16th Naples Workshop on Bioactive Peptides

The exciting role of peptides in life-science

Co-Chairmen

Giancarlo Morelli, Michele Saviano, Menotti Ruvo, Paolo Grieco

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16th Naples Workshop on Bioactive Peptides

THE EXCITING ROLE OF PEPTIDES IN LIFE SCIENCE

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

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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 7th

Session 1 - Peptides in Diagnostic and Therapeutic Applications

Chairmen: A.M. Papini – M. Chorev

10.30 ***Welcome addresses***

11.00 – 11.45 PL1 **Stephen B. H. Kent - University of Chicago –IL - USA**
Recent adventures in the synthesis of small protein toxins

11.45 – 12.05 O1 **Meritxell Teixidó - Institute for Research in Biomedicine (IRB Barcelona) - Spain**
Minichlorotoxins, a new family of blood-brain barrier peptide shuttles

12.05 – 12.25 O2 **Chiara Falciani - University of Siena – Italy**
The GAG-specific branched peptide NT4 reduces invasiveness of tumor cells and angiogenesis

12.25 – 12.45 O3 **Burkhard Bechinger - University of Strasbourg - France**
Cell-penetrating peptides with antimicrobial, transfection and transduction activities

12.45 – 13.05 O4 **Lihi Adler-Abramovich - Tel Aviv University - Israel**
Learning from nature to form new organic materials for tissue regeneration

13.05 - 14.15 ***Free Lunch***

Session 2 - Antimicrobial Peptides

Chairmen: L. Stella – S. Galdiero

14.15 – 14.55 PL2 **Martin Malmsten- Uppsala University – Sweden**
Nanoparticle and other delivery systems for host defence peptides

14.55 – 15.15 O5 **Marta De Zotti - University of Padua – Italy**
Analogs of the peptaibol trichogin as biopesticides

15.15 – 15.35 O6 **Dilip Shah - Donald Danforth Plant Science Center - USA**
Antifungal plant defensins MtDef4 and MtDef5:
Mechanisms of action and engineering transgenic peanut resistant to Aspergillus flavus and aflatoxin accumulation

15.35 – 15.55 O7 **Clara Pérez-Peinado - Pompeu Fabra University - Spain**
Mechanisms of bacterial membrane permeabilization of snake venom-derived peptides crotalicidin (Ctn) and Ctn[15-34]

15.55 - 16.15 ***Coffee break***

Session 3 – Peptides in Diagnostic and Therapeutic Applications

Chairmen: L. Moroder – E. Pedone

16.15 – 16.55 PL3 **Michael Chorev – Brigham & Women's Hospital, Harvard Medical School – USA**
Glycated CD59, a novel biomarker – from post-translational to translational"

16.55 – 17.15 O8 **Francesca Biscaglia - University of Padua - Italy**
Liver cancer cell targeting and SERRS imaging with peptide-functionalized Gold nanostructures

17.15 – 17.35 O9 **Lubomir Vezenkov - ENSCM/IBMM Montpellier - France**
Folded oligomers for cellular uptake and drug delivery

17.35 – 17.55 O10 **Francesco Saverio Di Leva – University of Naples - Italy**
Metadynamics-Driven discovery of cyclic pentapeptides as αvβ6 integrin-selective ligands

17.55 – 18.15 O11 **Kathrin Bellmann-Sickert - Leipzig University – Germany**
Modulation of Y1-receptor responses by chemically modified truncated NPY analogues

18.15 – 18.35 O12 **Gil Rosenman – Tel Aviv University - Israel**
Peptide Nanophotonics: from visible bionanodots to optical biochips

18.35 – 18.55 O13 **Carmen Lammi - University of Milan – Italy**
Lupin LILPKHSDAD (P5) peptide is a novel bi-functional inhibitor of PCSK9, a new target for the hypercholesterolemia treatment

18.55 – 19.15 O14 **Tom Grossman - VU University Amsterdam - Netherlands**
A chimeric cell-penetrating stapled peptide inhibits oncogenic Wnt signaling

19.30 ***Welcome Party***

FRIDAY, JUNE 8th

Session 4 – Antimicrobial Peptides
Chairmen: L. Stella – M. De Zotti

08.30 – 09.10 PL4 **Anne S. Ulrich - Karlsruhe Institute of Technology – Germany**
Membrane-active peptides and their photoswitchable analogues as antibiotics, anti-cancer agents, and cell penetrating carriers

09.10 – 09.30 O15 **Patricio Carvajal-Rondanelli - Universidad Católica de Valparaíso - Chile**
Understanding the antimicrobial properties/activity of an 11-residue Lys homopeptide by Alanine and Proline scan

09.30 – 09.50 O16 **Cristina Peggion – University of Padua - Italy**
Strategies of peptide-cotton bond formation for preparing biocompatible and antimicrobial fabrics

09.50 – 10.10 O17 **Markus Weingarth - Utrecht University - Netherlands**
Towards the native structure of the nisin: Lipid II pore

10.10 – 10.30 ***Coffee break***

Session 5 – Peptides in Diagnostic and Therapeutic Applications
Chairmen: P. Grieco – M. Saviano

10.30 – 11.10 PL5 **Victor Hruby – University of Arizona - USA**
Design of novel receptor selective peptide ligands for the melanocortin system: application to diagnosis and treatment of degenerative diseases

11.10 – 11.50 PL6 **Dale F. Mierke – Dartmouth College – NH – USA**
New adventures in NMR usage for peptide therapeutics

11.50 – 12.10 O18 **Marco van de Weert - University of Copenhagen – Denmark**
From drug substance to drug product: the challenges of peptide instability

12.10 – 12.30 O19 **Gianfranco Bocchinfuso – University of Rome Tor Vergata - Italy**
Protein tyrosine phosphatase SHP2 as a target for PTPN11-associated malignancies and other cancers

12.30 – 12.50 O20 **Sara Pellegrino – University of Milan – Italy**
Peptide Aptamers as an environmental friendly approach in the treatment of grapevine Plasmopora Vinicola downy mildew

12.50 - 14.15 ***Lunch on site***

PEPTIDE SHOWCASE

Chairmen: G. Morelli – L. Zaccaro

14.15 – 14.30 PS1 **Carolin Lechner – BACHEM - Switzerland**
Acetylation in Fmoc-SPPS

14.30 – 14.45 PS2 **Giorgio Marini – CEM - Italy**
Solid Phase Peptide Synthesis (SPPS) at Elevated Temperatures: Advances, Process Development, and Considerations

14.45 – 15.00 PS3 **Alessandro Pini – SETLANCE - Italy**
The antimicrobial peptide SET-M33. A case of peptide-based drug development

Session 6 – Peptide Mimetics

Chairmen: C. Toniolo – M. Ruvo

15.00 – 15.40 PL7 **Gilles Guichard - CNRS – University of Bordeaux – France**
α-Helix mimicry by interfacing oligourea foldamers with α-Peptides

15.40 – 16.00 O21 **Galia Maayan – Technion - Israel Institute of Technology - Israel**
Selective recognition and self-assembly by Cu(II) binding peptoids

16.00 – 16.20 O22 **Daniela Comegna – CNR Naples - Italy**
S-Glycosylated α-Peptoids: a versatile synthetic method towards potentially bioactive compounds

16.20 – 16.40 ***Coffee break***

16.40 – 17.00 O23 **Sharon Gilead - Tel Aviv University - Israel**
Amyloid formation by short peptides: from mechanistic insights to function

17.00 – 17.20 O24 **Jayanta Haldar – Jawaharlal Nehru Centre for Advanced Scientific Research - India**
Synthetic Mimics of Antimicrobial Peptides to Tackle Antimicrobial Resistance

17.20 – 17.40 O25 **Paul Alewood - University of Queensland, St Lucia - Australia**
Oxytocin Mimetics

17.40 – 18.00 O26 **Daniele Di Marino – University of Svizzera - Switzerland**
Targeting mRNA translation process to develop anticancer agents: a successful drug design study

18.00 – 19.00 ***Poster session***

Free evening

SATURDAY, JUNE 9th

Session 7 – Peptides in Chemical Biology
Chairmen: P. Grieco – C. Isernia

08.50 – 09.30 PL8 **Jeffrey W. Bode – ETH Zurich – CH**
Chemical Protein Synthesis with the KAHA Ligation

09.30 – 09.50 O27 **Claudia Bello - University of Florence – Italy**
Toward homogeneous glycoproteins via auxiliary-assisted sequential glycosylation and ligation of peptides

09.50 – 10.10 O28 **Tim Sewczyk - Leibniz University Hannover – Germany**
Immobilized proteases for industrial bioactive peptide production

10.10 – 10.30 ***Coffee Break***

Session 8 – Bioconjugation, Folding and Aggregation
Chairmen: P. Alewood – D. Marasco

10.30 – 11.10 PL9 **Aphrodite Kapurniotu - Technische Universität München – Germany**
Exploiting cross-amyloid peptide interactions to control amyloid self-assembly in Alzheimer's disease and type 2 diabetes

11.10 – 11.30 O29 **Maria Cristina Cringoli – University of Trieste – Italy**
Luminescent hydrogels from a tripeptide and carbon nanodots

11.30 – 11.50 O30 **Minying Cai – University of Arizona - USA**
Development of the MC1R Selective Ligands for the Melanoma Prevention

11.50 – 12.10 O31 **Evelina Parisi - University of Trieste – Italy**
Effects of chirality on tripeptide self-assembly

Session 9 – Peptides in Immunology
Chairmen: P. Rovero – A. Accardo

12.10 – 12.30 O32 **Sira Defaus – Pompeu Fabra University - Spain**
Expanding the potential and multivalency of the B2T synthetic peptide vaccine against foot-and-mouth disease virus

12.30 – 12.50 O33 **Kata Horvati - Eotvos L. University - Hungary**
Nanoparticulated multi-epitope conjugates as vaccine candidates against tuberculosis

12.50 – 13.10 034 **Luigi Buonaguro – Istituto Nazionale Tumori Naples - Italy**
Unique true predicted neoantigens (TPNAs) correlates with anti-tumor immune control in HCC patients

13.10 – 14.10 ***Free lunch***

Session 10 – Peptides in Chemical Biology
Chairmen: D.F. Mierke – L. Moroder

14.10 – 14.50 PL10 **David Chatenet - Centre INRS–Institut Armand-Frappier – Laval – Quebec – Canada**
Recent advances in the molecular and cellular pharmacology of the urotenssnergic system: new direction in the conception of UT ligands

14.50 - 15.10 O35 **Salvatore Di Maro – University of Campania - Italy**
Quick and Sound Solid-Phase Peptide Synthesis

15.10 – 15.30 O36 **Mattia Migliore – University of Rouen Normandie - France**
New insights in the structural characterization of turns in peptides: determination of NMR discriminatory parameters

15.30 – 15.50 O37 **Evelien Wynendaele - University of Ghent – Belgium**
Is there a role for quorum sensing peptides in cancer?

15.50 – 16.10 O38 **Adriano Mollica – University of Chieti - Italy**
CLIPS (Chemical LInkage of Peptides onto Scaffolds) technology applied to opioid peptides research

16.10 – 16.30 O39 **Federica Tonolo - University of Padua – Italy**
Protective effects of antioxidant milk-derived bioactive peptides on Caco-2 cell

16.30 - 17.00 ***Concluding Remarks***

17.00 – 23.00 ***Social events and Gala dinner***

POSTER SESSION

POSTER SESSION

Peptides in Diagnostic and Therapeutic Applications (P1-P17)
Antimicrobial peptides (P18-P24)
Peptide mimetics (P25-P31)
Peptides in Chemical Biology (P32-P39)
Bioconjugation, Folding and Aggregation (P40-P44)
Peptides in Immunology (P45-P47)

P1 Luisa CALVANESE
Mini-factors revealing VEGF receptors in imaging applications

P2 Sara LA MANNA
Triple-negative breast cancer: new potential therapeutics derived from SOCS3 protein

P3 Maria GALLO
Cell-penetrating peptides restricting oligomerization of G protein-coupled receptors: the CB1R-5HT2AR dimer

P4 Antonio MAZZOLENI
Synthesis and characterization of Glucosylated Peptides: toward selective plasmapheresis-based treatment of Multiple Sclerosis

P5 Annarita FALANGA
Synthesis and in vitro evaluation of fluorescent and magnetic nanoparticles functionalized with a cell penetrating peptide for cancer theranosis

P6 Annamaria SANDOMENICO
Chimeric Recombinant Antibody Fragment of anti_Nodal 3D1 for theranostic applications

P7 Carlo DIAFERIA
Multicomponent hydrogels based on Fmoc-FF for potential use as regenerative scaffolds

P8 Fernando FORMAGGIO
Synthesis of peptides potentially inhibiting the protein-protein interactions of the SHP2 phosphatase

P9 Sonia DI GAETANO
An integrin antagonist identified in the anophelin cE5 from the malaria vector Anopheles gambiae

P10 Emanuela IACCARINO
Therapeutic potential of novel Cripto-1 CFC small peptide mimetics in melanoma

P11 Antonella ACCARDO
Gd(III)-complex derivatives of aromatic oligopeptides as novel supramolecular MRI contrast agents

P12 Simona Maria MONTI
The Proteoglycan like domain of the tumour enzyme Carbonic Anhydrase IX is an Intrinsically Disordered Protein

P13 Fernando FORMAGGIO
Synthesis and spectroscopic characterization of analogs of the anticancer agent Culicinin D

P14 Chen LIU
Design and functional studies on analgesic peptides

P15 Stefania DE LUCA
Evaluation of HER2-specific peptide ligand for its employment as radiolabeled imaging probe

P16 Giancarlo MORELLI
Easy Formulation of Liposomal Doxorubicin modified with a Bombesin Peptide Analog for selective targeting of GRP Receptors over-expressed by Cancer Cells

P17 Domenica MUSUMECI
Synthesis, characterization and nucleic acid-binding ability evaluation of cationic nucleobase-decorated peptides

P18 Lucia LOMBARDI
Novel peptide biomaterials against biofilms

P19 Annarita FALANGA
Metallic nanoparticles: antibacterial activity and ecotoxicity

P20 Maria Luisa MANGONI
RGIn vivo efficacy of esculentin-1a derived peptides against Pseudomonas-aeruginosa induced pneumonia

P21 Floriana CAPPIELLO
Esc(1-21) and its diastereomer: antipseudomonal frog-skin derived peptides with multiple immunomodulatory properties

P22 Gianluigi FRANCI
The amphibian antimicrobial peptide temporin L inhibits in vitro herpes simplex virus type 1 infection, a continuous story

P23 Margherita DEGASPERI
In vitro and in vivo evaluation of D-BMAP18 peptides for the treatment of pulmonary infections in cystic fibrosis

P24 Marco SCOCCHI
Optimization of mammalian proline-rich antimicrobial peptides as small and effective inhibitors of bacterial protein synthesis

P25 Francesca CLERICI
Peptidomimetics based electrospun nanofibers

P26 B. FARINA
AIF(370-394)/CypA binding mode characterization: experimental and computational approaches

P27 Alessandra MONTI
Conformational stabilization of AIF(370-394) β-hairpin-like peptides

P28 Giuseppina SABATINO
Conformationally Constrained Analogues of Amyloidogenic Segments of Islet Amyloid Polypeptide

P29 Concetta AVITABILE
Self-Assembling of Fmoc-GC Peptide Nucleic Acid Dimers into Highly Fluorescent Aggregates

P30 Annalisa BORTOLOTTI
Characterization of the mechanism of membrane perturbation by small antimicrobial norspermidine-based peptidomimetics

P31 Ali Munaim YOUSIF
Peptoid-Peptide Hybrid Backbone of Urotensin-II(4-11): Synthesis and Biological Evaluation

P32 Maria MOCCIA
PNA based miR-34a mimics: a physico-chemical study and assessment in Neuroblastoma

P33 Militsa YANEVA
Synthesis and characterisation of stapled α-helical peptide that binds to G-quadruplex nucleic acid

P34 Emma FENUDE
Conformational Properties of Gluten Exorphins GE-A and its Role when Encrypted in Precursor Protein

P35 Aaron D. MARTIN
VEGF receptor binding studies of PlGF[87-101] peptide analogues

P36 Feliciana REAL FERNANDEZ
Peptides to study HIV-1 and CCR5 interactions by surface plasmon resonance

P37 Luciano PIRONE
The discovery of GRP78 as a novel KCTD15 interactor

P38 Davide ESPOSITO
Crystal structure of human carbonic anhydrase II in complex with a membrane-impermeant, isoform selective inhibitor

P39 Samaneh AHMADI
Theoretical study on the stability of amphi-ionophore cystine-based cyclopeptide stereoisomers with anions and cations

P40 Mariano VENANZI
Fluorescence studies of therapeutic peptides: the Semaglutide case

P41 Nunzianna DOTI
Structural and functional characterization of microbial transglutaminase in denaturing conditions

P42 Giuseppina FOCA'
Site-specific modification of antibody fragments by microbial transglutaminase

P43 Gianluca D'ABROSCA
Influence of metal ions on folding mechanism and self-association propensity of high homologous proteins

P44 Cristina PEGGION
Synthesis and conformational investigation of hetero-chiral sequential oligopeptides based on the (alphaMe)Aze/Ala dyad

P45 Hendrik RUSCHE
Antibody Epitope of human alpha-Galactosidase A revealed by affinity-mass spectrometry

P46 Mar FORNER
Novel foot-and-mouth disease vaccine platforms based on the successful B2T prototype: exploring multivalency and enhancing efficacy

P47 Lorenzo ALTAMORE
Use of peptides mimetics of proteins for characterisation of immune response in different pathological conditions: a powerful approach

PLENARY LECTURES

Recent adventures in the synthesis of small protein toxins

Stephen B.H. Kent

University of Chicago, Illinois 60637, USA

Ts1 is a beta scorpion toxin and Ts3 is an alpha scorpion toxin. Both proteins are isolated from the venom of the Brazilian scorpion Tityus serrulatus.[1] Efficient total chemical synthesis based on native chemical ligation was used to confirm the correct covalent structures of the Ts1 and Ts3 polypeptide chains. Triazole-forming 'Click' chemistry was used to fluorescently label Ts1 for studies of the voltage-gated sodium ion channel Nav1.4.[2] Racemic protein crystallography was used to determine the structure of the Ts3 toxin protein molecule by X-ray diffraction.[3] ShK toxin is a cysteine-rich 35-residue protein isolated from the sea anemone Stichodactyla helianthus that is a ligand for the voltage-gated potassium ion channel.[4] An efficient total chemical synthesis of ShK was effected by native chemical ligation at a Gln-Cys site, and the X-ray structure was determined by racemic protein crystallography.[5] The stereochemical configurations of individual Thr and Ile residues in the ShK polypeptide chain were inverted, to give allo-Thr and allo-Ile residues, and the effects on the folding and stability were measured. Structures of the resulting ShK protein diastereomers were determined by quasi-racemic protein crystallography.[6] The reported biological activity of D-allo-ShK, in which all Thr and Ile residues were in the allo form, was reexamined.[7]
Several approaches to the synthesis of 'lasso' peptides will be discussed.
Reflections on the current state and future challenges in chemical synthesis of peptides and proteins will be presented.

References

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Nanoparticle and other delivery systens for host defense peptides

M. Malmsten[1,2]

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2 Department of Pharmacy, Uppsala University, P.O. Box 580, SE-752 32 Uppsala, Sweden

Due to rapidly increasing resistance development against conventional antibiotics, finding novel approaches for the treatment of infections has emerged as a key health issue. Antimicrobial peptides (AMPs) have attracted interest in this context, and there is by now a considerable literature on the identification such peptides, as well as on their optimization to reach potent antimicrobial and anti-inflammatory effects at simultaneously low toxicity against human cells. In comparison, delivery systems for antimicrobial peptides have attracted considerably less interest. However, such delivery systems are likely to play a key role in the development of potent and safe AMP-based therapeutics, e.g., through reducing chemical or biological degradation of AMPs either in the formulation or after administration, by reducing adverse side-effects, by controlling AMP release rate, by promoting biofilm penetration, or through achieving co-localization with intracellular pathogens.

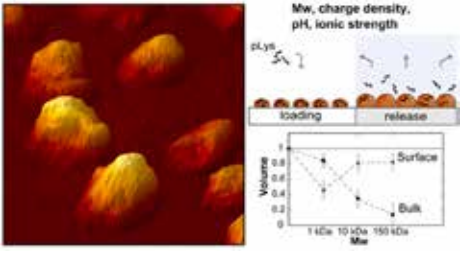

Figure 1. Surface-bound microgels as surface coatings for host defense peptides.

Here, an overview is provided of some of our recent work on delivery systems for antimicrobial peptides, including polymer nanogels[1,2], mesoporous silica[3], nanoclays/nanosheets[4], and quantum dots, with special focus on AMP-carrier interactions, as well as consequences of these for membrane interactions, as well as for antimicrobial and related biological effects of AMP-containing formulations.

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Glycated CD59, a Novel Biomarker from Post-translational to Translational

Michael Chorev

Laboratory for Translational Research, Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA

The discovery of glycated CD59 (GCD59) as a biomarker for glucose intolerance builds on the pathogenic role that the complement system plays in the development of complications of diabetes. Long lasting and frequent high levels of glucose lead to the glycation of CD59, a ubiquitous membrane-GPI anchored protein that inhibits the formation of the membrane attack complex (MAC). Glycation of CD59 results in its inactivation, namely loss of its capacity to protect cells from complement-mediated lysis. Inactivation of CD59 increases MAC deposition which induces the release of pro-inflammatory and pro-thrombotic cytokines that trigger inflammation, proliferation and thrombosis, as characteristically seen in the target organs of diabetes complications. The presentation will describe the development of all the molecular tools required for the construction of a reliable and quantitative assay to measure GCD59 in plasma and present the proof of concept for its clinical utility.

Membrane-active peptides and their photoswitchable analogues as antibiotics, anti-cancer agents, and cell penetrating carriers

Anne S. Ulrich[1,2], Erik Strandberg[1], Arianna Grau-Campistany[1], Parvesh Wadhwani[1], Marina Berditsch[2], Oleg Babii[2], Sergiy Afonin[1], Tim Schober[2], Jochen Bürck[1], Stephan Grage[1], Igor Komarov[3]

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3 Taras Shevchenko National University of Kyiv, IHT, 01601 – Kyiv, Ukraine

Membrane-active peptides are abundant in nature, with a multitude of functions ranging from host defence to cell-cell communication. Mechanistically, they can operate by inducing unspecific membrane damage, by assembly into discrete transmembrane pores, by eliciting membrane translocation, and/or by triggering membrane fusion. Using solid-state NMR structure analysis, we have resolved the membrane-bound architecture and dynamical behavior of several such representative types of peptides. By systematically varying their physicochemical properties (length, charge, hydrophobic moment, anchoring residues, etc.) and by screening them in different model membranes (bilayer thickness, spontaneous curvature, lipid composition, phase state, etc.), several key parameters could be identified that determine the membrane insertion and/or membranolytic effect of these amphiphilic sequences [1]. Namely, the spontaneous lipid curvature plays the most critical role besides hydrophobic mismatch [2,3], and some distinct features on the peptides - such as charge zippers or H-bond zippers - can stabilize their oligomeric transmembrane arrangement [4]. As specific examples, we have not only studied many derivatives of amphiphilic α-helical model peptides, but we also focused on the natural cyclic β-turn peptide gramicidin S (GS). This well-known dodecapeptide has numerous advantages over conventional antibiotics in terms of avoiding bacterial resistance, combatting biofilms, and synergistically treating local infections in humans. Yet, membranolytic side effects cannot be ignored, so we implemented a photoswitchable diarylethene functionality into the cyclic backbone of GS in order to allow switching its biological activity ON and OFF with VIS and UV light, respectively. This oxygen-free photodynamic approach was successful in widening the active concentration window for specific antimicrobial and anti-cancer applications of GS [5,6], and the general therapeutic potential of such photoswitchable systems will be discussed.

