15º Encontro Peptídico Ibérico
15th Iberian Peptide Meeting

10-12 February 2016

Faculdade de Ciências
Universidade do Porto
Porto • Portugal

Book of Abstracts

15th Iberian Peptide Meeting
15º Encontro Peptídico Ibérico

"Peptides: a world of possibilities"

Book of Abstracts

10th-12th February 2016, Porto, Portugal

Book of abstracts of the 15th Iberian Peptide Meeting
XV EPI – 10th-12th February 2016, Porto, Portugal
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Foreword

Peptides are increasingly relevant in many areas of industrial and academic R&D, from molecular biology to materials science, from synthetic chemistry to computational and structural biology, from food science to biotechnology, from early drug discovery to clinics… in summary, there seems to be no shortage in sight for the potential of peptides in science and technology. Peptide scientists in Iberia have been aware of these realities and challenges for at least three decades now.

In April 1988 several scientists from Spain (F. Albericio, D. Andreu, M. T. García-López, R. González-Muñiz, A. Negro, E. Nicolás) coincided at an Euchem meeting on Peptides in Port Camargue, near Montpellier. Over lunch and a few nightcaps shared with H. Maia, a chemistry professor from Braga, Portugal, they decided it was high time to have a meeting of peptide scientists in the Iberian Peninsula. They agreed that such a meeting would, at least for a start, skip a high-profile format and favor instead an inclusive formula that gave graduate students and postdocs a chance to sharpen their presentation skills before familiar, well-disposed listeners rather than the imposing audiences of larger meetings. Albericio and Andreu volunteered to organize the first edition, which was held the next fall in the relatively spartan settings of a youth hostel in Cabrera de Mar, on the coast north of Barcelona. With participants from Barcelona, Braga, Girona, Madrid and Valencia in an encouraging turnout, a second edition was planned for the following year in the Madrid area, organized by researchers from the University of Madrid and the CSIC. Then came meetings in León (1991), Gandía (1993)… and the rest, as it is said, is history (see table on the next page). From those modest beginnings, EPI (Encuentro Peptídico Ibérico, Iberian Peptide Meeting) has grown over three decades as the emblematic showcase for an entire generation of peptide-focused scientists from Portugal and Spain to divulge results, weave alliances and, more importantly, build up friendships. And friends outside the Iberian Peninsula eventually came aboard.

In 2016, the spirit of the XV EPI remains the same. All in all, we are thrilled to have you with us in Porto to enjoy an informal and stimulating atmosphere, gathering from senior scientists to undergraduate students to share their perspectives and experiences in Peptide Science.

WELCOME!

The organizing committee of the XV EPI

Iberian Peptide Meetings 1988-2016

Edition	Year	Venue	Chair(s)
1	1988	Cabrera de Mar	F. Albericio, D. Andreu
2	1989	Cercedilla	J. P. Albar, M. Bruix, J. V. Sinisterra
3	1991	León	A. Negro
4	1993	Gandía	B. Celda
5	1996	Girona	E. Bardají
6	1998	El Escorial	M. T. García-López, R. González-Muñiz, R. Herranz
7	2000	Valencia	E. Pérez-Payá
8	2002	Barcelona	M. Royo, E. Nicolás, I. Haro
9	2004	Porto	H. Maia
10	2006	Zaragoza	C. Cativiela, A. I. Jiménez
11	2008	Santiago de Compostela	J. L. Mascareñas, M. E. Vázquez
12	2010	Lisboa	M. Castanho, N. Santos
13	2012	Alicante	J. Villalaín, A. Ferrer-Montiel
14	2014	Bilbao	O. Millet
15	2016	Porto	P. Gomes

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University of Santiago de Compostela, Spain

SCIENTIFIC PROGRAM

Wednesday, February 10th

09:00-09:30 h – Registration

09:30-10:00 h – OPENING SESSION

Session 1

Chair: Oscar Millet

10:00-10:45 h – PLENARY LECTURE: Dek Woolfson, University of Bristol, UK
Using designed peptide modules to move into unexplored regions of protein structure/function space

10:45-11:15 h – COFFEE BREAK

11:15-12:30 h – ORAL COMMUNICATIONS

OC 1 - H. Zamora-Carreras: Exploring the chameleonic behaviour of peptides derived from the choline-binding domain of pneumococcal autolysin LytA

OC 2 - Carlos Serpa: Following pH jump-induced alpha-helix peptide (un)folding dynamics

OC 3 - Miquel Adrover: A detailed portrait of the folding pathway and dynamical behavior of neuromedin C

OC 4 - André Faustino: Conformational changes governing dengue virus capsid protein disordered *N*-terminal region and its inhibition by pep14-23

OC 5 - Jéssica Rodríguez: The AT-hook motif as minor groove anchor for synthetic DNA binders

12:30-14:00 h – LUNCH BREAK

Session 2

Chair: M. Cristina L. Martins

14:00-14:30 h – KEYNOTE LECTURE: Helena S. Azevedo, Queen Mary University of London, UK
The use of peptides for self-assembling biomaterials: instructive building blocks for constructing complexity and functionality in biomaterials

14:30-15:45 h – ORAL COMMUNICATIONS

OC 6 - Sofia C. Ribeiro: Peptide design for interfacial self-assembly of biomaterials for bone regeneration

OC 7 - Krystyna Duncan: Discovery of catalytic peptides via biocatalytic self-assembly

OC 8 - Fabíola Costa: Impact of different immobilization parameters on the immobilized antimicrobial peptide (Dhvar5) antibacterial activity

OC 9 - Ana S. Pina: A nanoscale magnetic antimicrobial platform

OC 10 - Akhilesh Rai: Design of potent antimicrobial and biocompatible surface using antimicrobial peptide

15:45-16:15 h – COFFEE BREAK

Session 3

Chair: David Andreu

16:15-16:45 h – KEYNOTE LECTURE: Meritxell Teixidó, Barcelona Institute for Science and Technology, Spain
Peptide nanoambulances for CNS drug delivery

16:45-18:15 h – ORAL COMMUNICATIONS

OC 11 - Vera Neves: A novel peptide platform derived from Dengue virus for drug delivery into the brain

OC 12 - P. Arranz-Gibert: Breaking barriers – the challenge of Blood-Brain Barrier (BBB) shuttles as therapeutic agents: gene therapy for Friedreich's ataxia

OC 13 - Patrícia Carvalho: Maximizing biomolecules signal detection for study of single protein-ligand interaction events

OC 14 - Armanda Santos: New BACE1 inhibitors for Alzheimer's disease treatment: evaluation of their eficacy in *in vitro* and *in vivo* models

OC 15 – Guillem Vazquez: Multifunctional short peptides for the design of anti-Alzheimer therapeutics

18:15-18:35 h – CEM LECTURE: Giorgio Marini, CEM Corporation
An improved coupling method for peptide synthesis at elevated temperature

18:35-19:15 h – WELCOME RECEPTION ("Porto de Honra")

19:15-20:00 h – Meeting of the EPI Steering Committee

Thursday, February 11th

Session 4

Chair: Rosario González-Muñiz

09:15-10:00 h – PLENARY LECTURE: Gilles Subra, University of Montpellier, France
Crafting new materials and polymers with hybrid peptide building blocks

10:00-10:45 h – ORAL COMMUNICATIONS

OC 16 - Jordi Solà: Dynamic pseudopeptides: generation of structural diversity inspired by Nature

OC 17 - Maria Lafuente: Adaptive processes in a topologically diverse dynamic library of pseudopeptides

OC 18 - Alexandra Plácido: Bioactive peptides of the Cry1Ab16 toxin from *Bacillus thuringiensis* by nanodevices films for potential biotechnological applications

10:45-11:15 h – COFFEE BREAK

Session 5

Chair: M. Eugenio Vázquez

11:15-12:30 h – ORAL COMMUNICATIONS

OC 19 - Carina Carvalho: Permeation of model membranes by Peptaibolin mimetics bearing different α,α-dialkylglycines

OC 20 - E. Fajardo-Sánchez: Dengue virus in the spotlight: capsid peptide binds specifically to a bilayer membrane model by Molecular Dynamics

OC 21 - Diana Lousa: When simulation and experiment fuse: analysing the interaction of the influenza fusion peptide with model membranes

OC 22 - Cláudia Fernandes: Selection of peptides for the efficient and mild affinity purification of retroviral particles

OC 23 - Marta Planas: Antimicrobial cyclic lipopeptides as agents for plant protection

12:30-14:00 h – LUNCH BREAK

Session 6

Chair: Susana Costa

14:00-14:30 h – KEYNOTE LECTURE: Miguel Vázquez, University of Santiago de Compostela, Spain
Bioactive metallopeptides derived from 2,2'-bipyridine

14:30-15:45 h – ORAL COMMUNICATIONS

OC 24 - Omar Brun: Selective derivatization of peptides containing *N*-terminal cysteines using 2,2-disubstituted cyclopent-4-en-1,3-diones

OC 25 - Rita Fernandes: Fluorine in peptide and protein engineering

OC 26 - J. Palà-Pujadas: Towards the chemical synthesis of the signaling protein Sonic Hedgehog

OC 27 - C. Díaz-Perlas: Total chemical synthesis of D-epidermal growth factor

OC 28 - Sira Defaus: Synthetic peptide vaccines against foot-and-mouth disease: success at last

15:45-16:15 h – COFFEE BREAK

Session 7

Chair: Nuno Santos

16:15-16:45 h – KEYNOTE LECTURE: Diana Gaspar, University of Lisbon, Portugal
Improving cancer therapy with anticancer peptides

16:45-18:15 h – ORAL COMMUNICATIONS

OC 29 - Filipe Vultos: In-111 labeled peptides towards the estrogen receptor for theranostic of breast cancer

OC 30 - Salvador Guardiola: Design, synthesis and biophysical study of peptide ligands targeting epidermal growth factor (EGF)

OC 31 - Célia Fernandes: Novel radiopeptides for molecular imaging of EGFR positive tumors

OC 32 - Anna Escolà: New insights into the design of somatostatin analogs: effect of the aromatic interaction into its 3D structure

OC 33 - Abigail Ferreira: Development of new peptide-gemcitabine conjugates for cancer therapy

19:15 h – MEETING DINNER @ CASA DA MÚSICA

Friday, February 12th

Session 8

Chair: Miguel Castanho

09:00-10:45 h – ORAL COMMUNICATIONS

OC 34 - J. García-Pindado: Bike peptides, a ride through the membrane

OC 35 - Cláudia Monteiro: Antimicrobial properties of short peptides derived from MSI-78

OC 36 - Mário Felício: Structure studies and mechanisms of action insights of two novel antimicrobial peptides

OC 37 - Carla Sousa: Ocellatins-PT antimicrobial peptides: structure, characterization, membrane interactions and anti-*Leishmania* activity

OC 38 - A. Revuelto-Pérez: A proteomimetic approach to disrupt protein-protein interactions of trypanothione reductase of *Leishmania infantum*

OC 39 - Pedro J. B. Pereira: The role of post-translational modifications on the activity of a specific thrombin inhibitor

OC 40 - Daniel Pulido: Peptide-targeted drug delivery systems of α-galactosidase A for the treatment of Fabry disease

10:45-11:15 h – COFFEE BREAK

Session 9

Chair: Miriam Royo

11:15-12:00 h – ORAL COMMUNICATIONS

OC 41 - C. Pérez-Peinado: Rational dissection of the rattlesnake peptide crotalicidin retrieves a fragment with enhanced antimicrobial and antitumor properties

OC 42 - Tiago Figueira: Quantitative characterization of peptide-lipid partition using surface plasmon resonance

OC 43 - Catarina Morais: Interaction of acylated $S4_{13}$-PV analogs with lipid membranes

12:00-12:45 h – PLENARY LECTURE: Margus Pooga, University of Tartu, Estonia
From cell penetration of transportan to receptor-mediated internalization of peptide nanoparticles

12:45-13:00 h – CLOSING SESSION

PLENARY SPEAKERS

Dek Woolfson

Professor Dek Woolfson took his first degree in Chemistry at the University of Oxford, UK. He then did a PhD at the University of Cambridge followed by post-doctoral research at University College London and the University of California, Berkeley. After 10 years as Lecturer through to Professor of Biochemistry at the University of Sussex, he moved to the University of Bristol in 2005 to take up a joint chair in Chemistry and Biochemistry.

Prof. Woolfson's research has always been at the interface between chemistry and biology, applying chemical methods and principles to understand biological phenomena. Specifically, his group is interested in the challenge of rational protein design, and how this can be applied in synthetic biology and biotechnology. His particular emphasis is on making completely new protein structures and biomaterials for applications in cell biology and medicine.

In 2011, Prof. Woolfson became the first recipient of the Medimmune Protein and Peptide Science Award of the Royal Society of Chemistry; and in 2014 he received a Royal Society Wolfson Research Merit Award. Prof. Woolfson is also Director of BrisSynBio, a £13.6M BBSRC/EPSRC-funded Synthetic Biology Research Centre.

Gilles Subra

Professor Gilles Subra graduated as a chemical engineer in 1994 from the Ecole Nationale Supérieure de Chimie de Montpellier (France). In 1999, he received his Ph.D. from Montpellier University under the supervision of Professor Jean Martinez in the field of combinatorial synthesis of libraries of α-MSH antagonists. He pursued his studies as an Institut Henri Beaufour postdoctoral fellow and set up the automated synthesis and analytical facility in Faculty of Pharmacy as a tool for the discovery of new neuropeptides.

In 2011, he obtained a full Professor position at the University of Montpellier and is currently working in Max Mousseron Institute for Biomolecules (IBMM). His research topics, situated at the interface of chemistry, biology and analytical science, include supported synthesis, design of chemical tools for improving detection of biomolecules by mass spectrometry and peptide-based polymers and materials with a special interest in hybrid bioorganic/inorganic materials.

Margus Pooga

Professor Margus Pooga graduated from University of Tartu with diploma in organic chemistry, and received PhD in biochemistry in 1998. After graduation he was employed at the Institute of Chemical Physics and Biophysics, and Estonian Biocentre up to 2005. In parallel, he worked with Professors T. Bartfai and Ü. Langel at Department of Neurochemistry of Stockholm University as a visiting scientist. In 2005 M. Pooga joined University of Tartu as senior researcher and was promoted to professorship in 2008.

M. Pooga is currently Professor of Chemical Biology, and Head of Department of Developmental Biology at the Institute of Molecular and Cell Biology, University of Tartu, Estonia.

Professor Pooga's research is focused on cell-penetrating peptides (CPP). He has participated in design of new CPPs, and his group has studied the mechanisms of CPPs, and CPP-mediated delivery of proteins and nucleic acids to cultured cells. His lab is also working on applications of CPP-assisted delivery of miRNA and siRNA to cells in culture and in animal models.

KEYNOTE SPEAKERS

Helena Azevedo

Professor Helena Azevedo received a MEng degree in Biological Engineering from the University of Minho (Portugal) and a Ph.D. from De Montfort University (UK). Then, she joined the 3B's Research Group at University of Minho as a postdoctoral researcher. In 2006, she was awarded with a Marie Curie fellowship which allowed her to work in the laboratory of Prof. Stupp at the Institute for BioNanotechnology in Medicine (Northwestern University, USA). From 2008-2013 she had a Research position at the 3B's Research Group as a principal investigator.

In 2013 she joined the School of Engineering and Materials Science at Queen Mary University of London (QMUL) as Senior Lecturer in Biomedical Engineering & Biomaterials where she is leading her own research group (http://www.sems.qmul.ac.uk/staff/?h.azevedo). Recently, she has been appointed Director of Operations of the new Institute of Bioengineering at QMUL. Her work focuses on self-assembling hyaluronan-peptide biomaterials for drug delivery, regenerative medicine, and biosensing.

Meritxell Teixidó

Dr. Meritxell Teixidó is Research Associate in the Design, Synthesis and Structure of Peptides and Proteins Group at the Institute for Research in Biomedicine in Barcelona, Spain. Her major interest lies in the field of peptide synthesis and the discovery of blood-brain barrier peptidic shuttles, in particular using these shuttles to deliver drugs, diagnostic agents and nanoparticles that otherwise cannot reach their target site inside the CNS unaided. In this regard, The research combines protease-resistant peptides, mass spectrometry techniques, and transport evaluation tools to achieve delivery systems of this nature. She has published around 30 papers and review articles and participated in 6 patents.

Since 2010, Dr. Teixidó has served as the Spanish national representative in the European Peptide Society. She has received scientific awards, such as the Bert L. Schram Award (ESCOM Science Foundation) for the most relevant poster presentation in drug discovery at the 29th European Peptide Symposium (2006), and the American Peptide Idol first prize at the 20th American Peptide Symposium (2007), Montreal, Canada. During her career, Meritxell Teixidó has participated in numerous research projects funded by national and international agencies. Likewise, she has taken part in long-term industrial collaborations with private funding (Laboratories Menarini, Farmhispania, Cancer Research Technology Ltd., Zyentia Ltd., Bioingenium).

Miguel Vázquez

Professor Miguel Vázquez López graduated in Chemistry in 1996 from the Universidad de Santiago de Compostela (USC), and obtained his PhD under the supervision of Prof. Manuel R. Bermejo, working on supramolecular helicates. In 2001 he joined Prof. Dante Gatteschi at the Università di Firenze (Florence, Italy), where he worked for six months in the study of the magnetic properties of metallosupramolecular assemblies. In 2002 he received a Marie Curie EU TMR fellowship and joined Prof. Luigi Fabbrizzi at the Università di Pavia (Pavia, Italy), where he worked for two years on the development of molecular sensors for anions and metallic ions.

In 2004 he joined the Department of Inorganic Chemistry of the Universidad de Vigo (Vigo, Spain) as a senior researcher, where he worked for two years on the development of coordination polymers. He returned to Santiago as a "Ramón y Cajal" researcher in 2006. In 2009 he made a short research stay of three month at the laboratory of Prof. Jean-Claude G. Bünzli at the Ècole Polytechnique Fédérale de Lausanne (Lausanne, Switzerland), where he worked on the development of NIR-luminiscent lanthanide coordination conjugates. He obtained his permanent position at the Inorganic Chemistry Department of the USC in 2011. In February 2011 he moved to the Center for Research in Chemical Biology and Molecular Materials (CIQUS) of the USC. He is author of more than 50 scientific papers in peer-reviewed international journals and board member of the Specialist Group of Chemical Biology of the Spanish Royal Society of Chemistry (RSEQ).

Diana Gaspar

Dr. Diana Gaspar graduated in Pharmaceutical Sciences from the University of Porto in 2006, and obtained her PhD degree in Pharmaceutical and Medicinal Chemistry at the University of Porto in 2010. Since 2011, she is a young researcher based at the Institute of Molecular Medicine (IMM Lisboa, Portugal), where she currently focuses on the study and development of biologically active peptides as new anticancer drugs. Her present research interests include also cell-to-cell physical and chemical communication in metastatic cancer combining a wide variety of experimental methodologies from biophysical to imaging techniques, such as atomic force microscopy (AFM).

In 2014 her work in biomechanics of breast cancer was awarded with the 2nd Edition of the Portuguese Breast Cancer Association - Associação Laço Research Grant 2014 for the study of breast cancer cells passage through the blood-brain-barrier and dissemination into the brain.

