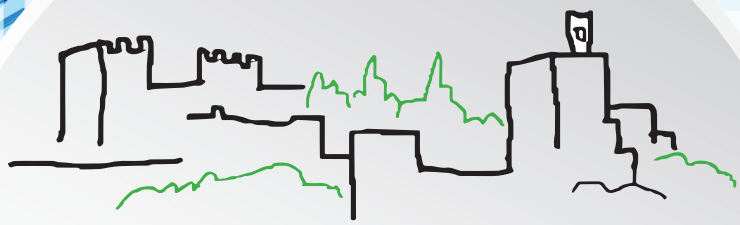
GRANADA

Book of Abstracts



February 19-21, 2020

V GEQB ChemBio
Group Meeting

Organized by:

CSIC

UNIVERSIDAD DE GRANADA

RSEQ

RSEQ Real Sociedad Española de Química

Organized by:

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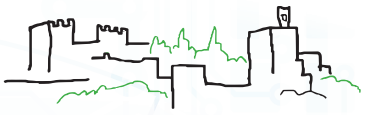
POSTER COMMUNICATIONS



February 19-21, 2020

V GEQB ChemBio Group Meeting

Welcome

WELCOME

On behalf of the Organizing Committee, it is our pleasure to invite you to the **V Chemical Biology Group Meeting (V GEQB)**, organized by the specialized group of Chemical Biology (GEQB) of the Spanish Royal Society of Chemistry (RSEQ).

The meeting, to be held at the auditorium of the Institute of Parasitology and Biomedicine López Neyra (CSIC) in Granada, **February 19-21, 2020**, has been envisaged as an opportunity for researchers in all areas of chemical biology to update into a single scientific event with a program that reflects cutting edge research in a large variety of inter-related areas. An impressive roster of internationally known speakers, from Italy, France, Netherlands, Spain and other European countries, as well as USA, has been enlisted for the plenary and invited lectures. These ones will be completed by a good number of oral, flash and poster communications to be selected from the submitted abstracts.

The biennial meetings of the RSEQ Chemical Biology Group started in 2012 in Santiago de Compostela and have successfully continued in Bilbao, 2014 (jointly with the 14th Iberian Peptide Meeting), Madrid, 2016 (this time jointly with the RSEQ specialized group on carbohydrates) and Barcelona, 2018 (jointly with the 16th Iberian Peptide Meeting).

We invite you to include the **V GEQB** in your plans and will be delighted if you can join us in this exciting occasion, with the attendant opportunity of expanding the scope of your research, meeting old and new colleagues and, why not, experiencing the vibrant city life of Granada.

See you in February at the V GEQB!!

With best personal regards,

Juan Carlos Morales Sánchez Co-chair

Sonsoles Martín Santamaría Co-chair

Ana Conejo García Co-chair

February 19-21, 2020

V GEQB ChemBio
Group Meeting

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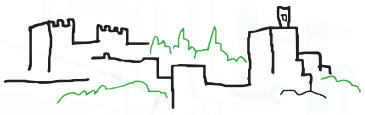

February 19-21, 2020

V GEQB ChemBio Group Meeting

Programme

Organized by: CSIC · UNIVERSIDAD DE GRANADA · RSEQ · RSEQ Real Sociedad Española de Química

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February 19-21, 2020

V GEQB ChemBio
Group Meeting

PROGRAMME
WEDNESDAY, February 19th, 2020

09:00-10:45 **REGISTRATION + COFFEE**

10:45-11:00 **GEQB – OPENING**

Mario Delgado, IPBLN-CSIC, Granada, Spain
Juan Carlos Morales Sánchez, IPBLN-CSIC, Granada, Spain

11:00-13:30 **SESSION 1**

Chair: Juan Carlos Morales Sánchez

11:00-11:45
Plenary Lecture *(sponsored by "Molecules", MDPI)*

A chemistry-based multidisciplinary strategy to semi-synthetic glycovaccines: from concept to first-in-human study and more in the context of *Shigella*
Laurence A. Mulard, Institute Pasteur, France