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Design of Novel Peptides and Peptidomimetics for Biological and Medical Applications

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It is now increasingly realized that peptides and peptidomimetics will be the drugs of the future. For this to become a reality, we will have to design peptides and peptidomimetics that are more stable, more bioavailable, more able to cross membrane barriers and more receptor/acceptor selective. We will discuss efforts in our laboratory and elsewhere that provide optimism that these goals can be met. The major approaches to this include: 1) enhanced computational methods; 2) conformational and topographical considerations; 3) multiple cyclization approaches; 4) glycosylation, N-methylation, lipidation and other modifications; 5) selectivity enhancement; and 6) multiple high throughput screening technologies and others. We will illustrate these approaches primarily examining the five very closely related melanocortin receptors that are involved in most of the key physiological functions necessary for a healthy life. The potential for application to important biological and medical problems will be emphasized. If time permits, a brief discussion of the need for and application of multivalency in drug design will be presented.

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New Adventures in NMR Usage for Peptide Therapeutics

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We will detail some techniques for the application of high resolution NMR in the identification and characterization of peptide binding to protein targets important for cancer therapeutics. These recent advances greatly enhance the identification of kinase inhibitors and peptide binders to molecular scaffolding proteins.

α-Helix Mimicry by Interfacing Oligourea Foldamers with α-Peptides

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Significant progress has been made towards the synthesis of non-natural oligomers with high propensity to fold into regular secondary structures (i.e. foldamers[1]). The ability to precisely and chemically control monomer sequences in these synthetic systems opens up unique opportunities for mimicking proteins and for creating new functions. In addition, foldamers are usually endowed with properties, i.e. structure predictability, chemical diversity, improved resistance to enzymatic degradation that make them well suited in the context of therapeutic applications.[2] However, the design of foldamers that bind to specific protein-surfaces for modulating protein-protein interactions and the elaboration of more sophisticated folded architectures such as tertiary and quaternary structures, resembling proteins in terms of size and shape (and ideally function) still remain challenging endeavours. Combining α-peptide and non-natural foldamer backbones in a single chain is a promising approach to create complex architectures and to redesign peptides and proteins by replicating or modulating their structures and functions. We have recently started to explore this concept of foldamer/α-peptide chimeras with aliphatic oligoureas, a class of foldamers[3] that adopt well-defined helical secondary structures akin to α-helices. In this presentation, we will show how key beneficial features of both species — such as natural epitope recognition of α-peptides and the innate helical stability of oligoureas — can be exploited in single chimeric constructs (i.e. block co-foldamers)[4] to generate original mimics of protein structures and α-helix mimics that specifically interact with protein surfaces.
Acknowledgements: This work has received support from the CNRS, Conseil Régional Nouvelle-Aquitaine (Project 20091102003) and ANR (ANR-12-ASTR-0024, ANR-12-BS07-0019 and ANR-15-CE07-0010). Constant support from UREkA, Sarl is also gratefully acknowledged.

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Chemical Protein Synthesis with the KAHA Ligation

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The chemical synthesis of proteins is a powerful tool for elucidating biological processes and has great potential for the preparation of the next generation of therapeutic proteins. Solid phase peptide synthesis (SPPS) can routinely provide peptide segments of about 40 amino acid residues but is not suited for the preparation of proteins. *Our group has developed a chemoselective amide-forming reaction of a-ketoacids and hydroxylamines (KAHA ligation), enabling the synthesis of proteins up to ~200 residues by combining unprotected peptide segments – without coupling reagents or side chain protecting groups.*

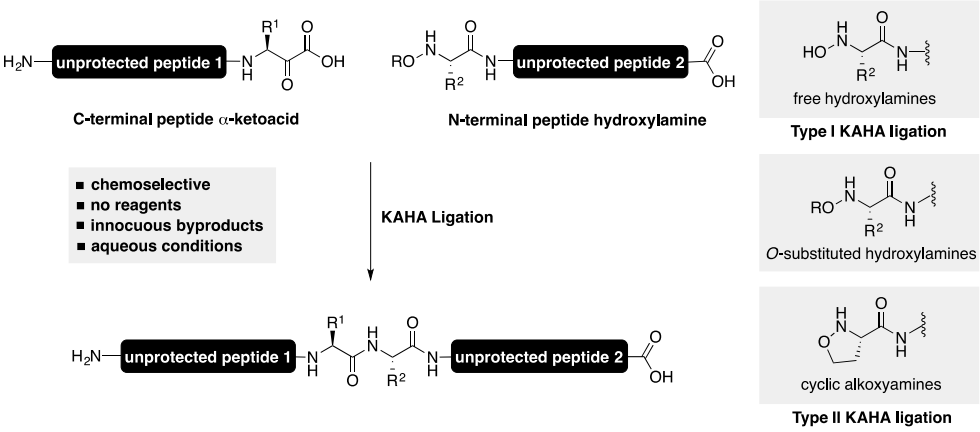

Figure 1. Chemoselective coupling of unprotected peptide segments with the a-ketoacid–hydroxylamine (KAHA) ligation. The KAHA ligation works with either substituted or unsubstituted hydroxylamines by distinct mechanisms. Cyclic hydroxylamines, particularly (S)-5-oxaproline, have proven to be the best combination of stability, reactivity, and synthetic access.

By establishing robust, scalable routes to the key linkers and building blocks, we can now routinely synthesize small proteins using Fmoc-SPPS – without the need for any non-standard workflows or post synthesis manipulations. Orthogonal protecting groups allow convergent protein synthesis in any direction, facilitating access to a wide variety of protein targets including synthetic enzymes, membrane-associated proteins, and molecular probes for biological processes.

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Exploiting cross-amyloid peptide interactions to control amyloid self-assembly in Alzheimer's disease and type 2 diabetes

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Amyloid self-assembly is linked to more than 30 devastating cell-degenerative diseases. Increasing evidence suggests that "cross-amyloid" interactions are able to modulate amyloidogenesis; such interactions might thus link pathogenesis of different amyloid diseases to each other. For instance, Alzheimer's disease (AD) and type 2 diabetes (T2D) appear to be linked to each other. A possible molecular link between the two diseases could be the interaction between their key amyloid polypeptides Aβ (AD) and islet amyloid polypeptide (IAPP).[1]

However, cross-amyloid interactions can be also used to design potent inhibitors of amyloid self-assembly. In fact, we have earlier designed conformationally constrained, non-amyloidogenic and bioactive analogs of full length IAPP, a glucose regulatory neuropeptide, as potent inhibitors of amyloid self-assembly of both IAPP and Aβ.[1-3]

More recently, we have developed a "hot-segment-linking" approach to design a series of conformationally constrained IAPP-derived segments as mimics of the IAPP interaction surface with Aβ.[4] These peptides, termed "interaction surface mimics" (ISMs), turned out to be nanomolar inhibitors of amyloid formation and cytotoxicity of Aβ, IAPP or both polypeptides.[4] Importantly, the nature of the linker moiety determines both ISM inhibitory potency and target selectivity.

Here I will present our studies on first and next ISM generations. Due to their favorable properties, these peptides are promising leads for anti-amyloid drugs and templates for peptidomimetics for targeting pathogenic amyloid self-assembly in AD, T2D or both diseases. In addition, our inhibitor design strategy could be used to design potent inhibitors of other pathogenic protein-protein interactions as well.

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Recent advances in the molecular and cellular pharmacology of the urotensinergic system: new direction in the conception of UT ligands

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The urotensinergic system, formed by a G protein-coupled receptor (GPCR) termed UT and two endogenous peptide ligands Urotensin II (UII, H-Glu-Thr-Pro-Asp-[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH) and Urotensin II-related peptide (URP, H-Ala-[Cys-Phe-Trp-Lys-Tyr-Cys]-Val-OH), is currently regarded as a potential key contributor to cardiovascular function. While multiple animal studies have shown the therapeutic potential of UT ligands for treatment of heart failure and atherosclerosis, their lack of efficacy in clinical studies points toward a greater understanding of UT pharmacology at both the molecular and cellular levels. UII and URP are cyclic peptides that share a common and strictly conserved bioactive cyclic core (-Cys-Phe-Trp-Lys-Tyr-Cys-) but differ by their extracyclic N-terminal residues. While sharing common biological activity, these two endogenous ligands appear to be functionally selective, which could be explained by distinct interactions with their cognate receptor.

In an effort to decipher the molecular and cellular pharmacology of this system, we implemented discrete replacements with natural and unnatural amino acids, and analyzed how these modifications impact binding, signaling profile and aortic ring contraction. Also, we have also explore the possibility that the functional selectivity observed with UII and URP might arise from the existence of UT oligomers.

Finally, we have investigated a new avenue that would allow the control of UT activation in the absence of endogenous ligands. These works have led to the discovery of unique bias agonists and allosteric modulators of the UT receptor but have also improved our understanging of the molecular and cellular pharmacology of this system.

ORAL PRESENTATIONS

Minichlorotoxins, a new family of blood-brain barrier peptide shuttles

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Brain delivery is one of the major challenges in drug development because of the high number of patients suffering from central nervous diseases (CNS) and the low efficiency of treatments available. Although the blood–brain barrier (BBB) prevents most drugs from reaching their targets, BBB-shuttle peptides offer great promise to safely overcome this formidable obstacle. Peptides which are experiencing a golden era are receiving growing attention because of their lower cost, reduced immunogenicity, and higher chemical versatility than traditional Trojan horse antibodies to be used as BBB-shuttles, as we have recently reviewed.[1-3]

Over the last years, we have reported the use of BBB-shuttles inspired in peptides found in venoms. We have minimized apamin, a neurotoxin from bee venom, by reducing its complexity, toxicity and immunogenicity, while preserving brain targeting, active transport, and protease-resistance leading to MiniAp-4.[4]

In this communication, a recently discovered new family of peptides able to cross the BBB, MiniChlorotoxins (MiniCTXs) will be presented. They are derived from Chlorotoxin (CTX), a disulphide-rich stable peptide derived from the venom of the Israeli scorpion Leirus quinquestriatus, which is able to enter the brain and bind specifically to tumour tissue. MiniCTXs are the result of the research performed to decipher the minimal part of CTX that maintains transport capacity and but abolishing its toxicicity. Presenting unpublished results on their potential application in the field of delivery of macromolecules and/or nanoparticles as promising future therapies for CNS disorders that require crossing the BBB.

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The GAG-specific branched peptide NT4 reduces invasiveness of tumor cells and angiogenesis

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NT4 is a branched peptide that selectively addresses cancer cells. It proved to be efficient in human cancer cell and tumor tissue selective-binding. It has been successfully used, once conjugate to cytotoxic units to kill cancer cells in vitro and in vivo[1-2], and as a tracer ex-vivo and in vivo, once coupled to fluorescent probes. NT4 selectivity is due to its nanomolar affinity for heparan sulfate proteoglycans[2], HSPGs, which are also well established modulators of cancer cell differentiation, tumor invasiveness and angiogenesis[3].
We investigated which kind of effects, mediated by its binding to HSPG, the peptide produced on endothelial and tumor cells in terms of modulation of invasiveness and angiogenesis. The growth of HUVEC endothelial cells was enhanced by FGF2, as expected, and NT4 reduced this triggered-growth to the control levels, in a dose dependent way. Migration of HUVEC on collagen or fibronectin was completely inhibited by NT4. NT4 also inhibits tube formation, particularly when the phenomenon is increased by FGF2 (Figure 1).
In different human cancer cells, NT4 also interfered with directional migration, inducing disorganization of actin filaments and stress fibers and increasing the number filopodia. Besides the peptide inhibited collagen degradation by cancer cells.
NT4 proved to reduce angiogenesis by impairing endothelial cells growth, migration and tubes formation. It also showed to decrease invasive phenotype of tumor cells by reducing migration and collagen degradation[4].

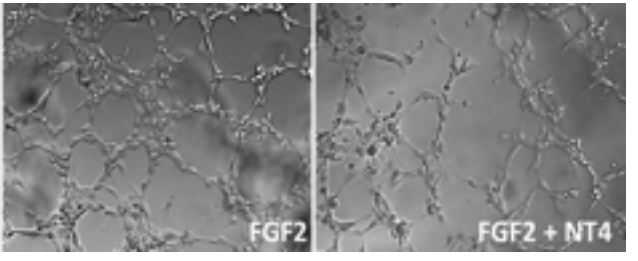

Figure 1. NT4 inhibits HUVEC tube formation.

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Cell-penetrating peptides with antimicrobial, transfection and transduction activities

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A family of histidine-rich peptides LAH4 was designed using linear cationic peptides such as magainins as a template. These designed peptides have been shown to exhibit considerable antimicrobial, nucleic acid transfection as well as cell penetrating activities. In contrast to their natural templates their membrane interactions are strongly pH dependent. The delivery of cargo by these peptides is complex, involving many steps, which we investigated on a structural and biophysical level.
Recently, vectofusin-1, a member of the family of LAH4 peptides has been shown to spontaneously self-assemble into helical coiled-coil structures, spherical aggregates, that further assemble into annular and extended nanofibrils and hydrogels as a function of phosphate and in a pH-dependent manner. This bears considerable interest for the design of biomaterials.
Furthermore, the peptide has a strong capacity to enhance the gene transfer by lenti- and adeno associated viruses into the cell interior. Thereby, the fibres formed by this relatively short have therapeutic applications ranging from monogenic and infectious diseases to cancer, by enhancing transduction levels of target cells and reducing the amount of lentivirus for greater safety and reduced costs. Vectofusin-1 promotes the entry of several retroviral pseudotypes into target cells when added to the culture medium, without cytotoxicity. These associate with viral particles allowing them to be easily pelleted. These fibrils have a unique coiled-coil α-helical structure whereas most other viral transduction enhancers form β-amyloid fibrils. Our observations define vectofusin-1 as a member of a new class of α-helical lentiviral transduction enhancers. Its coiled-coil fibril formation is reversible which bears considerable advantages in handling the peptide in conditions well-adapted to Good Manufacturing Practices and scalable gene therapy protocols.

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Learning from nature to form new organic materials for tissue regeneration

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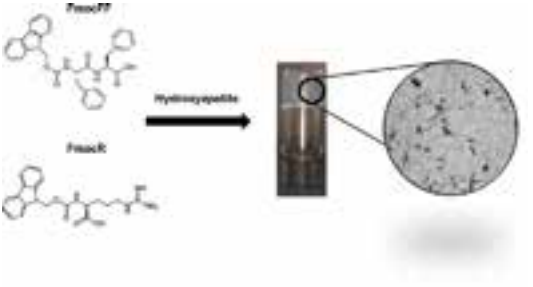
Molecular self-assembly is a key direction in current nanotechnology based material science fields. In this approach, the physical properties of the formed assemblies are directed by the inherent characteristics of the specific building blocks used. Molecular co-assembly at varied stoichiometry substantially increases the structural and functional diversity of the formed assemblies, thus allowing tuning of both their architecture as well as their physical properties.

In particular, building blocks of short peptides and amino acids can form ordered assemblies such as nanotubes, nanospheres and 3D-hydrogels. These assemblies were shown to have unique mechanical, optical, piezoelectric and semiconductive properties. Yet, the control over the physical properties of the structure has remained challenging. For example, controlling nanotube length in solution is difficult, due to the inherent sequential self-assembly mechanism. Another example is the control of 3D-hydrogel scaffold's physical properties, including mechanical strength, degradation profile and injectability, which are important for tissue engineering applications.

Here, in line with polymer chemistry paradigms, we applied a supramolecular polymer co-assembly methodology to modulate the physical properties of peptide nanotubes and hydrogel scaffolds. Utilizing this approach with peptide nanotubes, we achieved narrow nanotube length distribution by adjusting the molecular ratio between the two building blocks; the diphenylalanine assembly unit and its end-capped analogue. In addition, applying a co-assembly approach on hydrogel forming peptides resulted in a synergistic modulation of the mechanical properties, forming extraordinary rigid hydrogels. Furthermore, we designed organic-inorganic scaffold for bone tissue regeneration (Figure 1).

This work provides a conceptual framework for the utilization of co-assembly strategies to push the limits of nanostructures physical properties obtained through self-assembly.



Analogs of the peptaibol trichogin as biopesticides

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Fungi belonging to the genus Trichoderma are distributed worldwide and have been used successfully in field trials against many crop pathogens. They produce peptaibols, a peculiar family of peptides, as part of their defense system against other microorganisms. Such secondary metabolites are known for their plant-protection properties: they (i) possess antimicrobial activity, (ii) act as stimulants of plant defences and growth (iii) elicit plant production of volatiles to attract natural enemies of herbivorous insects. Moreover, peptides are ecofriendly compounds that are degraded by enzymes to nontoxic amino acids. With this presentation, we show our progress towards the exploitation of naturally occurring peptides of the peptaibols family as biopesticides. With such compounds, we can circumvent both the health hazards and the unreliable effectiveness in open field connected with the use of antagonistic microorganisms as biological control agents, while keeping the biomolecules responsible for their beneficial effects. Our peptides have been tested (alone or in combination) in vitro against the fungi Botrytis cinerea and Penicillum italicum and the bacterium Pectobacterium carotovorum, some of them considered priority pests for fruits, vegetables and medicinal plants across European countries. We found that in particular an analog of the peptaibol trichogin is able to completely inhibit the growth of B. cinerea and other pathogens for over a week at low micromolar concentrations.

Antifungal plant defensins MtDef4 and MtDef5: Mechanisms of action and engineering transgenic peanut resistant to Aspergillus flavus and aflatoxin accumulation

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Fungal pathogens impose major constraints on crop yields globally. Host defense peptides have evolved in plants to protect from the damaging effects of fungal pathogens. Defensins are sequence divergent cysteine-rich antifungal peptides of innate immunity expressed in all plants. They exhibit potent antifungal activity in vitro and therefore have potential for use in transgenic crops for enhanced resistance to fungal pathogens. MtDef4 and MtDef5 are two sequence-divergent apoplast-localized defensins expressed in Medicago truncatula. MtDef4 is a monomeric defensin of 47 amino acids, whereas MtDef5 is a novel bi-domain defensin containing two monomeric domains linked by a 7-amino acid peptide. These defensins differ from each other in sequence, net charge and hydrophobicity. MtDef4 inhibits the growth of several filamentous fungi including Fusarium graminearum at micromolar concentrations. In contrast, the bi-domain MtDef5 inhibits the growth of these fungi at submicromolar concentrations. Two ascomycete fungi N. crassa and F. graminearum respond differently to MtDef4 challenge[1]. Membrane permeabilization is required for the antifungal activity of MtDef4 against F. graminearum but not against N. crassa. MtDef4 is internalized by these fungi, but is targeted to different subcellular compartments in each fungus. In contrast, MtDef5 rapidly permeabilizes the plasma membrane of both fungi and induces accumulation of reactive oxygen species. It is also internalized by these fungi, but uses spatially distinct modes of entry into these fungi. It co-localizes with cellular membranes, travels to nucleus and becomes dispersed in other subcellular locations[2]. MtDef4 binds to plasma membrane resident bioactive phospholipid phosphatidic acid (PA), whereas MtDef5 binds to several phospholipids but with strong preference for phosphatidylinositol monophosphates, PI3P, PI4P and PI5P. MtDef5 forms oligomers in presence of PIP, PI and PA[1,2]. Thus, MtDef4 and MtDef5 exhibit different modes of antifungal action and have strong potential for use as unique antifungal agents in transgenic crops.
Aflatoxins, secondary metabolites produced by Aspergillus flavus, are extremely toxic carcinogenic compounds. Aflatoxin contamination caused by A. flavus infection of peanuts poses a major threat to public health in developing countries of sub-Saharan Africa and Asia. Transgenic peanut lines overexpressing apoplast-targeted MtDef4 have been generated. When challenged with A. flavus, peanut seeds expressing this defensin exhibit strong resistance to this pathogen and accumulate extremely low levels of aflatoxins[3]. This is the first study to demonstrate highly effective biotechnological strategy for successfully generating transgenic peanuts that are near-immune to aflatoxin contamination, offering a panacea for food safety for people in developing countries.

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Mechanisms of bacterial membrane permeabilization of snake venom-derived peptides crotalicidin (Ctn) and Ctn[15-34]

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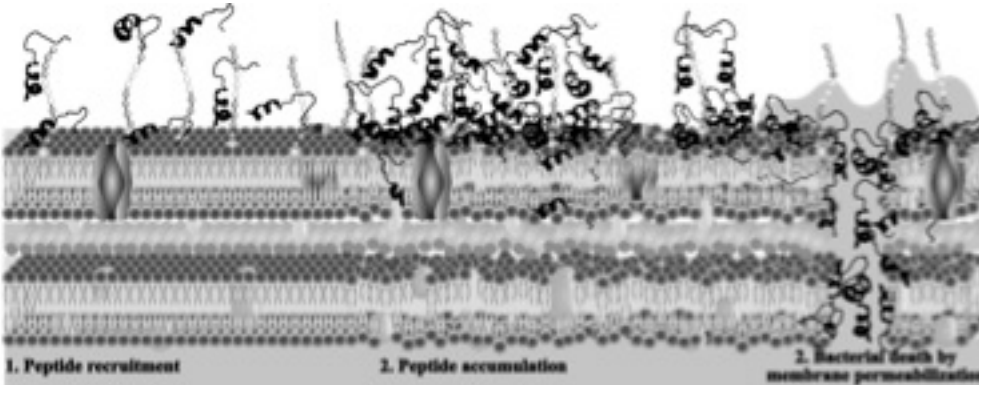
Against the alarming rise of multi-resistant bacteria, antimicrobial peptides (AMPs) have emerged as a promising therapeutic choice, due to their potency, broad spectrum and mechanisms of action that minimize the appearance of resistance. In this context, we recently identified a new cathelicidin-like AMP from the venom gland of the South American Crotalus durissus terrificus rattlesnake, named crotalicidin (Ctn), which demonstrated potent antimicrobial and antitumoral properties. Subsequent studies allowed us to identify a minimal motive, Ctn[15-34], with enhanced selectivity and serum stability while preserving activity. In the present study[1] we investigate the antibacterial mechanism of both Ctn and Ctn[15-34], focusing on their membrane-disruptive properties. Although both peptides are shown to permeabilize E. coli membranes in a three-step process, i.e., (1) recruitment, (2) accumulation, (3) membrane disruption, slight differences between them were detected by time-resolved flow cytometry, suggesting different modes of action. Additionally, the effect of the peptides on bacterial cells was directly visualized by atomic force microscopy, and their bacterial surface localization by confocal microscopy. Ctn[15-34] demonstrated preference for vesicles that mimic bacterial or tumoral cell membranes, confirming its potential as an anti-infective lead.