PLENARY LECTURES

PL 1 – Dek Woolfson
Using designed peptide modules to move into unexplored regions of protein structure/function space

PL 2 – Gilles Subra
Crafting new materials and polymers with hybrid peptide building blocks

PL 3 – Margus Pooga
From cell penetration of transportan to receptor-mediated internalization of peptide nanoparticles

PL 1 - Using designed peptide modules to move into unexplored regions of protein structure/function space

Dek Woolfson

Schools of Chemistry and Biochemistry, & BrisSynBio, University of Bristol, United Kingdom

Nature has almost certainly only shown us a glimpse of what is possible structurally and functionally with polypeptide chains. To help us explore past the confines of natural protein structures, and into what has been termed the *dark matter of protein space*, we have developed a toolkit of *de novo* peptides.[1] These can be used as building blocks for the rapid construction of new protein structures and supramolecular assemblies. This talk will demonstrate the utility of this approach to make nanoscale protein pores,[2,3] and peptide-based nanocages.[4] Potential applications of these structures and materials span nanoscience, synthetic biology and biotechnology.

References

1. J.M. Fletcher, A.L. Boyle, M. Bruning, G.J. Bartlett, T.L. Vincent, N.R. Zaccai, C.T. Armstrong, E.H.C. Bromley, P.J. Booth, R.L. Brady, A.R. Thomson, D.N. Woolfson. *ACS Synthetic Biology* **2012**, *6*, 240-250.
2. N.R. Zaccai, B. Chi, A.R. Thomson, A.L. Boyle, G.J. Bartlett, M. Bruning, N. Linden, R.B. Sessions, P.J. Booth, R.L. Brady, D.N. Woolfson. *Nature Chemical Biology* **2011**, *7*, 935-941.
3. A.R. Thomson, C.W. Wood, A.J. Burton, G.J. Bartlett, R.B. Sessions, R.L. Brady, D.N. Woolfson, *Science* **2014**, *346*, 485-488.
4. J.M. Fletcher, R.L. Harniman, F.R.H. Barnes, A.L. Boyle, A. Collins, J. Mantell, T.H. Sharp Antognozzi, P.J. Booth, N. Linden, M.J. Miles, R.B. Sessions, P. Verkade, D.N. Woolfson. *Science* **2013**, *340*, 595-599.

PL 2 - Crafting new materials and polymers with hybrid peptide building blocks

Jeremie Ciccione,[a,c] Cecile Echalier,[a,c] Coline Pinese,[a] Said Jebors,[a,c] Xavier Garric,[a] Muriel Amblard,[a] Vincent Humblot,[b] Tao Jia,[d] Jean-Luc Coll,[d] Ahmad Mehdi,[c] Jean Martinez,[a] Gilles Subra[a]

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Affording biological properties to a material is one of the challenges the chemists have to tackle, not only to improve the efficiency of existing devices such as implants, dressings, catheters, etc. but also to propose new solutions to unlock some limitations in medicine and biotechnology. Materials prepared from or modified by peptides are particularly attractive due to the nearly unlimited range of functionalities and bioactivities afforded by the peptide moiety. However, most of synthetic approaches to obtain functional materials rely on non-covalent coatings or post-grafting realized by multistep ligation chemistry. In this context, we envisioned a bottom-up strategy to introduce bioactive peptides in polymers or materials. The approach relies on the synthesis of hybrid blocks displaying one or several hydroxysilane groups.[1] The condensation of such moieties proceeds chemoselectively, at room temperature, in aqueous conditions and is compatible with the handling of complex biomolecules. Interestingly, any type of peptides but also biopolymers and dyes can be silylated and thus can participate to the covalent formation of the material. Grafting on surface of glass,[2] silica,[3] titanium and silicone can be performed straightforwardly but direct synthesis of three dimensional hybrid materials can be also operated in a single step by sol gel process.[4] Depending on the ratio, the position of the silane group(s), and the structure of the hybrid blocks, comb-like,[5] and linear polymers,[6] hydrogels, membranes, multifunctional nanoparticles and organized materials were obtained. Several applications will be presented during this lecture including cell-adhesive hydrogels, antibacterial silicone catheters and multi ligands nanoparticules for cancer imaging.

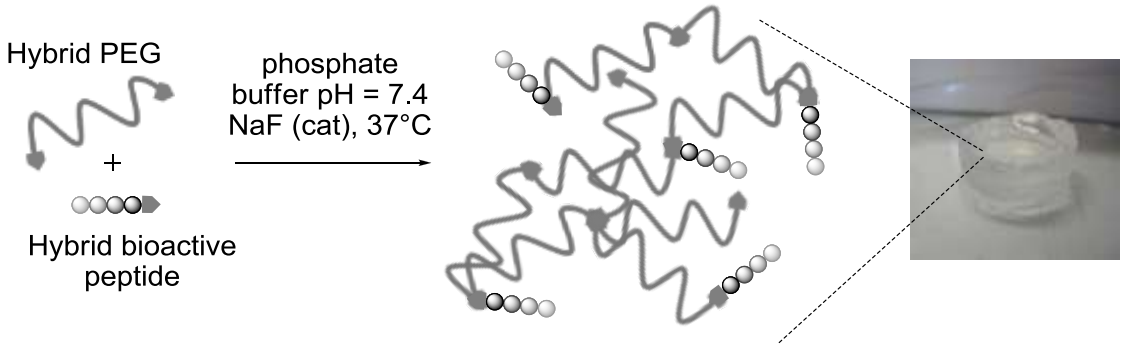

Figure 1. One step synthesis of peptide-PEG hydrogels.

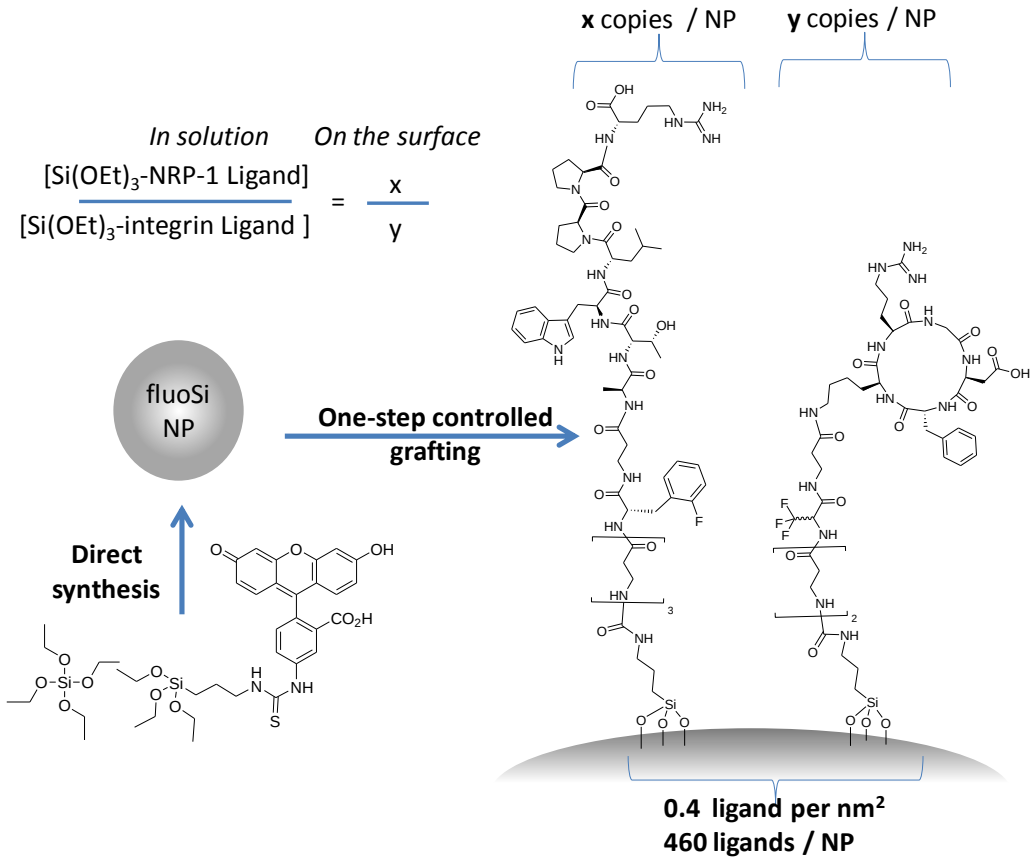

Figure 2. One step synthesis of multifunctional Si NPs.

References

1. J. Martinez, G. Subra, A. Mehdi, S. Jebors, C. Enjalbal, L. Brunel, *et al*. Materiaux hybrides peptide-silice, WO2013190148 A1, 2013.
2. S. Jebors, C. Enjalbal, M. Amblard, A. Mehdi, G. Subra, J. Martinez, *J. Mater. Chem. B* **2013**, *1*, 2921-2925.
3. S. Jebors, C. Enjalbal, M. Amblard, G. Subra, A. Mehdi, J. Martinez. *Tetrahedron* **2013**, *69*, 7670-7674.
4. S. Jebors, S. Cecillon, C. Faye, C. Enjalbal, M. Amblard, A. Mehdi, *et al*. *J. Mater. Chem. B* **2013**, *1*, 6510-6515.
5. S. Jebors, C. Pinese, B. Nottelet, K. Parra, M. Amblard, A. Mehdi, *et al*. *J. Pept. Sci*. **2015**, *21*, 243-247.
6. S. Jebors, J. Ciccione, S. Al-Halifa, B. Nottelet, C. Enjalbal, C. M'Kadmi, *et al*. *Angew. Chem. Int. Ed.* **2015**, *54*, 3778-3782.

PL 3 - From cell penetration of transportan to receptor-mediated internalization of peptide nanoparticles

Margus Pooga

Institute of Molecular and Cell Biology, University of Tartu, Estonia

Chimeric cell penetrating peptide (CPP) transportan (TP) was obtained by fusion of a neuropeptide fragment with mastoparan from wasp venom. Like other CPPs, TP can cross the barrier of biological membranes in model systems, but in order to gain access to the interior of living cells, it mainly harnesses endocytosis. Transportan and its shorter analogue TP10 are capable of delivering into cells various types of hydrophilic macromolecules, ranging from peptides and oligonucleotides to proteins and plasmids, and even viral particles. Transduction of selected cargoes to cells by transportans can be achieved by using either covalent coupling or non-covalent strategy, and the latter has turned out to be more efficacious. Novel TP10-based peptides of PepFect (PF) and NickFect (NF) family that were designed for cellular delivery of nucleic acids (NA), condense NA to uniform nanoparticle-like complexes with 30-80 nm in diameter. The formed nanoparticles are recognized by type A scavenger receptors (SR-A), which trigger their uptake by macropinocytosis and caveolin-mediated endocytosis. The inhibition of SR-A or their down-regulation diminishes cellular uptake of PF-NA nanoparticles and completely abolishes the activity of delivered nucleic acid. In cells PFs promote the liberation of nucleic acid cargo from entrapping endosomal vesicles to cytoplasm, and in case of splicing-switching oligonucleotides and pDNA, PepFects facilitate their translocation to cell nucleus. PepFects carry nucleic acids to their target sites also *in vivo*, e.g. delivery of miR146a mimic by PF6 alleviates inflammatory response in atopic dermatitis model in mice.

KEYNOTE LECTURES

KN 1 – Helena Azevedo
The use of peptides for self-assembling biomaterials: instructive building blocks for constructing complexity and functionality in biomaterials

KN 2 – Meritxell Teixidó
Peptide nanoambulances for CNS drug delivery

KN 3 – Miguel Vázquez
Bioactive metallopeptides derived from 2,2'-bipyridine

KN 4 – Diana Gaspar
Improving cancer therapy with anticancer peptides

KN 1 - The use of peptides for self-assembling biomaterials: instructive building blocks for constructing complexity and functionality in biomaterials

Helena S. Azevedo

School of Engineering & Materials Science and Institute of Bioengineering
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Biomaterials research has witnessed substantial progress in the last two decades and has shifted from bionert to smart bioactive biomaterials. Biomaterials are now designed rationally with controlled structure and dynamic functionality to integrate with native tissues and promote regeneration. Molecular self-assembly allows control of biomaterial properties from the molecular to the macro-scale (Figure 1), and this strategy is known as "bottom-up" approach.

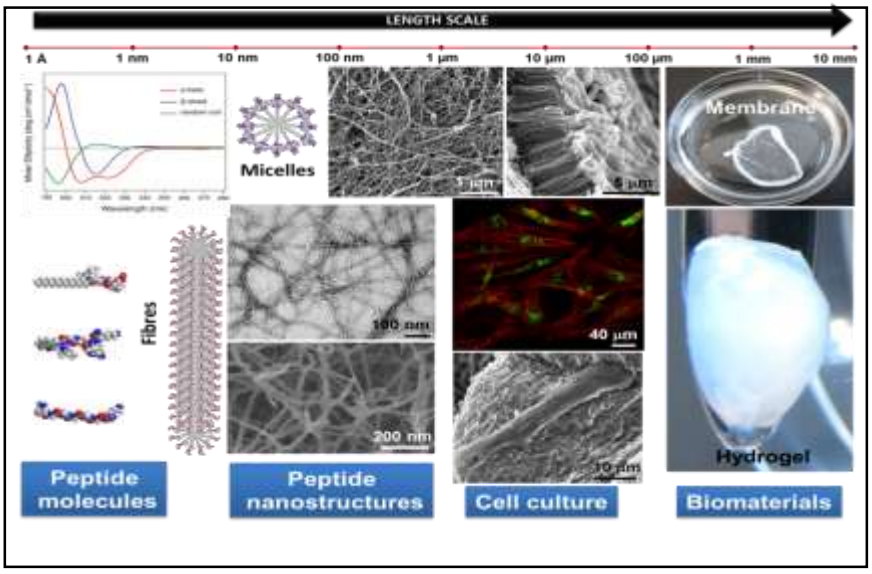

Figure 1. Bottom-up fabrication of biomaterials using self-assembling peptides.

In our group, we use biological inspiration and tools of chemistry, physics and engineering to develop biomaterials by self-assembly with particular properties and functions for applications in biomedicine. We have elected peptides as building blocks for self-assembly because they are readily accessible through chemical synthesis; the information required for their self-assembly is encoded within their sequence; their self-assembly is spontaneous (simple method to develop biomaterials), instantaneous (milliseconds) and reproducible (defined stable structures). Furthermore, by varying systematically the chemical structure (e.g. sequence size and nature of the amino acid side groups) during synthesis it is possible to adjust the self-assembling properties of the building blocks, as well as to produce a variety of diverse nanostructures. In this communication, we will present our "bottom-up" efforts to develop innovative biomaterials (membranes, capsules, hydrogels) designed for specific biomedical applications (drug delivery, cell culture).

References: D.S. Ferreira, Y.-A. Lin, H. Cui, J.A. Hubbell, R.L. Reis, H.S. Azevedo. Adv. Healthcare Mater. **2015**, *4*, 602-612; A.C. Mendes, K.H. Smith, E. Tejeda-Montes, E. Engel, R.L. Reis, H.S. Azevedo, A. Mata. Adv. Funct. Mater. **2013**, *23*, 430-438; D.S. Ferreira, A.P. Marques, R.L. Reis, H.S. Azevedo. *Biomaterials Sci.* **2013**, *1*, 952-964; D.S. Ferreira, R.L. Reis, H.S. Azevedo. *Soft Matter* **2013**, *9*, 9237-9248; A.C. Mendes, E.T. Baran, P. Lisboa, R.L. Reis, H.S. Azevedo. *Biomacromolecules* **2012**, *13*, 4039-4048; R.M. Capito, H.S. Azevedo, Y.S. Velichko, A. Mata, S.I. Stupp. *Science* **2008**, *319*, 1812-1816.

KN 2 - Peptide nanoambulances for CNS drug delivery

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The brain is protected by a barrier of cells that tightly regulates the transport of substances into this organ. The essential protective function of the blood-brain barrier (BBB) is also a red light for 98% of drug candidates for the treatment of the central nervous system (CNS). Over recent years we have worked extensively on the use of peptides as BBB-shuttles (or Nanoambulances) to carry drugs that cannot cross the BBB unaided and therefore cannot reach the CNS.[1]

Initially, we focused our efforts on passive diffusion peptide Nanoambulances.[2-5] In these studies, we achieved molecules with 2-4 amino acids that are efficient at carrying drugs such as L-dopa, baicalin, GABA, ... In some cases, the Nanoambulance plays a dual role and once inside the brain acts as an enzyme inhibitor.[6] In spite of their potential use for small molecules, passive diffusion shuttles have limitations for transporting macromolecules (proteins, mAbs, nanoparticles). This limitation prompted us more recently to focus on the use of peptides recognized by receptors as actively transported Nanoambulances.[7-10]

In this communication, we will present a set of protease-resistant peptides with the capacity to transport cargoes of distinct sizes and types across the BBB. In addition, these peptides do not show acute toxicity and their immunogenicity risk is low. Our Nanoambulances bring with them the possibility to fulfil an unmet clinical need, namely the treatment of CNS disorders.

References

1. M. Malakoutikhah *et al. Angew. Chem. Int. Ed*. **2011**, *50*, 7998-8014.
2. M. Teixidó *et al. J. Am. Chem. Soc.* **2007**, *129*, 11802-11813.
3. M. Malakoutikhah *et al. J. Med. Chem*. **2008**, *51*, 4884-4889.
4. M. Malakoutikhah *et al. J. Med. Chem.* **2010**, *53*, 2354-2363.
5. P. Arranz-Gibert *et al. J. Am. Chem. Soc.* **2015**, *137*, 7357-7364.
6. M. Teixidó *et al. Biopolymers- Pept. Sci.* **2013**, *100*, 662-674.
7. R. Prades *et al. Biomaterials* **2012**, *33*, 7194-7205.
8. B. Oller-Salvia *et al. Biopolymers – Pept. Sci.* **2013**, *100*, 675-686.
9. R. Prades *et al. Angew. Chem. Int. Ed*. **2015**, *54*, 3967-3972.
10. B. Oller-Salvia *et al. Angew. Chem. Int. Ed.* **2015**, DOI: 10,1002/anie:201508445.