11:45-12:15 **Invited Lecture**

Impact of Biophysical Techniques on Drug Discovery
Juan Félix Espinosa, Lilly, Madrid, Spain

12:15-12:45 **Invited Lecture**

Turning stem cells into a medicine
Mario Delgado, IPBLN-CSIC, Granada, Spain

12:45-13:15 **Oral Communications**

OC1. Glycodendropeptides as potential synthetic vaccines against allergy
Javier Rojo, IIQ-CSIC, Seville, Spain

OC2. Development of new streamlined saponin vaccine adjuvants: molecular-level insights into saponin conformation correlating with adjuvant activity
Alberto Fernández-Tejada, Cicbiogune, Bilbao, Spain

13:15-13:30 **Flash Communications**

FC1. Pablo Valverde, NMR Studies of the specific interaction of DC-SIGN with the blood group A/B antigens

FC2. Beatriz Fernández de Toro, Avenues to characterize the interactions of extended N-glycans with proteins by NMR spectroscopy: the influenza hemagglutinin case

FC3. Manuel González Cuesta, Enzyme regulators with self-delivery capabilities

13:30-15:00 **LUNCH**

15:00-16:30 **SESSION 2**

Chair: Rosario Sánchez Martín

15:00-15:30 **Invited Lecture**

Multi-Scale Approaches to Computer-Assisted Enzyme Design
Gonzalo Jiménez-Oses, CICBiogune, Bilbao, Spain

15:30-16:00 **Invited Lecture**

Efficient and Sustainable Synthesis of Muramic and Glucuronic acid Glycodendrimers as Dengue Virus Antagonists
María José Hernáiz Gómez, Universidad Complutense, Madrid, Spain

16:00-16:15 **Oral Communication**

OC3. sp2-Iminosugar glycolipid mimetics as immunoregulatory agents
Elena M. Sánchez, University of Seville, Spain

16:15-16:30 **Flash Communications**

FC4. Alejandra Matamoros Recio, Computational approaches to the structure and activation mechanism of Toll-like receptor 4

FC5. José Antonio Laz-Ruiz, TDesign and development of novel nanodevices for biomedical applications

FC6. María Pia Lenza, Deciphering the N-glycan profile and interactions of FcεRIα by NMR

16:30-17:00 **COFFEE BREAK + POSTER SESSION 1**

17:00-18:30 **SESSION 3**

Chair: Ignacio Alfonso

17:00-17:30 **Invited Lecture**

Designing the 3D microenvironment of cells for the bioengineering of humanized tissues
Joao Mano, University of Aveiro, Portugal

17:30-18:00 **Invited Lecture**

Discovery of Novel Spirocycles as Inhibitors of the Mitochondrial Permeability Transition Pore Opening for the Treatment of Myocardial Reperfusion Injury
Delia Preti, University of Ferrara, Italy

18:00-18:15 **Oral Communication**

OC4. Interaction of amyloid-β with functionalised gold nanoparticles
Patrick Gámez, University of Barcelona, Spain

18:15-18:30 **Flash Communications**

FC7. **Reyes Núnez Franco,** Computational prediction of activity-enhancing mutations by allosteric networks optimization

FC8. **María Dolores Moya Garzón,** Development of salicylate derivatives with double GO/LDHA inhibitory activity as pharmacological approach for the treatment of primary hyperoxaluria type 1

FC9. **Marta Pazo Pascual,** Lipid responsive helical oligoalanines for cell penetration

18:30-19:00 **MEETING – GEQB**

THURSDAY, February 20th, 2020

09:00-11:00 **SESSION 4**
Chair: José Luis Mascareñas

09:00-09:45 **Plenary Lecture** *(sponsored by "Pharmaceuticals", MDPI)*
The Chemistry-medicine continuum: approaches to HIV/AIDS eradication and to enhanced immuno-therapy
Paul Wender, Stanford University, USA

09:45-10:15 **Invited Lecture**
To be announced
María Jesús Vicent, Centro de Investigación Príncipe Felipe, Valencia, Spain