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Liver Cancer Cell Targeting and SERRS Imaging with Peptide-Functionalized Gold Nanostructures

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The targeted delivery of biocompatible nanoparticles to malignant tumours has become a powerful tool in cancer nanomedicine for diagnosis and therapy. This is due to the high sensitivity and specificity they exhibited when conjugated with active targeting ligands such as antibodies, peptides and small molecules. The hepatitis B virus PreS1 (21-47) sequence has been recently identified as a specific ligand of the Squamous Cell Carcinoma Antigen-1 (SCCA1), a member of the ovalbumin-family of serine protease inhibitor, overexpressed in the majority of liver cancers[1]. In this context, the aim of the present study was to synthesize PreS1-functionalized gold nanostructures for targeting and imaging of liver tumours. Nanostructures were prepared from naked gold nanoparticles, obtained by laser ablation of a gold target in water. Nanostructures were encoded with a Raman Reporter, to achieve highly intense Surface-Enhanced Raman Resonance Scattering (SERRS) signals, and coated with peptide and polymer ligands presenting a thiol group for conjugation to gold[2]. Since the organization of the targeting unit on the nanoparticles surface could affect the antigen recognition[3], the orientation of the targeting peptide on nanostructures was investigated by synthesizing some peptide analogues. Peptides were synthesized on solid phase by an optimized protocol and conjugated to nanostructures, directly or through a PEG spacer, exploiting the affinity of gold for the thiol group of the ligand. Nanostructures targeting properties were assessed by recording, cell by cell, the SERRS signals on hepatocellular carcinoma cells, overexpressing or not the antigen. Nanostructures coated with PEG alone or with an antibody specific for SCCA1 were used as negative and positive control, respectively. Once verified the peptide stability in serum and the absence of cytotoxic effects induced by the nanostructures, the targeting activity was further evaluated in vivo on healthy mice genetically modified to overexpress SCCA1 in liver. The results of in vitro and of in vivo experiment will be presented.

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Folded oligomers for Cellular Uptake and Drug Delivery

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Many potential drugs are ineffective because of their inability to cross certain biological membranes, such as the lipid bilayer or the blood-brain barrier. Once inside the cell, those compounds often have to find a local address, also known as cell compartment, but unfortunately often they will lose their way and find themselves trapped in the ''wrong neighbourhoods'' (cell organelles). Cell penetrating peptides (CPP) gave to a certain extent an answer to this problem by delivering a large array of bioactive molecules. While potent, those compounds share some of the general peptide drawbacks like low bioavailability, enzymatic degradation and in the case of polycationic compounds sometimes toxicity. In our group, we sought to develop an alternative class of compounds based on structurally organized oligomers also known as foldamers. We designed and synthesized a first generation of vectors for cellular penetration composed of dipeptide mimetics that had the particularity to be non-charged and were used to deliver dies[1], a mass spectrometry tag[2] and an anti-cancer drug[3,4] to the endo-lysosomal compartment. Recently we have developed a second generation of amphipathic vectors that adopt a ribbon structure, possess low cationic content and target the cytosol compartment. These oligomers were used to deliver a die and a pro-apoptotic peptide inside MDA-MB-231 cancer cells.[5,6] This class of compounds exhibited remarkable enzymatic degradation resistance and very high cellular uptake compared to the reference CPP Penetratin.

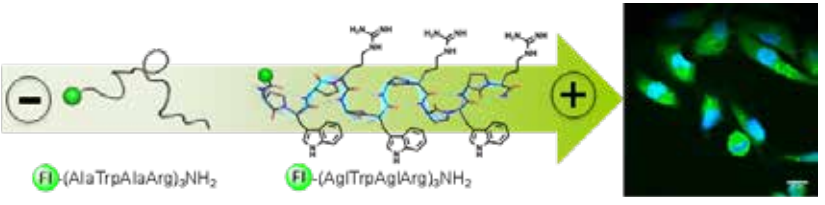

Figure 1. Figure caption example

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Metadynamics-Driven Discovery of Cyclic Pentapeptides as αvβ6 Integrin-Selective Ligands

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Over the last decades, arginine-glycine-aspartate (RGD) binding integrins[1] have been considered attractive targets for cancer diagnosis and therapy[2]. Among these, the αvβ6 integrin has recently emerged as a prognostic marker for several tumors[3], and thus the development of selective, low molecular weight ligands of this receptor is now of great demand. Here, a metadynamics[4]-driven design strategy allowed us to successfully transform a helical nonapeptide[5] into a cyclic pentapeptide endowed with remarkable αvβ6 potency and specificity. NMR and docking studies elucidated the reasons for the high affinity and selectivity of this compound, paving the way for the rational design of new αvβ6-specific small sized peptides or even organic molecules. Finally, our peptide was further developed into a PET tracer for specific αvβ6 in vivo mapping, prompting its future employment for medical applications.

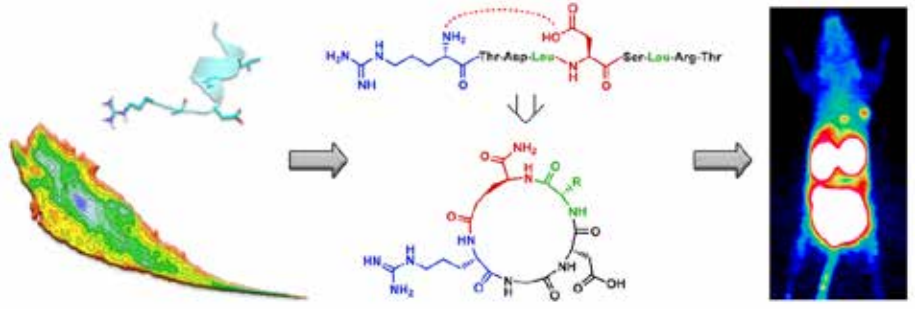

Figure 1. From metadynamics-assisted design to in vivo application of cyclic pentapeptdes as αvβ6 integrin-selective ligands.

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Modulation of Y1-receptor responses by chemically modified truncated NPY analogues

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G-protein coupled receptors can activate a multitude of signaling pathways after activation. Predominantly, they stimulate G-proteins that induce or inhibit cAMP formation, release of calcium, formation of inositolphosphate or other downstream signals. G-protein activation is silenced by arrestin-mediated internalization of the receptor followed by either degradation or recycling. However, under certain circumstances, both the G-protein as well as the arrestin pathway can be activated or silenced separately and independently, a process known as biased signaling. One component responsible for such a behavior is the receptor ligand. We have developed truncated neuropeptide Y analogues that bind specifically to the Y1 receptor subtype. These peptides have been modified N-terminally with different chemical moieties in order to elucidate their impact on modulating the receptor response. One subset of peptides was able to induce G-protein signaling comparable to native NPY, but did not or only marginally show arrestin recruitment giving rise to biased agonism; another subset induced both G-protein and arrestin signaling, thus comprising the first fully active truncated Y1 selective NPY analogues so far. These studies give insights into how receptor signaling can be modulated by ligand modification.

Peptide Nanophotonics: From Visible Bionanodots to Optical Biochips

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Encoding of human genome enabled development of new bionanomaterials self-assembled from chemically synthesized biomolecules. These bioinspired nanostructures opened the avenue for wide application fields such as tissue engineering, new drugs and nanotechnology.
In this work we report on a new concept of thermally-induced reconformation of peptide secondary structure in bioinspired nanoensembles. This biological phase transition of refolding native α-helix-like structure into antiparallel β-sheet network is followed by deep modification of basic physical properties such as elementary symmetry, piezoelectric, linear and non-linear optical and electronic. We will focus on recently observed a new effect of visible fluorescence of peptide/protein β-sheet nanostructures[1,2]. This structure-sensitive effect has the same physical origin as visible fluorescence found in amyloid fibrils associated with neurodegenerative diseases[3]. It is attributed to intermolecular hydrogen bonds of antiparallel β-sheet structure. Proposed new concept of peptide secondary structure modification has been applied for development of new visible multicolour bionanodots with quantum yield reaching ~30%.

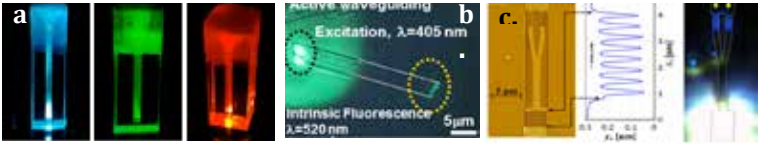

Figure 1. **a.** Multicolor visible FFF-Nanodots; **b.** Active waveguiding in peptide tape; **c.** Peptide 1x2-power Y-optical splitter with grating couplers at all three ports

Another application of this biophotonic phenomenon is peptide integrated optics[4]. It is based on a new nanotechnology which combines bottom-up controlled deposition of peptide wafers of a large area and top-down high resolution patterning for fabrication of peptide optical waveguides (POW) and peptide-based optical devices. Found reconformation of peptide secondary structure enables to switch mode of waveguiding from passive to active regime. Multifunctional optical properties such as nonlinear optical, electrooptical effects and visible fluorescence in these bioinspired peptide nanomaterials, switchable passive-to-active light waveguiding make these POW attractive for application in implantable biophotonic chips towards health monitoring, biomedical diagnosis, light-activated therapy and optical communication with embedded biosensors.
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Lupin LILPKHSDAD (P5) peptide is a novel bi-functional inhibitor of PCSK9, a new target for the hypercholesterolemia treatment

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) has been recently identified as a new target for hypercholesterolemia treatment[1]. Interestingly, we have observed that in mild hypercholesterolemic subjects, which had consumed lupin protein (30 g/day) for 4 weeks, the final circulating PCSK9 level had been reduced by 8.5% versus baseline value. In addition, we have also provided evidences related to the mechanism of action by which lupin peptides may modulate PCSK9 in HepG2 cells[2]. In this context, we have demonstrated that LILPKHSDAD (P5), a peptide deriving from peptic lupin protein hydrolysis and absorbable at intestinal level, is able to modulate the PCSK9 target with a dual mechanism of action. In particular, P5 peptide reduces PCSK9 production and secretion through a decrease of HNF1-alpha in HepG2 cells and an absorbed lupin peptide is able to inhibit the protein-protein interaction between PCSK9 and the LDL receptor with an IC50 value equal to 1.6±0.33 μM. All these evidences contribute to explain the beneficial hypocholesterolemic effects of lupin peptide, which were observed in clinical studies and opening a new area of investigation on plant proteins.

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A chimeric cell-penetrating stapled peptide inhibits oncogenic Wnt signaling

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Ligands that selectively bind to biomolecular targets are a prerequisite for most strategies aiming at the elucidation or modulation of biological processes. The discovery of these ligands can be very challenging in particular when aiming for interfaces of protein–protein interactions. Giving the huge amount of available structural data, peptide binding epitopes provide a rich source of inspiration for novel ligands.[1] Preorganization of such peptides into their bioactive conformation or the use of rigid peptidomimetic scaffolds have been applied to increase binding affinity. However, there is a lack of approaches for the stabilization of irregular and complex peptide structures.[1] In addition, the design of peptide-derived bioactive compounds is often hampered by their low cellular uptake and biostability. Addressing these challenges, my lab focuses on the design of peptide-inspired ligands for an application in proximity-induced chemical reactions[2] and as inhibitors of protein-protein interactions.[3-6]

Here, I report the design of a cell-penetrating, peptide-derived inhibitor of the interaction between β-catenin and TCF transcription factors,[6] an interaction crucially involved in Wnt signaling. The Wnt signaling pathway plays a critical role in cell proliferation and differentiation, thus it is often associated with diseases such as cancers. Unfortunately, although attractive, developing anti-cancer strategy targeting Wnt signaling has been challenging given that the most attractive targets are involved in protein–protein interactions. Our approach combines peptide stapling to optimize proteolytic stability, with lessons learned from cell-penetrating peptide (CPP) design to maximize cellular uptake resulting in NLS-StAx-h, a selective, cell permeable, stapled peptide inhibitor of oncogenic Wnt signaling.

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Understanding the Antimicrobial Properties/Activity of an 11-residue Lys Homopeptide by Alanine and Proline Scan

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Previous work demonstrated that lysine homopeptides adopt a polyproline II (PPII) structure. Those lysine homopeptides with odd number of residues, especially with 11 residues (K11), were capable of inhibiting the growth of a broader spectrum of bacteria than those with an even number[1,2]. Confocal studies also determined that K11 was able to localize exclusively in the bacterial membrane, leading to cell death[2]. In this work, the mechanism of action of this peptide was further analyzed focused on examining the structural changes in bacterial membrane induced by K11, and in K11 itself when interacting with bacterial membrane lipids. Moreover, alanine and proline scans were performed to K11 for identifying relevant positions in structure conformation and antibacterial activity. To do so, circular dichroism spectroscopy (CD) was conducted in saline phosphate buffer (PBS) and in lipidic vesicles, using large unilamellar vesicles (LUV), composed of 2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) or bacterial membrane lipid. Antimicrobial activity of K11 and their analogs was evaluated in Gram-positive and Gram-negative bacterial strains. The scanning electron microscopy (SEM) micrographs of S. aureus exposed to the Lys homopeptide at MIC concentration showed blisters and bubbles formed on the bacterial surface, suggesting that K11 exerts its action by destabilizing the bacterial membrane. CD analysis revealed a remarkably enhanced PPII structure of K11 when replacing some of its central residues by proline in PBS. However, when such peptide analogs were confronted to either DMPG-LUV or membrane lipid extract-LUV, tendency to form PPII structure was severely weakened. On the contrary, K11 peptide showed a remarkably enhanced PPII structure in the presence of DMPG-LUV. Antibacterial tests revealed that K11 was able to inhibit all tested bacteria with a MIC value of 5 μM, while proline and alanine analogs have a reduced activity on Listeria monocytogenes. Besides, the activity against V. parahaemolyticus was affected in most of the alanine-substituted analogs. However, lysine substitutions by alanine or proline at position 7 did not alter the activity against all tested bacterial strains, suggesting that this position can be screened to find a substitute amino acid yielding a peptide with increased antibacterial activity. These results also indicate that the PPII secondary structure of K11 is stabilized by the interaction of the peptide with negatively charged phospholipids in the bacterial membrane, though not being the sole determinant for its antimicrobial activity.

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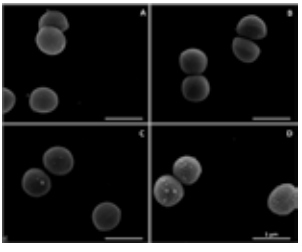

Strategies of peptide-cotton bond formation for preparing biocompatible and antimicrobial fabrics

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The need to develop new biocompatible fabrics for a variety of applications is greatly promoting academic and industrial research. In this connection, we prepared cotton-based, antibacterial textiles characterized by the covalent attachment of short peptides.
Peptide covalent grafting on the cotton surface was achieved in different ways, but always exploiting the naturally occurring hydroxyl groups of cellulose. In the most classical approach, we transform the hydroxyl group into an amine for the easy attachment of amino acids and peptides. In search for green and mild reactions, we exploited also chemoselective ligations through an oxime or a thioazolidine ring in aqueous solution. With this last approach we were able to link an octapeptide, derived from the N-terminal domain of a dermaseptin 1S mutant, known for its antimicrobial properties. In this example, we used the chemo-enzymatic, TEMPO-mediated oxidation of the hydroxyl groups into aldehydes by means of laccase in mild acidic aqueous conditions. The subsequent reaction with the β-aminothiol of a Cys-peptide gave a stable covalent bond (Figure 1).

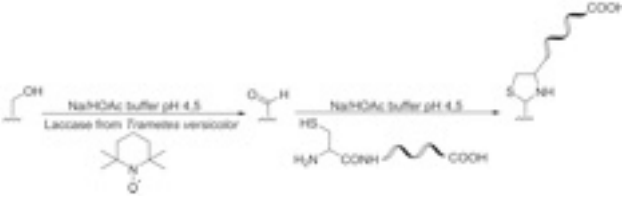

Figure 1. Laccase mediated cotton oxidation and chemoselective reaction with a Cy-peptide

We characterized our cotton-peptide samples by means of FT-IR, UV-Vis and XPS and determined their antimicrobial activity against Staphylococcus aureus and Escherichia coli. Interestingly, some of the materials gave promising results against the Gram positive strain, responsible for most hospital-acquired infections.

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Towards the native structure of the nisin: Lipid II pore

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The alarming rise of resistant bacteria urgently calls for the design of new antibiotics that are robust to resistance development. Ideal templates could be peptide-antibiotics that specifically target the membrane-anchored bacterial cell wall precursor Lipid II at irreplaceable pyrophosphate groups. Indeed, these peptides kill even the most refractory bacteria at nmol concentrations without detectable resistance. However, due to the challenge of studying small drug–receptor complexes in membranes, structural data on peptide : Lipid II interaction are scarce and only available in highly artificial media such as micelles or organic solvents. In consequence, the native peptide-binding modes such as pore formation could never be visualized, and the pharmaceutically relevant states are unknown. Altogether, this lack of knowledge critically limits the design and use of Lipid II binding drugs.
On the example of nisin, the preeminent Lipid II-binding peptide-antibiotic, we present the first high-resolution study on peptide : Lipid II complexes in liposomes and directly in native cell membranes. Using cutting-edge sensitivity-enhanced solid-state NMR, we present substantial progress towards the structure of the native nisin : Lipid II pore complex. Our study also demonstrates that previously obtained data in micelles do not correspond to a physiological state of nisin (Figure 1).[1]

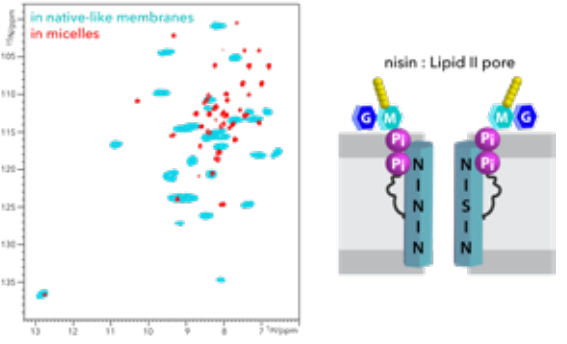

Figure 1. left: Comparison of NMR spectra of the nisin : Lipid II complex in native-like membranes (in blue, solid-state NMR) and micelles (red, solution NMR). Left: Illustration of the nisin : Lipid II pore

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From drug substance to drug product: the challenges of peptide instability

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Drug substances, compounds which exert a desired pharmacological effect, are hardly ever the sole constituents of any marketed drug product. A variety of additives (excipients) are often present to assure the drug substance can exert its activity during the intended life-time of the product.
Peptides have shown to be interesting drug substances, with potential increased potency and efficacy compared to small molecules[1]. However, their larger size and limited membrane permeability means that they are generally administered by injection. At the same time most peptides have limited physicochemical stability in solution. A common issue is the formation of aggregates/fibrils, resulting in a loss of active material, potential clogging of needles, and a visually unappealing product that the user is likely to reject.
In this lecture the challenges in understanding and preventing peptide aggregation/fibrillation will be illustrated using a few selected pharmaceutically relevant peptides[2,3]. Specifically, challenges will be shown in understanding the aggregation/fibrillation behaviour of the uncharged peptide carbetocin, which forms insoluble amorphous aggregates upon prolonged stress, but fibrils in the presence of some charged surfactants[2]. A charged model peptide behaved oppositely, changing its aggregation behaviour from fibrillation to amorphous aggregation in the presence of certain salts[3]. The limited available literature on peptide stabilisation, along with the analytical challenges in understanding peptide-peptide interactions, means the formulation scientist has limited rational formulation strategies available. To assure pharmacologically relevant peptides can be transformed into useful drug products, a more concerted effort is needed to design rational formulation strategies.

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Protein tyrosine phosphatase SHP2 as a target for PTPN11-associated malignancies and other cancers

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SHP2 is a tyrosine phosphatase required for the activation and modulation of several metabolic pathways (i.e. ERK/MAP kinase). Its structure is characterized by the presence of the catalytic domain (PTP) and of two SH2 domains (N-SH2 and C-SH2), which bind protein sequences containing a phosphotyrosine (pY).[1] Under basal conditions, the protein attains a closed, autoinhibited conformation, where the PTP domain is partially blocked by the N-SH2 domain, and thus its activity is very low. On the other hand, gain of function mutations destabilize the N-SH2/PTP interface, causing the abnormal activation of SHP2 and leading to developmental disorders, juvenile leukemia, and other malignancies.[2] Several evidences indicate that increased affinity for signaling partners is the critical event for abnormal activation of the RAS/MAPK pathway. We are currently developing oligopeptides aimed at inhibiting this interaction.
Using a combined molecular dynamics (MD) and experimental approach, we identified some short sequences of 8-9 amino acids with nM affinity for the N SH2 domain. We are currently working on further optimization of the sequence and developing an effective strategy to deliver our peptides within cells, using cell-penetrating peptides as carriers.

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Peptide Aptamers as an environmental friendly approach in the treatment of grapevine *Plasmopora Vinicola* downy mildew

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The oomycete phylum, especially members of the genus *Phytophthora* and *Plasmopora* , comprise some of the most problematic crop pathogens that represent a threat for global food security.[1] In particular, *Plasmopora Vinicola* is the causal agent of grapevine downy mildew.[2] Oomycete infections are currently controlled by frequent applications of copper-based compounds on crops, but the repeated and massive use of these compounds leads to environmental pollution, residual toxicity and adverse effects on human health.[3] The discovery of innocuous and reliable alternatives to traditional inorganic fungicides remains challenging.

Here we present an environmental friendly approach for crop protection based on the development of peptide aptamers. In particular, small peptides that inhibit cellulose synthase, a vital enzyme involved in oomycete cell wall formation and cell stability, were identified by a yeast two-hybrid assay. A library of peptide aptamers was synthesized and tested *in vitro* on leaf disk bioassays (Figure 1). A 8-mer peptide was found active at micromolar concentration, causing histological and ultrastructural alterations on pathogen cell wall as evicted by Scanning Electron Microscopy analysis.