KN 3 - Bioactive metallopeptides derived from 2,2'-bipyridine

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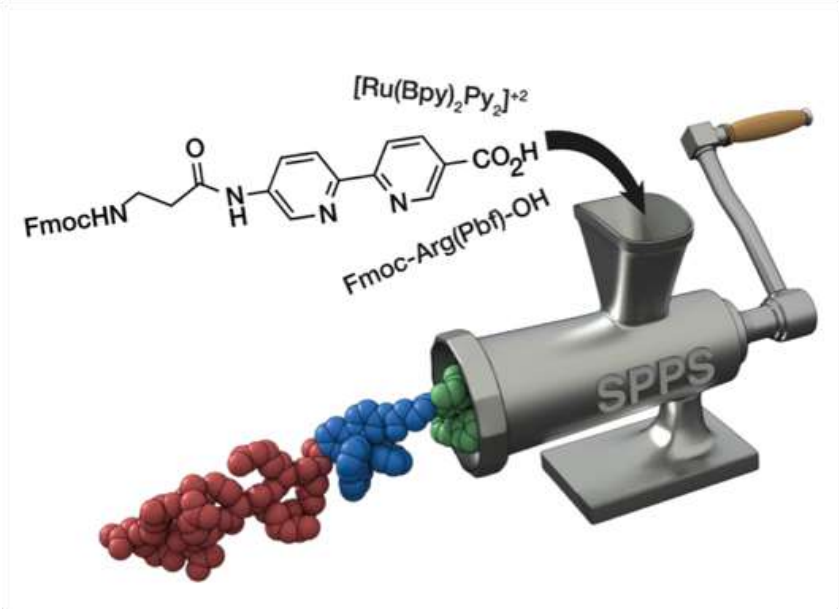
Chemical biology is an established research field that aims to the application of chemical tools and methodologies to the study and modification of complex biological systems. Despite its enormous development over the last decade, the contributions of coordination and metallosupramolecular chemistry in this area remain relatively scant. One possible reason for this underdevelopment is that the fundamental chemical differences between biomolecules and most ligands used in these areas hamper the integration of metal complexes into biological systems. Moreover, the stiffness and slowness of classical solution covalent chemistry, traditionally used to synthesize organic ligands, difficults quick and easy access to these molecules in order to make a thorough study to relate functionality and structure of their derived metal complexes. To bridge this gap we are working on the application of peptides derived from the classical chelate 2,2'-bipyridine as potential precursors of bioactive metal complexes. We present herein the main results of our research in this field.[1]



References

1. a) Rama, G.; Ardá, A.; Maréchal, J.-D.; Gamba, I.; Ishida, H.; Jiménez-Barbero, J.; Vázquez, M. E.; Vázquez López, M. *Chem. Eur. J*. **2012**, *18*, 7030-7034; b) Gamba, I; Salvadó, I.; Rama, G.; Bertazzon, M.; Sánchez, M. I.; Sánchez-Pedregal, V. M.; Martínez-Costas, J.; Brissos, R. F.; Gamez, P.; Mascareñas, J. M.; Vázquez López, M.; Vázquez, M. E. *Chem. Eur. J*. **2013**, *19*, 13369-13375; c) Gamba, I; Rama, G; Ortega-Carrasco, E.; Maréchal, J.-D.; Martínez-Costas, J.; Vázquez, M. E.; Vázquez López, M. *Chem. Commun*. **2014**, *50*, 11097-11100; d) Gamba, I.; Salvadó, I.; Brissos, R.F.; Gamez, P.; Brea, J.; Loza, M. I.; Vázquez, M. E.; Vázquez López, M. *Chem. Commun*. **2016**, *52*, 1234-1237; e) Gamba, I.; Rama, G.; Ortega-Carrasco, E.; Berardozzi, R.; Sánchez-Pedregal, V. M.; Di Bari, L.; Maréchal, J.-D.; Vázquez, M. E.; Vázquez López, M. *Dalton Trans*., **2016**, 45, 881-885.

KN 4 - Improving cancer therapy with anticancer peptides

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There have been important achievements in what concerns cancer chemotherapy, however the low levels of selectivity and efficacy of conventional treatments together with resistance development urges us to seek innovative therapeutic approaches that can increase lifetime and reduce suffering of oncologic patients. Although antimicrobial peptides (AMPs) have been essentially studied as potential alternatives for fighting infectious diseases, their use as anticancer peptides (ACPs) either alone or in combination with conventional drugs in cancer therapy has been regarded as a strategy worth exploring. As unique molecules, when compared to the actual chemotherapeutic arsenal available for cancer treatment, ACPs show a variety of modes of action that seem to co-exist in some types of cancer. Studies focused on ACPs' mechanisms of action are crucial for optimizing drug development, thus our group has been dedicated to this endeavor by studying the activity of either synthesized or natural ACPs using innovative combinations of biophysical techniques and seeking to reduce the uncertainty in the prediction of antitumor activity based on ACPs structures.

The human neutrophil peptide-1 (HNP-1) is an endogenous AMP implicated in different cellular phenomena such as tumor proliferation. The presence of HNP-1 in the serum/plasma of oncologic patients turns this peptide into a potential tumor biomarker. By monitoring cells' biophysical and nanomechanical properties of solid and hematological tumors in the presence of HNP-1, a mechanism of action for this particular anticancer agent was proposed. The experimental approach of this work relied on the combination of spectroscopic and atomic force microscopy (AFM) techniques for probing solid and non-solid tumor cells' viability and biomechanical properties before and after peptide contact. Flow cytometry, surface charge density and AFM studies reveal HNP-1 preferential activity towards solid tumors. The use of AFM in oncology allows using parameters such as the cell membrane structure, morphology and stiffness and consequently pinpoints the cellular structures damaged by the chemotherapeutic agent. These damages ultimately dictate the ability of the tumor cells to migrate and invade different organs after treatment. As previously observed in our research with a synthetic anticancer peptide, cell death occurs without full neutralization of the cancer cell membrane, a distinctive characteristic for ACPs' mode of action relative to AMPs. The obtained results also point to a translocation of the peptide to the interior of the cell and a posterior nuclear DNA and cytoskeleton damage with consequent tumor cell collapse due to an apoptotic process.

Acknowledgements
This work was supported by a grant from Laço. D.G. and J.F. acknowledge FCT-MEC for fellowships SFRH/BPD/73500/2010 and SFRH/BD/70423/2010, respectively.

ORAL COMMUNICATIONS

OC 1 - Exploring the chameleonic behaviour of peptides derived from the choline-binding domain of pneumococcal autolysin LytA

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Structural characterization of peptides encompassing the sequences of choline-binding repeats (CBR) led us to observe a chameleonic behaviour in a 14-residue peptide.[1] This peptide, LytA(239-252), is derived from the CBR3 of the choline-binding domain (CBD) from the pneumococcal choline-binding protein LytA, an autolysin performing its catalytic function in the bacterial peptidoglycan layer. LytA(239-252) forms a stable native-like β-hairpin both in aqueous solution and in the presence of trifluoroethanol (TFE), but, surprisingly, it adopts a stable amphipathic α-helical structure in both zwitterionic (dodecylphosphocholine, DPC) and anionic (sodium dodecylsulphate, SDS) micelles, and also in small unilamellar vesicles. Considering the significant differences in the side chain distribution between the β-hairpin and the helical structure, we proposed that the remarkable amphipathicity of the α-helix may be a requirement for the interaction and stabilization of the peptide structure in micelles or lipid vesicles. To confirm this proposal and get further in the understanding of the chemical-physical properties responsible for the structural duality of peptide LytA(239-252), we proceeded to analyse by CD and solution NMR the effects of different medium conditions on the structure of the related peptide LytA(259-272), corresponding to the CBR4 from the CBD of LytA, and highly similar to LytA(239-252) (57.1% identity), and of some specially designed LytA(239-252) variants. Also, we are studying LytA(239-272), a peptide containing both mentioned CBR3 and CBR4 sequences, and the linker between them. These results will be described in this communication.

References

1. H. Zamora-Carreras, B. Maestro, E. Strandberg, A.S. Ulrich, J.M. Sanz, M.A. Jiménez. *Chem Eur J.* **2015**, *21*, 8076-8089.

OC 2 - Following pH-jump induced alpha-helix peptide (un)folding dynamics

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One of the major challenges in the field of biophysical chemistry is to understand the mechanisms of protein folding, i.e. how an unstructured polypeptide chain can rapidly adopt a unique, densely packed, three dimensional structure. Our aim is to study the earliest conformational events related to protein folding and unfolding, such as the formation of isolated helical segments, reverse turns and β-hairpins.

Protein and peptide structure are influenced to a large extent by the charge state of ionizable groups on the side chains of several amino acids. In the present work, we use a laser-pulsed pH-jump technique to induce peptide (un)folding, a methodology in which a suitable photo-triggered acid generator deprotonates very quickly, resulting in long-lived and reversible pH-jump in the environment. These proton gradients protonate relevant residues of the peptide, producing change in the amino acid charge status and peptide equilibrium, consequently leading to peptide conformational changes. Those structural changes are accompanied by a variation in the overall solution volume, inducing a pressure wave. Using Time-Resolved Photoacoustic Calorimetry,[1] detection of the pressure wave allows the determination of the enthalpy, kinetics and volume changes accompanying the (un)folding of the peptide with a single method.

A protein fragment analogue of the C-Peptide from bovine pancreatic ribonuclease-A (RN80) and known to form an α-helix was studied.[2] CD and NMR spectroscopy were used in the study of the pH-dependence of RN80 α-helix formation, revealing a bell-shaped curve with a maximum near pH 5. The main forces stabilizing the short alpha-helix peptide are the salt-bridge formed between Glu-2 and Arg-10 and the π-stacking interaction involving His-12 and Tyr-8.

The amino acids identified as mainly involved in ionic equilibria of RN80 were studied. The processes of proton release from the photoacid and protonation of the amino acids were discriminated by their time scales. Volume changes in the protonation of the negatively charged carboxylate group of glutamic acid model compounds (expansion) are opposite to that observed in the protonation of the uncharged imidazole ring of acetyl-L-histidine methylamide (contraction). Time-resolved Photoacoustic Calorimetry at different temperatures provided the Arrhenius parameters for these protonation reactions, which have rate constants that differ by one order of magnitude at room temperature. The time scales of proton release, amino acid protonation and early unfolding events were used to distinguish between these processes. We observed a concentration-independent volume expansion in the sub-microsecond range (~370 ns), assigned to the unfolding of the RN80 α-helix, whereas a microsecond contraction is associated with α-helix folding. Following kinetics, thermochemistry and structural volume changes on a nanosecond to microsecond time scale allowed us to map the RN80 α-helix conformational dynamics.

References

1. T. Gensch, C. Viappiani. *Photochem. Photobiol. Sci.* **2003**, *2*, 699.
2. K. R. Shoemaker. *Proc. Natl. Acad. Sci. U.S.A* **1985**, *82*, 2349.

Acknowledgments

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OC 3 - A detailed portrait of the folding pathway and dynamical behavior of neuromedin C

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Neuromedin C (NMC) is an endogenous decapeptide (GNHWAVGHLM-NH_2) that exerts a variety of biological functions both on the central nervous system and in the gastrointestinal tract through its interaction with the bombesin receptor subtype-2 (BB_2R).[1] Therefore, BB_2R antagonists have pharmacological potential to treat disorders that appear as a result of NMC dysfunction or misregulation.[2,3] However, the efficient design of such antagonists requires a detailed understanding of the structural features of NMC, which hitherto are unknown.

We have used NMR spectroscopy to determine the conformational ensembles of NMC in aqueous solution, at five different TFE/water percentages to decode its folding pathway upon lipid/receptor interaction, and under its SDS micelle bound state that likely resembles the receptor-triggered conformation. NMC displays a disordered but well-defined backbone architecture, which undergoes a progressive coil-to-α-helix transition in the presence of increasing TFE concentrations, first at the C-terminus and then at the N-terminus. NMC also adopts a C-terminal α-helical conformation when it binds to SDS micelles. The formation of this complex is directed by thermodynamically favored hydrophobic interactions that occur concomitant with the unfavorable deprotonation of His^8 and its further insertion into the micelle.[4]

Moreover, we have observed that NMC in the presence of 60% TFE displays a structure similar to that adopted when it is embedded into the SDS micelles. Therefore, this constitutes a unique example to discriminate the folding and the binding effects on the residue level dynamics. NMR relaxation data have demonstrated that the acquisition of the micelle bound α-helical conformation constrains the NMC flexibility more than the confinement itself.[4]

References

1. H. Ohki-Hamazaki, M. Iwabuchi, F. Maekawa, *Int. J. Dev. Biol.*, **2005**, *49*, 293-300.
2. D.C. Damin, F.S. Santos, R. Heck, M.A. Rosito, L. Meurer, L.M. Kliemann, R. Roesler, G. Schwartsmann, *Dig. Dis. Sci.*, **2010**, 55, 2203-2210.
3. B.A. Nock, R. Cescato, E. Ketani, B. Waser, J.C. Reubi, T. Maina, *J. Med. Chem.*, **2012**, *55*, 8364-8374.
4. M. Adrover, P. Sanchis, B. Vilanova, K. Pauwels, G. Martorell, J. J. Pérez, *RCS Adv.*, **2015**, *5*, 83074-83088.

OC 4 - Conformational changes governing dengue virus capsid protein disordered *N*-terminal region and its inhibition by pep14-23

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Dengue virus (DENV) infection affects millions of people and is becoming a major global disease for which there is no specific available treatment. Pep14-23 is a recently designed peptide, based on a conserved segment of DENV capsid (C) protein.[1-3] This peptide inhibits the interaction of DENV C with host intracellular lipid droplets (LDs),[2,3] essential for viral replication.[1,4] Moreover, pep14-23 also inhibits DENV C interaction with very low-density lipoproteins,[5,6] which may prevent lipoviroparticle formation. Here,[7] combining bioinformatics and biophysics, we analyzed pep14-23 structure and ability to bind different phospholipids, relating that information with the full-length DENV C protein. We show that pep14-23 acquires α-helical conformation upon binding to anionic phospholipid membranes, displaying an asymmetric charge distribution structural arrangement (Figure 1). Structure prediction for the *N*-terminal segment reveals four viable homodimer orientations that alternatively shield or expose the DENV C hydrophobic pocket (Figure 1). Taken together, these findings suggest a new biological role for the disordered *N*-terminal region, which may function as an autoinhibitory domain mediating DENV C interaction with its biological targets. The results fit with our current understanding of DENV C and pep14-23 structure and function, paving the way for similar approaches to understanding disordered proteins and improved peptidomimetics drug development strategies against DENV and similar Flavivirus infections.

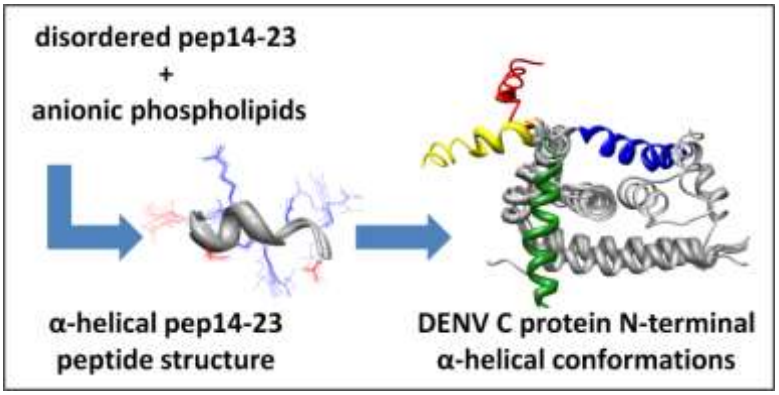

Figure 1. Both the intrinsically disordered *N*-terminal region of DENV C protein and the inhibitor peptide pep14-23 (based on that same region) acquire alpha-helical structure upon interaction with anionic phospholipids.

References

1. F.A. Carvalho *et al. J. Virol.* **2012**, *86*, 2096-2108; 2. I.C. Martins *et al. Biochem. J.* **2012**, *444*, 405-415; 3. I.C. Martins *et al.* International Patent Office Nr WO2012/159187, 2012; 4. M.M. Samsa *et al. PLoS Pathog.* **2009**, *5*, e1000632; 5. A.F. Faustino *et al. Nanomedicine: NBM* **2014**, *10*, 247-255; 6. A.F. Faustino *et al. Sci. Rep.* **2015**, *5*, 10592; 7. A.F. Faustino *et al. ACS Chem. Biol.* **2015**, *10*, 517-526.

OC 5 - The AT-hook motif as minor groove anchor for synthetic DNA binders

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In cells, the regulation of the protein expression is mainly carried out at the transcription stage and is dependent on the action of specialized proteins called transcription factors (TFs) that can recognize specific DNA sequences and control the rate of transcription. Owing to its relevance, there is a great interest on the development of non-natural DNA binding TFs. We report the development of binary chimeric DNA binding peptides made of the DNA binding fragment of a natural TF (basic region of a bZIP protein or a monomeric zinc finger module) and an AT-hook peptide motif. The resulting binary peptide conjugates display high DNA affinity and excellent sequence selectivity for sites of 9 base pairs. The AT-hook motif also favors the cell internalization of the conjugates.[1] Furthermore, the AT-hook can serve as a minor groove anchor for the DNA-contacting regions of two transcription factors (GCN4 and GAGA). The resulting ternary chimera, which represents a new, non-natural DNA binding motif, binds with high affinity and selectivity to a long composite sequence of 13 base pairs (TCAT-AATT-GAGAG), interacting with DNA through one face of the double helix (Figure 1).[2]

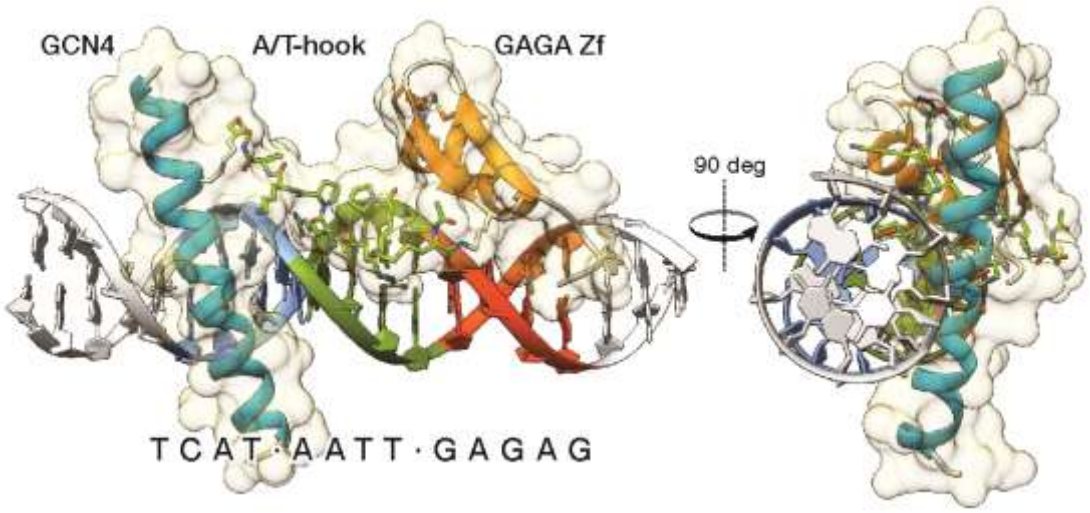

Figure 1. Model of the interaction between the tripartite construct and the target composite DNA sequence. The image on the right shows the interaction of the three modules along the DNA axis covering one side of the double helix.

References

1. J. Rodríguez, J. Mosquera, J. R. Couceiro, M. E. Vázquez, J. L. Mascareñas, *Chem. Sci*. **2015**, *6*, 4767-4771.
2. J. Rodríguez, J. Mosquera, R. García-Fandiño, M. E. Vázquez, J. L. Mascareñas, submitted.