10:15-10:45 **Invited Lecture**
Structural Studies of Weak Protein-Ligand Complexes by NMR: Novel Saturation Transfer Difference (STD) NMR Approaches
Jesús Angulo, Universidad de Sevilla , Spain

10:45-11:00 **Flash Communications**

FC10. **Javier Valverde Pozo,** A fluorescence ICT sensor for aminopeptidase detection in live cells

FC11. **Joan Miguel-Ávila,** Transition Metal Promoted Cycloadditions in Biologic Media

FC12. **Esther Jiménez Moreno,** Acetals as acid-sensitive cleavable linkers for the design of antibody-drug conjugates

11:00-11:30 **COFFEE BREAK + POSTER SESSION 1**

11:30-13:30 **SESSION 5**
Chair: Maria Jesús Vicent

11:30-12:00 **Invited Lecture**
Bio-inspired Materials Based on Simple Peptidic Derivatives
Beatriz Escuder, Universitat Jaume I, Castellón, Spain

12:00-12:30 **Invited Lecture**
Nature-inspired DNA-based nanodevices for diagnostic and drug-delivery applications
Francesco Ricci, University of Rome, Italy

12:30-13:00 **Invited Lecture** *(sponsored by SEQT)*

Development of synthetic Toxin-Antitoxin-based therapeutic systems for cancer treatment
Guillermo de la Cueva, Bionand, Spain

13:00-13:30 **Oral Communications**

OC5. Chemo-Enzymatic Total Synthesis of Sorbicillinoid Natural Products
Anna Sib, ICIQ Tarragona, Spain

OC6. Applying dynamic chemistry for miR-21 direct detection and quantification
Antonio Delgado -González, University of Granada, Spain

13:30-15:00 **LUNCH**

15:00-16:30 **SESSION 6**
Chair: Óscar Millet

15:00-15:30 **Invited Lecture** *(Young Investigator GEQB Award)*

Supramolecular Chemistry Synthesizing Artificial Biology
Javier Montenegro, CIQUS, Santiago de Compostela, Spain

15:30-16:15 **Oral Communications**

OC7. Dendrimers as a powerful tool in biomedicine. Applications in tissue regeneration processes and as biomarkers
Yolanda Vida, University of Málaga, Spain

OC8. Ruthenium-catalyzed redox isomerizations inside living cells
Christian Vidal, CIQUS, Santiago de Compostela, Spain

OC9. Fighting Resistances: Photoswitchable Antimicrobials Fully Operated under Visible Light
Xavier Just-Baringo, IRB, Barcelona, Spain

16:15-16:30 **Flash Communications**

FC13. **Salvador Guardiola,** Target-based de novo design of heterochiral cyclic peptides against the PD-1/PD-L1 interaction

FC14. **Guillermo Bañuelos** Sánchez, Specific Reverse Transcriptase Inhibitors for Mammalian LINE-1 Retrotransposons

FC15. **Raik Artschwager,** Towards the chemo-enzymatic synthesis of a N-glycomimetic library for the targeting of human DC-SIGN

16:30-17:00 **COFFEE BREAK + POSTER SESSION 2**

17:00-18:30 **SESSION 7**
Chair: Ana Conejo

17:00-17:45 **Oral Communications**

OC10. Dynamic stereoselection of kinetically-inert DNA-binding metallopeptide cylinders
Miguel Vázquez-López, CIQUS, Santiago de Compostela, Spain

OC11. GaudiMM: Opening New Horizons in Molecular Modeling of Chemobiological Systems
Jean-Didier Maréchal, UAB, Barcelona, Spain

OC12. Quinolimide-based probes: New tools to study protein-protein interactions
Juan Antonio González-Vera, University of Granada, Spain

17:45-18:30 **Plenary Lecture**
Size matters! Controlling the cellular microenvironment yields new insight into the inner working of cells
Wilhelm T. S. Huck, Radboud University, The Netherlands