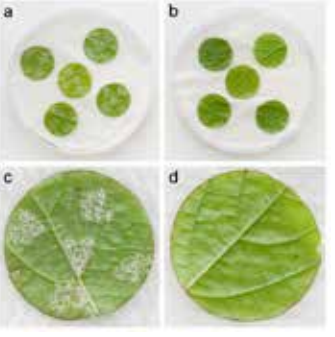

Figure 1 Untreated (a and c) and treated (b and d) grapevine leaf disks

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Selective recognition and self-assembly by Cu(II) binding peptoids

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Peptoids – N-substituted glycine oligomers – are peptidomimetics capable of forming stable helical structures that resemble the polyproline type helices. Peptoids can be easily synthesized on solid support, using a method that employs primary amines, thus avoiding protection and de-protection steps. Moreover, peptoids are biocompatible and both their sequences and secondary structures exhibit high stability. Capitalizing on the great versatility of the peptoid backbone, we generate peptoids having metal-binding ligands in a specific positions along the sequence, and investigate their interactions with biologically relevant metal ions towards biomimetic function.[1-5] Here we will describe a rationally designed helical peptoid bearing two distinct metal binding ligands at positions i and *i*+3, which enables selective recognition of one or two metal ions depending on its environment.[6] Using various spectroscopic techniques, we demonstrate (1) the selective intramolecular binding of Cu^{2+} and its extraction from a mixture of neighboring metal ions in high concentrations, and (2) the selective intermolecular binding of two ions, namely Cu^{2+} and either Zn^{2+}, Co^{2+} or Fe^{3+}, generating hetero-bimetallic peptoid duplexes. We show that these unique recognition processes are controlled by both the sequence and the structure of the peptoid. Further on, we will present the self-assembly of three peptoid trimers bearing a bipyridine ligand and a polar group (N-ethyl-R, R = OH or OCH_3 or NH_2) by Cu^{2+} coordination. X-ray diffraction analysis revealed unique, highly symmetric, binuclear cyclic or aqua-bridged binuclear cyclic structures, formed by the self-assembly of two peptoid molecules with two Cu^{2+} ions. Studies of the crystals in acetonitrile solution showed that the first two macrocycles disassemble to their corresponding monometallic complexes, while the third one, having the largest number of intermolecular hydrogen bonds, retain its unique structure in solution.

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S-Glycosylated α-Peptoids: a versatile synthetic method towards potentially bioactive compounds

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Peptoids are peptidomimetics whose side chain is shifted on the nitrogen atom instead of on the α-carbon as in peptides.[1] This modification confers on them stability towards proteolysis and their easy synthetic access makes them very intriguing compounds. At the same time, glycosylation is widely investigated for tuning the biological activity of non-naturally glycosylated peptides and proteins, since carbohydrates play an essential role in the interaction with the environment and mediate many cell-cell recognition processes, such as signaling, cell growth and differentiation, immune response, and inflammation.[2] In this regard, the synthetic entry to glycosylated α-peptoids is an interesting topic in organic chemistry and opens the way to the construction of glycopeptidomimetic libraries with potentially relevant biological applications. The submonomeric peptoid solid-phase synthesis is a very straightforward strategy and it has been already developed to obtain S-glycosylated peptoids[3] containing thio-linked sugars known to be more resistant toward the hydrolytic action of glycosidases with respect to their *O*-analogues.

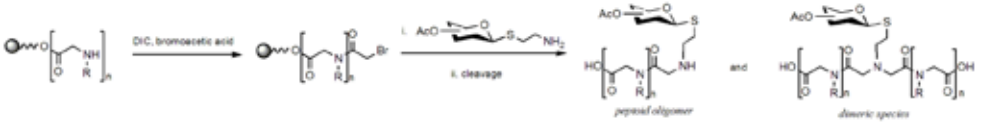

Figure 1. Submonomeric Solid Phase Synthesis of S-Glycopeptoids

However, the updating of this previous work unveiled a few synthetic problems connected with this method, in particular the formation of a "sugar-bridge" dimeric byproduct (Fig. 1). This observation tempted us to carry out a deep study on the reaction conditions either to reduce the side reaction increasing the final yields of the desired oligomers, or to force the formation of these interesting byproducts for the development of new S-glycopeptoids.

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Amyloid formation by short peptides: from mechanistic insights to function

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Two decades ago, the research concerning the formation of amyloid fibrils was focusing mainly on the understanding of their pathological role in diverse devastating diseases and on how to arrest them. However, in recent years, the amyloid world has expanded to include many more aspects of amyloid fibrils, such as natural-functional amyloids and synthetic amyloid-like nanostructures.
The common feature dominating all of these phenomena is a spontaneous and accurate process of self-assembly of proteinous building blocks into well-ordered structures at the nano-scale. Scanning of amyloidal sequences by peptide array methodology resulted in mechanistic insights regarding important molecular determinants within amyloidogenic polypeptides which are responsible for recognition and self-assembly. Then, the identified recognition motives were the basis for rational design of peptide inhibitors as well as short peptide mimetics of amyloid forming proteins.
Using a minimalistic reductionist approach, very short peptides down to dipeptides were found to be able to form amyloid-like structures and also other architectures such as nanotubes and nanospheres. This new class of short peptides was found to possess distinct mechanical, optical, piezoelectric and electronic properties that can be exploited for nanotechnological applications. Today, the research that started in quest of the mechanism of amyloid formation, evolved into the material sciences field, exploring the use of self-assembled bio-inspired building blocks for the fabrication of quantum dots, semiconductive nanowires and more.

Synthetic Mimics of Antimicrobial Peptides to Tackle Antimicrobial Resistance

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The emergence of antimicrobial resistance (AMR) compounded with biofilm formation has put many lives at risk. To address this situation, our group has engineered amino acids based small-molecular and macromolecular synthetic mimics of antimicrobial peptides (AMPs) which displayed potent activity against multi-drug-resistant (MDR) bacteria, without triggering the development of resistance against them.[1-3] Through our design we have addressed some of the limitations of AMPs such as stability, toxicity and ease of synthesis. Importantly, they revealed anti-biofilm property and showed potent *in-vivo* activity against various infection models (such as; skin-infection, thigh-infection, burn and surgical-site wound infection) without showing much *in-vivo* toxicity.[4-6] Some of the small-molecular AMP mimics also showed activity against fungi, malarial parasites, and Ebola virus. Moreover, they are capable to re-sensitize the obsolete antibiotics (such as tetracycline, rifampicin and erythromycin) to multi-drug resistant Gram-negative NDM-1 class of bacteria both *in-vitro* and *in-vivo*.[6,7] These membrane-targeting AMP mimics engineered by our group have immense potential to be developed as possible therapeutics in the era of antimicrobial resistance.

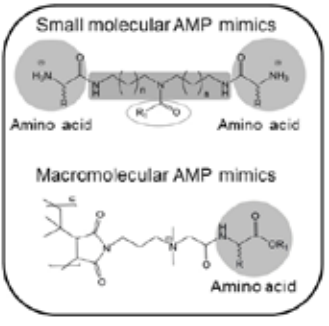

Figure1. Small-molecular and macromolecular synthetic mimics of AMPs

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Oxytocin Mimetics

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Poor oral availability and susceptibility to reduction and protease degradation is a major hurdle in peptide drug development for peripheral or central nervous system candidates. In contrast, druggable receptors in the gut present an attractive niche for peptide therapeutics. Here we demonstrate, in a mouse model of chronic abdominal pain, that oxytocin receptors are significantly upregulated in nociceptors innervating the colon. Correspondingly, we have developed novel chemoselective strategies to engineer non-reducible stable oxytocin analogues that are equipotent to native oxytocin. Moreover, we describe single atom modifications to oxytocin disulfide bridges produce ligands with improved selectivity across species. Nuclear magnetic resonance structural analysis of native oxytocin and the seleno-oxytocin derivatives reveals that oxytocin has a pre-organized turn structure in solution, in marked contrast to earlier X-ray crystallography studies. These seleno-oxytocin analogues potently inhibit colonic nociceptors both in vitro and in vivo in mice with chronic visceral hypersensitivity. Our findings have important implications for the clinical use of oxytocin analogues and disulfide-rich peptides in general.

Targeting mRNA translation process to develop anticancer agents: a successful drug design study

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The molecular interaction between the eukaryotic translation initiation factor 4E (eIF4E) and the eukaryotic translation initiation factor 4G (eIF4G) is essential for the translational machinery assembly and consequently for the initiation step of mRNAs translation[1]. Higher concentration of eIF4E causes increased translation of mRNAs that has been found to be involved in different types of cancer[1]. Inhibiting the eIF4E-eIF4G interaction represents a possible solution to reduce cell proliferation with beneficial effect on cancer progression.[2] A well-characterized group of proteins called 4E-binding proteins (4E-BPs) is known to inhibit and regulate the interaction between eIF4E and eIF4G.[2] The cytoplasmic Fmrp interacting protein 1 (CYFIP1) is a member of the 4E-BP family and interacts with eIF4E through a α-short helix.[3,4,5] The eIF4E binding region in CYFIP1 shows low conservation among the other 4E-BPs, therefore assuming a specific binding mode.[6] Here we illustrate a rational approach to design a stapled CYFIP1 derived peptide (SCD) able to interact selectively with eIF4E showing promising anticancer activity. First, we have performed classical molecular dynamics simulations (MD) to identify the best position on the CYFIP1 scaffold to introduce the staple modification. Afterwards, we have used a novel free energy method developed in our group called funnel metadynamics (FM)[7] to characterize the binding free-energy surface (FES) of the newly synthesized peptide SCD to eIF4E. Our calculations disclose the SCD binding mode to eIF4E with an estimate of the binding free energy of ~ -7 kcal/mol. Furthermore, our results show that the triazole-bridged macrocyclic scaffold (stapled) stabilizes the structure of the peptide, however not interfering with the binding mode. Prompted by the promising theoretical data, we have synthesized the SCD peptide and tested it on MDA-MB-231 human breast cancer cell line. The experimental results show that the SCD peptide is not cytotoxic and shows encouraging anticancer activity targeting selectively the translational machinery. Further experiments are foreseen to characterize in more detail the pharmacological activity of SCD. Our study shows how to combine with success standard and cutting-edge computational techniques in drug design, paving the way to further investigations on peptide/protein interaction in systems of pharmacological interest.

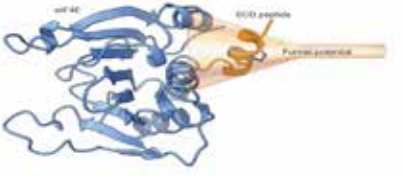

Figure 1. Representation of the funnel metadynamics (FM) setup. The three-dimensional (3D) structure of the eIF4E and SCD peptide are represented in blue and orange cartoons respectively. The funnel potential is also shown.

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Toward homogeneous glycoproteins via auxiliary-assisted sequential glycosylation and ligation of peptides

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Having access to homogeneous proteins carrying complex posttranslational modifications (PTMs) is essential for studying the role of the PTMs in protein function and misfunction.
We established a strategy for the synthesis of peptides carrying multiple and complex PTMs, focusing at first on the preparation of homogenously glycosylated peptides. The tumor marker MUC1, a protein abundantly O-glycosylated in his extracellular domain, was chosen as synthesis target. Chemically synthesized mucin peptides are conjugated to a photocleavable ligation auxiliary[1], obtained via multistep synthesis, that supports native chemical ligation (NCL) and carries a PEG polymer. This facilitates effective enzymatic glycosylation and recovery of the resulting glycopeptides without the need for chromatographic steps[2]. It also gives access to glycosylated peptide α-thioesters that are otherwise inaccessible[3]. The conjugates are linked to each other via auxiliary-mediated NCL and the ligation products are recovered as unprotected glycopeptides after UV irradiation.

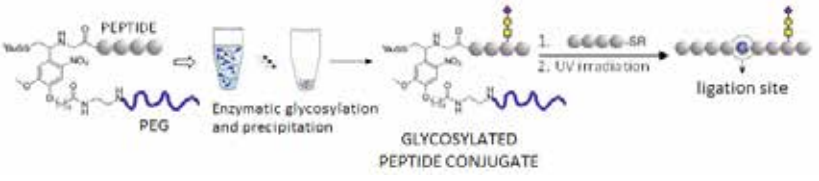

Figure 1 The PEGylated auxiliary supports sequential glycosylation and easy recovery of glycopeptides and mediates NCL. The native glycopeptide is obtained after photocleavage.

Currently a library of mucin polypeptides with multiple glycosylations is assembled that will be used in proteomic studies to provide new insights into the role of glycosylation in mucin functions and cancer progression. At the same time we are expanding the approach to sequential ligation[4] in combination with sequential glycosylation, to gain access to larger, more complex homogenous glycoproteins. These developments also require extension of this method towards ligation at sites different from glycine and to other PTMs, in order to broaden it toward the synthesis of any kind of homogeneously posttranslationally modified protein.

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Immobilized proteases for industrial bioactive peptide production

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Bioactive Peptides are known to have a wide variety of biofunctional properties. They have anti-hypertensive, immunomodulatory, antimicrobial or antioxidative activities. Bioactive peptides can be produced and activated by proteolytic enzymes. The majority of all bioactive peptides have not been fully identified und characterized yet. Most can be obtained from natural protein sources like milk or plant proteins.
The majority of industrial productions use cost-intensive batch processes with varying product and peptide compositions. By immobilizing proteases (like subtilisin) on functionalized ceramic capillaries, the resulting peptide composition can be regulated by flow-rate, pH and the immobilized protease. Furthermore, energy consuming terminations such as heat treatment or pH-shift are not required. In addition to the production of defined hydrolysates, this approach is particularly suitable for peptide mapping and proteolysome analysis.

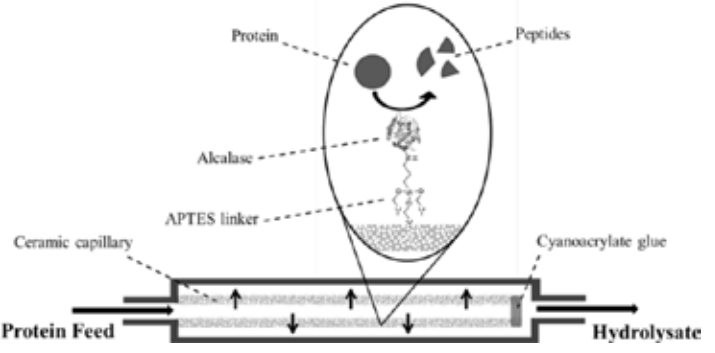

Figure 1. Capillary module for continuous flow dependent hydrolysis

In this scalable system a protein feed is digested depending on the residence time in the capillary, hence depending on the flow rate and enabling the continuous generation of reproducible peptide compositions, so-called peptide fingerprints, for the production of selected bioactive peptides. The peptides are further characterized and investigated regarding their bioactive properties.

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Luminescent hydrogels from a tripeptide and carbon nanodots

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Hydrogels based on self-organization of short peptides into fibrils are attractive for the development of biocompatible and biodegradable materials, useful to the creation of novel therapeutic paradigms in nanomedicine.[1] However, high heterogeneity of nanostructure dimensions is a known issue that is due to the hierarchical mechanism of self-assembly, which proceeds uncontrolled from fibrils to their bundling into fibers of various thicknesses. In this work, we addressed this issue by limiting bundle-formation for a self-assembling D, L-tripeptide with the addition of carbon nanodots as an element interfering with self-assembly. We evaluated the effects of the nanodots on the supramolecular organization of the tripeptide into supramolecular hydrogels at physiological conditions.[2]

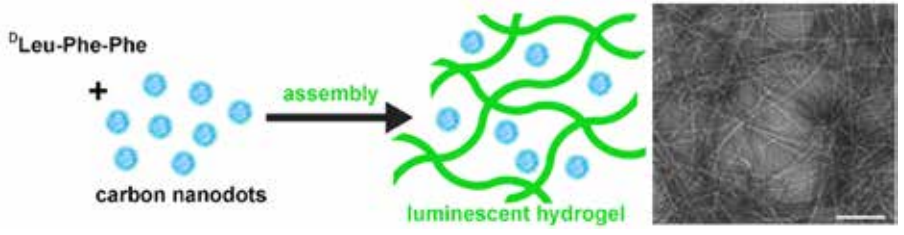

Figure 1. Self-assembly and TEM micrograph of hydrogel containing peptide and carbon nanodots. Scale bar = 200 nm.

This combination of these two elements allowed to obtain nanostructured hydrogels, whereby the nanodots enriched the systems with their fascinating fluorescence properties.[3] Moreover, their addition allowed fine-tuning of the viscoelastic properties of the final materials, which were composed of thinner fibrils with narrow diameter distribution, relative to the systems bearing the peptide alone. These new systems feature a number of potential applications, including sensing and use as biomaterials.

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Development of the MC1R Selective Ligands for the Melanoma Prevention

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Seeking effective treatments of melanoma is very hot in current era of science and technology. However, prevention of melanoma has been neglected. Here, we propose developing selective hMC1R selective ligands for the melanoma prevention agents. We have been very successful in developing selective hMC1R ligands. Lately we first successfully developed only natural amino acid made peptide, [Leu^3, Leu^7, Phe^8]-γ-MSH-NH_2 which is a potent selective hMC1R agonist; and many other hMC1R selective agonists which are more druggable and bioavailable. *In vivo* studies demonstrated these peptides can cause immediately pigmentation. The natural skin color can be resumed less than 20 hours. The high selectivity of the [Leu^3, Leu^7, Phe^8]-γ-MSH-NH_2 for the hMC1R and shorter half-life provides a safer and reduced side-effect agent for the prevention of melanoma skin cancer. This research will be more applicable and benefit for most of people for skin cancer prevention.

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Effects of chirality on tripeptide self-assembly

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Self-assembly of short peptides into hydrogels can be a useful approach to mimic large protein biomolecules, such as those composing the extracellular matrix or enzymes, with minimalist building blocks.[1,2] It is sufficient to introduce a D-amino acid in a L-tripeptide to achieve self-organised nanostructured hydrogels from non-gelling L-analogues.[3] The reasons for such divergent behaviour have been obscure up to now. In this work, the different behaviour of homochiral and heterochiral tripeptides were analysed in detail as a continuum from single molecules up to the macroscopic materials, revealing the significance of a defined spectroscopic signature in peptide conformation, and its role in the effective packing of tripeptides into supramolecular structures. The proposed rational design of self-assembling tripeptides containing both D- and L-amino acids is supported by both *in silico* and experimental data, including single-crystal XRD, that allow to link macroscopic properties of the final soft materials to fine structural details of their building blocks. Cell viability was assessed on fibroblast cells revealing potential applications as biomaterials. We believe this work significantly expands our understanding of chirality effects in the self-assembly behaviour of simple molecules, and as a such is highly valuable for the rational design of supramolecular systems.

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Expanding the potential and multivalency of the B2T synthetic peptide vaccine against foot-and-mouth disease virus

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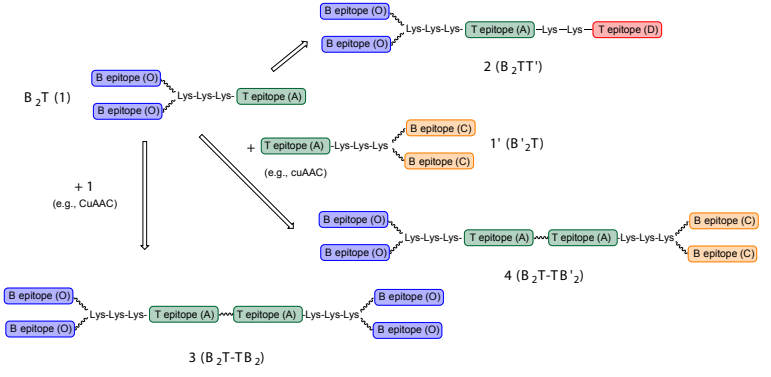
Foot-and-mouth disease virus (FMDV) causes a highly transmissible infection of pigs and other animals, recognized as the animal disease with the direst economic effects worldwide. High incidence rates in large areas of Eurasia, Africa or Latin America stress the need for effective ways to control FMD, among which safe, marker vaccines (i.e., allowing to tell infected from vaccinated animals) are viewed as the most sensible option, with peptide-based vaccines receiving growing attention in this regard. In 2016 we reported the full protection of swine against FMD by vaccination with B_2T (**1**), a platform displaying two and one copies, respectively, of FMDV B- and T-cell peptide epitopes in a branched fashion.[1] To the known advantages of peptide vaccines (safety, marker nature, fine-tuning to various strains, easy shipping and storing), B_2T adds highly efficient synthesis by thiol-ene conjugation of prepurified modules, confering it fast adaptability in emergency responses to new outbreaks. We are now exploring the potential of the B_2T design to provide enhanced performance in terms of, e.g., lower dosage, longer-lasting protection and/or multivalency. To this end, strategies such as tandem display of >1 T-cell epitope (**2**), or back-to-back fusion of homologous (**3**) or heterologous (**4**) B_2T units by chemoselective reactions (e.g., CuAAC) are being studied. Results from trial vaccinations of Swiss mice and swine will be discussed.



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Nanoparticulated multi-epitope conjugates as vaccine candidates against tuberculosis

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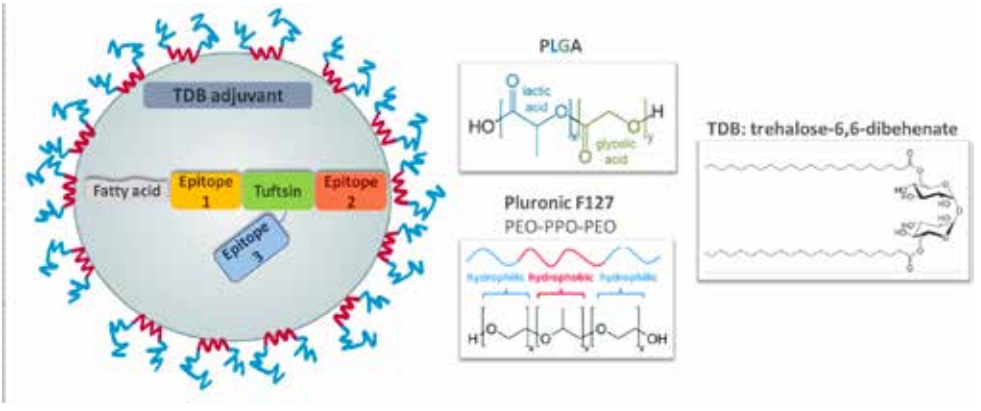
The most challenging factor in preventing tuberculosis (TB) is the ability to provide immunity against the multiple stages of the pathogen and provide cross-protection within the subtypes. Immunization with a multivalent subunit vaccine, which combines multiple antigens derived from different stages of the pathogen's life cycle, hold promise for overcoming the major obstacles.



In this study, promiscuous epitopes of immunodominant proteins were conjugated to a lipo-peptide carrier by using chemoselective coupling techniques. As delivery platform, poly (D,L-lactic-co-glycolic acid) (PLGA) nanoencapsulation was applied and in order to enhance the immunstimulatory effect, a bacterial cell wall component (TDB) was incorporated. Nanoparticulated vaccine conjugates and relevant controls were administered (sc.) to BALB/c mice and the induced immune response were evaluated. Synthesis, analytical and structural characterization together with the comparison of the different conjugates and the effect of encapsulation and adjuvant incorporation will be discussed in the presentation.

Acknowledgement

This study was supported by Hungarian Scientific Research Fund (OTKA 115431, 124077), MedInProt Protein Science Research Synergy Program and Bolyai János Research Fellowship of the Hungarian Academy of Sciences.