OC 6 - Peptide design for interfacial self-assembly of biomaterials for bone regeneration

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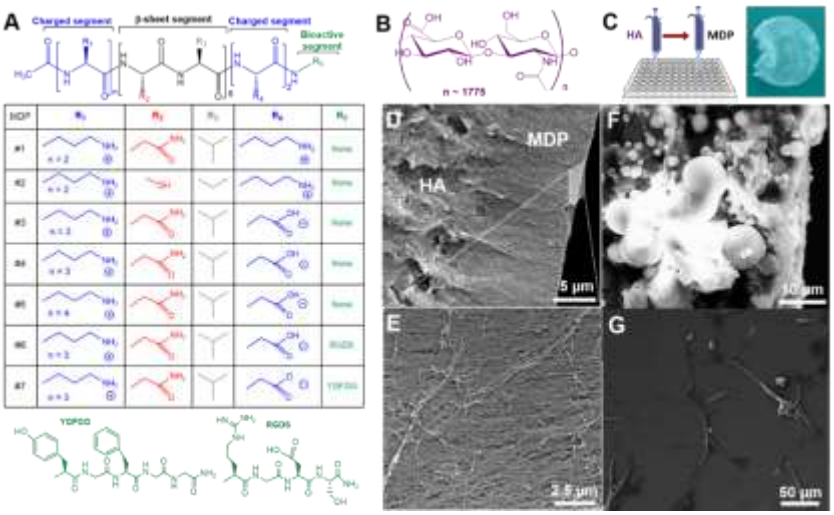
Peptide self-assembly is gaining increasing interest to precisely engineer innovative biomaterials. Through rational design, multiple interacting domains can be integrated into the peptide molecule to drive their self-assembly into well-defined structures and engage with cell surface integrins to promote specific cell responses (e.g. adhesion or differentiation). Multi-domain peptides (MPDs) were first designed by the Hartgerink,[1,2] and consist of an ABA block motif in which the central B block is composed of alternating hydrophilic and hydrophobic amino acids and block A is composed of a variable number of charged residues. Charge screening of the A block, favours the assembly of the B block and intermolecular hydrogen bonding among the peptide backbone stabilizes the peptide assemblies. We report the design of new MPDs (Figure-A) to interact with the anionic polymer hyaluronan (HA, Figure-B) and form interfacial membranes displaying distinct bioactive epitopes aiming to promote mineralization (EE), cell adhesion (RGDS) and osteogenic differentiation (YGFGG). Variations in the number of charged residues in the A block and in the central block were implemented to investigate the interaction with HA and the microarchitecture of the final membrane. All MPDs were successfully synthesized and purified, and shown to have beta-sheet secondary structure. Membranes were obtained by self-assembly (Figure-C) and SEM analysis of the membrane cross-section revealed distinct levels of organization (Figure-D) and a nanofibrillar structure at the surface (Figure-E). *In-vitro* mineralization studies revealed the ability of the membranes to nucleate calcium phosphate mineral on their surface (Figure-F) and cell culture on the membranes, using primary human periosteal cells, showed a large number of elongated and well-spread cells adhered to the membrane (Figure-G). We anticipate that these highly organized biomimetic membranes can provide biochemical signals for stem cell growth and differentiation and be used to promote bone regeneration.



References: 1. H. Dong *et al. J. Am. Chem. Soc.* **2007**, *129*, 12468-12472; 2. L. Aulisa *et al. Biomacromolecules* **2009**, *10*, 2694-2698. **Ackowledgements:**This work was supported by the Portuguese Foundation for Science and Technology under the scope of the project PTDC/CTM-BIO/0814/2012 and by the European Regional Development Fund (ERDF) through the Operational Competitiveness Programme "COMPETE" (FCOMP-01-0124-FEDER-028491).

OC 7 - Discovery of catalytic peptides via biocatalytic self-assembly

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Efficient and effective catalysis is key to biological processes and many chemical reactions. Nature has evolved impressive catalysts; however, they have severe drawbacks of complexity, limited stability and restriction to catalyse biological reactions in water. This brands them non-ideal for industrial applications. On the other hand, the design and discovery of efficient synthetic catalysts has been limited, questioning whether this reductionist approach can be utilised for challenging reactions. The number of possible synthetic sequences which potentially possess the desired function is huge; therefore it is a challenge to identify appropriate sequences. Herein, a combined methodology is described for the selection of catalytic phages utilizing phage display[1] and well characterized self-assembling gelators.[2]

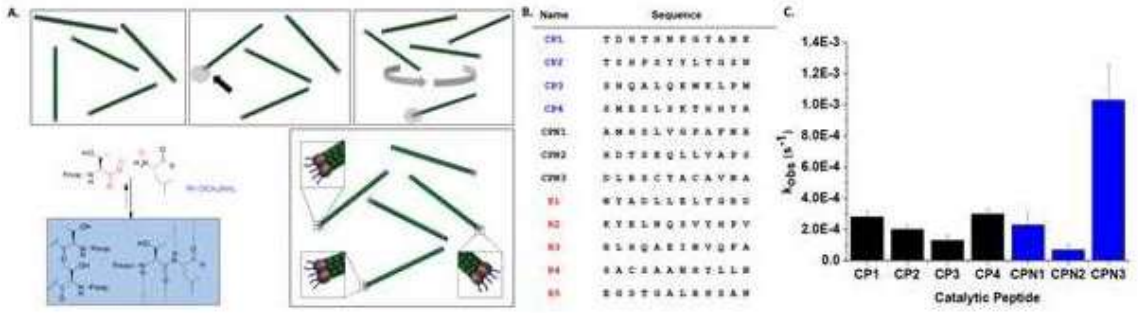

Figure 1. A. Catalytic gelation in discovery of catalytic phages and reaction scheme of catalytic condensation of amino acid derivatives to form the product which locally assembles. **B.** Peptide sequences selected by catalytic self-assembly combined with phage display. CP1-4 were selected using Fmoc-Thr and Leu-OMe; CPN1-3 were selected using Fmoc-Thr and Leu-NH2. **C.** Turnover data for hydrolysis of p-nitrophenyl acetate by free peptides (based on a 10 minute time course) with 0.5 mM peptide, 6 mM *p*-nitrophenyl acetate (0.018 mM CPN3).

This new combinatorial technology allows for discovery of selective catalytic peptides for the acceleration of slow chemical reactions based on the catalytic turnover rather than binding.[3] Seven peptides, consisting of twelve amino acids each, were identified and all were shown to possess the ability to hydrolyze amide and ester bonds under physiological conditions. Although catalytic activities observed were low, an insight into the relevance towards catalyst design can be provided by these minimalistic structures. CPN3 was highlighted as the most efficient esterase catalyst and the free peptide sequence was investigated further to obtain a better understanding of the mechanism of action. CPN3 is a random, unassembled peptide and an alanine sweep reveals that the cysteine residues are key for catalysis. The esterase activity of this peptide compares favourably with previously reported histidine containing catalysts[4] and is the first reported peptide catalyst which does not contain histidine as a catalytic residue. The peptides identified in this study have the tendency to behave as small molecule catalysts rather than an enzyme since binding does not play a role here.

References: 1. G. P. Smith, V. A. Petrenko. *Chem. Rev*. **1997**, *97*, 391; 2. R.J. Williams, A.M. Smith, R. Collins, N. Hodson, A.K. Das, R.V. Ulijn. *Nat. Nanotechnol*. **2009**, *4*, 19; 3. Y. Maeda, N. Javid, K. Duncan, L.S. Birchall, K.F. Gibson, D. Cannon, Y. Kanetsuki, C. Knapp, T. Tuttle, R.V. Ulijn, H. Matsui. *J. Am. Chem. Soc*. **2014**, *136*, 15893; 4. K. Duncan, R.V. Ulijn. *Biocatalysis* **2015**, *1*, 67.

OC 8 - Impact of different immobilization parameters on the immobilized antimicrobial peptide (Dhvar5) antibacterial activity

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Antimicrobial peptides (AMPs) are a new class of promising agents highly selective, with a broad spectrum of activity, and with a low tendency to induce resistance. AMPs immobilization onto biomaterials surface is a promising strategy to avoid bacterial colonization.[1] However, a correct peptide orientation and exposure from the surface is essential to maintain AMP antimicrobial activity. To that end we used Dhvar5 that is derived from Histatin5.[2] The aim of the work was to study the immobilization of Dhvar5, testing different peptide orientations (*C* and *N*-terminal) and different spacers with different flexibilities, in order to create an antimicrobial surface. Also, a surface with adsorbed Dhvar5 was produced for comparison reasons. Chitosan was the polymer chosen for peptide tethering, due to its antimicrobial properties and readiness to be functionalized.

Dhvar5-derived peptides were produced in order to introduce different spacers with a terminal cysteine for subsequent immobilization. Chitosan ultrathin films obtained through spin-coating on gold substates (Au) were modified with *N*-acetyl cysteine (NAC), followed by peptide immobilization through disulfide bridge formation using oxidative conditions. A toolbox of surface characterization techniques (IRRAS, ellipsometry, water contact angle) corroborated the success of the tethering reaction. Peptide quantification by the phenathrenequinone (PHQ)[3] assay showed a surface density of around 2.0 ng/mm^2. Bacterial adhesion assays with a methicillin-resistant *S. aureus* strain (MRSA), using LIVE/DEAD® Bacterial Viability Kit (Baclight™), showed a reduction on the number of adhered bacteria onto modified surfaces, comparing to Au control surfaces. This reduction was strikingly high on the *N*-terminal tethering using the longer 6-aminohexanoyl (AHA) spacer used. No difference was observed between spacers with different flexibilities (AHA vs dipeptide GlyGly). Surface with adsorbed Dhvar5 enhanced bacteria adhesion comparing to control.

Efficacy assays demonstrated that covalent immobilization of Dhvar5 exposing its cationic end, improves the chitosan coating antimicrobial effect by decreasing MRSA colonization. This effect was enhanced when longer spacers were used independently of their flexibility. In opposite, immobilized Dhvar5 exposing its hydrophobic end has no effect on bacterial adhesion to chitosan, and when adsorbed in a random orientation even induces bacterial adhesion to chitosan coating.[4]

References

1. F. Costa *et al. Acta Biomater.* **2011**, *7*, 1431-1440; 2. H.P. Stallmann *et al. Peptides* **2005**, *26*, 2355-2359; 3. R.E. Smith, R. Macquarrie. *Anal. Biochem.* **1978**, *90*, 246-255; 4. F. Costa *et al. Biomaterials* **2015**, *52*, 531-538.

Acknowledgements

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OC 9 - A nano-scale magnetic antimicrobial platform

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Infectious diseases associated with drinking-water and wastewater remediation are a major concern of our modern society. The use of antimicrobial peptides (AMPs) present advantages over the existing wastewater treatments as the lack of by-product formation (e.g. chlorine) and development of multidrug-resistance pathogens (e.g. antibiotics).[1]

A novel magnetic antimicrobial support has been developed where iron oxide magnetic nanoparticles (MNPs) were combined with an AMP, RWRWRW.[2] Poly(ethylene) glycol-coated MNPs were functionalized with $(RW)_3$ and its antimicrobial activity was evaluated against *Escherichia coli* k-12 DSM498, a biological indicator of water contamination, and *Bacillus subtilis* 168, employing a novel high-throughput method. A minimal inhibitory concentration (MIC) of 500 μM was found for both strains, with a visible bactericidal effect. This nano-scale magnetic antimicrobial platform presents a wide range of applications from to environmental to biomedical applications. The new screening protocol can be applied for the screening and discovery of new AMPs and materials with AMP properties. It shows a minimal consumption of reagents and materials, and allows the online quantitative monitoring of antimicrobial agents, as opposed to the current methodologies.

References

1. T. Blin, V. Purohit, J.R.M. Leprince, T. Jouenne, K. Glinel. *Biomacromolecules* **2011**, *12*, 1259-1264.
2. A.S. Pina, A.L. Batalha, C.S.M. Fernandes, M.A. Aoki, A.C.A. Roque, A.C.A. *Water Research* **2014**, *66*, 160-168.

OC 10 - Design of potent antimicrobial and biocompatible surface using antimicrobial peptide

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Implantation of medical devices is growing widely and therefore accompanied by an increasing incidence of device-associated infections, leading to multi-billion dollar burden on health care systems all over the world. The use of antimicrobial peptides (AMPs) is highly advantageous over conventional antibiotics due to its broad-spectrum activity, selectivity, and minimal bacterial resistance so far.[1] In recent years, AMPs have been chemically immobilized on surfaces of medical devices to render them with antimicrobial properties.[2-5] However, the study regarding the activity of immobilized AMPs maintained in the presence of serum remains elusive. Surfaces having immobilized cationic peptides are susceptible to be adsorbed by plasma proteins with the subsequent loss of antimicrobial activity. Furthermore, with the exception of very few studies that have determined the cytotoxicity of surfaces in mammalian cells,[4,6] the effect of the immobilized AMP on human cells is relatively unknown.

Here, we have developed an elegant and robust antimicrobial coating based on covalent immobilization of AMP Cecropin-Mellitin (CM) on gold nanoparticles coated surfaces. A high amount of CM peptide (110 μg/cm^2) is immobilized on thiol-PEG-amine coated surface compared to previously reported amount (> 10 μg/cm^2) so far. The immobilized CM peptides have potent antimicrobial activity against both Gram-positive and negative bacteria in human serum along with low probability to induce the resistance to bacteria. It is found that the immobilized CM induces the permeability to outer and inner membrane of reporter *E. coli* bacteria in 5 and 20 min after contact respectively. The described coating technology can be translated on to gold nanoparticles coated Ti surface with the similar fashion of antimicrobial activity. Importantly, the coating is non-toxic to human endothelial and fibroblast cells (HUVECs and NDHF) cultured on top along with no initiation of platelet and compliment activation, indicating the coating is bio and hemo-compatible. We envisage that the described antimicrobial coating may be promising to prevent medical device associated infection.

References

1. M. Zasloff. *Nature* **2002**, *415*, 389.
2. M. Bagheri, M. Beyermann, M. Dathe. *Antimicrob. Agents Chemother.* **2009**, *53*, 1132.
3. G. Gao, K. Yu, J. Kindrachuk, D. E. Brooks, R. E. Hancock, J. N. Kizhakkedathu. *Biomacromolecules* **2011**, *12*, 3715.
4. G. Z. Gao *et al. Biomaterials* **2011**, *32*, 3899.
5. V. Humblot, J. F. Yala, P. Thebault, K. Boukerma, A. Hequet, J. M. Berjeaud, C. M. Pradier. *Biomaterials* **2009**, *30*, 3503.
6. M. Nakamura, T. Iwasaki, S. Tokino, A. Asaoka, M. Yamakawa, J. Ishibashi. *Biomacromolecules* **2011**, *12*, 1540.

OC 11 - A novel peptide platform derived from Dengue virus for drug delivery into the brain

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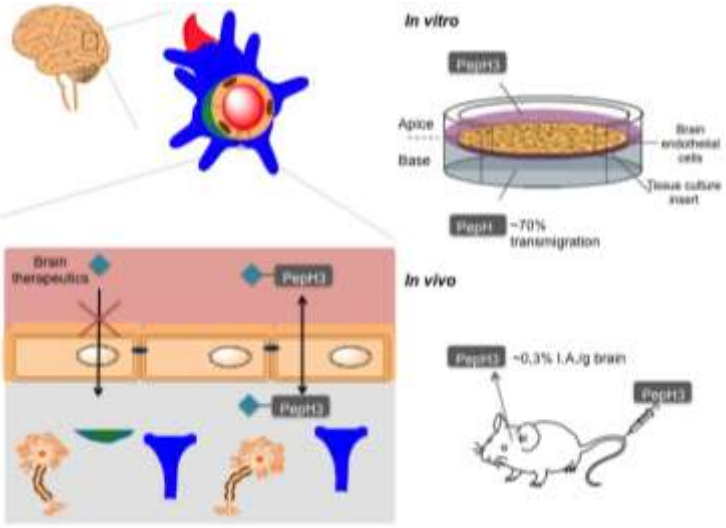
Neuropharmaceutics are the largest potential growth sector of the pharmaceutical industry. Despite significant developments in understanding the brain and the great advances in medical technology, many CNS associated diseases, such as Alzheimer's and Parkinson's diseases, stroke, and brain tumors, remain beyond the reach of conventional therapies. Effectiveness of CNS therapeutic molecules if often limited by the fact that following systemic administration they do not reach the target site in the brain, the brain is protected from the systemic circulation by the blood-brain barrier (BBB). The BBB is a natural defense against circulating toxic and infection agents that also prevents most therapeutic compounds from reaching the brain.Several strategies have been proposed to enhance transport of drugs across the BBB, including invasive techniques and receptor mediated transcytosis. Both strategies are severely limited, by the permanent damage caused to the BBB or local expression and saturation of receptors. Therefore, new drug delivery systems are required. We have developed innovative peptides able to translocate the BBB. A pool of small peptides derived from specific domains of Dengue virus type 2 capsid protein (DEN2C) were synthesized and radiolabeled with two radioisotopes (Tecnetium and Gallium). These peptides have characteristics commonly found in cell penetrating peptides (CPP), such as being cationic and lipophilic. In an *in vitro* model of the BBB, two peptides, pepH1 and pepH3, revealed high translocation rate after 24 h, therefore being considered promising candidates for drug delivery to the brain. *In vivo* biodistribution profiles reveal an improved brain uptake for pepH3, followed by brain release. The results are consistent with biophysical studies obtained for pepH3 showing that pepH3 interacts with anionic membranes, similarly to what is found in brain endothelial cells. Currently, the peptide is being coupled to a therapeutic antibody to test its efficacy as a drug delivery system to the brain.



OC 12 - Breaking barriers – the challenge of Blood-Brain Barrier (BBB) shuttles as therapeutic agents: gene therapy for Friedreich's ataxia

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Blood-brain barrier (BBB) shuttles[1] are a promising solution for therapeutics not able to reach the brain parenchyma, thus allowing treatment of central nervous system (CNS) diseases. Such molecules are able to cross the BBB through either passive[2] or active[1] transport mechanisms. While passive BBB shuttles are useful to carry small organic molecules, actively transported BBB shuttles by endocytic mechanisms can carry larger molecules, ranging from proteins to supramolecular structures such as organic nanoparticles and viral particles. Given their capacity to overcome the BBB, these shuttles may provide the key to the treatment of diseases affecting the CNS. In this regard, Friedreich's Ataxia (FRDA)[3] is a neuromuscular disease affecting mainly the CNS, heart, and muscles. This disorder affects around 1 in 50,000 people and is caused principally by a triplet expansion repeat in intron 1 of the frataxin gene, a mitochondrial protein encoded in chromosome 9. This oversized intron leads to a condensed DNA conformation, namely "sticky DNA",[4] which blocks transcription, and subsequently to low expression levels of frataxin. The characteristics of FRDA facilitate gene therapy since only an increase in the levels of frataxin is required and it is not necessary to correct the genome. In this respect, herpes simplex virus type 1 (HSV-1) particles containing a bacterial artificial chromosome (BAC) with the whole frataxin gene[5] have been reported to allow *in vivo* expression of the naturally occurring diverse isoforms of this protein in each cell type on demand – *i.e*. regulated by alternative splicing. Nevertheless, these viral particles are not able to cross the BBB. Our group envisaged an approach whereby such therapeutic agents conjugated to actively transported BBB shuttles provide effective treatment for FRDA in the CNS. Here we will report on a study of the applicability of such shuttles to transport 200-nm viral particles through the BBB for the treatment of FRDA.