20:30 **CONFERENCE DINNER**

FRIDAY, February 21st, 2020

09:00-11:00 **SESSION 8**

Chair: Sonsoles Martín Santamaria

09:00-9:45 **Plenary Lecture**

Breaking the limits in understanding glycan recognition by NMR
Jesús Jiménez-Barbero, Cicbiogune, Bilbao, Spain

09:45-10:15 **Invited Lecture**

The design of mGlu2 NAMs for neuropsychiatric disorders and studies towards potential tracers for PET imaging
Michiel Van Gool, Janssen, Toledo, Spain

10:15-10:45 **Invited Lecture**

Conformational landscapes in enzyme design
Silvia Osuna, Universitat di Girona, Spain

10:45-11:05 **Flash Communications**

FC16. **Lucía Tapia,** Efficient tyrosine trapping by the supramolecular protection from Src phosphorylation

FC17. **Mantas Liutkus,** Modular Artificial Light Harvesting Systems

11:05-11:30 **COFFEE BREAK + POSTER SESSION 2**

11:30-13:30 **SESSION 9**

Chair: Eugenio Vázquez

11:30-12:30 **Oral Communications**

OC13. Non-coding RNAs delivered by Magnetic and Gold Nanoparticles for the treatment of Uveal Melanoma
Álvaro Somoza, IMDEA, Madrid, Spain

OC14. G-quadruplex DNA Structures as Drug Targets for Small Molecules
Jorge González García, University of Valencia, Spain

OC15. Studying the Reaction Mechanism of Orotate Phosphoribosyltransferase by means of X-ray Crystallography and Computational Simulations
Maite Roca, Universitat Jaume I, Castellón, Spain

OC16. *(Best PhD thesis GEQB Award)*
To be announced

12:30-13:00 **Invited Lecture**

Bioorthogonal turn-on photosensitizers
Olalla Vázquez, Philipps-Universität Marburg, Germany

13:00-13:30 **Invited Lecture**

NMR and biosynthetic gene cluster analysis strategies for the determination of the structure and full absolute configuration of complex microbial natural products
Fernando Reyes, Fundación Medina, Granada, Spain

13:30-13:45 **CLOSING REMARKS & BEST GEQB FLASH & POSTER AWARDS**

13:45-15:00 **MINI-LUNCH**

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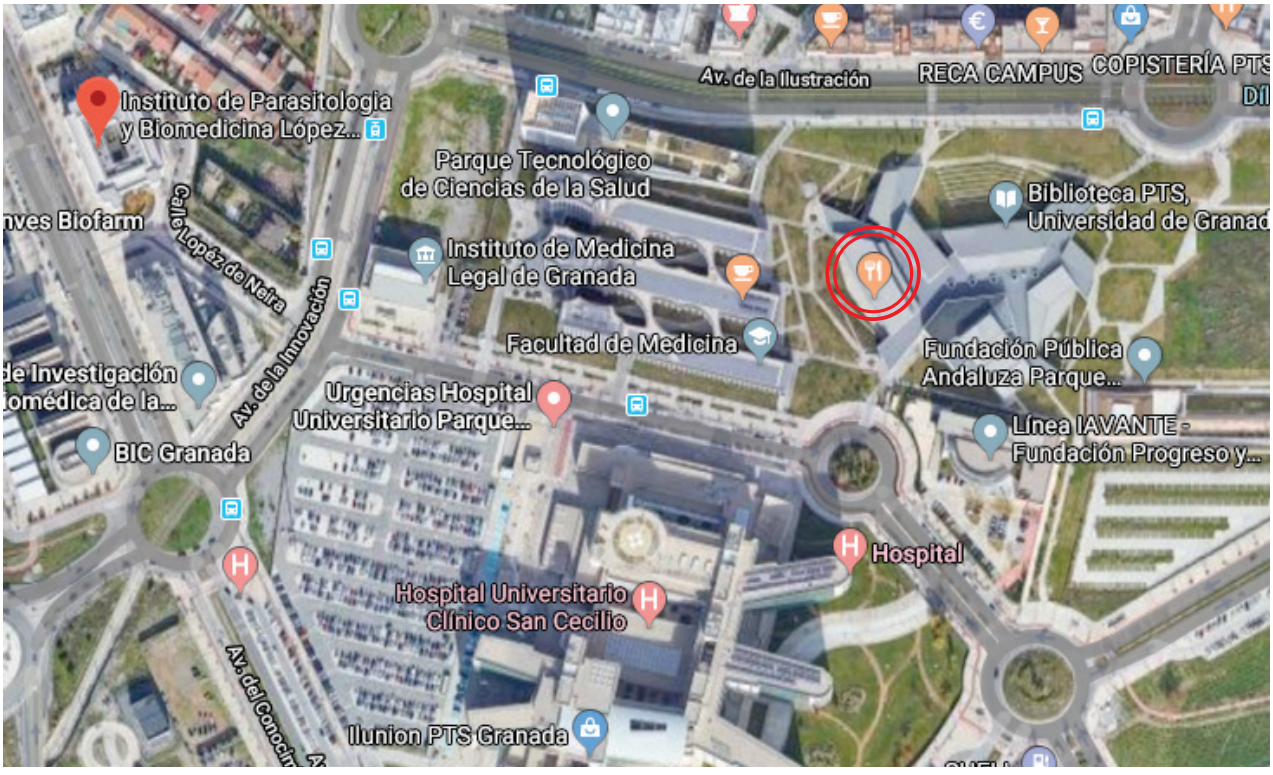
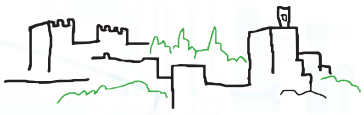
Granada Metro Map