Unique true predicted neoantigens (TPNAs) correlates with anti-tumor immune control in HCC patients

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A novel prediction algorithm is needed for the identification of effective tumor associated mutated neoantigens. Only those with no homology to self wild type antigens are true predicted neoantigens (TPNAs) and can elicit an antitumor T cell response, not attenuated by central tolerance. To this aim, the mutational landscape was evaluated in HCV-associated hepatocellular carcinoma.
Liver tumor biopsies and adjacent non-tumor liver tissues were obtained from 9 HCV-chronically infected subjects and subjected to RNA-Seq analysis. Mutant peptides were derived from single nucleotide variations and TPNAs were predicted by comparison with corresponding wild-type sequences, non-related self and pathogen-related antigens. Immunological confirmation was obtained in preclinical as well as clinical setting.
The development of such a novel algorithm resulted in a handful of TPNAs despite the large number of predicted neoantigens. Furthermore, TPNAs may share homology to pathogen's antigens and be targeted by a pre-existing T cell immunity. Cross-reactivity between such antigens was confirmed in an experimental pre-clinical setting. Finally, TPNAs homologous to pathogen's antigens were found in the only HCC long-term survival patient, suggesting a correlation between the pre-existing T cell immunity specific for these TPNAs and the favourable clinical outcome.
The new algorithm allowed the identification of the very few TPNAs in cancer cells, and only those targeted by a pre-existing immunity strongly correlated with long-term survival. Only these represent the optimal candidates for immunotherapy strategies. Figure 1. Figure caption example

Quick and Sound Solid-Phase Peptide Synthesis

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The growing interest in protein-protein interactions (PPIs) role in various biological processes has sustained the reassessment of peptides not solely as tools to dissect protein functions but also as potential drug candidates.[1] The latter has boosted the development of synthetic strategies, that can smoothly provide large peptide libraries. In this respect, the development of both microwave (MW)[2] and flow-assisted (FC)[3] synthetic approaches has substantially extended the opportunities for the synthesis of either a growing number of peptide modifications or peptides endowed with "difficult sequences". Nevertheless, both these techniques require the use of appropriate *apparati*, that can be expensive and unaffordable for many laboratories. In the light of these considerations, herein we describe the development of a facile, low-cost, sustainable and efficient methodology for peptide synthesis by applying the ultrasound wave irradiation to the classical SPPS. Our approach is inspired by the well-documented use of ultrasound irradiations (US) in chemistry, also known as "sonochemistry", as effective method to activate reagents in both homogenous and heterogeneous systems.[4] Considering the few examples of US chemical transformations in SPPS reported so far, it has not yet been proven whether or not ultrasounds could be extensively applied to assist Fmoc/*t*Bu-SPPS in lowering reaction times and/or reagent excess without affecting yields and degree of purity of the final compounds. Herein, by systematically investigating the effects of ultrasound waves on each single step of canonical peptide synthetic protocols, we were able to dramatically reduce both reaction time and reagent excess required to assemble a peptide. Noteworthy, no significant effect of ultrasound irradiation was noticed on the main side reactions typical of the solid phase peptide assembly, including aspartimide formation and Cys and His racemization. Finally, the efficiency of our strategy was challenged by synthesizing several known difficult sequences, including $ACP_{(65-74)}$, Aib-Enkephaline and JR-10, providing results comparable to those reported in literature for MW- and FC-assisted protocols.

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New insights in the structural characterization of turns in peptides: determination of NMR discriminatory parameters

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During the last decades, there has been an increased interest in the use of peptides as therapeutics. The knowledge of the 3D structure of these molecules is crucial to gain a full understanding of their structure-activity relationships and to identify the motifs involved in partner recognition and binding[1]. Peptide flexibility makes solution NMR coupled to molecular modelling the privileged method for structural studies.
It has been shown that helices and turns constitute the preferential recognition patterns of bioactive peptides by their targets. Despite their prevalence, turns have always been more challenging to characterize by NMR than α-helices, because of their non-periodic nature and their heterogenic structures. Indeed, several classes of turns, characterized by a different number of residues and exhibiting different geometries, exist in polypeptide structure. In the literature, the establishment of the NMR characteristic data (H-H distances and scalar couplings) of these different structural motifs is based on a rather ancient analysis of poly-alanine chains[2], which does not take into account all the types of turns or the influence of the nature of the side-chain on these structures.
The aim of this work is to provide additional and new structural information, via molecular modelling calculations, to better characterize each type of turns. We focused on β-turns (4 residues) and γ-turns (3 residues), which are the most encountered turns in peptide structures.
Specific H-H distances were measured in a set of turns selected from PDB X-ray protein structures. New characteristic signatures that permit to identify, by NMR, all types of β-turns and of γ-turns were found. Particular attention has been paid to the proline structural role in these secondary structures.
To complete the analysis, the $^{3}J_{HN\text{-}H\alpha}$ scalar coupling constant of the central residue of γ-turns or the two central residues of β-turns was calculated[3]. For most of the β-turns, the set of coupling constants allowed to discriminate one turn from another.
Taken together, signatures allowing to discriminate all types of β-turns and γ-turns by different distances and scalar coupling constants were established. These new data will enable to refine the NMR structural description of turns in peptides and might be applied to peptidomimetic studies.

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Is there a role for quorum sensing peptides in cancer?

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Several diseases, including colorectal cancer, inflammatory bowel disease, cystic fibrosis, multiple sclerosis and psychiatric/mental disorders, are associated with alterations in the human microbiota composition. However, to date, the mechanisms by which the microbiota influence these health conditions are not yet fully understood; bacterial metabolites such as short chain fatty acids (SCFAs), toxins or DNA-damaging reactive oxygen species (ROS) are currently being investigated as potential mediators for this association.
In vitro studies from our group have shown that quorum sensing peptides, *i.e.* bacterial metabolites which are constitutively produced and used by bacteria as inter-bacterial communication molecules, are also able to functionally modify in a selective way cancer cells: different peptides were found to promote tumor metastasis by initiating tumor cell invasion and angiogenesis[1,2]. These results were now confirmed *in vivo* as well: the investigated quorum sensing peptides, *i.e.* EntF-metabolite (*Enterococcus faecium*, SNLVECVFSLFKKCN) and EDF-derived (*Escherichia coli*, NWN) peptide, were found to promote tumor metastasis in Swiss nude mice, which were orthotopically injected with luciferase-transfected HCT8/E11 cells, by bioluminescent imaging, as well as macroscopic and microscopic investigation of the tissues. These results thus indicate a causal relationship between microbially produced quorum sensing peptides and cancer metastasis.

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CLIPS (Chemical LInkage of Peptides onto Scaffolds) technology applied to opioid peptides research

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CLIPS (Chemical LInkage of Peptides onto Scaffolds) technology[1] is a novel and still unexplored versatile method for constraining and functionalizing the 3D-conformation of peptides. This novel cyclization type involves the cyclization of linear peptides via reaction with a small scaffold like dibromoxylenes. The bromine anchor points react exclusively with the thiols of the Cys or Pen in the peptide and attach to the peptide via multiple thio-ether bonds.

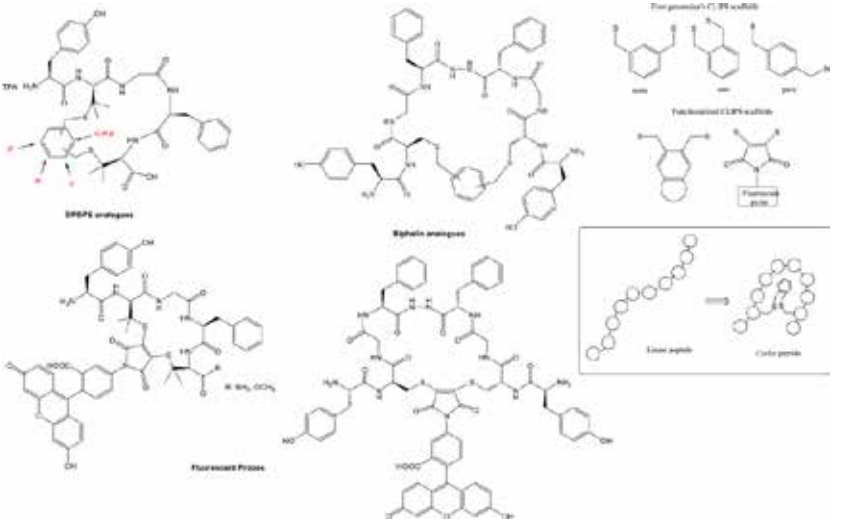

Figure 1. CLIPS Scaffolds

Six cyclic analogues of DPDPE, biphalin and three fluorescent probes were prepared and characterized as μ/δ-opioid receptors agonists.[2,3] The novel biphalin and DPDPE derivatives were tested by in vitro and by in vivo animal model of pain.

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Protective effects of antioxidant milk-derived bioactive peptides on Caco-2 cell

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Milk-derived bioactive peptides have been identified as potential ingredients of functional considering their various functions that result in antihypertensive, antimicrobic and antioxidant effects. In particular, this study is focused on their antioxidant activity in a cellular model. Four synthetic peptides, **N-6-R, A-7-R, K-8-K, A-11-M**, whose sequences correspond to human and bovine caseins, were used to evaluate the potential antioxidant role of the bioactive peptides in signalling pathways. The obtained results showed that TrxR and GR activities were preserved against oxidative stress by the four bioactive peptides, in particular by **K-8-K, A-7-R** and **A-11-M**. Similarly to what observed in enzymatic activity analysis, **K-8-K** and **A-7-R** protected cells against ROS production induced by TbOOH. Finally, we observed that bioactive peptides, in particular **K-8-K**, decreased lipid peroxidation in Caco-2 cells.

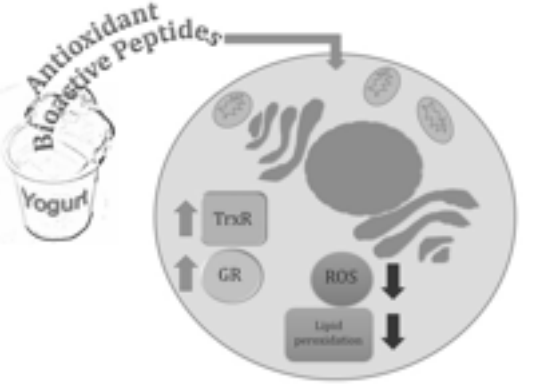

Figure 1. Antioxidant effects of milk-derived bioactive peptides in Caco-2 cells

On the whole, we have shown that the four bioactive peptides (**N-6-R, A-7-R, K-8-K, A-11-M**) exert a protective effect in Caco-2 cells against oxidative stress. The obtained results highlight a possible role of the four peptides on modulation of gene expression. They could be involved in the regulation of some redox pathways and we plan to further analyse the relationship between the bioactive peptides and transcription factors.

POSTER PRESENTATIONS

Mini-factors revealing VEGF receptors in imaging applications

L. Calvanese[1], A. Caporale[1], G. Focà[2], E. Iaccarino[3], A. Sandomenico[1,2], N. Doti[1,2,] I. Apicella[4], G.M. Incisivo[2], S. De Falco[4], L. Falcigno[1,2,5], G. D'Auria[1,2,5] and M. Ruvo[1,2]

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Vascular Endothelial Growth Factors (VEGF) and their corresponding receptors, mainly VEGFR1 and VEGFR2, are part of the molecular machinery that supports angiogenesis, i.e. the formation of new capillary from pre-existing blood vessels. Angiogenesis, normally involved in physiological processes, also plays a relevant role in numerous human pathologies. Even though several angiogenesis-targeting therapies are on the market[1], finding strategies to hit pathological processes without interfering with physiological ones, still remains a challenging goal in medicine. Several cancer cells overexpress VEGFR1 and VEGFR2 and their soluble ligands[2,3]. That correlates with enhanced ability of endothelial cells to form new blood vessels and the capability to support blood supply to growing tumors. Developing tools to detect VEGF receptors, *in vivo* as well as *in vitro*, is useful to discriminate between healthy/unhealthy cells in cancer diagnosis and/or predicting tumor-sensitivity to anticancer therapies[5]. Under this heading, we have designed small peptides able to selectively bind VEGF receptors. Peptide systems have been designed to be small, cyclic and to host the retro-inverted version of triplets of residues known to be essential for VEGF receptors recognition. We named them 'mini-factors'[6]. Mini-factors do bind with different specificity and affinity VEGF receptors without affecting receptor activity as usually observed with other binders having neutralizing capability. Mini-factors with best binding properties have large difference of affinity toward VEGFR1 compared to that towards VEGFR2. They have been successful tested as molecular probes for sensing receptors on cell surface[6], supporting their use in cancer tissues detection by imaging techniques.

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Triple-negative breast cancer: new potential therapeutics derived from SOCS3 protein

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Suppressor of cytokine signaling (SOCS) proteins are a family of negative feedback regulators of cytokine signaling mediated by the JAK-STAT pathway[1]. Only two members of this family, SOCS1 and SOCS3, contain a kinase inhibitory region (KIR) crucial for the inactivation of JAKs, leading to suppression of inflammatory cytokines[2]. It has been suggested that SOCS proteins can play pivotal roles in development and progression of cancers. Further, in triple negative breast cancer (TNBC) subtype the proteolytic degradation of SOCS3 protein causes the activation of inflammatory cytokines and, as a whole, recombinant SOCS3 demonstrated able to prevent TNBC tumour growth and metastasis by suppressing inflammatory cytokines[3]. In this study we designed several SOCS3'mimetics derived from the N-terminal region of SOCS3 encompasses KIR and ESS domain that interface the complex with JAK2. These peptides were characterized by Circular Dichroism and Surface Plasmon Resonance spectroscopies. Moreover, the activity of one sequence, named KIRESS, which contained crucial residues for the complex SOCS3/JAK2 was investigated in vivo in mouse xenografts of MDA-MB-231-luci tumours as model of human TNBC subtype. KIRESS peptide demonstrated capable to eliminate pulmonary metastasis and showed a significant reduction of primary tumour growth.
KIRESS peptide can be considered as a starting point to create, through structural and chemical modifications, compounds with high affinity and stability as potential therapeutics in TNBC[4].

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Cell-penetrating peptides restricting oligomerization of G protein-coupled receptors: the CB1R-5HT2AR dimer

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G protein-coupled receptors (GPCRs) have been classically described as monomeric transmembrane (TM) receptors that form a ternary complex between a ligand, the GPCR and its associated G protein. However, it is now well accepted that many GPCRs form, in addition to functional monomeric structures, higher-order oligomeric complexes constituted by a number of equal (homo) or different (hetero) monomers (Figure 1A). GPCR heteromers are defined as novel signaling units with functional properties different from homomers and represent a completely new field of study. For instance, when coexpressed in specific brain regions, CB_1R and $5HT_{2A}R$ form heteromeric structures (Figure 1B) that have been related to (tetrahydrocannabinol-linked) effects such as memory impairment, anxiety and dependence.[1] In the present study, in order to identify the functional properties of the CB_1R-$5HT_{2A}R$ heterodimer, we used synthetic peptides with the amino acid sequence of the TM domains of CB_1R, fused to a cell-penetrating sequence derived from HIV TAT, to disrupt the formation of the heteromer. These peptides were tested in Bimolecular Fluorescence Complementation assays in cells expressing receptors fused to two complementary halves of YFP, and in their ability to modify cAMP and p-ERK1/2 signaling when both receptors are co-activated with ligands. In addition, we used molecular dynamics simulations of the CB_1R-$5HT_{2A}R$ heterodimer to identify hot-spots on the dimer interface. Thus, we also report on designed peptides fused to a CPP vector that effectively interfere with this predicted CB_1R-$5HT_{2A}R$ interface.

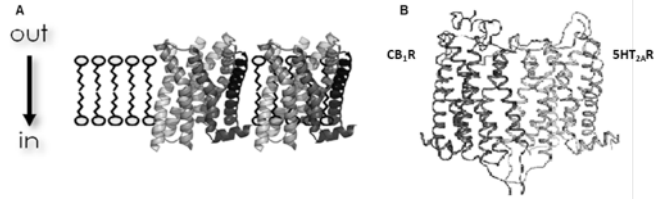

Figure 1. Representation of GPCRs oligomers in biological bilayers (A). Crystal structure of CB_1R and $5HT_{2A}R$ heteromers (B).

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Synthesis and characterization of Glucosylated Peptides: toward selective plasmapheresis-based treatment of Multiple Sclerosis

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Multiple Sclerosis (MS) is a neurodegenerative disease presumably involving an antibody-mediated mechanism in the damage of myelin sheath surrounding the axons in the central nervous system. Since the aetiology is still unknown and the therapies are feeble, isolating the specific autoantibodies is a major goal to understand and treat this complex pathology. Previous studies already assessed that the glucosylation of the *H. Influenzae* adhesin protein HMW1 on asparagine residues in consensus sequons (N-X-T/S) is necessary for protein secretion and efficient adherence to host cells, hence vital to assure infectivity of the bacterium.[1] Recently, the presence of N-Glucosyl epitopes in H. influenzae C-terminal adhesin fragment HMW1ct (1205-1526) was proven to be essential for the identification of the highest affinity antibodies in MS, showing a very specific recognition. Hence, hyperglucosylated HMW1ct is the first example of an N-glucosylated antigen that can be considered a relevant candidate for triggering pathogenic antibodies in MS.[2]
From a fundamental point of view, finding the minimal epitope recognized by antibodies and understanding the impact of glucosylation on adhesin sequons is a crucial task. To do so, a collection of adhesin-derived sequences was synthesized through solid-phase peptide synthesis and analysed by CD and NMR techniques. These peptides contain differently glucosylated consensus sequences that were able to recognize antibodies in patients' sera. Moreover, these putative antigens were loaded onto polymeric scaffolds with the aim to obtain multivalent macromolecules ideally able to snare selectively circulating antibodies. Indeed, the development of a tentacle-like, antigen-decorated polymer is a promising stratagem for the isolation and characterization of reactive antibodies found in the sera of patients suffering from autoimmune diseases. For this scope, the achievement of synthetically accessible, peptide-based microarchitectures may provide a great step forward both in the treatment and in the comprehension of MS.

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Synthesis and in vitro evaluation of fluorescent and magnetic nanoparticles functionalized with a cell penetrating peptide for cancer theranosis

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The enormous developments in nanotechnology have brought significant advancements to the production of multifunctional therapeutics for the prevention and/or control of diseases. For instance, superparamagnetic iron oxide nanoparticles (SPIONs) constitute an important platform of nanomaterials that are widely used for biomedical applications such as imaging[1], cell labelling[2], hyperthermia, and drug and gene delivery. We rationally designed and synthesized multifunctional nanoparticles (NPs) composed of a SPION core, cyanine fluorescent dye emitting in far red, polyethylene glycol (PEG5000) coating, and the membranotropic peptide gH625, from the cell-penetrating peptides (CPP) family. The peptide sequence was enriched with an additional cysteine so it can be involved as a reactive moiety in a certain orientation- and sequence-specific coupling of the CPP to the PEG shell of the NPs.
The in vitro evaluation performed by using fluorescence confocal spectral imaging showed that after a short incubation duration SPIONs-PEG-CPP uptake was 3-fold higher than that for SPIONs-PEG. The CPP also drives the subcellular distribution of a higher NP fraction towards low polarity cytosolic locations. Therefore, the major cellular uptake mechanism for the peptide-conjugated NPs should be endocytosis. Enhancement/acceleration of this mechanism by gH625 appears promising because of potential applications of SPIONs-PEG-gH625 as a multifunctional nanoplatform for cancer theranosis involving magnetic resonance imaging, optical imaging in far red, drug delivery, and hyperthermia.

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Chimeric Recombinant Antibody Fragments of anti-Nodal 3D1 mAb for theranostic applications

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Nodal is a potent embryonic morphogen belonging to the TGF-beta superfamily. Typically, it binds to the Alk4/ActRIIB receptor complex in the presence of the co-receptor Cripto-1. Nodal expression is restricted to embryonic tissues and human embryonic stem cells, whereas it is absent in normal adult cells[1]. In the last years, Nodal has been indicated as a diagnostic biomarker and as a therapeutic target for several types of cancer. Indeed, Nodal overexpression is associated with a large number of human solid tumours (i.e metastatic melanoma, breast, pancreatic cancer) and its intracellular signalling controls tumorigenesis promoting uncontrolled proliferation, differentiation and invasive phenotypes[1]. Nodal validation as biomarker and as therapeutic target needs potent reagents for its binding and neutralization of its activity, for this purpose we have generated an anti-Nodal monoclonal antibody named 3D1 and its therapeutic efficacy has been proven in aggressive melanoma both *in vitro* and *in vivo* models[2,3]. We have generated a partly humanized, recombinant Fab of 3D1 to obtain new molecules that may better penetrate tissues and have faster clearance for imaging purposes; also, the lack of the Fc domain and of the carbohydrate portion reduces toxic effects on the immune system and facilitates its preparation. The 3D1 Fab has been designed and prepared with specific sites for chemical (unpaired cysteines) or enzymatic (transglutamination) modifications[4]. We present the biochemical characterization of the 3D1 recombinant chimeric Fab efficiently produced in the *E. Coli* host using the periplasmic expression strategy. Expression and purification conditions have been optimized. Biochemical characterizations have confirmed the folding (Circular Dichroism) and the identity (ESI TOF mass spec) of purified *r*Fab 3D1. Preliminary binding data show that it recognizes the *rh*Nodal protein with an affinity 100 fold decreases compared to that the whole 3D1 antibody. This chimeric Fab fragment could be employed as scaffold to generate new properly engineered Nodal targeted-theranostic agents[5] i.e. nanoparticles for imaging purposes and therapy of melanoma and other Nodal positive tumours.

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Multicomponent hydrogels based on Fmoc-FF for potential use as regenerative scaffolds

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Gels are multi-component materials with long mechanical relaxation times that can be deformed with modest stresses. The low molecular weight peptide Fmoc-FF (N-fluorenylmethyloxycarbonyl diphenylalanine) shows the propensity to self-assemble in stable hydrogels.[1] Gelation process in short/ultrashort peptides are associated with the formation of space spanning structures formed by self-organization through hydrogen bonding and π-stacking interactions. Recently, we reported the synthesis and the structural characterization of the hexapeptide PEG_8-(FY)3, containing a polyethylene glycol (PEG) decoration and an alternation of Tyr and Phe residues. Above 1.0 %wt this peptide was able to generate soft hydrogels with good *in vitro* cytocompatibility.[2] It has been previously reported that the co-assembly of two building blocks into one ordered structure can promote the formation of a new composite material exhibiting new architectures, higher and tuneable mechanical properties, higher stability and improved biofunctionality.[3] According to these considerations, here we report the synthesis and the formulation of novel peptide-based multicomponent hydrogels composed of Fmoc-FF/PEG_8-(FY)3 mixed at different volume/volume ratios (2/1 and 1/1). In order to evaluate the effect of PEG moiety, we also prepared mixed hydrogels in which PEG_8-(FY)3 was replaced by (FY)3 analogue, lacking of the PEG moiety. Mixed hydrogels were prepared by the "solvent-switch" method. Results indicate that the kinetics formation of the hydrogels is affected by both the relative ratio between the peptides and the PEG. Rheology analysis highlighted the improved mechanical features of co-assembled gels. The possibility to sustain prolonged release of a model hydrophilic drug (Doxorubicin) was also observed. Coupling all these features with the demonstrated improved biological profiles, these peptide-based materials could be proposed as exogenous scaffold for hard tissue engineering.