References

1. R. Prades, B. Oller-Salvia, S. M. Schwarzmaier, J. Selva, M. Moros, M. Balbi, V. Grazú, J.M. de La Fuente, G. Egea, N. Plesnila, M. Teixidó, E. Giralt. *Angew. Chemie Int. Ed.* **2015,** *54*, 3967-3972.
2. P. Arranz-Gibert, B. Guixer, M. Malakoutikhah, M. Muttenthaler, F. Guzmán, M. Teixidó, E. Giralt. *J. Am. Chem. Soc.* **2015,** *137*, 7357-7364.
3. J.B. Schulz, S. Boesch, K. Burk, A. Durr, P. Giunti, C. Mariotti, F. Pousset, L. Schols, P. Vankan, M. Pandolfo. *Nat. Rev. Neurol.* **2009,** *5*, 222-234.
4. R.D. Wells. *FASEB J.* **2008,** *22*, 1625-1634.
5. A. Gimenez-Cassina, R. Wade-Martins, S. Gomez-Sebastian, J.C. Corona, F. Lim, J. Diaz-Nido. *Gene Ther.* **2011,** *18*, 1015-1019.

OC 13 - Maximizing biomolecules signal detection for study of single protein-ligand interaction events

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Amyloid fibrils are formed via the amyloidogenesis process, by which peptides or proteins monomers spontaneously self-associate into highly ordered aggregates with quasi-crystalline structures.[1-7] Mature amyloid fibrils, often associated with human neurodegenerative pathologies such as Alzheimer's and Parkinson's disease, are in most cases relatively innocuous, as shown by us.[1-5] In fact, amyloid fibrils even have physiological roles, including in humans (reviewed in [5-7]). Toxicity is mostly due to precursor aggregates, oligomers and protofibrils.[1-3] Importantly, the likelihood of amyloidogenesis can be predicted from the amino acid sequence.[4] The fibrils rich in β-sheet architecture provides them high stability and mechanical strength, allowing chemical reactions to occur in their vicinity without affecting them.[5-7] Given this low toxicity, ordered and stable structure, which can be predicted and manipulated to produce diverse topographies, amyloid fibrils have been suggested as potential novel biomaterials for nanotechnology and nanomedicine, namely as bioactive gels and in biosensing.[5-7]

References

1. International Patent Office, Patent Nr WO/2008/028939A1.
2. I.C. Martins *et al. EMBO J*. **2008**, *27*, 224-233.
3. I. Kuperstein *et al. EMBO J*. **2010**, *29*, 3408-3420.
4. Maurer-Stroh *et al. Nat. Methods* **2010**, *7*, 237-242.
5. C.A.E. Hauser *et al. Chem. Soc. Rev*. **2014**, *43*, 5326-5345.
6. I. Cherny *et al. Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 4062-4092.
7. E. Gazit. *Nanomedicine (Lond)* **2014**, *9*, 2433-2436.

OC 14 - New BACE1 inhibitors for Alzheimer's disease treatment: evaluation of their eficacy in *in vitro* and *in vivo* models

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Alzheimer's disease (AD) is the most common dementia worldwide and at present an effective therapy is an unmet clinical need. It is generally accepted that accumulation of amyloid-β protein (Aβ) in the brain parenchyma represents an early incident on a cascade of events that ends in neurodegeneration and dementia, and thus, Aβ is considered as the etiologic agent of the disease. The formation of Aβ requires the initial cleavage of the amyloid precursor protein (APP) by the β-secretase enzyme (BACE1), followed by the activity of the γ-secretase over the ensuing transmembrane fragment. So far, only one BACE1 inhibitor previously developed reached the phase II/III clinical trials. Therefore, the goal of this study is the design and development of a new peptide inhibitor of BACE1, a key enzyme for the production of amyloid-β peptide (Aβ), which we expect will overcome some of the limitations of previous BACE1 inhibitors that hindered their clinical use. The compounds we designed were screened for their ability to inhibit BACE1 by an *in vitro* cell-free assay. The compounds IC_{50} was determined and the two most promising drugs were then evaluated for their ability to inhibit BACE1 and reduce endogenous Aβ production in a cellular model of AD (Neuro-2A cells overexpressing APPswe, N2A-APPswe). The levels of secreted Aβ40 and Aβ42 as well as the levels of the soluble fragment sAPPβ were assessed by ELISA and Western blot, respectively, after incubation of N2A-APPswe cells with the compounds for 24 hours. In these conditions, 100 µM of compound 5 reduced the levels of Aβ40 and Aβ42 by 74.0 % and 84.2 %, respectively, whereas 100 µM of compound 6 reduced Aβ40 and Aβ42 levels by about 74 %.Accordingly, both compounds induced a reduction in the levels of sAPPβ. Moreover, a 24 h incubation with the new BACE1 inhibitors (compound 5 and 6) at the IC_{50} concentration observed in N2A-APswe cells, did not induce citotoxicity as assessed by the MTT assay, caspase 3/7 activity and LDH release.

The efficacy of the selected compounds to inhibit BACE1 was also tested in the 3xTg AD mouse model. We observed that both compounds (1.25 mg/kg) reduced plasma Aβ40 by about 30% and, at a dose of 5.0 mg/kg, reduced plasma Aβ42 by 30%, as assessed by sandwich ELISA 24 h after a single administration to 4 months-old mice. Regarding brain soluble Aβ levels, 1.25 mg/kg of the compounds 5 and 6 reduced Aβ40 by 50% and 28 %, respectively, while both compounds reduced Aβ42 by about 28% without altering APP and BACE1 levels, indicating the decrease in Aβ is due to BACE1 enzymatic inhibition. Moreover, the compounds decreased the soluble APP cleavage fragment sAPPβ brain levels whereas sAPPα levels remain unchanged, suggesting they inhibit BACE1 selectively.

Taking into account our preliminary results, we expect to develop a new BACE1 inhibitor that will be able to delay the onset and progression of the disease since it will prevent Aβ production and the subsequent neurotoxic events triggered by Aβ accumulation.

Acknowledgements

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OC 15 - Multifunctional short peptides for the design of anti-Alzheimer therapeutics

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Alzheimer's disease (AD), a neurodegenerative disorder that eventually leads to the death of the patient, is nowadays the most common cause of dementia in the elderly population. Its main symptoms are memory loss, cognitive decline, mood changes and difficulty in remembering new information. Due to the massive socioeconomical impact of this disease, the development of effective treatments and improved diagnostic techniques has become of vital importance.

After many years of research, it is generally accepted that aggregated forms of Aβ peptide play a key role in the advent and progression of the disease.[1,2] Extracellular senile plaques of fibrillar Aβ are found in affected brain tissues of AD patients, although soluble oligomeric aggregates are considered the most toxic species. Metals such as Cu(II) and Zn(II), whose homeostasis is altered in AD brains, are thought to interact with the Aβ protein, promoting its aggregation and generating oxidative stress. Recent investigations targeting such metal ions have shown promising results.[3]

Herein, we present the synthesis and properties of a series of chelating short peptides which may act as metal protein attenuating compounds, ROS inhibitors and/or fluorescence-based metal sensors.

References

1. P. Faller, C. Hureau, O. Berthoumieu. *Inorg. Chem*. 2013, *52*, 12193
2. P. Gamez, A. B. Caballero. *AIP Advances* 2015, *5*, 092503-1
3. K. J. Barnham, A. I. Bush. *Chem. Soc. Rev*. 2014, 43, 6727

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OC 16 - Dynamic pseudopeptides: generation of structural diversity inspired by Nature

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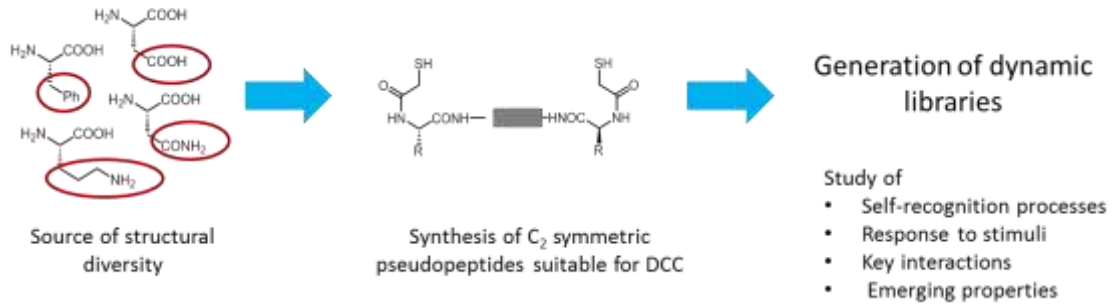
The broad structural diversity of naturally occurring amino acids provides a priceless tool to build bioinspired artificial systems. Amino acids are the main building blocks that form the complex machinery that Nature uses in living systems, for example in recognition, transport or catalysis. Inspired by Nature, chemists have also designed a number of artificial structures with remarkable properties such as molecular receptors, intertwined structures or molecular motors, to name a few. Combining the attractive properties of amino acids with synthetic moieties gives rise to an increased number of structural possibilities with different functions. However, in order to create and study new, complex structures, chemists usually face synthetic limitations. In this context, Dynamic Covalent Chemistry (DCC) provides an excellent methodology to generate many new structures from a (limited) set of building blocks. Combining the principles of DCC with the structural variety of amino acids in pseudopeptidic systems, we can construct Dynamic Chemical Libraries (DCL) with a broad range of properties. This diversity in terms of structure, hydrophobicity or charge that arises from the side chain of amino acids allows the study of molecular interactions, self-assembly processes or the response to stimuli in minimalistic systems, in a bioinspired approach.



References

1. J. Li, P. Nowak, S. Otto. *J. Am. Chem. Soc.* **2013**, *135*, 9222-9239.
2. J. Atcher, A. Moure, I. Alfonso. *Chem. Commun.* **2013**, *49*, 487-489.
3. J. Solà, M. Lafuente, J. Atcher, I. Alfonso. *Chem. Commun.* **2014**, *50*, 4564-4566.
4. M. Lafuente, J. Atcher, J. Solà, I. Alfonso. *Chem. Eur. J.* **2015**. DOI: 10.1002/chem.201501415.

OC 17 - Adaptive processes in a topologically diverse dynamic library of pseudopeptides

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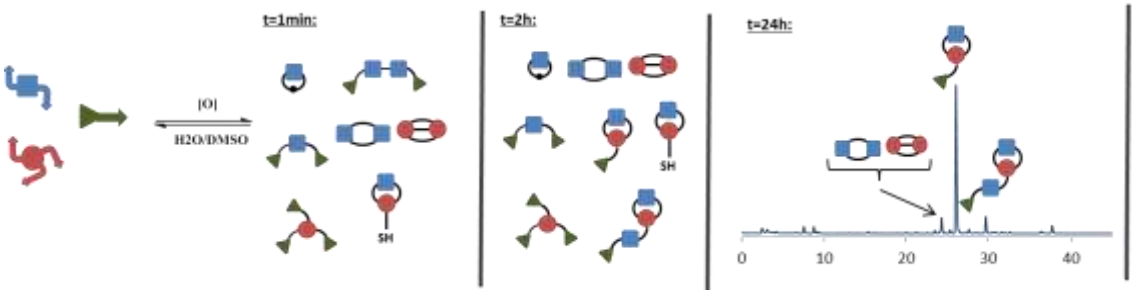
The Dynamic Combinatorial Chemistry (DCC) proposes the creation of a mixture of compounds (Dynamic Combinatorial Library, DCL) inter-connected through reversible chemical processes. The changes of concentration of species in a DCL contain valuable information about changes in stability, with the corresponding implications in the recently emerged topic of Systems Chemistry and the concept of molecular evolution. One of the most widely used chemical connections to generate DCLs is the disulfide bond, mainly from molecules with two reacting sites (bipodal), and thus directed towards the generation and interconversion of cyclic oligomers.

In this work, we studied the effect of combining mono-, di- and trithiols in the same DCL in order to expand the structural and topological diversity. We will show how the reaction mixture evolves from a complex combination of compounds to the almost exclusive formation of a major species through error-check and correction processes. This adaptation proceeds by delicate non-covalent intramolecular interactions, which are sensitive to structural and environmental effects.



References

1. a) P.T. Corbett, J. Leclaire, L. Vial, K.R. West, J.-L. Wietor, J.K.M. Sanders, S. Otto. *Chem. Rev.* **2006**, *106*, 3652-3711; b) J.H.R. Reek, S. Otto. Dynamic Combinatorial Chemistry, Wiley-VCH, Weinheim, 2010.
2. a) R.F. Ludlow, S. Otto. *Chem. Soc. Rev.* **2008**, *37*, 101-108; b) J. Li, P. Nowak, S. Otto. *J. Am. Chem. Soc.* **2013**, *135*, 9222-9239.
3. J. Atcher, A. Moure, I. Alfonso. *Chem. Commun.* **2013**, *49*, 487-489.
4. S. Otto, R.L.E. Furlan, J.K.M. Sanders. *Science* **2002**, 297, 590-593.
5. S.P. Black, J.K.M. Sanders, A.R. Stefankiewicz. *Chem. Soc. Rev*. **2014**, *43*, 1861-1872.
6. J. Solà , M. Lafuente, J. Atcher, I. Alfonso. *Chem. Commun*. **2014**, *50*, 4564-4566.

OC 18 - Bioactive peptides of the Cry1Ab16 toxin from *Bacillus thuringiensis* by nanodevices films for potential biotechnological applications

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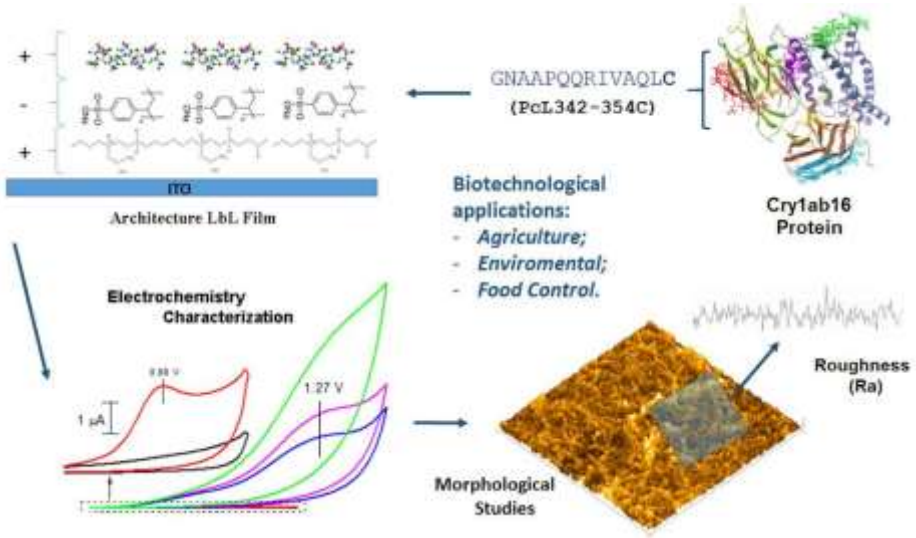
Peptides are potential candidates to meet the needs of the modern world in relation to diagnosis, disease monitoring, quality control in industry, and more recently, detection of genetically modified organisms (GMOs) and food security through the development of biosensors.[1] Cry1Ab16 is a toxin of crystalline insecticidal proteins family that has been widely used in GMOs to gain resistance to pests. Recently, many studies have focused on evaluating the potential environmental impact of this toxin, including the impact on aquatic environments. Moreover, this toxin has been detected in maternal and fetal samples during pesticide exposure studies related to genetically modified food. For the first time, in this study, peptides derived from the immunogenic Cry1Ab16 toxin (from *Bacillus thuringiensis*) were immobilized as layer-by-layer films.[2] Given the concern about food and environmental safety, a peptide with immunogenic potential, PcL342-354C, was selected for electrochemical characterization. In addition, an attempt was made to optimize the electrochemical signal of the peptide through interspersion with different natural or synthetic polymers. Finally, the system was characterized using various morphological and spectroscopic techniques such as AFM, UV-Vis, XRD, and FTIR. An ITO/PEI/$(PSS/PcL342\text{-}354C)_n$ film proved to be an excellent candidate for applications in electrochemical sensors and other biotechnological applications for GMOs and environmental indicators.



References

1. A. Plácido, *et al.* Novel Strategies for Genetically Modified Organism Detection, in: E.I. Academic Press (Ed.) Genetically Modified Organisms in Food Production - Safety, Regulation and Public Health, Waltham, MA, USA, 2015, pp. 119-131; 2. E.A. de Oliveira Farias, *et al. Mater. Sci. Eng. B. Solid.* **2015**, *200*, 9-21.

Acknowledgements

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OC 19 - Permeation of model membranes by Peptaibolin mimetics bearing different α,α-dialkylglycines

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Some peptides are able to interact with the lipid bilayer membrane of bacteria, being able to modify and even permeate the membrane. Such is the case of Peptaibols, a family of natural antimicrobial peptides bearing α,α-dialkylglycines such as Aib, Iva and Deg in their composition. These stereochemically hindered amino acids yield peptides with more defined conformations and more resistant to biodegradation,[1] usually adopting highly helical secondary structures that directly correlates to their mechanism of action, and hence antimicrobial properties, which rely on the permeation of the cell membrane and formation of ionic channels.[2] Recent in silico studies suggest that membrane affinity might be increased by the substitution of the Aib residues by more structurally constrained and more hydrophobic α,α-dialkylglycines.[3]

Bearing these facts in mind, we now report the membrane permeation studies of a model peptide, Peptaibolin (Ac-Leu-Aib-Leu-Aib-Phol), which is the shortest member of the peptaibols family, and several mimetics incorporating unnatural α,α-dialkylglycines (Deg, Dpg, Ac6c) at the native Aib positions.[4] The model membranes were based on small unilamellar vesicles composed of phosphatidylcholines (egg lecithin, DPPC), phosphatidylglycerols (DPPG, DOPG) and cholesterol, at different compositions and ratios, containing a fluorescent probe encapsulated in their aqueous interior, in order to monitor the permeation process by fluorescence spectroscopy. The obtained results revealed a correlation between the length and bulk of the side chain of the unnatural α,α-dialkylglycines and the ability of the corresponding peptide to permeate the model membranes.

References

1. S. Aravinda, N. Shamala, P. Balaram. *Chemistry & Biodiversity* **2008**, *5*, 1238-1262.
2. H. Duclohier. *Chemistry & Biodiversity* **2007**, *4*, 1023-1026.
3. T. G. Castro, N. M. Micaêlo. *J. Phys. Chem. B* **2014**, *118*, 649-658.
4. V.I.B. Castro, C.M. Carvalho, R.D.V. Fernandes, S.M.M.A. Pereira-Lima, E.M.S. Castanheira, S.P.G. Costa. *Tetrahedron* **2015**, in press.

