, Bioactivity Evaluation and Computational Studies

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Influenza is a highly contagious, acute respiratory illness, which represents one of the main health issues worldwide. Even though some antivirals are available, the alarming increase in virus strains resistant to them highlights the need to find new drugs.[1] Previously, Superti et al. deeply investigated the mechanism of the anti-influenza virus effect of bovine lactoferrin (bLf) and the role of its tryptic fragments (the N- and C-lobes) in antiviral activity.[2] Recently, through a truncation library, we identified the tetrapeptides, Ac-SKHS-NH_2 (**1**) and Ac-SLDC-NH_2 (**2**), derived from bLf C-lobe fragment 418–429, which were able to bind hemagglutinin (HA) and inhibit cell infection in a concentration range of femto- to picomolar.[3] Starting from these results, in this work, we initiated a systematic SAR study on the peptides mentioned above, through an alanine scanning approach. We carried out binding affinity measurements by microscale thermophoresis (MST) and surface plasmon resonance (SPR), as well as hemagglutination inhibition (HI) and virus neutralization (NT) assays on synthesized peptides. Computational studies were performed to identify possible ligand–HA interactions. Results obtained led to the identification of an interesting peptide endowed with broad anti-influenza activity and able to inhibit viral infection to a greater extent of reference peptide.

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