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Synthesis of peptides potentially inhibiting the protein-protein interactions of the SHP2 phosphatase

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Mutations of the SHP2 phosphatase appear to be involved in cell proliferation mechanisms that occur in different tumor forms. Molecular dynamics studies and use of peptide libraries have allowed the identification of potential inhibitors of the protein-protein interactions of SHP2, able to block the activation of the cascade process in which the enzyme is involved.[1] In particular, in this work we focused on two short sequences, amenable of an easy chemical synthesis:

-V-L-pY-M-Q-P-L-N-G-R-K-
-G-L-N-pY-I-D-L-D-L-

where pY stands for tyrosine O-phosphate. Following standard procedures of solid-phase synthesis, we prepared a series of peptides modelled after the two sequences above mentioned. The 3D-structures of the peptides synthesized were investigated by means of circular dichroism and 2D-NMR. In most cases, we observed unordered structures.
We also tested the behaviour of our peptides in the presence of two hydrolytic enzymes: chimotrypsin and pronase-A. All peptides are degraded by pronase-A, but they display some resistance to chimotrypsin, probably due to the presence of the phosphate moiety.
We are currently investigating the ability of our peptides to bind to the SHP2 phosphatase.

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An integrin antagonist identified in the anophelin cE5 from the malaria vector *Anopheles gambiae*

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A RGD motif was identified in the N-terminal region of cE5, a potent salivary thrombin inhibitor from the African malaria vector *Anopheles gambiae*[1,2]. The tripeptide RGD is known for its involvement in binding to some integrins[3]. Integrins are a family of cell adhesion receptors located on cell membranes that regulate different crucial cellular functions and have been implicated in tumour progression[4]. The expression levels of integrins appeared to be correlated with the migration ability of cells making them attractive targets for cancer therapy. A peptide (APQ30) encompassing the first 30 amino acids residues of the protein cE5 and including the RGD tripeptide was tested in cell adhesion assays and found to inhibit $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins mediated adhesion[1]. A shorter peptide (APQ16), strongly conserved among members of the *A. gambiae* species complex and including only the first 16 residues, retained adhesion inhibitory properties, however with enhanced specificity toward $\alpha_v\beta_5$[1]. In addition, migration and invasion assays showed its capacity to inhibit the invasiveness of the malignant cell lines HepG2 and MDA-MB231. Altogether our data point to APQ16 as a new promising candidate as theranostic agent.

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Therapeutic potential of novel Cripto-1 CFC small peptide mimetics in melanoma

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The diagnosis of melanoma is increasing and current therapies for advanced disease remains unsuccessful. Thus, research aimed at developing novel targeting approaches is needed. Cripto-1 (CR-1), an epidermal growth factor (EGF-like) developmental morphogen, functions as a co-receptor for the tumor growth factor (TGF-beta) related molecule, Nodal[1]. CR-1/Nodal signalling pathways can induce cell survival, proliferation and migration of cancer cells. We hypothesize that interference with CR-1/Nodal receptor binding dynamics will reduce downstream Nodal-dependent signalling events important for cancer growth and enhance the effects of other anti-cancer drugs used in combination[2]. To test this hypothesis we have developed small peptides that mimic CR-1 CFC binding domain and prevents CR-1 from binding to the ALK4 receptor necessary for proper CR-1 co-receptor function for Nodal. We found that in vitro treatment of human melanoma cells with our prototype CFC mimetic B3 reduced viability and negatively affected cell cycle progression. We also show that treatment with B3 resulted in reduction of Nodal-dependent signaling via Smad2/3 and ERK1/2 as a result of the loss of CR-1 co-receptor function. These results provide the scientific rationale for the development of anti-CR-1/Nodal therapeutics that can synergize with or complement current treatment approaches in melanoma.

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Gd(III)-complex derivatives of aromatic oligopeptides as novel supramolecular MRI contrast agents

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Supramolecular contrast agents (CAs) can be prepared by self-assembling of monomeric units containing two different portions: i) a chelating agent able to allow kinetically and thermodynamically stable coordination of paramagnetic metal ions (like Gd^{3+} for T_1 positive CAs) for diagnostic applications in MRI, ii) a hydrophobic portion (one or more alkyl chains or a peptide sequence) able to prompt the self-assembling in water.[1] Due to the well-known capability of the diphenylalanine and of its strictly related derivatives (FFF, FFFF, Fmoc-FF, Boc-FF) to self-assemble in a large variety of supramolecular nanostructured materials,[2] FF sequence can be used as hydrophobic portion to prepare supramolecular CAs. Gd-complex can be alternatively positioned at the end or at the center of the aromatic framework. The water solubility can be improved by insertion of PEG moiety of different length. FF derivatization with Gd-complexes causes a drastic decrease of the self-assembling capability. The elongation of the aromatic framework[3] and/or the replacement of Phe with others non-coded amino acids,[4] having a more extended aromatic side chain (e.g: naphthylalanine), are two possible strategies to restore monomer interactions.

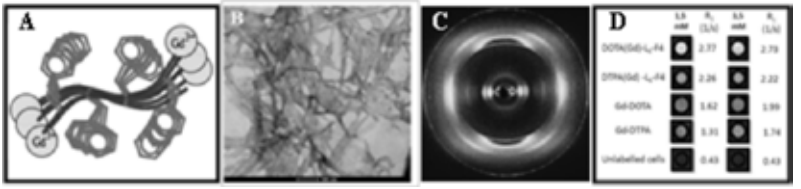

Figure 1: A) Schematic representation of a supramolecular CA based on tetra-phenylalanine Gd-conjugate, B) TEM image of self-assembled Gd(III)-derivative; C) WAXS diffraction pattern acquired on the solid fiber and D) T1-weighted images and observed relaxation rates of pellets of cells.

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The Proteoglycan like domain of the tumour enzyme Carbonic Anhydrase IX is an Intrinsically Disordered Protein

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Human Carbonic Anhydrases (hCAs) are ubiquitous zinc-enzymes, which catalyze the reversible hydration of carbon dioxide to bicarbonate ion and proton.[1] One of the most interesting members of the hCAs family is the transmembrane protein hCA IX which is present in a limited number of normal tissues and overexpressed in several malignant human tumours. For this reason it is considered a cancer target. hCA IX is a multidomain enzyme consisting of a N-terminal proteoglycan (PG)-like domain, a catalytic domain, a transmembrane region and a intracytosolic tail. Despite the importance of CA IX in tumor progression and spreading,[2,3] few studies have been reported on the biochemical and structural features of the proteoglycan like domain. Here, we report for the first time the full characterization of this domain, providing insights into its structural and functional features. PG domain has been produced at high yields in *E.coli* cells and characterized by means of biochemical, biophysical, and molecular dynamics studies. Results showed that it belongs to the family of intrinsically disordered proteins, being globally unfolded with only some local residual polyproline II secondary structure. The observed conformational flexibility may have several important roles in tumour progression, facilitating interactions of hCA IX with partner proteins assisting tumour spreading and progression.

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Synthesis and spectroscopic characterization of analogs of the anticancer agent Culicinin D

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Culicinin D is an anticancer peptaibol, non-ribosomally synthesized by the fungus *Culicinomyce clavisporus*. It exhibits selective toxicity towards MDA468 breast tumor cells with an IC_{50} value of less than 6 nM.[1,2] As all peptaibols, its sequence is characterized by the presence of the non-coded residue Aib (α-aminoisobutyric acid), a C-terminal 1,2-aminoalcohol and an acylated N-terminus. The primary structure of Culicinin D is as follows: But-Pro-**(*R*)-AHMOD**-Aib-Aib-**AMD**-Leu-Aib-Leu-βAla-**APAE**, where **(*R*)-AHMOD** is 2-amino-6-hydroxy-4-methyl-8-oxo-decanoic acid, **AMD** is 2-amino-4-methyldecanoic acid, and **APAE** is 2-(2-aminopropylamino)ethanol (Figure 1).

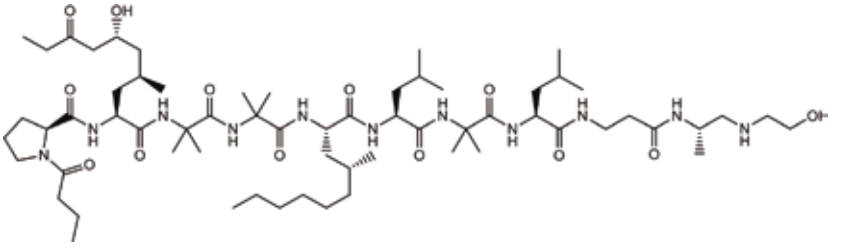

Figure 1. Sequence of the naturally-occurring anticancer peptaibol *Culicinin* D

Culicinin D is a promising lead compound for the development of peptide-based drugs, as it conjugates an anticancer activity in the low nanomolar range with the presence of as many as seven non-coded residues out of ten. This latter feature should ensure a very good proteolytic stability. However, its total synthesis is very difficult,[2,3] impairing its appealing to pharmaceutical companies.
In this contribution, we describe our attempt at simplifying the native sequence, while preserving the side-chain main features, such as polar moieties and steric hindrance. The design, synthetic strategy, and characterization of three *simplified* analogs are reported, together with preliminary results on their antitumor activity.

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Design and functional studies on analgesic peptides

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Opioids are the first-line medicines for pain relief, but they also pose a number of problems[1]. So the goal of the US Department of Health and Human Services (HHS) is to cope with the crisis [2]. To solve this problem, we built the conotoxins database and then used an AI algorithm to filter the database. Conotoxin ImI was selected from the database. By identifying the relationship between conotoxin and Acetylcholine receptor (AchR), a series of peptides were designed and synthesized. MST results showed that the affinity between α7 AchR and peptides was higher than the natural peptide. Detection of Ca^{2+} in Dorsal root ganglion cells with Flow cytometry and patch clamp, Compared with natural peptides, the peptides can decrease the intracellular calcium concentration of DRG cells significantly. The hot-plate model is used to detect the biological activity *in vivo* and the peptides we designed can significantly increase the pain threshold of the hot-plate. And our research can helped to design new analgesic strategies.

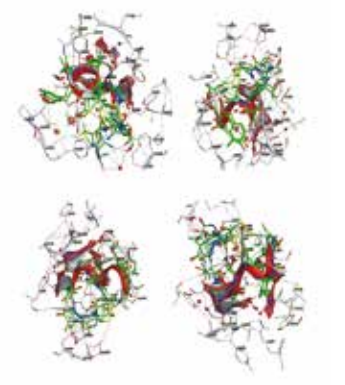

Figure 1. Figure caption example

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Evaluation of HER2-specific peptide ligand for its employment as radiolabeled imaging probe

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HER2 transmembrane receptor is an important target in immunotherapy treatment of breast and gastroesophageal cancer[1,2]. Molecular imaging of HER2 expression may provide essential prognostic and predictive information concerning disseminated cancer and aid in selection of an optimal therapy[3]. Radiolabeled low molecular weight peptide ligands are particularly attractive as probes for molecular imaging, since they reach and bind to the target and clear from non-target organs and blood stream faster than bulky antibodies4. In this study, we evaluated a potential HER2-imaging probe, an A9 nonapeptide, derived from the trastuzumab-Fab portion[4]. Its cellular uptake was investigated by mass spectrometry analysis of the cytoplasmic cellular extracts. Moreover, based on in-silico modeling, DTPA chelator was conjugated to N-terminus of A9. 111In-labeled A9 demonstrated nanomolar affinity to HER2-expressing BT474 cells and favorable biodistribution profile in NMRI mice. This study suggests that the peptide A9 represents a good lead candidate for development of molecular probe, to be used for imaging purposes and for the delivery of cytotoxic agents.

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Easy Formulation of Liposomal Doxorubicin modified with a Bombesin Peptide Analog for selective targeting of GRP Receptors over-expressed by Cancer Cells

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Development of peptide-targeted liposomes represents a great advance in the use of liposomal drugs for cancer therapy. Several studies, both *in vitro* and *in vivo* from many laboratories worldwide confirm the efficacy of this approach. Many efforts have been putted in the individuation of the best receptor/peptide pair, in liposome design and in industrial development to obtain the most efficient medicines in the fight against cancer. However, many critical issues are still unsolved in the development of these advanced drug delivery systems. Even if several compounds have been prepared at lab scale for preliminary *in vitro* and *in vivo* studies, the industrial development could be very difficult and expensive1.

Independently from the preparation mode, either by using the pre-formulation or the post-formulation method, the de-novo preparation of peptide targeted liposomal drugs needs of several complex steps that use expansive products such as GMP and highly pure peptide derivatives. Moreover, the peptide labelled liposomal drugs could be affected by short solution stability due to the poor peptide stability in buffered solution at physiological pH; this will influence the expiration date of the formulated drug and it will force the producing pharma companies in developing more sophisticated preservation procedure such as lyophilizing steps and drug loading only on the reconstituted compound.

We reported on a new method for an easy preparation of peptide targeted liposomal doxorubicin based on direct modification of the commercial liposomal drug Doxil®. The method uses a three vials kit and uses a two steps procedure that could be easily performed by clinicians directly before patient administration of the liposomal drug. Analytical data confirm the requested specifics for the final product such as: 1) the absence of free peptide in solution; 2) the properties (shape and size) of the peptide modified liposomes similar to that of Doxil® liposomes; 3) the doxorubicin content of the liposomes in line with the amount of Doxorubicin loaded in Doxil®. We tested our method for labeling Doxil® liposomes with a Bombesin analog peptide for the development of targeted liposomal doxorubicin to be used in GRP overexpressing tumours such as ovarian and breast cancers. Preliminary animal studies confirmed that in mice treated with Bombesin labelled liposomal drugs, prepared according the developed method, tumor growth is low, with an improved efficacy of the product respect to mice treated with non-modified Doxil® or with saline solution.

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Synthesis, characterization and nucleic acid-binding ability evaluation of cationic nucleobase-decorated peptides

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A large number of chimeric compounds that bear DNA nucleobases connected to amino acid moieties (**nucleoamino acids, NBAs**) are reported in literature[1,2] and can constitute the building blocks for the assembly of synthetic **nucleobase-containing peptides** (**nucleopeptides**), investigated for their potential applications in biomedicine and biomaterial fields.[2] The presence of both nucleobases and peptide backbone in nucleopeptides offers the possibility of binding nucleic acids[2] or protein targets,[3] modulating important cell regulatory processes,[3,4] as well as functioning for DNA/RNA delivery into cells.[5] In this context, in the last decades we realized nucleobase-decorated artificial peptides in which the nucleobases were connected via short linkers to diamino acid moieties (Fig. 1), and evaluated their biological activity.[2,3]

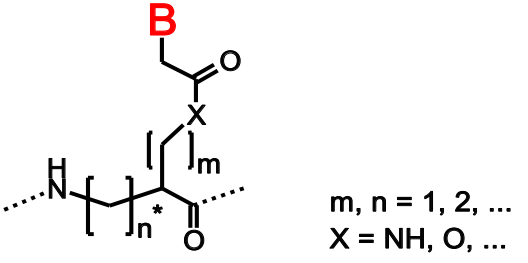

Figure 1. Nucleobase-containing peptides

Here, we describe the synthesis and characterization of **positively charged nucleopeptides**, as well as the evaluation of their biomolecular recognition properties towards both single and double stranded DNA and RNA targets assessed by CD and UV spectroscopies. Furthermore, SEM characterization of nucleopeptide/nucleic acid complexes will be reported for the first time.

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Novel peptide biomaterials against biofilms

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The extensive use of antibiotics for human and animal care has resulted in the development of bacterial resistance towards antibiotics. Recently, great attention is devoted to the possibility to build self-assembling peptide structures with antibacterial activity but most studies are limited to self-assembling entities to verify their eventual antibacterial activity.[1,2] The objective of this research project is to have a versatile nanosystem composed of a self-assembling sequences with antimicrobial activity due to the presence of antimicrobial peptides (AMPs). For this purpose, we used two different sequences one shorter which should aid the self-assembling process and the other longer and containing two moieties, the one serving for the assembly and the one serving as antimicrobial. The antimicrobial peptide WMR[3] was used in this study and properly modified in order to be inserted the self-assembling structure. We focus on the possibility of exploiting the multivalent presentation of AMPs on self-assembled nanostructures to improve antibiofilm activity. We used several techniques for a physico-chemical characterization of the self-assembled structures and performed experiments on microorganisms to test the capability to inhibit the biofilm growth or promote the eradication. The study has demonstrated that our peptide structures are promising tool against biofilms.

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Metallic nanoparticles: antibacterial activity and ecotoxicity

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Healthcare is considered one of the most significant domains of nano-biotechnology. The use of metal nanostructures paves the way for numerous applications in this field such as antibacterial paints, antibacterial patches or even antibacterial films. The possibility to synthesize various kind of metallic nanoparticles and the ever-increasing ability to control their size as well as structure to improve antibacterial activity has played an important role. Despite their advantages, leakage of heavy metals represents the major problem responsible of their toxicity[1]. Coating with antimicrobial peptides represents an emerging strategy to reduce toxicity and enhance antibacterial activity.[2]
The presence of nanoparticles and released metal ions in the aquatic environment is considered to be amongst the greatest environmental concerns because of their potential harmful effects on both aquatic organisms and humans via direct or indirect exposure. Among freshwater aquatic organisms, the crustacean Daphnia magna is often the best choice for toxicity evaluations thanks to its high sensitivity, short life cycle, easiness of manipulation in laboratory. In addition, it is widely present in diverse freshwater lakes and ponds, and plays a key role in transfer of energy and nutrients to upper food webs, representing also an important ecological sentinel species.
In this study we evaluated the ecotoxicity of quantum dots and Au nanoparticles coated with indolicidin, one of most common antibacterial peptide.

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In vivo efficacy of esculentin-1a derived peptides against Pseudomonas-aeruginosa induced pneumonia

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Pulmonary infections due to the Gram-negative bacterium *Pseudomonas aeruginosa* remain one of the major cause of morbidity and mortality[1], either in intensive care units or in ventilated patients, as well as in cystic fibrosis (CF) sufferers[2]. In parallel, the decrease in the pharmaceutical industry research pipeline for novel antimicrobial agents has resulted in an urgent need for the discovery of new strategies to address the vital problems of infectious diseases[3]. Naturally occurring antimicrobial peptides (AMPs) or their derivatives stand for an appealing source for the generation of new therapeutics. Recently, the frog skin-derived AMP Esc(1–21) and its diastereomer Esc(1–21)-1c were found to possess potent *in vitro* antipseudomonal activity[4]. Here, they were first shown to preserve the barrier integrity of airway epithelial cells better than the human AMP LL-37. Furthermore, Esc(1–21)-1c was more efficacious than Esc(1–21) and LL-37 in protecting host from pulmonary bacterial infection after a single intra-tracheal instillation at a very low dosage of 0.1 mg/kg causing 2-log reduction in the number of lung bacterial cells and was accompanied by less leukocytes recruitment and reduced inflammatory response. Importantly, in contrast to what reported for other AMPs, the peptide was administered at 2 hours after bacterial challenge to better reflect the real life infectious conditions[5]. To the best of our knowledge, this is also the first study investigating the effect of AMPs on airway-epithelia associated genes upon administration to infected lungs. Overall, our data highly support advanced preclinical studies for the development of Esc(1–21)-1c as an efficacious therapeutic alternative against pulmonary *P. aeruginosa*

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Esc(1-21) and its diastereomer: antipseudomonal frog-skin derived peptides with multiple immunomodulatory properties

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Multidrug-resistant bacterial infections represent one of the most complex global health challenges. Particularly, pulmonary infections by the opportunistic pathogen *Pseudomonas aeruginosa* are the main cause of mortality especially in cystic fibrosis (CF) patients[1]. For this reason, alternative strategies to conventional antibiotics are needed and antimicrobial peptides (AMPs) represent new promising compounds for antimicrobial therapy. Our recent studies showed that the frog-skin derived AMP Esc(1-21) and its diastereomer Esc(1-21)-1c not only display antimicrobial activity against *P. aeruginosa* biofilm [2] or *Pseudomonas* cells internalized in human bronchial epithelial cells (CFBE)[3], but also the capacity to stimulate migration of CFBE [3]. Persistent bacterial infections often result in the development of lesions, especially in the lungs of CF sufferers; therefore, the capacity of AMPs to accelerate wound repair of the bronchial epithelium is extremely advantageous to restore lung functionality. By performing fluorescence studies on CFBE, we demonstrated that Esc(1-21) or Esc(1-21)-1c induce typical morphological changes associated with a migratory phenotype, such as cytoplasmic protrusions developing into lamellipodia. By treating cells with the cell proliferation blocker hydroxyurea, cell migration was not affected, indicating that cell proliferation is not essential for the peptide-induced cell migration. Moreover, we showed that the mechanisms underlying the peptide-induced cell migration imply an epidermal growth factor receptor (EGFR) mediated signaling pathway[3] and that metalloproteinases are involved in EGFR trans-activation. Finally, we found out that both peptides are able to inhibit cyclooxygenase-2 (COX-2) synthesis in murine macrophages stimulated by *P. aeruginosa* lipopolysaccharide, indicating their anti-inflammatory activity. Based on these interesting biological properties, further studies are conceivable to develop these peptides as new drugs with multiple functions.

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The amphibian antimicrobial peptide temporin L inhibits in vitro herpes simplex virus type 1 infection, a continuous story

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Antimicrobial drugs is lacking of innovative therapies leading the possibility to develop resistance mechanism in the major part of microorganism. In this scenario the World Health Organization (WHO) defines as priority the discovery of new drugs able to fight the superbugs that are emerging. Not only bacteria, parasite and fungi are acquiring new "escape" mechanisms, also the viruses adopt new strategies in order to survive. For instance the Herpes Simplex Virus type 1 (HSV-1) strain resistant to acyclovir and its modifications are nowadays a reality. In this scenario the characterization and the develop of innovative antimicrobial therapies represent a mission for the researcher. Among them an important class of source as new antimicrobial agent is represented by the peptides (AMP).
These peptides share the amphipathic nature, the positive charges and the presence of hydrophobic amino acid residues.[1,2] Concerning the fact that several peptides among this class were full characterized for their antibacterial activities, we investigate the potential of a peculiar AMP of Temporin group: Temporin L (TL). These peptides are an huge class of AMPs that count around 100 members with a defined range of size[3]. Despite there are several papers on their activity and mechanisms of action on prokaryotic models, few data are reported on viruses. Temporin L is, until today, the only temporin that showed antibacterial activity against Gram negative and has, unfortunately, also a high hemolytic property. For this reason several modification in the primary structure of TL were achieved in order to increase the antibacterial activity with the counterpart in toxicity reduction. Here we reported for the first time the Temporin L derivate ([Pro3, DLeu9] TL1) and its modified (indicated as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) evaluation as inhibitor of HSV-1 infection in in-vitro model. To test the antiviral activity of the peptides, and to identify the precise site of action, four types of experiments were set up: the co-treatment test, virus pre-treatment, cells pre-treatment and post-treatment. Then we evaluated the membrane fusion properties defining the phase of infection interferences of these peptides and prelude to the mechanism of action.