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OC 20 - Dengue virus in the spotlight: capsid peptide binds specifically to a bilayer membrane model by Molecular Dynamics

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Dengue virus (DENV) C protein is crucial for viral assembly in order to ensure specific encapsidation of its genome. DENV C protein associates with intracellular membranes through a conserved hydrophobic domain and accumulates in endoplasmic reticulum-derived lipid droplets which could provide a platform for capsid formation during assembly. In a previous work,[1] we described a region in DENV C protein which induced a nearly complete membrane rupture of several membrane model systems, which was coincident with the theoretically predicted highly hydrophobic region of the protein (peptide DENV2C6). In this work we have performed a molecular dynamics (MD) study of the interaction of a peptide corresponding to this DENV C region with different membrane model systems. Unrestrained all-atom MD was carried out using NAMD 2.9 by using the CHARMM36 force field for the lipid molecules and the CHARMM general force field for the peptide. We have used a Highly Mobile Membrane-Mimetic (HMMM) lipid bilayer model for the MD. We show that DENV2C6 is capable of binding specifically to membranes and that this binding is modulated by lipid composition through specific lipid-peptide interactions (Figure 1). These data corroborate our previous results and open a new avenue for the development and identification of peptides, which might be used as new drugs against the DENV life cycle, through the modulation of membrane structure.

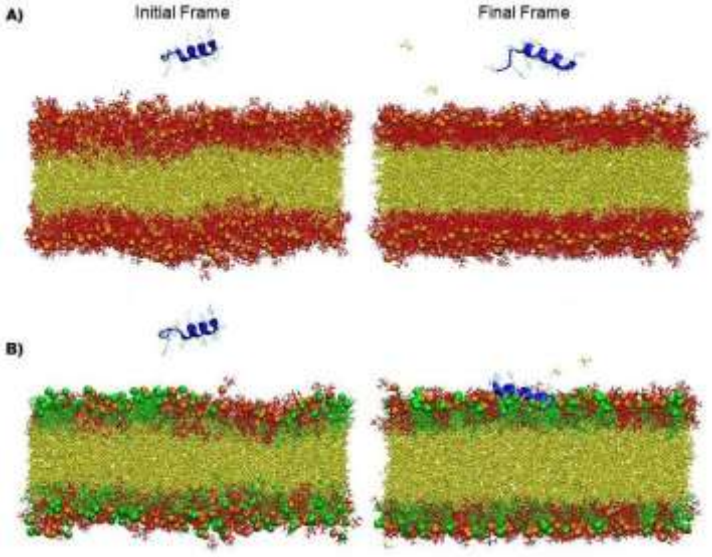

Figure 1. The initial (t=0 ps) and final (t=110 ns) snapshots of the HMMM model membrane systems containing the peptide (Blue) and (**A**) PC and (**B**) PC/PA phospholipids. Phospholipid phosphate atoms are depicted in VDW drawing style whereas the rest of molecules are depicted in licorice drawing style. PC and PA are shown in red and green respectively, the organic solvent molecules are shown in yellow.

References

1. H. Nemésio, M.F. Palomares-Jerez, J. Villalaín. *Mol. Membr. Biol.* **2013**, *30*, 273-87.

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OC 21 - When simulation and experiment fuse: analysing the interaction of the influenza fusion peptide with model membranes

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The emergence of an influenza pandemic is one of the biggest health threats of our time and, therefore, there is an urgent need to develop vaccines and drugs against a broad spectrum of influenza viruses (IV). A promising strategy to combat IV is to inactivate the fusion process between the viral and host membranes, which is mediated by the surface protein hemagglutinin (HA). During this process, the *N*-terminal region of HA, known as fusion peptide (FP), inserts into the host membrane. Although it has been shown that the FP plays a crucial role in the fusion process, the molecular effect of the peptide remains unclear.

In order the shed light into this problem, we used a combination of state-of-the-art experimental and simulation techniques to analyse the WT influenza FP and four mutants. Fluorescence based methods were used to analyse the partition coefficient of the WT and mutant peptides in model membranes, and their ability to promote lipid-mixing was analysed using a (FRET)-based assay. To rationalize the results obtained in these experiments, we analysed the energy landscape of the peptides by performing bias-exchange metadynamics (BE-META) simulations. This allowed us to characterize the conformational properties of the WT peptide in a model membrane and understand how this structure is affected by the mutations studies. This study also elucidated the factors that explain the reduced activity of the mutants, which contributes to a better understanding of the role of the influenza FP in the fusion process.

Figure 1. The WT and four mutants of the influenza fusion peptide.

OC 22 - Selection of peptides for the efficient and mild affinity purification of retroviral particles

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Retroviral particles are expensive to manufacture, mostly due to the downstream processing steps which result in low recoveries (~30%) and concentration factors. The fragility of the lipid membrane layer that harbors envelope glycoproteins, often critical for infectivity, greatly reduces the titers of infectious particles. The identification of scalable and cost-effective strategies for viral purification and removal is paramount. In this work, a dodecapeptide phage-display library was panned against retrovirus like particles expressing the envelope protein Ampho4070A (VLPs-AMPHO) and VLPs without the target protein, used as a negative control (VLPs). A depletion/selection panning protocol was successfully used to deal with the structural complexity of the target, and a total of three distinct peptide sequences displaying preferential binding towards VLPs-AMPHO were found. Peptide 3 (CAAALAKPHTENHLLT), which appeared as the lead candidate, was synthesized and immobilized onto two purification matrices (cross-linked agarose and magnetic particles). The matrices selectively bound VLPs-AMPHO and in both cases recovery yields higher than 90% were obtained when employing mild elution conditions.

OC 23 - Antimicrobial cyclic lipopeptides as agents for plant protection

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In the last decades the use of antimicrobial peptides in plant protection has been widely studied due to their excellent biological properties. This family of peptides display a wide spectrum of activity against bacteria and fungi, selectiviy towards microbial targets, and a low frequency in developing microbial resistance. A subfamily of antimicrobial peptides that has emerged in recent years includes lipopeptides. They consist of a linear or cyclic peptide sequence, either cationic or anionic to which a fatty acid moiety is covalently attached. Lipopeptides have been shown to exhibit significant antibacterial and antifungal activity. Moreover, the addition of a lipophilic acyl chain to antimicrobial peptides has been demonstrated to be a useful strategy to increase their membrane affinity, thus improving their activity. In addition, lipopeptides have also been described to be active against plant pathogens. For instance, natural cyclic lipopeptides isolated from *Bacillus* strains have well-recognized antimicrobial activity against phytopathogenic microorganisms.

Within our efforts of finding new agents to control plant pathogens, in a previous study we designed and synthesized a library of cyclic decapeptides with general structure c(X5-Phe-X_3-Gln) where X is Lys or Leu. From this library we identified c(Lys-Lys-Leu-Lys-Lys-Phe-Lys-Lys-Leu-Gln) (BPC194) with high activity against the plant pathogenic bacteria *Erwinia amylovora*, *Xanthomonas vesicatoria* and *Pseudomonas syringae*. This peptide also exhibited low hemolysis. The antimicrobial activity of BPC194 and the excellent properties described for lipopeptides prompted us to design and synthesize lipopeptides derived from this cyclic decapeptide. We prepared a family of cyclic lipopeptides by acylating BPC194 at Lys5 with a range of fatty acids. We also analyzed if the position of the hydrophobic chain influenced the biological activity. Futhermore, taking into account that we have previously observed that the presence of a D-amino acid or of a His residue generally renders less hemolytic peptides, we decided to include in this study cyclic lipopeptides incorporating a D-Lys, a D-Phe or a His. All cyclic lipopeptides were screened against the above phytopathogenic bacteria, and the hemolytic activity against red blood cells and the phytotoxicity in tobacco leaves were also determined. The solid-phase synthesis as well as the results of the biological activity will be presented and discussed. The best cyclic lipopeptides are candidates useful not only to combat plant pathogens but also to be applied in other fields.

OC 24 - Selective derivatization of peptides containing *N*-terminal cysteines using 2,2-disubstituted cyclopent-4-en-1,3-diones

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The reaction of thiols with maleimides has been extensively used for the synthesis of all types of conjugates. Although the usefulness of this reaction is beyond question, some issues still remain unsolved. It is known that the resulting 4-alkylsulfenylsuccinimides are prone to hydrolysis and thiol-exchange reactions, which cause the formation of different regioisomeric succinamates and premature cleavage of the conjugate, respectively.

We envisaged that 2,2-disubstituted cyclopent-4-ene-1,3-diones (CPDs) could perform as maleimide analogs, and decided to investigate the stability of the generated Michael-type adducts with peptides containing cysteine at different positions. Interestingly, we found that the reaction between CPDs and cysteines with a free amine rendered a stable, not yet described adduct. On the contrary, the reaction with other cysteines (i.e. peptides with *C*-terminal and internal cysteines) formed a Michael-type adduct that remained in equilibrium with the starting materials (Figure 1).

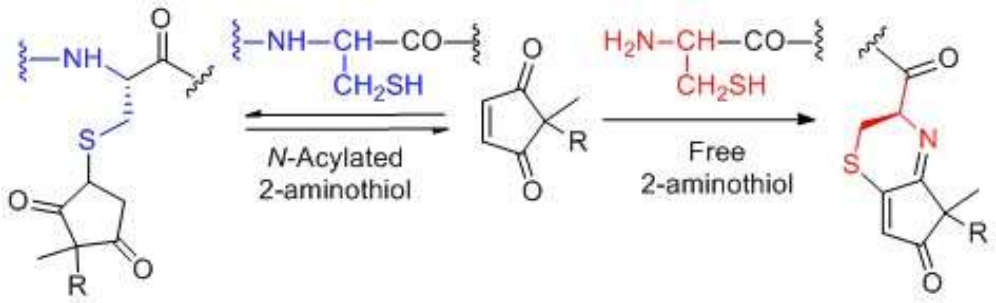

Figure 1. Differential reactivity of CPDs with 1,2-aminothiols and other thiols.

We have taken advantage of this differential reactivity to selectively label peptides containing *N*-terminal cysteines in the presence of other cysteine-containing peptides. Furthermore, regioselective double derivatization of peptides containing two differently placed cysteines was achieved by means of a tandem reaction with a CPD and a maleimide. The incorporation of a carboxyl-derivatized CPD to a peptide chain proved to be effective using standard peptide coupling, deprotection and purification conditions. This peptide was, afterwards, successfully conjugated to a PNA (Peptide Nucleic Acid) containing an *N*-terminal cysteine. Homocysteine (1,3-aminothiol) was found to react with CPDs in a reversible manner.

OC 25 - Fluorine in peptide and protein engineering

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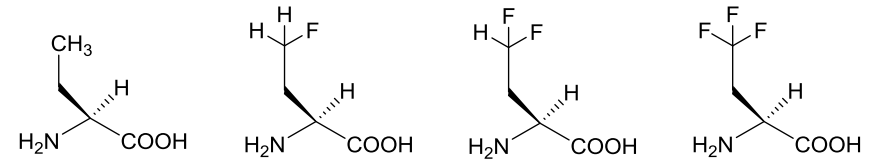
Fluorine's low polarizability and strong inductive effect make it an attractive tool in the design of non-canonical amino acids. Substituting side chain hydrogen atoms of amino acids with fluorine alters their chemical properties, such as hydrophobicity, acidity/basicity and reactivity. Thus, substitution of canonical amino acids with fluorinated analogues provides an opportunity for modulating the structure and activity of peptides and proteins. Incorporation of fluorinated amino acids has been shown to significantly affect protein folding and stability, peptide-protein and protein-protein interactions.[1]

To systematically investigate these effects (*S*)-2-aminobutyric acid (Abu) derivatives with increasing fluorine content (Fig. 1) were incorporated into proteins and into peptides based on the helical coiled-coil motif. Here, we present an overview of the past decade´s research from our group in the field of fluorinated amino acids and their use as a tool in protein engineering.[2-4]



(*S*)-2-aminobutyric acid (**Abu**) | (*S*)-Monofluoroethyl glycine (**MfeGly**) | (*S*)-Difluoroethyl glycine (**DfeGly**) | (*S*)-Trifluoroethyl glycine (**TfeGly**)

Figure 1. Structures of (*S*)-2-aminobutyric acid (Abu) and its fluorinated analogues.

References

1. M. Salwiczek, E.K. Nyakatura, U.I.M. Gerling, S. Ye, B. Koksch. *Chem. Soc. Rev.* **2012**, *41*, 2135-2171
2. U.I.M. Gerling, M. Salwiczek, C.D. Cadicamo, H. Erdbrink, S. Grage, P. Wadhwani, A. Ulrich, M. Behrens, G. Haufe, C. Czekelius, B. Koksch. *Chem. Sci.* **2014**, *5*, 819-830
3. V. Asante, J. Mortier, G. Wolber, B. Koksch. *Amino Acids* **2014**, *46*, 2733-2744
4. S. Ye, B. Loll, A. A. Berger, U. Mülow, C. Alings, M. Wahl, B. Koksch. *Chem. Sci.* **2015**, *6*, 5246-5254

OC 26 - Towards the chemical synthesis of the signaling protein Sonic Hedgehog

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Chemical protein synthesis (CPS) has become a potent tool for the synthesis of proteins, especially those which are not easily accessed by DNA recombinant methods, such as proteins with posttranslational modifications. The key reaction in CPS is Native Chemical Ligation, which implies a chemoselective ligation between two unprotected peptide fragments in aqueous solution. It requires a cysteine residue at the *N*-terminus of one peptide, and a thioester moiety at the *C*-terminus of the other fragment.[1,2] Several strategies have been developed for the synthesis of *C*-terminal thioester peptides using Fmoc-SPPS.[3] In our group thioester peptides are obtained following the *N*-acylurea approach which gives *N*-acyl-benzimidazolinone (Nbz) peptides as precursors of thioesters (Fig. 2 a,b).[4,5] We have developed new derivatives from the *N*-acylurea linker which are acylated with different chloroformiates (Fig. 2c). This new linker allows the presence on the same peptide of a cysteine residue at the *N*-terminal position and a thioester precursor at the *C*-terminus. We have used the new derivatives to perform Kinetic Controlled Ligations. All this methodology has been used for the synthesis of the natural protein Sonic Hedgehog, a lipoprotein with a palmitic residue at the *N*-terminus and a cholesterol molecule at the *C*-terminus (Fig. 1). The interest of this protein relies on its important biological functions in embryonary development.[6] Aberrant activity of Sonic Hedgehog has been related to several diseases including medulloblastoma, prostate and skin cancer, which makes it a potential therapeutic target.

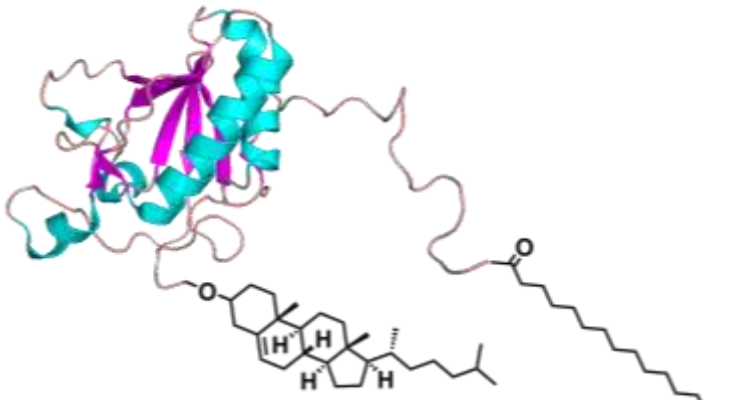

Fig. 1 Structure of Human Sonic Hedgehog N-terminal domain

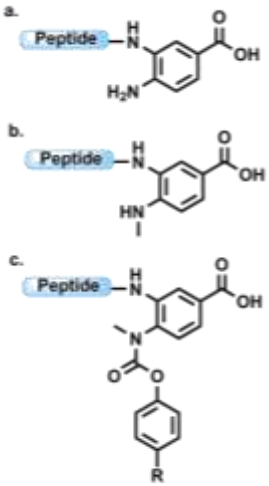

Fig. 2 a. Nbz linker; b. MeNbz linker; c. MeNbz linker derivatives

References

1. P.E. Dawson, T.W. Muir, I. Clark-Lewis, S.B. Kent. *Science* **1994**, *266*, 776.
2. S.B. Kent. *Chem. Soc. Rev.* **2009**, *38*, 338-351.
3. F. Mende, O. Seitz. *Angew. Chem. Int. Ed*. **2011**, *50*, 1232-1240.
4. J.B. Blanco-Canosa, P.E. Dawson. *Angew. Chem. Int. Ed.* **2008**, *47*, 6851-6855.
5. J.B. Blanco-Canosa, B. Nardone, F. Albericio, P.E. Dawson. *J. Am. Chem. Soc*. **2015**, *137*, 7197-7209.
6. M. Varjosalo, J. Taipale. *J. Genes & Development* **2008**, *22*, 2454-2472.

OC 27 - Total chemical synthesis of D-epidermal growth factor

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Epidermal growth factor (EGF) is a predominant regulator of angiogenesis. Uncontrolled growth of blood vessels plays a pathogenic role in several disorders including cancer and intraocular neovascular diseases. The development of improved antagonists of EGF, preventing the interaction with its receptor, is an important current objective in medicinal chemistry. With the mirror image phage display method, new D-peptide ligands of EGF can be obtained, which would be resistant to proteolytic digestion. This method involves the chemical synthesis of the mirror image form of the natural protein molecule.
EGF is a 53-amino acid protein with three intramolecular disulfide bonds. The stepwise synthesis of D-EGF gives rise to secondary reactions and provides low yields. For this reason, the combination of solid phase peptide synthesis (SPPS) and native chemical ligation (NCL) was applied to the synthesis of D-EGF. In this way, the EGF sequence was divided in two segments and they were synthesized with a cysteine and a thioester, respectively. After overcoming the synthetic challenges of this type of peptides, the two segments were linked using NCL. Finally, with the folding/disulfide bond formation, D-EGF was obtained. The chemical accessibility of D-EGF will make possible the use of mirror image phage display to search for new antagonists of EGF.

OC 28 - Synthetic peptide vaccines against foot-and-mouth disease: success at last

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Peptide-based vaccines would appear as the ideal alternative to conventional (e.g., inactivated whole-virus) vaccines, because they are safe (no infectious agent involved), versatile (readily adaptable to emergent outbreaks) and cost-effective (reliable, reproducible production and scale-up by chemical synthesis; simple storage and transport). These advantages are however offset by problems such as the difficulty in definition and chemical reproduction of epitopes, the usually low immunogenicity of peptides, or the often intricate relation between host-pathogen interaction and immune response, all of which partly explain why only a handful of peptide vaccines have attained therapeutic status.

Among the different pathogens targeted by peptide vaccines, foot-and-mouth disease virus (FMDV), arguably the economically most devastating animal disease worldwide, has received considerable attention. We have recently described vaccine candidates, generically known as BnT, consisting of a T-cell epitope branching out via a Lys tree into multiple (n= 2, 4) copies of a B-cell epitope. This particular arrangement of B and T epitopes on a single molecular platform is shown to confer full protection against FMDV challenge in both swine and cattle. The presentation will discuss various aspects in the development of this vaccine, particularly issues such as epitope orientation and multiplicity, chemical ligation methods or adjuvanticity. Insights on the uptake of the peptide by immune cells and on its *in vivo* localization will also be provided.

OC 29 - In-111 labeled peptides towards the estrogen receptor for theranostic of breast cancer

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The application of new strategies for diagnosis and targeted therapies has played important role in the improvement of breast cancer (BC) survival rate. The estrogen receptor (ER) status has been a well-established biomarker for BC prognosis and for guiding treatment. Despite recent advances there is still a need for more effective diagnostic and therapeutic tools mainly because of the heterogeneity of BC. Therefore, the search for novel ER targeting ligands has been a demanding task.[1]

ER proliferative effects in BC are mediated by a complex network of genomic and non-genomic actions. Based on that knowledge, peptidomimetics have been described which specifically interfere with the ER mediated mechanisms.[2] Peptides containing the sequence LXXLL have demonstrated high affinity towards the ER and therefore could be of potential value in the development of targeted specific radiopharmaceuticals.

In the present work three small peptides (8 to 9 amino acids) containing the LXXLL domain have been conjugated to the bifunctional chelator DOTA and radiolabeled with indium-111 (^{111}In). The choice of this radionuclide was based on the simultaneous gamma ray and Auger electron emission, which offers the possibility of both SPECT imaging and targeted radiotherapy.

The ER binding affinities of the peptide conjugates were evaluated by a fluorescent polarization assay. The ^{111}In-labeled peptides were obtained in high radiochemical yield and purity. The in vitro stability of the radiolabelled peptides were evaluated by HPLC. The lipophilicity (log P) of the radiolabelled peptides was measured by the "shake flash" method. Cellular uptake of the radiolabelled peptides was assessed in human breast cancer cell lines, such as MCF-7 (ER positive) and MDA-MB-231 (ER negative). Biodistribution studies of the ^{111}In-labeled peptides were also performed in female mice.

References

1. A. Cervino *et al. Nucl. Med. Biol.* **2013**, *40*, 581-91.
2. G. Leclercq *et al. Curr. Pharm. Des.* **2011**, *17*, 263.

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OC 30 - Design, synthesis and biophysical study of peptide ligands targeting epidermal growth factor (EGF)

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Epidermal growth factor receptor (EGFR) is a key target in chemotherapy. Some drugs acting on the receptor are currently in use; however, drug resistance, which causes tumour relapse, calls for the discovery of alternative inhibitors. Relying on existing structural data and using several approaches, like docking and receptor hotspot mimicry, we have designed novel peptides directed at EGF, the main growth factor ligand of EGFR (Figure 1). An array of biophysical techniques have been used to characterize the structure and interaction of these ligands with the target protein. The different design methods have been successful in identifying peptides able to bind EGF, and their capacity to inhibit the interaction between EGF and EGFR has also been studied. This new approach, based on targeting the smaller companion of a protein-protein interaction, can be envisaged as a parallel drug design strategy to design a new class of binders that could serve as part of a potential multidrug cancer therapy.

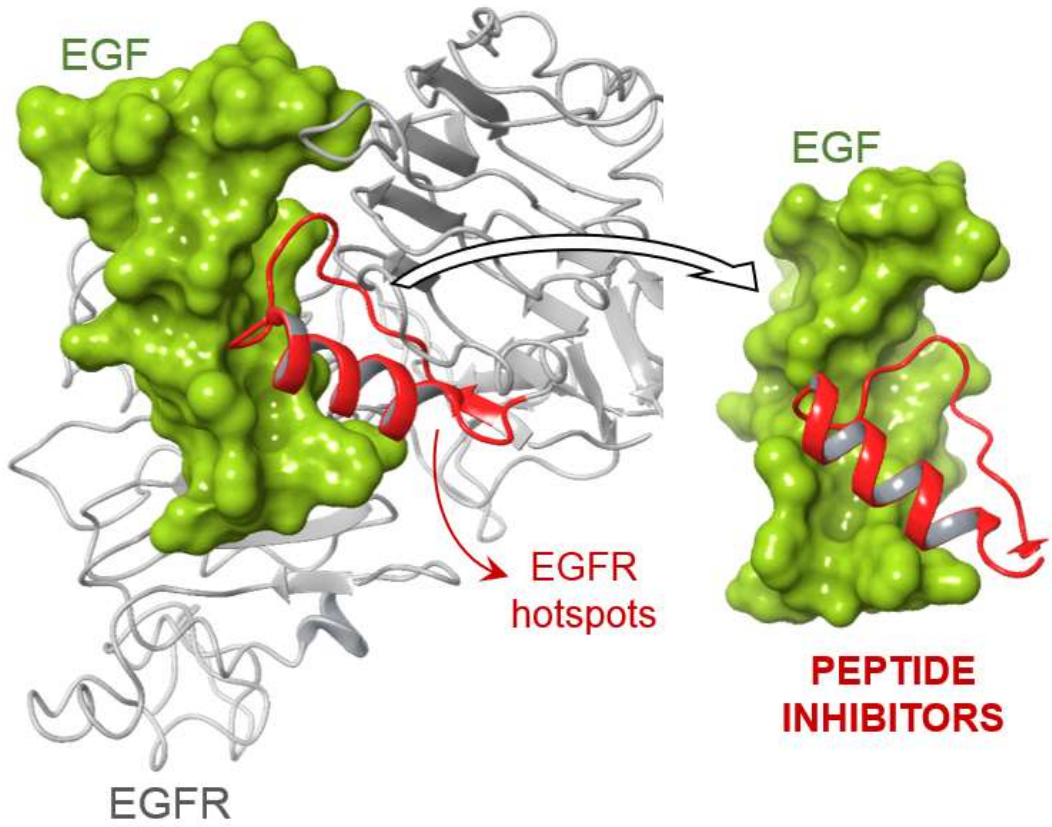

Figure 1. A novel class of peptide ligands against EGF has been designed using computer-aided docking and EGFR hotspot mimicry. Their binding to EGF has been biophysically studied and their ability to disrupt the EGF-EGFR interaction has been demonstrated *in vitro*.

OC 31 - Novel radiopeptides for molecular imaging of EGFR positive tumors

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The epidermal growth factor receptor (EGFR) plays a key role in the development and evolution of cancer, being an attractive target for cancer therapy. Consequently, several therapeutic agents have been designed for targeting this receptor, namely monoclonal antibodies (e.g., cetuximab) and tyrosine kinase inhibitors (TKi). Therefore, the non-invasive evaluation of EGFR status by molecular imaging modalities, such as positron emission tomography (PET) or single photon emission tomography (SPECT) may assist the selection of patients who are expected to benefit from anti-EGFR targeted therapy and to monitor their response to personalized cancer treatment. Hence, remarkable efforts are being devoted to find suitable radiotracers for *in vivo* imaging of EGFR. In this context, there has been a growing interest to identify small peptides to specifically target the extracellular domain of EGFR. From such efforts the novel peptides GE11 (YHWYGYTPQNVI) and D4 (LARLLT) were identified to bind to EGFR-overexpressing tumor cells *in vitro* and *in vivo*.

In the present work we have synthesized GE11 and D4 derivatives that have been coupled to DOTA and NOTA-based chelating agents through spacer chains of variable length. The resulting conjugates were successfully radiolabeled with $^{67/68}$Ga. The radiopeptides were obtained in high specific activity, high radiochemical yield and high radiochemical purity. The *in vitro* stability of the radiopeptides was evaluated by HPLC and their lipophilicity (log *P*) was determined. The radiopeptides were characterized by HPLC upon comparison with the corresponding inactive gallium complexes.

The biological behavior of the radiolabeled peptides was evaluated by internalization studies in tumor cell lines with different EGFR densities. Biodistribution studies of the most promising radiopeptides were performed in tumor-bearing mice.

References
K.C. Cuneo *et al. Pharmacology & Therapeutics* **2015**, *154*, 67–77.

Acknowledgements
The authors would like to thank Fundação para a Ciência e Tecnologia for financial support through projects EXPL/QEQ-MED/1950/2013, EXCL/QEQ-MED/0233/2012 and UID/Multi/04349/2013. Ana Gonçalves thanks FCT for a BI grant.

OC 32 - New insights into the design of somatostatin analogs: effect of the aromatic interaction into its 3D structure

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Somatostatin, also known as somatotropin release-inhibiting factor (SRIF), is a 14-amino-acid natural peptide. In clinical practice, somatostatin is currently used as a gastric anti-secretory drug to treat growth hormone secretion disorders and endocrine tumors. It is involved in multiple biological functions mediated by direct interactions between it and at least five characterized G-protein-coupled receptors, called SSTR1-5. These receptors differ in their tissue distribution and pharmacological properties. It is known that the molecule-crucial region for the interaction with these receptors is the one that includes Phe7-Trp8-Lys9-Thr10 residues (pharmacophore). It is also known that those residues form a β-hairpin stabilized by aromatic interactions between Phe6 and Phe11 side chains.[1] The vast majority of the analogs synthesized are shorter ones (6-8 residues), which mimic that pharmacophore; this is the case for octreotide,[2] lanreotide,[3] vapreotide,[4] and pasireotide,[5] the analogs which have been commercialized until now (Figure 1).

Our approach consists of modifying the sequence of the original structure but maintaining the 14-residue peptide (Figure 2). Previous work done in the group allowed us to obtain, for the first time, an accurate 3D view of some 14-residue somatostatin analogs where aromatic interactions between amino acids (AA) 6, 7 and 11 are key to the conformational stability of the peptide.[6]

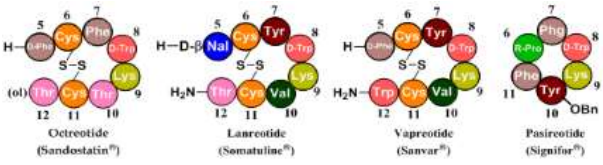

Figure 1.- Hexa- and octa-peptides commercialized until now.

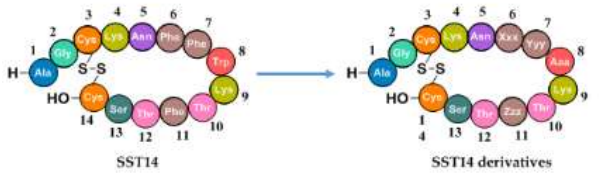

Figure 2.- General strategy for the synthesis of new 14-amino acids somatostatin analogs.

We will describe the effect of the non-natural aromatic AA L-3-(3',5'-dimethylphenyl)alanine (Dmp) in either positions 6, 7 and 11 in comparison with other AA previously obtained in our group such as L-3-mesitylalanine (Msa).[6] We will also introduce the non-natural aromatic AA D-3-(3'-quinolyl)alanine (Qla) in position 8 to change the electronic properties of the indole ring and clarify if the N-H bond is strictly necessary for activity with all receptors.[7]

References

1. M. Knappenberg, *et al. Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol*. **1982**, *700*, 229; 2. S.W.J. Lamberts, *et al. N. Eng. J. Med*. **1996**, *334*, 246; 3. J. Marek, *et al. Eur. J. Endocrinol*. **1994**, *131*, 20; 4. P.M. Girard, *et al. AIDS* **1992**, *6*, 715; 5. C. Bruns *et al. Eur. J. Endocrinol*. **2002**, *146*, 707; 6. P. Martín-Gago, *et al. Angew. Chem. Int. Ed.* **2012**, *51*, 1820; 7. R. Ramón *et al. ChemBioChem* **2011**, *12*, 625.

Acknowledgments

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OC 33 - Development of new peptide-gemcitabine conjugates for cancer therapy

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In developed countries, cancer is one of the major causes of death. Gemcitabine (2',2'-difluorodeoxycytidine), commercially available as Gemzar® by Eli Lilly and Company, is a nucleoside analogue which has been proven efficient against a wide range of solid tumors.[1] The use of gemcitabine hydrochloride was approved by the FDA in 1996 as first-line treatment for patients with locally advanced (non-resectable Stage II or Stage III) or metastatic (Stage IV) pancreatic adenocarcinoma or for patients previously treated with fluorouracil (5-FU). Gemcitabine is activated *in vivo* via phosphorylation of its 5'-monophosphate by deoxycytidine kinase, and is subsequently phosphorylated by intracellular kinases to the triphosphate form.[2] However, gemcitabine may be deaminated to its inactive uridine metabolite, 2',2'-difluorodeoxyuridine, by cytidine deaminase, which is present at high levels in both human plasma and liver.[3]

This communication aims at showing the chemical modification of gemcitabine and subsequent conjugation to Cell Penetrating Peptides (CPP), in an effort to facilitate delivery of that drug into cancer cells, taking advantage of the fact that CPP are able to efficiently pass through cell membranes while being non-cytotoxic and carrying a wide variety of cargos inside cells.[4] Two different CPP were synthesized by Solid Phase Peptide Synthesis (SPPS), purified and characterized chromatographically (HPLC and LC-MS/MS). Gemcitabine was successfully modified and conjugated to both CPP. The stability of the hydrolysable bonds was studied in PBS buffer at physiological pH and temperature and the results show a different time-dependent kinetics of gemcitabine release for both conjugates. The effect of these conjugates on the growth of the three cell lines was also preliminarily evaluated by SRB assays.[5]

References

1. E. Moysan, G. Bastiat, J.P. Benoit. *Mol. Pharm*. **2013**, *10*, 430-444.
2. P. Huang, S. Chubb, L.W. Hertel, G. B. Grindey, W. Plunkett. *Cancer Res*. **1991**, *51*, 6110-6117.
3. J.R. Kroep, C.J. van Moorsel, G. Veerman, D.A. Voorn, R.M. Schultz, J.F. Worzalla, L.R. Tanzer, R.L. Merriman, H.M. Pinedo, G.J. Peters. *Adv. Exp. Med. Biol.* **1998**, *431*, 657-660.
4. D.M. Copolovici, K. Langel, E. Eriste, U. Langel. *ACS Nano* **2014**, *8*, 1972-1994.
5. A. Ferreira, I. Fernandes, N. Mateus, P. Gomes, N. Vale. Submitted.

Acknowledgements

We thank the Fundação para a Ciência e Tecnolgia (FCT, Portugal) for financial support through IF00092/2014 and UID/Multi/04378/2013 projects. Thanks are further due to ON.2 and FCUP for co-funding refurbishment of the Porto Peptide Synthesis Facility (POP-UP) through operation NORTE-07-0162-FEDER-000111. NV is also grateful to FCT for holding IF position 00092/2014.

OC 34 - Bike peptides: a ride through the membrane

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There are several examples in nature of cyclic peptides which display a biaryl or biaryl ether motif in their structures. Among them, complex polycyclic compounds with interesting biological activities such as vancomycin or aciculitins are found.[1,2] This common feature acts as a conformational constraint rendering the desired rigidity, as well as, enhancing their metabolic stability, potency and selectivity.[3] Furthermore, the biaryl motif provides a site for aromatic-aromatic or π-cation interactions with the residues present on protein surfaces.

Our goal is to synthesize medium-sized biaryl bicyclic peptides containing 5 or 6 amino acids which display a biaryl bond between the side-chains of two aromatic residues in the sequence. This novel kind of biaryl stapled-like peptides can be used as scaffolds and be decorated with the most suitable residues so as to allow recognition between them and a protein target. Regarding the permeability of these compounds, the introduction of positively charged residues and the presence of the hydrophobic biaryl bridge are expected to favour their cellular uptake. Guanidinium groups present in the side-chain of arginine-containing peptides also became interesting for us in order to target p53 tetramerization domain. According to previous experience in our group, calixarenes with this feature were able to act as "pharmacological chaperones" by interacting with p53 and different mutants stabilizing the native tetrameric assembly of the proteins.[4] This fact is highly relevant, since mutations in this domain leads to the development of different types of cancer due to the loss of the structure and/or activity of the proteins.

References

1. P. Lloyd-Williams, E. Giralt. *Chem. Soc. Rev.* **2001**, *30*, 145-157.
2. L. Feliu, M. Planas. *Int. J. Pept. Res. Ther.* **2005**, *11*, 53-97.
3. N.K. Pandit. Introduction to the pharmaceutical sciences, Lippincott Williams&Wilkins, 2007.
4. S. Gordo, V. Martos, E. Santos, M. Menéndez, C. Bo, E. Giralt, E. de Mendoza. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 16426-16431.

OC 35 - Antimicrobial properties of short peptides derived from MSI-78

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Antimicrobial peptides (AMP) are a class of broad-spectrum antibiotics known by their ability to disrupt bacterial membranes and their low tendency to induce bacterial resistance, arising as excellent candidates to fight bacterial infections. In this study, we aimed at designing short 12-mer and 17-mer AMP, derived from MSI-78 (22 residues), a highly effective and broad spectrum synthetic AMP, by truncating the peptide at the *N*- and/or *C*-termini while spanning the MSI-78 sequence with 1 amino acid shifts. These designed peptides were evaluated regarding antimicrobial activity against selected gram-positive *Staphylococcus* strains and the gram-negative *Pseudomonas aeruginosa*.

The 17-mer peptide, MSI-78(4-20) (KFLKKAKKFGKAFVKIL), demonstrated to be as effective as MSI-78 against the strains tested while being more selective toward bacterial cells, as it was less toxic to Red Blood Cells (RBC) than MSI-78. As such, MSI-78(4-20) represents an improved version of the lead peptide MSI-78. Regarding the 12-mer peptides, a reduction in antimicrobial activity was observed, an effect that was more pronounced for gram-positive *Staphylococcus* strains. However, the 12-mer peptide CEM1 (GIGKFLKKAKKF) arose as a good candidate to fight *P. aeruginosa* infections. Biophysical studies support a mechanism of action for MSI-78(4-20) and CEM1 based on the disruption of the bacterial membrane permeability barrier, which in turn leads to loss of membrane integrity and ultimately to cell death. These features point to a mechanism of action similar to the one described for the lead peptide MSI-78.

Acknowledgements
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OC 36 - Structure studies and mechanisms of action insights of two novel antimicrobial peptides

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Increased resistance to conventional antibiotics has become a major worldwide problem, due to the inefficiency against resistant bacteria. The need to find new antimicrobial alternatives has become imperative with the emerging of multi-resistant bacteria. One of the most promising possibilities is the use of natural antimicrobial peptides (AMPs), which play an important role in the innate immune response of different organisms. AMPs are small cationic amphipathic molecules and their mechanisms of action are not yet fully understood. Some physicochemical properties play an important role in their mode of action, namely their amphipathic conformation upon interaction with biomembranes and their positive net charge, which allows them to interact preferentially with negatively charged biomembranes, such as those from bacteria. Until now, AMPs demonstrate a low propensity for drug resistance development.

Two different AMPs (Pa-MAP 1.5 and 1.9) were synthetically created based on an antifreezing peptide from the antarctic fish *Pleuronectes americanus*. These peptides showed promising therapeutic results against Gram-negative bacteria. *In silico* simulations revealed the preference of both for anionic membranes. These results were confirmed by dynamic light scattering, surface plasmon resonance and zeta-potential measurements. Membrane leakage assays using lipid vesicles composed by POPC:POPG and POPC:POPS showed that Pa-MAP 1.5 and 1.9 efficiently induce membrane disruption at concentrations as low as 0.30 μM. Studies using the fluorescent probes DPH, TMA-DPH, di-8-ANEPPS and Laurdan, either using model vesicles or *Escherichia coli* cells, showed that membrane properties are altered by the peptides. Atomic force microscopy (AFM) imaging of *E. coli* cells treated with either of the peptides demonstrate the peptide-induced changes on cell shape, increased surface roughness and release of the cellular content. The overall effect of both peptides can be translated in cell death (flow cytometry assays). The results obtained suggests that, although these two AMPs have several similarities regarding sequence and structure, they act through different mechanisms of action toward the target cells.