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In vitro and in vivo evaluation of D-BMAP18 peptides for the treatment of pulmonary infections in cystic fibrosis

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Patients with cystic fibrosis require pharmacological treatment against chronic lung infections due to antibiotic resistant pathogens. The common antibiotics are not sufficient and so new compounds are required. Among the leading compound for developing new drugs, the antimicrobial peptides are promising because of the broad spectrum of activity and the slow rate in acquired resistance. In order to obtain a peptide stable in the pulmonary environment we truncated and modified using D-amino acids, the bovine antimicrobial peptide BMAP27, synthesizing the D-BMAP18[1]. It is an α-helical peptide which has a MIC_{90}= 16 μg/ml against *Pseudomonas aeruginosa* CF-isolates[2] and has a good activity against their biofilm. We tested with success the bactericidal activity of *D*-BMAP18 toward *P. aeruginosa* in CF- sputum by co-treatments with DNase and NaCl. We also tested its *in vitro* and *in vivo* toxicity observing a non-negligible toxicity when it was intratracheally administrated to mice[3], a side effect that may be linked to this route of administration. Preliminary test on a systemic infection model in *Galleria mellonella* (wax moth) are in progress to verify this hypothesis. In order to improve its efficacy and to minimize undesired side effects, we are also trying new routes of administration in mice and the design of a modified *in situ* activable D-BMAP18 prodrug. The final aim is to obtain a safe drug for the treatment of pulmonary infections in cystic fibrosis.

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Optimization of mammalian proline-rich antimicrobial peptides as small and effective inhibitors of bacterial protein synthesis

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Among the molecules proposed as leading candidate for the development of new antibiotics, the proline-rich antimicrobial peptides (PrAMPs) emerged because of their high antimicrobial potency and low cytotoxicity. Recently, the mode of action of several PrAMPs has been described in great detail. It is known that they mainly enter the bacterial cytosol through the inner membrane transporters SbmA.. Then it has been shown that PrAMPs block the activity of the bacterial ribosomes and impair the functions of chaperons like DnaK, therefore killing pathogens by the lethal block of their protein synthesis[1]. Now, the upcoming frontier is the optimization of PrAMPs and their transformation in new antibiotic drugs. We are working on some mammalian PrAMPs in order obtain molecules with improved antimicrobial potential, and fitting the needs of a large scale production by pharmaceutical companies. To this aim, we are testing many fragments and mutants of these peptides, to i) identify the residues of the proline-rich sequences that are crucial for the protein synthesis inhibition in bacteria, and ii) find the shortest sequence needed to provide an antibiotic effect, scaling down the difficulty and the cost of the PrAMPs production.

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Peptidomimetics based electrospun nanofibers

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Electrospinning is a simple and versatile technique used for the fabrication of continuous micro and nanofibers. This approach is inexpensive, scalable, reliable and mainly used from polymer solutions and polymer melts.[1] Nonpolymeric molecules can usually not be electrospun, as only polymer solutions or melts are sufficiently viscous to provide the required degree of molecular entanglement.[2] However, recent studies have demonstrated that high molar mass polymers are not essential for production of uniform electrospun fibers but that sufficient intermolecular interactions acting as chain entanglements is the primary criterion.[3] Recently it was demonstrated that the dipeptide phenylalanine-phenylalanine (FF), and two Fmoc derivatives, *i.e.* Fmoc glycine (Fmoc-Gly) and Fmoc-phenylalanyl-glycine (Fmoc-Phe-Gly), in spite of their small size, can assemble by electrospinning to nanofibers basing solely on noncovalent interactions.[2,4]

A study on the electrospinnability of short peptide chains containing natural amino acids (Gly, Ala, Leu, Val) together with a heterocyclic scaffold properly functionalized, is here reported. The above compounds were dissolved in high concentration in HFIP and the experiments executed on a <5 μl scale using microliter electrospinning technique and on 0,5 ml scale using a conventional setup in laboratory scale. Obtained fibers were characterized with different techniques: optical microscopy, SEM, AFM and Raman and FT-IR spectroscopy. The fibers appear continuous, with a diameter between 400 and 700 nm and with a full cross-section demonstrating for the first time the possibility to develop electrospun nanofibers from unnatural short peptides.

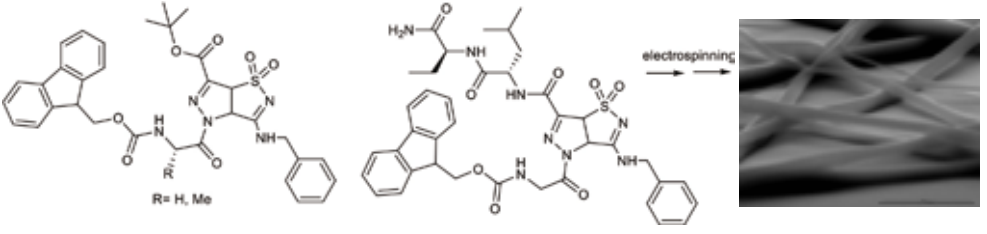

Figure 1. Electrospun peptidomimetics and an example of nanofibers obtained

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AIF(370-394)/CypA binding mode characterization: experimental and computational approaches

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The complex formation between AIF and CypA proteins following oxidative stress in neuronal cells plays a crucial role in neuronal cell death by a mechanism independent of caspases activation.[1,2] Through extensive *in vitro* analyses, molecular insights into AIF(Δ1-121)/CypA interaction have been gained.[3,4] AIF(Δ1-121), the apoptogenic form of AIF, interacts with CypA mainly through the amino acid region spanning residues 370-394. The corresponding synthetic peptide AIF(370-394) binds CypA with a K_D of about 12 μM and inhibits complex formation in vitro with a IC_{50} of 5 μM.[3] The delivery of this peptide in neuronal cells induces neuroprotection against high doses of glutamate by inhibiting the AIF-CypA axis.[4] Furthermore, we have demonstrated that the AIF(370-394)-binding region on CypA is included in that of AIF(Δ1-121), indicating that the peptide represents a simplified model to study the protein-protein interaction.[4]
In this framework, now we provide new information on AIF(370-394)/CypA binding mode, by coupling NMR spectrometry, mutagenesis studies and direct binding experiments. AIF(370-394) amino acids crucial for the interaction with CypA have been identified by saturation transfer difference (STD-NMR) technique and confirmed by mutagenesis studies. Moreover, a conformation of the peptide-bound to CypA has been also suggested by trNOESY-NMR spectroscopy. Finally, the structural insights obtained have been used to generate a model of the complex AIF(370-394)/CypA. These data have a great importance for identifying first-in-class selective inhibitors of the AIF/CypA complex, to be used in therapeutic approaches.

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Conformational stabilization of AIF(370-394) β-hairpin-like peptides

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Synthetic peptides are widely used as chemical tools to modulate protein-protein interactions (PPI[1] which are often mediated by well-defined secondary structure elements, including α-helices and β-structures (β-turns and/or β –hairpin).[1] Often these molecular recognition motifs encompass short peptide sequences (8-12 residues), which make them amenable to subsequent modifications. However, the majority of peptides reproducing small region of proteins rarely assume in solution the native conformations, limiting their bioactivity.
With the aim to stabilize a β-hairpin conformation by interstrand covalent bridges made through chemoselective reactions, we have extensively studied, modified and characterized the 24 amino acids bioactive peptide AIF(370-394) to obtain β-harpin model systems.[2-5]
In particular, we have investigated the conformational features of a series of highly constrained bi-cyclic analogues containing both disulphide and 1,4-disubstituted 1,2,3-triazole bridges.[6] Comparative biochemical assays have also been carried out to evaluate the ability of the new analogues to bind the target protein CypA[3-5] and to obtain structure-activity relationship insights.

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Conformationally Constrained Analogues of Amyloidogenic Segments of Islet Amyloid Polypeptide

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Pancreatic islet amyloid is a characteristic feature of type 2 diabetes and islet amyloid polypeptide (IAPP) is its major protein component. As the conformational transition of α-helical IAPP conformations to β-sheet ones underlies IAPP amyloidogenesis[1,2], we designed a series of peptides in which we attempted to stabilize the α-helical structure in the amyloidogenic IAPP segments IAPP(8-18) and IAPP(8-28) via i-to-i+4 side chain-to-side chain cyclization. Analogues B1cyclo and B2cyclo exhibited higher α-helical propensities than their linear precursors while the low solubility of B3cyclo and B4cyclo prohibited their study. Importantly, significant α-helix stabilization was observed in B5cyclo; by contrast, B6cyclo had a high β-sheet forming propensity and lower α-helical content than the linear precursor. Most importantly, B6cyclo but not B5cyclo was found to be able to suppress IAPP amyloid formation and cytotoxicity. Thus, B6cyclo is a promising candidate for the development of inhibitors of IAPP amyloidogenesis and related cell-damage. Limited proteolysis experiments using insulin-degrading enzyme (IDE) and/or trypsin to hydrolyse IAPP either in the absence or in the presence of B6cyclo were then performed. IDE is a zinc metalloprotease able to degrade several different substrates including insulin, β-amyloid peptides (Aβ) and IAPP. These experiments[3] coupled with LC-MS analysis allow for identification of the IAPP region involved in the interaction with the B6cyclo peptide.

Analogues (8-18)	
B1cyclo	Ac-cyclo(DTQRK)ANFLVH-NH_2
B2cyclo	Ac-ATQRLAcyclo(DFLVK)-NH_2
B3cyclo	Ac-Cyclo[Nle(ε-N3)-TQR-Pra]ANFLVH-NH_2
B4cyclo	Ac-Cyclo[Ala(β-N3)-TQR-Pra]ANFLVH-NH_2
Analogues (8-28)	
B5cyclo	Ac-cyclo(DTQRK)ANFLVHSSNNFGAILS-NH_2
B6cyclo	Ac-ATQRLAcyclo(DFLVK)SSNNFGAILS-NH_2

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Self-Assembling of Fmoc-GC Peptide Nucleic Acid Dimers into Highly Fluorescent Aggregates

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Self-assembly is a process in which components, either separate or linked, spontaneously form ordered aggregates. The use of short peptides or modified nucleotides as building blocks for the aggregates is particularly intriguing for the production of new materials, electronic nanodevices, and biosensors[1]; Many studies about nucleopeptides, composed of amino acids connected to the bases through a methylene carbonyl linker at the N-terminus or on the side chain, reported their abilities to self-assemble in different ways (depending on the pH or on the addition of metal ions)[2], while the ability of peptide nucleic acids (PNA) to aggregate has been very little explored[3].
In this work we investigated the self-assembling properties of a PNA dimer, conjugated at the N-terminus to a fluorenylmethoxycarbonyl group. This PNA dimer forms nano-aggregates at low concentration in $CHCl_3$/CH^3OH mixtures, which retain very interesting fluorescent properties (high quantum yield in the visible region with lifetimes on the nanosecond scale). These features make them promising materials for applications in optoelectronics.

The self-assembling properties of Fmoc-GC dimers

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Characterization of the mechanism of membrane perturbation by small antimicrobial norspermidine-based peptidomimetics

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Bacteria are showing increasing resistance against available antibiotics. Naturally occurring antimicrobial peptides (AMPs) are promising molecules to fight multi-resistant microbes. These cationic and amphipathic peptides show strong antimicrobial activity, killing bacteria mainly by perturbing their membranes, without being significantly toxic towards human cells. Notwithstanding the potential of AMPs in the fight against drug resistance, their therapeutic application presents some limitations, such as their susceptibility to proteolytic degradation. At the same time, the rational design of protease-resistant peptidomimetic molecules with the same properties of AMPs, is complicated by the fact that multiple equilibria (self-assembly, water-membrane partition, insertion in the bilayer, pore formation, etc.) influence the membrane-perturbing activity, and any modification in molecular properties perturbs all of them to different extents. Spectroscopic approaches, in particular fluorescence methods, are extremely powerful in the characterization of the behavior of AMPs or peptidomimetics in interaction with lipid bilayers[1]. By using these techniques, together with molecular dynamics simulations, we studied the mechanism of action of an antimicrobial norspermidine-based peptidomimetic (Nor Trp) [2], which is active in the low μM range, without being toxic. We characterized aggregation, membrane binding, insertion in the membrane, pore formation and bilayer perturbation. Overall, our findings provide a clear picture of the interaction of NorTrp with lipid bilayers, and of its mechanism of membrane perturbation.

Figure. Scheme of the multiple equilibria involved in peptide-membrane interactions (left panel); chemical structure of NorTrp (right panel)

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Peptoid-Peptide Hybrid Backbone of Urotensin-II$^{(4-11)}$: Synthesis and Biological Evaluation

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Urotensin-II (U-II), a somatostatin-like neuropeptide, was originally isolated from the urophysis, a neuroendocrine gland of the teleost fish *Gillichthysmirabilison*. The presence of U-II in human body has been documented within both vascular and cardiac tissues[1]. In vascular beds containing high levels of the receptor of U-II (UT)has been observed a potent vasoconstriction effect. Human U-II (*h*U-II) is the most potent vasoconstrictor reported ten times more potent than endothelin-1[1]. *h*U-II is a cyclic peptide, ETPD[CFWKYC]V-OH, established as the natural ligand of an orphan G-protein coupled receptor (UT).Peptoid monomers are artificially designed building blocks composed of N-subtituted glycine (NSG) monomers whose side chains are appended to the nitrogen atom rather than the α-carbon.Zuckermann has realized the first experimental paper describing the synthesis of oligomers of N-substituted glycine residues[2]. Due to this unique chemical structure, peptoids have several advantages over peptides: Larger selection of side chains; It was shown that the achiral centers in the N-alkyl glycine monomers could promote secondary structures in solution; better solubility and cell permeability; the N-alkylated backbone was shown to be resistant to enzymatic degradation[1]. Based on these interesting properties, peptoids have been used in wide areas such as cell-penetrating properties and antimicrobial activitiesas well as mimics of lung surfactant proteins[2]. Interestingly, peptoids were also used as active site ligands of G protein-coupled receptors (GPCRs) such as melanocortin system and somatostatin analogues[3]. Herein, the urotensin system composed by UT as GPCR model and its minimal active ligand UII$^{(4-11)}$, was subject of our sought for new ligands bearing peptoid-peptide hybrid backbone and new structure-activity relationship studies.

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PNA based miR-34a mimics: a physico-chemical study and assessment in Neuroblastoma

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Neuroblastoma (NB) is a solid tumour responsible for the 15% of childhood cancer deaths.[1] The amplification of the MYCN proto-oncogene with subsequent MYCN protein overexpression represents the most significant biomarker occurring in ~25% of all NB tumours.[1] MYCN is a transcription factor which has a crucial role in normal brain and neural development.[2] MicroRNAs (miRNAs), have emerged as a novel class of potent regulatory molecules;[3] being involved in many biological processes like protein coding gene expression, proliferation, differentiation, apoptosis and stem cell self-renewal, and also acting as modulators in human tumour initiation and progression.[4] It is not surprising that dysregulation of miRNAs has been involved in a variety of pathologies including several types of cancer.[4] In particular, miR-34a has been proven to be a general tumour suppressor (lymphoma, lung cancer, neuroblastoma *etc*) and the most potent regulator of MYCN protein level, since it directly targets the 3'UTR MYCN mRNA resulting in inhibition of the MYCN protein.[5] Peptide Nucleic Acids (PNAs) have been identified as suitable analogues to develop miRNA-34a mimics with enhanced properties such as binding affinity/stability and resistance to enzymatic degradation.[6] The aim of this study was to identify the minimum portion of PNA-based miR-34a mimics to successfully inhibit MYCN. PNA constructs of different length were designed including (a) a seed sequence and/or 3'-supplementary binding site recognising 3'UTR region of MYCN mRNA (b) a peptide carrier to enhance cellular uptake (c) a spacer (d) a fluorescent tag. The PNA-based miR-34a mimics were prepared by microwave assisted solid phase peptide synthesis; a detailed physico-chemical study of the PNA/RNA (3'UTR MYCN mRNA) complex was performed by UV/CD spectroscopy and by all-atoms Molecular Dynamics simulations by using GROMACS. In the end, cellular uptake and biological activity of the PNA based mimics was tested in vitro on Neuroblastoma cell lines.

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Synthesis and characterisation of stapled α-helical peptide that binds to G-quadruplex nucleic acid

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G-quadruplexes (G4) are four-stranded nucleic acid structures consisting of G-tetrads formed by circularly hydrogen-bonded guanines[1]. Studies had shown that G4 structures play important roles in multiple vital cellular processes such as DNA replication, transcription, translation and telomere maintenance.

The RHAU helicase (also known as DHX36 gene product, or G4R1, from human) is from the DEAH (Asp-Glu-Ala-His)-box family of RNA helicases that play role in hematopoiesis, heart development[2] and spermatogenesis. We have previously reported a solved structure of an 18-amino acids peptide, identified as the G4-binding domain of RHAU, in a complex with a G4[3]. The secondary structure of the RHAU18 peptide is determined to be an α-helical.

In this study, in order to improve the stability of RHAU18 and study the binding affinity to G4, we have synthesized and purified various stapled RHAU18 analogues in house. Two amino acids at *i*, *i+4* positions on the same face of a native peptide sequences were chosen and substituted with glutamic acid (Glu) and lysine (Lys). This stapling strategy involves the formation of a lactam bridge.

The characterization of peptide structures was performed using circular dichroism (CD) and nuclear magnetic resonance spectroscopy (NMR), which confirmed the enhanced α-helicity of all stapled RHAU18 analogues. CD analysis of a series of RHAU18 peptides shows improved stability upon stapling. The interaction and binding affinity of stapled RHAU18 analogues with G4 is studies by gel electrophoresis.

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Conformational Properties of Gluten Exorphins GE-A and its Role when Encrypted in Precursor Protein

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Many kinds of bioactive peptides have been found in the enzymatic digest of food proteins; some of these are of animal origin, such as β-casomorphin and hemorphins, some are of plant origin such as gluten exorphins. Interestingly, opioid peptides derived from animal proteins are mostly μ-receptors selective, while those of plant origin are mostly δ-receptors selective. Activities profiles obtained with a cyclic enkephalin analogue and its linear correlate revealed that μ- and δ-receptors differs on their conformational requirements. Gluten exorphins are opioid peptides isolated from enzymatic digest of wheat gluten and can be classified into three groups according to their structure, namely, gluten exorphin A, GE-A, gluten exorphin B, GE-B, gluten exorphin C, GE-C. There are two members of GE-A which are GE-A5 (GYYPT) and GE-A4 (GYYP). The structure of GE-A is unique in that, unlike most opioid peptides which have Tyr in their N-terminus, GE-A has a Gly in N-terminus (essential for opioid activity). In our work on synthetic opioid peptides we studied the conformational behaviour in solution of conventionally protected gluten exorphins sequences.[1] These product have been synthesized, purified, and then analysed by NMR spectroscopy.[2] We described here the chloroform solution NMR analysis of Boc-GYYP-OMe (**1**) and Boc-GYYPT-OMe (2). The initial NMR analysis of the synthetic tetrapeptide **1** clearly indicate the coexistence of two different conformation in equilibrium. The results on the conformational characterization of both structures will describe in this work. Furthermore, we analyse the influence of conformational characteristics of tetrapeptide **1** on the structure of wheat glutenin. The understanding of the role and organization structural of the natural protein permitted the design of delivery systems containing the bioactive sequence.

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VEGF receptor binding studies of PlGF[87-101] peptide analogues

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The Placental Growth Factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family that selectively binds to and activates VEGF receptor 1 (VEGFR1), plays a key role in pathological angiogenesis during ischemia, inflammation and cancer.[1] The binding of PlGF to VEGFR-1 also activates VEGFR-2, either displacing VEGF-A from VEGFR-1 and making it available for the binding to VEGFR-2,[1] or through a transphosphorylation mechanism.[2] Data *in vivo*, demonstrated that PlGF-deficient mice showed an attenuated responses to VEGF in pathological angiogenesis,[3] and that PlGF is necessary for angiogenic events in adult tissues during ischemia, inflammation and cancer.[4] Previous experiments of mutagenesis and structural analysis showed that the PlGF-VEGFR1 complex formation is mediated a the β-harpin region of PlGF spanning residues Gln87 and Val100.[5] An analogue peptide designed on this region provided a proangiogenic activity *in vitro* and *in vivo* and unlike PlGF full-length protein interacts with both VEGFR receptors, VEGFR1 and VEGFR2.[6] These findings makes the β-harpin region of PlGF an ideal starting point, for the development of a novel peptide-based compound for therapeutic applications. In this frame, here we reported the design and the structural and functional characterization of analogues of the PlGF β-harpin region 87-101, in order to better characterize its interaction with receptors and improve the activity of the wild type peptide. The new peptides have been extensively characterized by SPR for binding to both VEGFR-1 and VEGFR-2 and on HuVEC cells to assess the activity. Some modified peptides display receptor binding ability and suppression of VEGF-induced cell proliferation. Our data provide new molecular insights for designing new compounds with therapeutic relevance in the angiogenic process.

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Peptides to study HIV-1 and CCR5 interactions by surface plasmon resonance

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The Human Immunodeficiency Virus (HIV) attacks $CD4^+$ cells of the blood and lymphoid tissues, such as $CD4^+$ T cells, $CD4^+$ monocytes/macrophages and dendritic cells. During the process of HIV infection, the viral surface glycoprotein gp120 binds initially the receptor CD4 and then the chemokine coreceptor CCR5 of the host cell, in particular, its extracellular loop 2 (ECL2) and N-terminal (Nt) regions. The main fragment of glycoprotein gp120 involved in the interaction with CCR5 is its third hypervariable loop (V3 loop). The use of artificial nanosystems mimicking CCR5 receptor holds a promise for the treatment of HIV infection during antiretroviral therapy.[1]
We performed the synthesis of two different cyclic peptide analogues of the viral V3 loop in order to carry out binding and kinetic studies with CCR5 peptide fragments ECL2 and Nt as HIV-host interaction model. At this purpose, the linear peptides were synthesized using both automatic microwave-assisted solid phase peptide synthesis (SPPS) and manual SPPS, following Fmoc/tBu strategy. One of the V3 loop peptides was not achieved due to problems encountered at the step of the disulfide bridge formation. The second V3 loop peptide, ECL2 and Nt were purified and characterized. The peculiarities of their structure and interaction were studied by CD spectroscopy and surface plasmon resonance (SPR) experiments.
The V3 loop peptide was immobilized on a sensor chip CM-5 type, then ECL2 and Nt peptides (both separately and as a mixture) were flowed over SPR biosensor surface at different concentrations. The kinetic and affinity experiments were analysed and fitted to theoretical models to calculate the corresponding affinity constants. Results showed that both ECL2 and Nt peptides interact with the V3 loop peptide, observing higher affinity for ECL2 peptide compared with Nt. Indeed, ECL2 peptide appeared the principal contributor to the binding when a peptide mixture was studied.
These results highlight CCR5 peptides as interesting CCR5-mimicking candidates when coupled to nanoparticles (NPs) as biomimetic systems. NPs bearing CCR5 peptide fragments are able to bind with high affinity gp120 HIV protein and some inflammatory chemokines.[2] Particularly, herein we selected the peptide ECL2 as the best candidate due to its highest affinity to V3 loop peptide.