OC 37 - Ocellatins-PT antimicrobial peptides: structure, characterization, membrane interactions and anti-Leishmania activity

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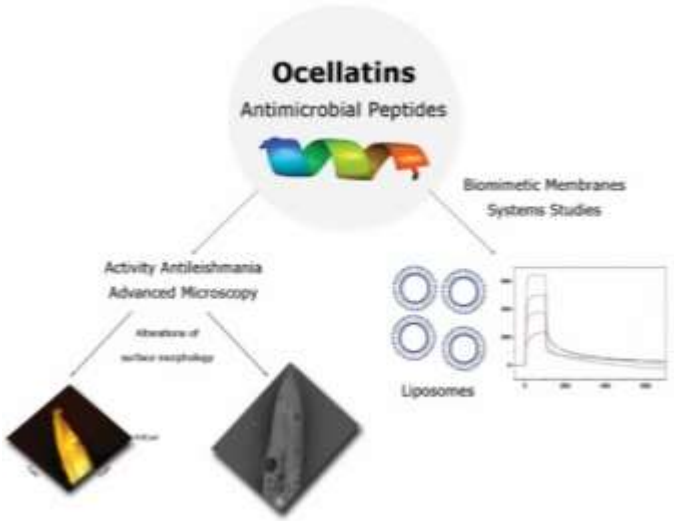
Antimicrobial peptides (AMPs) are ubiquitous molecules as a part of innate immunity. Ocellatins are a family of AMPs isolated from the skin secretions of the genus Leptodactylus with antibacterial activity. Recently, we isolated eight new ocellatin peptides from the skin secretion of *Leptodactylus pustulatus* and determined their amino acid sequences by de novo sequencing and cDNA cloning. All Ocellatin-PT peptides exhibited low antimicrobial activity but caused membrane perturbation in *E. coli*. Moreover, there was no haemolytic activity and cytotoxicity to murine fibroblasts at the determined minimal inhibitory concentrations (MICs).[1]



In this work, we continued the study of these Ocellatin-PT peptides, testing their antileishmania activity to promastigotes and axenic amastigote forms of *Leishmania infantum*, describing the effect of these peptides on parasite membranes using atomic force microscopy (AFM) and scanning electron microscopy (SEM) and testing its cytotoxicity against peritoneal macrophages. Moreover, we characterized by circular dichroism (CD) the secondary structure of Ocellatin-PT peptides and we further explored the different interaction affinity of these peptides for bacteria, parasite and mammalian cell membranes, using surface plasmon resonance (SPR). All peptides, except OcellatinPT2, showed moderate activity against promastigote form of *Leishmania infantum*, but only Ocellatin-PT4 and OcellatinPT6 showed activity against the axenic amastigote state. AFM and SEM images confirmed the perturbation of the membrane of promastigote form of *Leishmania infantum*, caused by Ocellatin-PT1 and Ocellatin-PT8. Selectivity for parasite *versus* human cell was also found, with all Ocellatin-PT peptides showing low toxicity against peritoneal macrophages. In summary, binding affinities calculated by SPR show a higher affinity of both Ocellatin-PT1 and Ocellatin-PT8 for bacteria and leishmania lipid membranes compared to mammalian lipid membranes.

References

1. M.M. Marani, *et al.*. *J. Nat. Prod*. **2015**, *78*, 1495-1504.

OC 38 - A proteomimetic approach to disrupt protein-protein interactions of trypanothione reductase of *Leishmania infantum*

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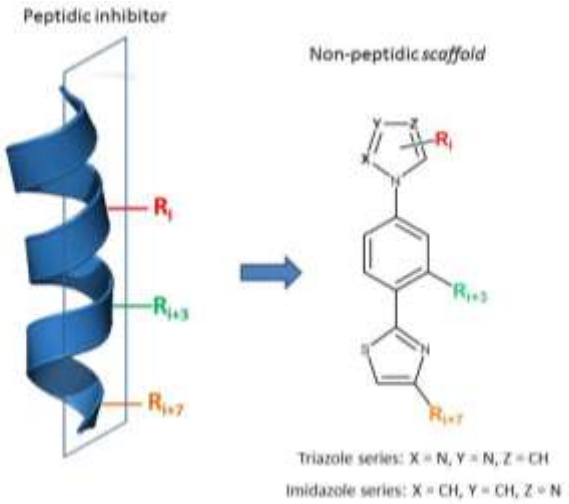
Current treatment of leishmaniasis, a neglected disease caused by *Leishmania* protozoan, requires administration of toxic and poorly tolerated drugs.[1] Trypanothione Reductase (TryR), an enzyme exclusive and essential for parasite survival, is a validated[2] and an attractive target that allows a selective action on the parasites. The biologically active form for the enzyme is a homodimer, and this offers a unique mode of inhibition, that is, by interfering with protein-protein interactions (PPIs) between both subunits. Molecular modeling and site-directed mutagenesis studies showed that Glu436 and Gln439 (located in an α-helix from P435 to M447) were hotspots for dimer stabilization. Based on these results, in our group, a series of peptides and peptidomimetics were designed and synthetized, mimicking the α-helix containing these hotspots, that resulted in potent inhibitors both of the activity and the dimerization of the enzyme.[3,4] However, use of these compounds as antileishmanials is hampered by their susceptibility to proteolysis and their poor bioavailability.



In order to improve the drug-like properties of the above peptide-based prototypes, we herein report the first non-peptide helical mimetics prepared following a proteomimetic approach to disrupt TryR protein-protein interactions. Different heterocyclic scaffolds were used to project the key side-chains at the i, i+3 and i+7 positions (essential to the PPI) in a spatial orientation similar to that in the native helix (Figure). These proteomimetics may result in molecules with potent activity and better pharmacokinetics than the peptide-based prototypes. Their design, synthesis and biological evaluation will be presented.

References

1. T.S. Tiuman, *et al*. *Int. J. Infect. Dis* **2011**, *15*, 525-532; 2. P. Baiocco, *et al*. *J. Med. Chem*. **2009**, *52*, 2603-2612; 3. M. Toro, *et al. ChemBioChem* **2013**, *14*, 1212-1217; 4. P.A. Sanchez-Murcia, *et al*. *RSC Adv*. **2015**, *5*, 55784-55794.

OC 39 - The role of post-translational modifications on the activity of a specific thrombin inhibitor

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The mammalian blood clotting system comprises a complex signal amplification cascade, with sequential activation of serine proteinase zymogens. The last proteinase in the cascade, Factor IIa / thrombin, plays a central role in the regulation of the hemostatic process given its pro- and anticoagulant functions. In result of their unique lifestyle, sidestepping the hemostatic mechanisms of their hosts is a vital need for hematophagous animals. To this effect, hematophagous parasites evolved an impressive arsenal of specific and highly effective inhibitors targeting the serine proteinases of blood clotting. Due to its central role in hemostasis, thrombin is the most common target of natural anticoagulants. Indeed, a number of unique mechanisms of thrombin recognition and inhibition have been identified among anticoagulants from hematophagous animals.[1,2] The still poorly characterized group of small (32-80 amino acids) cysteine-less thrombin inhibitors is a particularly intriguing class of natural anticoagulants. Belonging to MEROPS families I53, I64, I72, I74, I76, and I77, these flexible peptides can specifically bind to thrombin and effectively impair blood clotting. The mechanisms of action of some of these molecules started to be unveiled recently,[3-5] again revealing an unexpected diversity.

The sleeping sickness vector tsetse fly produces the smallest thrombin inhibitor described to date, with only 32 amino acid residues.[6] Despite its small size, this molecule – termed TTI – can specifically recognize and inhibit thrombin. However, native TTI was found to be three orders of magnitude more effective than recombinant or synthetic variants of the inhibitor at impairing thrombin's activity.[7] Using a combination of custom chemical synthesis and biochemical and structural characterization, we demonstrated that the increased efficiency of native TTI is due to the presence of two sulfated tyrosine residues, a specific post-translational modification that dramatically modulates the interaction between the inhibitor and its target proteinase.

References

1. M.A. Corral-Rodríguez, S. Macedo-Ribeiro, P.J.B. Pereira, P. Fuentes-Prior. *Insect Biochem. Mol. Biol.* **2009**, *39*, 579-595.
2. M.A. Corral-Rodríguez, S. Macedo-Ribeiro, P.J.B. Pereira, P. Fuentes-Prior. *J. Med. Chem.* **2010**, *53*, 3847-3861.
3. A.C. Figueiredo, D. de Sanctis, R. Gutiérrez-Gallego, T.B. Cereija, S. Macedo-Ribeiro, P. Fuentes-Prior, P.J.B. Pereira. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E3649-E3658.
4. C.Y. Koh, S. Kumar, M. Kazimirova, P.A. Nuttall, U.P. Radhakrishnan, S. Kim, P. Jagadeeswaran, T. Imamura, J. Mizuguchi, S. Iwanaga, K. Swaminathan, R.M. Kini. *PLoS ONE* **2011**, *6*, e26367.
5. A.C. Figueiredo, D. de Sanctis, P.J.B. Pereira. *PLoS ONE* **2013**, *8*, e71866.
6. M. Cappello, P.W. Bergum, G.P. Vlasuk, B.A. Furmidge, D.I. Pritchard, S. Aksoy. *Am. J. Trop. Med. Hyg.* **1996**, *54*, 475-480.
7. M. Cappello, S. Li, X. Chen, C.B. Li, L. Harrison, S. Narashimhan, C.B. Beard, S. Aksoy. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 14290-14295.

OC 40 - Peptide-targeted drug delivery systems of α-galactosidase A for the treatment of Fabry disease

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Fabry disease is a rare genetic lysosomal storage disease (LSD), characterized by a disorder of the glycosphingolipid metabolism due to a deficient lysosomal α-galactosidase A (GLA) activity. This enzymatic deficiency causes progressive accumulation of the glycosphingolipid globotriaosylceramide (Gb3) in the lysosomes of kidneys, heart, skin and brain, leading to multi-systemic clinical symptoms. The current treatment, which has demonstrated positive effects in patients, is the enzyme replacement therapy (ERT), in which a recombinant protein (GLA) is periodically administered. However, ERT presents some drawbacks such as the short half-life of the enzyme, making it necessary to administer high amounts of protein, the appearance of immunogenicity related side effects, and the high cost of the treatment.

One potential strategy to overcome these limitations consists in the use of targeted delivery systems. In this work, two approaches for the delivery of GLA enzyme have been developed. One of them consists in the preparation of GLA loaded nanoliposomes by DELOS-SUSP methodology, based on the depressurization of a CO_2-expanded liquid solution of lipids and/or surfactants into an aqueous phase. The other approach is based on the PEGylation of the enzyme, a widely used strategy that usually results in a longer half-life and activity of the therapeutic enzyme *in vivo*.

A relevant feature for the potential therapeutic efficacy of these systems is to deliver them to the lysosomes of endothelial cells, the main cell type affected by Gb3 accumulation. To achieve this, targeting peptides have been incorporated to both drug delivery systems. Namely, two peptides have been tested: a) a cyclic RGD peptide, a well-known ligand for integrins, overexpressed in endothelial cells affected by Fabry disease, and b) a cell penetrating γ-peptide developed in our research group with high capacity to target the lisosomes. The incorporation of these peptides in both vehicles resulted in a better cell internalization of the therapeutic enzyme, as well as in a better efficacy in reducing Gb3 accumulation in cells.

OC 41 - Rational dissection of the rattlesnake peptide crotalicidin retrieves a fragment with enhanced antimicrobial and antitumor properties

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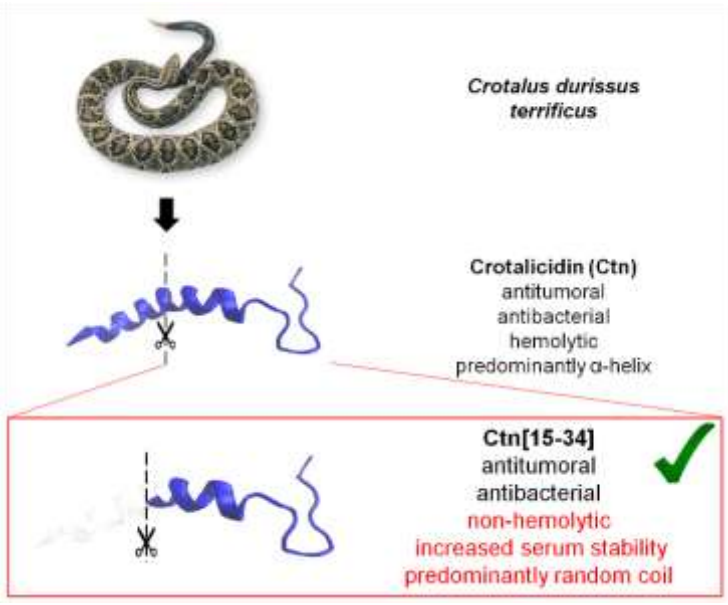
In recent decades, antimicrobial resistance has become a global health problem due to the abuse and misuse of antibiotics. The rise of multi-resistant bacteria calls for alternatives to classical antibiotics, with novel mechanisms of action. In this context, antimicrobial peptides (AMPs) are a promising choice by their potency, broad spectrum and mechanisms of action that minimize the appearance of resistances. Among AMPs, cathelicidins are a widely studied family whose unifying feature is a highly conserved cathelin-like domain in their precursor form. In mammals, cathelicidins are expressed mostly in circulating neutrophils, where they are stored as pro-peptides in specific granules. The functionally active cathelicidin peptides are released after cleavage of the pro-region by specific enzymes such as elastase or serine proteases. Our group has recently identified a new cathelicidin-like AMP named crotalicidin (Ctn) in the venom of the South American rattlesnake *Crotalus durissus terrificus*. In order to define a minimal structural motif with enhanced pharmacological properties, we have performed an *in silico* elastase dissection of the Ctn sequence yielding fragments, Ctn[1-14] and Ctn[15-34].

The presentation will report the synthesis, structural analysis and biological activity of Ctn, Ctn[1-14] and Ctn[15-34]. The solution structure of Ctn (α-helical *N*-terminus, disorganized *C*-terminus) is maintained in the fragments. Toxicity assays on bacteria, tumor and healthy cells show Ctn as the most toxic peptide, with antibacterial and antitumor activities in the low μM range and also toxic to healthy eukaryotic cells.



Despite its highly helical, amphipathic structure, Ctn[1-14] is innocuous, while surprisingly Ctn[15-34] preserves most of the antibacterial and antitumor activity of the parent peptide. More interestingly, Ctn[15-34] is highly selective against pathogenic cells and far more stable in human serum than Ctn. Taken together, these results suggest that Ctn[15-34] as a promising antimicrobial and/or antitumor lead.

OC 42 - Quantitative characterization of peptide-lipid partition using surface plasmon resonance

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The mechanism of action of bioactive peptides frequently involves interaction with lipid membranes. Membrane active peptides, such as antimicrobial peptides and cell penetrating peptides, concentrate within membranes and/or translocate them to perform their biological functions. Other peptides such as antiviral fusion inhibitors, concentrate at the membrane surface to promote target recognition and binding. Thus, there is an increasing need for optimized quantification methods that measure relevant peptide-membrane interaction parameters, namely partition constants (K_p), independently of peptide properties or function.

Surface plasmon resonance (SPR) is a label-free molecular binding detection technique for which there are no rigorous quantitative models of analysis of partition data. We have developed a mathematical partition model for flow-based SPR data treatment that enables quantitative *K*p determination. The model uses easily accessible sensorgram response data from Biacore L1 sensor chip experiments to correlate lipid deposition levels with drug binding data. We have performed experimental clarification of specific model assumptions and defined its limitations. The model accurately determined the *K*p of enfuvirtide, an anti-HIV-1 fusion inhibitor peptide (Figure 1), and kyotorphin, an endogenous analgesic peptide involved in drug optimization, towards POPC lipid membranes and predicted the respective saturation conditions.

We also successfully analyzed peptide-membrane unbinding data and retrieved kinetic parameters relative to membrane partition, further improving the dynamic application of the technique.

Our work represents a novel mathematical approach to improve the applicability of conventional SPR data to solute-membrane interaction studies. With the increasing interest in membrane active peptides and peptide mediated drug delivery, SPR applications might become valuable tools to predict peptide phase partition.

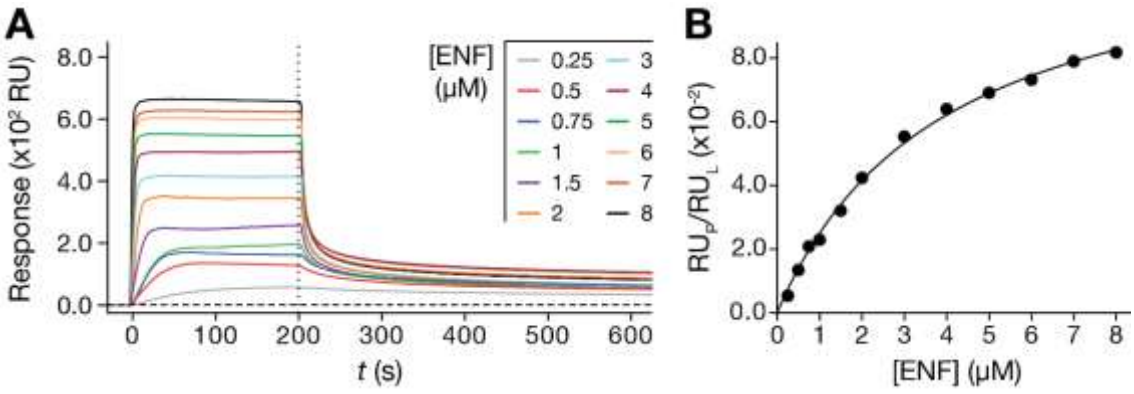

Figure 1. Application of the SPR partition model to enfuvirtide (ENF) (**A**) SPR time-resolved sensorgrams of enfuvirtide interacting with deposited POPC SUV. (**B**) Binding response data fitting with the SPR partition equation. Response values were acquired at t = 200 s.

OC 43 - Interaction of acylated $S4_{13}$-PV analogs with lipid membranes

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The therapeutic application of nucleic acids for the treatment of genetic disorders requires vehicles to deliver them at their intracellular sites of action in order to promote the correction of the abnormal phenotype. For this purpose, the vehicle should be able to surpass cellular membranes. Cell penetrating peptides (CPPs) have been developed for nucleic acid delivery due to their recognized ability to transpose membranes. In our laboratory, the ability of the $S4_{13}$-PV CPP to mediate the intracellular delivery of small interference RNA (siRNA) was significantly improved through the incorporation of acyl groups at the N-terminus of the peptide, the lauroyl group showing to be the most advantageous.

Aiming at gaining insights into the molecular mechanisms responsible for the different properties of the acylated $S4_{13}$-PV analogs in terms of their performance as siRNA delivery systems, a comprehensive biophysical characterization of the interaction of the acylated $S4_{13}$-PV analogs with model membranes has been carried out. In order to unravel how each peptide affects membrane physical properties, different experimental approaches were implemented, namely by using differential scanning calorimetry, ^{31}P-NMR and assays of calcein release. Additionally, surface- pressure/area experiments using monolayers were performed to assess the effect of the acyl chain length of acyl-$S4_{13}$-PV CPP on negatively charged membranes.

Altogether, our results might contribute for the establishment of structure-activity relationships, towards a rational design of new and efficient peptide-based nucleic acid delivery systems.

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