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The discovery of GRP78 as a novel KCTD15 interactor

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KCTD15 is a member of the KCTD (K^+ Channel Tetramerization Domain) family, a class of proteins frequently implicated in crucial physio-pathological processes[1]. In the last few years, several lines of evidences suggest that KCTD15 is an obesity-linked protein in humans. Although these findings have important implications for obesity control, the molecular mechanism underlying the KCTD15 action in these processes is still unknown. This is likely due to the lack of structural and molecular information regarding this protein. To fill this gap, KCTD15 was here heterologously expressed and biophysically characterized[2]. Its spectroscopic characterization shows that the folded region of the protein corresponds to the residues 56-234. This portion, which assumes a pentameric state, is also endowed with a remarkable thermal stability (Tm 65 °C). To validate the correct folding of KCTD15, the binding with the well-known interactor Ap2α[3] was evaluated by Microscale Thermophoresis (MST). The inability to bind the N-terminal region of Cullin3, previously reported[4],was chosen as negative control in the MST experiment. The availability of large amounts of stable recombinant protein also made possible a functional proteomic approach in 3T3-L1 cells to search for novel KCTD15 interactors. These investigations led to the discovery of GRP78 as a novel KCTD15 partner and the validation of the interaction has been achieved by immunoprecipitation. Furthermore, the presence in *Drosophila* of a GRP78 homologue corroborates the physiological role played by the complex KCTD15-GRP78 in the adipogenesis process and indicates that it is evolutionarily conserved. Present results also suggest that KCTD15 may be a new target for obesity control.

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Crystal structure of human carbonic anhydrase II in complex with a membrane-impermeant, isoform selective inhibitor

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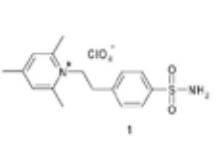
Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous metallo-enzymes which catalyze the reversible hydration of carbon dioxide to bicarbonate and a proton ($CO_2 + H_2O = HCO_3^- + H^+$)[1]. Human CAs (hCAs) are involved in a large number of physiological processes and their abnormal levels and/or activities have been often associated with different diseases. In particular, the isoform IX (hCA IX) is considered one of the most important therapeutic target for different aggressive cancers. Indeed, in tumor hypoxic conditions it is strongly overexpressed on the malignant cells surface, contributing to their proliferation, adhesion and cell invasion[2]. Unfortunately, the high amino acid conservation existing among the human isoforms has made it difficult to design isoform specific CA inhibitors (CAIs)[1]. An interesting class of isoform-selective CAIs is constituted by the pyridinium salts of benzene-sulfonamides[3]. These compounds, thanks to their cationic nature, are membrane-impermeant[3], resulting potential candidates for targeting the extracellular active site of hCA IX. Among the pyridinium containing sulfonamides, compounds 1 and 2 were shown to possess low nanomolar affinity for hCA IX, and also to be less effective inhibitors of widespread, off target isoforms hCA I, II and IV[4].



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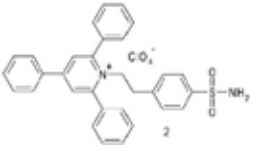

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Here we report a comprehensive kinetic and crystallographic study on compounds 1 and 2 as CAIs. In particular, the crystal structure of the hCA II/2 adduct and its comparison with that of hCA II/1 complex, previously characterized[5], demonstrated that the substituents of the pyridinium ring play a key role in determining the conformation of the inhibitor within the CA active site and, consequently, the binding affinity to the enzyme. These findings provide useful insights for the design of more isozyme-selective inhibitors mainly targeting the tumor-associated hCA IX.

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Theoretical study on the stability of amphi-ionophore cystine-based cyclopeptide stereoisomers with anions and cations

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Cyclic peptides are a large class of natural compounds which attracted a great deal of attention, especially as potential amphi-ionophores.[1] The stability of amphi-ionophore cystine-based cyclopeptide (TCP) stereoisomers in complexation with achiral substance (Li^+, Na^+, K^+, F^-, Cl^-, and Br- ions) were studied by quantum chemical calculations at the M06-2X/6-311++ G(d,p) level of theory. The nature of the intermolecular interaction between TCPs and ions were studied by natural bond orbital analysis (NBO)[2] and the quantum theory of atoms in molecules (QTAIM)[3] methods. According to the size of the ions and configuration of TCPs, the location of ions (within or above the TCPs) and the types of formed intermolecular interactions are different.

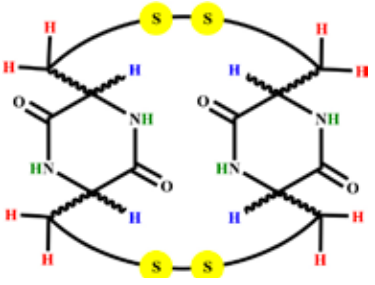

Figure 1. Tricyclopeptide (TCP)

The results revealed that the free TCP7 and TCP8 enantiomers are the most stable structures among all TCPs. This is the case for the corresponding ion-containing complexes except Li^+, in which formed the most stable complex with TCP3. Moreover, the calculated energies of the optimized complexes containing the same ion shows different stability order as compared to the free TCPs.

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Fluorescence studies of therapeutic peptides: the Semaglutide case

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Glucagon-like peptide 1 (GLP-1) is a 30 amino acid long hormone produced by intestinal enteroendocrine L-cells, featuring unique glucose-dependent insulinotropic effects. Unfortunately, endogenous GLP-1 is rapidly degraded by dipeptidyl peptidase-4 (DPP-IV), endopeptidase 24.11 and renal clearance, resulting in a half-life of approximately 2 minutes. For this reason, intense research activity has been dedicated to the design of GLP-1 analogs, showing higher stability and longer retention times in the plasma.
Semaglutide is a GLP-1 analog, resistant to DPPP-IV and demonstrating enhanced insulinotropic effects. It is characterized by the substitution of Ala-8 with an Aib residue, and the derivatization of Lys-26 with a polymeric chain, comprising two 2-polyethilene glycol groups, a Glu residue and a C18 lipid chain (Figure 1)

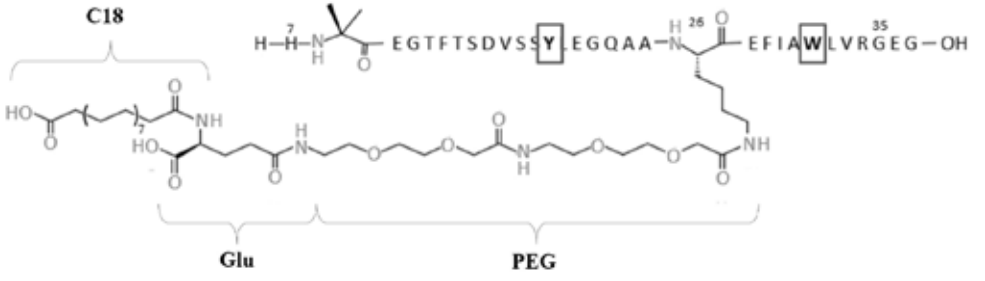

Figure 1. Molecular formula of Semaglutide

We applied steady-state and time-resolved fluorescence techniques to study the aggregation properties of Semaglutide, determining its critical aggregation concentration (20 μM) and the hydrodynamic radius of the aggregates it forms in aqueous solution at micromolar concentrations.
We also found that a very slow process leads to a morphological rearrangement of the Semaglutide aggregates, characterized by the emergence of a long-wavelength fluorescence emission.
The origin of this emission, already reported in the literature and tentatively assigned to the formation of peptide mesoscopic structures, is at present under intense scrutiny in our laboratory.

Structural and functional characterization of microbial transglutaminase in denaturing conditions

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Transglutaminases (TG) catalyze transglutamination reactions on glutamines.[1-3] These enzymes and in particular the microbial TG (MTG), are largely exploited for modifying proteins in food and other biotechnological applications.[1-3] They can also be used in the pharmaceutical field for the site-specific modification of proteins and peptides to introduce agents like PEGs that can make them more soluble in water and protect from protease degradation and undesired immune responses.[1-3] Moreover, they can be used for protein labelling to introduce fluorescent probes, radionuclides, chelating agents for diagnostics and therapy, or functional molecules such as DNA and lipid fragments, or chemical groups for the immobilization on solid supports.[1-3] Therefore, the optimization of experimental reaction conditions for the MTG-mediated chemical modification of macromolecules, such as proteins and/or nucleic acids, for theranostic purposes, is gaining an ever increasing interest. In this frame, we have performed a structural and functional characterization of MTG, in different conditions of pH and in the presence of different chaotropic agents, such as urea and guanidinium chloride. The structural stability of MTG has been evaluated by means of Circular Dichroism (CD) and fluorescence measurements and the ability to perform transglutamination reactions between peptide substrates has been evaluated in the different conditions. Data show that the enzyme is able to properly work also under extreme conditions but with less favourable kinetics and reduced selectivity.
Information obtained in this study are very useful for expanding the application of MTG to the derivatization of relevant therapeutic proteins and peptide that are normally unreactive toward the enzyme.

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Site-specific modification of antibody fragments by microbial transglutaminase

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Antibody engineering technologies are constantly advancing to improve the clinical effectiveness of monoclonal antibodies (mAbs), antibody fragments (monomeric and dimeric Fab) and antibody's surrogates.[1] Among these strategies, the enzymatic modification of antibodies permits a more precise control of conjugation reactions compared to chemical approaches mostly based on different reactivity of amino acid side chains.[2] As an example, we have set up a procedure for the site-specific derivatization of monomeric fragments (Fab') of Herceptin antibody using a mixed chemical-enzymatic approach based on the use of microbial transglutaminase (MTG). Transglutaminases are a family of proteins that catalyze the formation of a stable isopeptide bond between the γ-carboxyamide group (acyl donor) of a glutamine and the ε-amino group (acyl acceptor) of a lysine.[3]
The approach is based on alkylation of the C-terminal hinge cysteines generated by pepsin fragmentation with ad-hoc designed short peptides containing on one end a bromine atom and on the other a consensus sequence[4] specifically recognized by MTG to perform a transglutamination reaction. The consensus sequence can be easily conjugated with any tag containing a lysine and a new reactive group for orthogonal chemical reactions or a reporter molecule for site specific Fab' labelling. Products obtained have been characterized by SDS-PAGE and LC-MS to assess purity and identity. Moreover, the binding affinity of derivatized Fab' to the recombinant Her2 has been determined by ELISA assays and compared to that of the intact Herceptin antibody. The optimized protocol can provides about 60÷70% of derivatized full active antibody fragment.

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Influence of metal ions on folding mechanism and self-association propensity of high homologous proteins

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Using three isostructural proteins of the prokaryotic zinc finger family as model systems ($Ml_{452-151}$ lacking zinc binding and $Ml_{153-149}$ and Ros87 that bind a structural metal ion), we aim at contributing to the knowledge about the mechanisms by which metal ions perturb proteins structure and function, folding mechanism and self-association propensities[1,2]. The prokaryotic zinc finger domain[3] shows a $\beta\beta\beta\alpha\alpha$ globular fold that, while including a $\beta\beta\alpha$ motif similar to the eukaryotic domain, is stabilized by an extensive hydrophobic core of 15 amino acids and uses different combinations of amino acids to coordinate the structural metal ion when present[4]. We will discuss how the presence of structural metal can influence structure and function of this domain and how the metal recruitment can modify the folding pathway of these relatively small domains, control conformational accessibility to aggregation-prone states and change aggregation kinetics. While these model domains have little direct disease-relevance, implications of our findings should be of broad general interest as many disease-relevant proteins bind metal ions, which could similarly influence their structures, folding pathways and aggregation.

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Synthesis and conformational investigation of hetero-chiral sequential oligopeptides based on the (αMe)Aze/Ala dyad

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Conformationally restricted α-amino acids are able to promote specific main-chain conformations in peptides. One of them, (S)-C^{α}-methyl azetidine-2-carboxylic acid [(S)-(αMe)Aze], when combined in *homo-chiral* dipeptide sequences with (S)-Ala, is known to produce multiple γ-turns.[1] Recently, some of us developed a practical synthesis of both (αMe)Aze enantiomers.[2] By use of (R)-(αMe)Aze we also synthesized the hetero-chiral sequential oligopeptide series listed below.
In a recent 3D-structural study, we showed that in the crystal state the hetero-chiral tripeptide adopts a regular type-II β-turn (Figure 1). This unexpected outcome seems to indicate that sequence chirality might be a dominant feature in directing type of backbone folding in these sequential oligopeptide series. This conformational discrepancy between hetero- and homo-chiral peptide series appears to be confirmed by our initial investigation in solution by use of FT-IR absorption, NMR, and CD spectroscopies.

Boc-[(*S*)-Ala-(*R*)-(αMe)Aze]$_n$-OMe (n = 1-3)
Boc-[(*R*)-(αMe)Aze-(*S*)-Ala]n-OMe (n = 2,3)
Boc-[(*S*)-Ala-(*R*)-(αMe)Aze]n-(*S*)-Ala-OMe (n = 1,2)

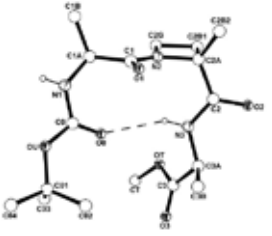

Figure 1. X-Ray diffraction structure of Boc-(*S*)-Ala-(*R*)-(αMe)Aze-(*S*)-Ala-OMe

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Antibody Epitope of human α-Galactosidase A revealed by affinity-mass spectrometry

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Alpha-galactosidase (αGal) is a lysosomal enzyme that hydrolyses the alpha-galactosyl moiety from glycosphingo-lipids. Mutations in the αGal genes lead to defect enzymes resulting in substrate accumulation and pathophysiology. The deficiency of αGal, called Fabry's Disease (FD), belongs to the lysosomal storage diseases. Effective treatment of FD has been developed by enzyme replacement therapy (ERT) by infusion of recombinant enzyme to increase enzyme levels and reduce accumulated substrate. Immuno-reactivity and IgG antibody formation are major, therapy-limiting complications of ERT. Here we report the antibody epitope identification of human αGal, αGal(309-332), using a combination of proteolytic excision of the immobilized immune complex. The epitope peptide, αGal(309-332) was synthesized by solid-phase peptide synthesis; its affinity was determined by SPR (KD, 39 x 10-9 M) nearly identical to that of the full length enzyme (KD, 16 x 10-9 M). Proteolytic excision- mass spectrometry is shown to be an efficient tool for epitope identification of immunogenic lysosomal enzymes. Since the full length enzyme and the antibody epitope showed comparable binding affinities, this provides a basis for reversing immunogenicity.

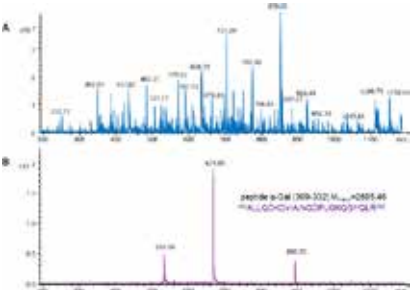

Figure 1. ESI-Ion Trap mass spectrum of supernatant fraction after tryptic digestion (A) and mass spectrum of epitope fraction (B)

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Novel foot-and-mouth disease vaccine platforms based on the successful B_2T prototype: exploring multivalency and enhancing efficacy

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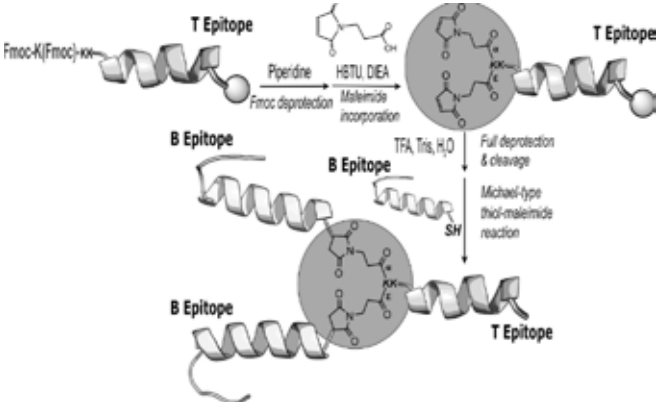
Classical vaccines against foot-and-mouth disease virus (FMDV) based on inactivated virus, while remaining the standard of care, have shortcomings such as the need for cold storage/shipping, lack of cross-reactivity among serotypes, or the risk that mishandling the highly infectious agent may cause its release to the environment with potentially catastrophic outcomes. Many such limitations are avoided in (subunit) vaccines devoid of infectious agent, among which those based on synthetic peptides are particularly relevant here. We have developed a peptide-based construction (B_2T) combining 2 and 1 copies, respectively, of B- and T-cell FMDV peptide epitopes into a single molecular platform. The resulting vaccine candidate conferred full protection of swine against virus challenge[1]. Our attention remains focused on B_2T-based constructs, with a view to addressing issues such as single dose administration (no boost), lasting immunity, multivalency, etc. In this communication we will describe our progress towards such goals. Synthetic strategy and experimental data will be reported, including specific details (cleavage, maleimide incorporation, thiol-ene, azide-alkyne couplings, side reactions, etc.).



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Use of peptides mimetics of proteins for characterisation of immune response in different pathological conditions: a powerful approach

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Peptides are a powerful tool for the identification of antibodies and for the study of chemical and biological mechanisms involved in several disorders. We present the synthesis and the screening of different libraries of peptides related to some specific diseases for in-depth studies of these pathological conditions. In particular:
Type 1 Diabetes: our data show a cross-reactivity of T1D and LADA autoantibodies in patient sera against two *h*GAD-deriving peptides and one Coxsackievirus-deriving peptide[1]. These results suggest a possible correlation between viral infections and the onset of T1D.
Psoriasis: we preliminarily highlighted a specific interaction between autoantibodies in psoriatic arthritis and rheumatoid arthritis patient sera and the endogenous antimicrobial peptide LL37[2].
Fabry disease: through affinity measures we successfully identified a promising alpha-galactosidase epitope involved in the autoimmune mechanism which lead to the Fabry disease[3].
Rett syndrome: our preliminary data show a correlation between hyperglucosylated adhesin and Rett syndrome, suggesting a plausible involvement of *Haemophilus influenzae* in its pathogenesis[4].
Allergic reactions to antibiotics: We synthesized and tested selected peptides derived from albumin (HSA), modified with penicillin G or amoxicillin, obtaining preliminary results about the possible role of aberrant modifications in allergic reactions to antibiotics[5].

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A study of mAb 3D1-Nodal peptides interaction by STD NMR spectroscopy

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Nodal is a member of the transforming growth factor beta (TGF-β) superfamily expressed during early embryonic development, but reactivated in several advanced-stage cancers. Thus, the presence of Nodal in cancer cells and its absence in normal tissues, makes Nodal an ideal biomarker and a potential antitumor target for inhibiting compounds. The correlation between the inhibition of Nodal signaling and the decrease of aggressive cancer cell features has been well established both in vitro and in vivo, as recently reviewed [1]. Canonical Nodal signaling occurs via binding to a receptor complex consisting of the Cripto-1 co-receptor and type I (ALK4/7) and type II (ActRIIB) activin-like kinase receptors. The Nodal binding region to Cripto-1 was identified in the sequence 44-67, which include the pre-helix loop and the H3 helix of Nodal [2]. This Nodal fragment was targeted with a high affinity monoclonal antibody (mAb), namely 3D1 [3, 4].

In order to define the antibody-antigen recognition at atomic level, an epitope mapping analysis was carried out by performing a series of saturation transfer difference (STD)-NMR experiments that involved 3D1 mAb with the native sequence Nodal44-67wt and some mutants, together with the two fragments reproducing only the pre-helix (Nodal44-56) and helix (Nodal53-67) regions [5].

Our results delineate the antibody-antigen contact atoms and confirm the information previously obtained at lower peptide concentrations by immune-enzymatic methods i.e. the minimal epitope recognized by 3D1 encompasses the region 46-52, corresponding to the pre-helix loop of Nodal.

These structural information can greatly help in the designing of more specific and improved mAbs for the inhibition of Nodal:Cripto-1 in therapeutic settings for cancers.

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PEPTIDE SHOWCASE

Acetylation in Fmoc-SPPS

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Acetylation steps in solid-phase peptide synthesis (SPPS) are considered common practice in the scientific community as a means to overcome the formation of deletion sequences, caused by incomplete couplings during individual coupling cycles. Certain manufacturing processes stipulate routine acetylation (aka routine capping) as part of each single synthesis cycle in order to increase crude product quality and/or to gain additional insight into the synthesis of peptides that are difficult to access, especially during early development phases. Acetic anhydride is the reagent of choice that is typically used for such purposes. In this presentation, recommendations are given on how to utilize such procedures. All advantages notwithstanding, the introduction of acetic acid related reagents into SPPS processes bears the risk of undesired acetylations, typically accompanied with yield loss. Strategies to prevent such side reactions will be presented accordingly.

Solid Phase Peptide Synthesis (SPPS) at Elevated Temperatures: Advances, Process Development, and Considerations

Giorgio Marini

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

Our previous research improved coupling efficiency and speed. The result, difficult and long sequences are effectively synthesized in a fraction of the time using much less solvent [1]. Advances have been made which further reduce the cycle time to under 4 min, offer an overall solvent reduction greater than 90 % compared to other SPPS processes, and are readily scalable to generate up to 200 mg purified peptide in a single run. As an example, a 20mer at 0.3 mmol scale, can be synthesized in little as an hour. This unique chemistry, which is ideal and readily applicable for developing peptide vaccines for personalized medicine, will be discussed.

Rapid scale-up for clinical trials and peptide production has been accomplished using similar technology. Crude purity from R&D to production scale is preserved if not improved and unwanted side reactions such as epimerization and aspartimide formation are easily controlled. The result, easier purification and reduced labor cost. Cycle times at the production scale range from 10 – 60 min with the capability to produce up to 1 KG crude peptide in a single batch. Several examples, including process development, will be presented.

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The antimicrobial peptide SET-M33. A case of peptide-based drug development

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The peptide SET-M33 is currently under preclinical development for the set up of a new antibacterial agent against the most important Gram-negative pathogens.[1] It is a synthetic molecule produced in tetra-branched form, that makes the peptide particularly stable for in vivo use. Its mechanism of action involves LPS binding and membrane permeation.[2] In in-vivo models of P. aeruginosa infections the peptide enabled a survival percentage of 60-80% in sepsis and lung infections when injected i.v. or by nebulization.

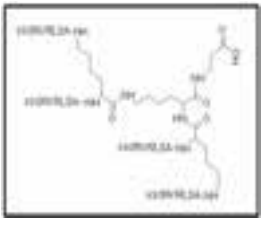

Figure. The tetra-branched peptide SET-M33

The peptide is also able to neutralize LPS thus inhibiting the expression of inflammatory cytokines. This produces a strong anti-inflammatory effect as demonstrated in vivo in models of pulmonary infections. [3] Plasma clearance, biodistribution, acute toxicity, synergistic activity with traditional drugs,[4] and resistance selection profiles in comparison with molecules already used in the clinical practice, have been evaluated. The conjugation of SET-M33 to nanoparticles based on different carriers (dextran, poly-lactide-co-glycolide, and others) is under evaluation for the improved delivery and slow release of the molecule administered by aerosol or systemically. Preclinical tests including ADME, safety pharmacology and manufacturing processes are in the last stages of development, thus SET-M33 is expected to enter into clinical trials in the next 18 months.

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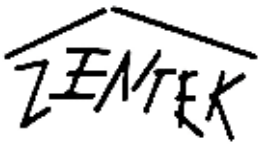

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