Venue Map





February 19-21, 2020

V GEQB ChemBio Group Meeting

Plenary Lecture

A CHEMISTRY-BASED MULTIDSCIPLINARY STRATEGY TO SEMI-SYNTHETICGLYCOVACCINES: FROM CONCEPT TOFIRST-IN-HUMAN STUDY AND MORE IN THE CONTEXT OF *SHIGELLA*

Laurence A. Mulard

[a] Institut Pasteur, Chimie des Biomolécules, 28 rue du Dr Roux, 75 724 Paris Cedex 15, France
[b] CNRS UMR3523, Institut Pasteur, 75015, France

Shigellosis, or bacillary dysentery, caused by the enteroinvasive bacteria *Shigella*, remains one of the top diarrheal diseases in children under five.[1] Species/serotype diversity and geographical distribution strongly support the need for a multivalent vaccine against*S. flexneri*. Epidemiological data suggest that protection against re-infection is mainly achieved by antibodies specific for the O-antigen (O-Ag) moiety of the lipopolysaccharides (LPS). In the search for a highly immunogenic *Shigella* vaccine able to generate protective immunity in young children in low and middle income countries, we have engaged into the development of immunogens consisting of synthetic fragments of a selectedpanel of O-Ags covalently linked *via* single point attachment to carrier proteins as a possible alternative to detoxified *Shigella* LPS-protein conjugates.

A multidisciplinary strategy interfacing medicinal chemistry, immunochemistry and structure-based vaccinology was implemented.Sets of "protective" epitopes were identified by use of a diversity of well-defined synthetic oligosaccharides representing fragments of the O-Ags of interest. Protein conjugates of the most promising oligosaccharideswereevaluated for their immunogenicity in mice. SF2a-TT15, a tetanus toxoid (TT) conjugate encompassing a synthetic hapten corresponding to three basic repeating units of the O-Ag from *S. flexneri* 2a (SF2a), the most prevalent *Shigella* serotype, was developed as described.[2] In preclinical studies, SF2a-TT15 has been shown to induce anti-LPS bactericidal antibodies. A GMP batch was produced and a first-in-human, single-blinded, observer-masked randomized, dose escalation, placebo-controlled study was conducted to assess safety and immunogenicity in healthy adult volunteers.[3]

With the first rationally designed synthetic oligosaccharide conjugate vaccine candidate in hand for the most prevalent *Shigella* serotype, this presentation provides an overview of our strategy for a broad coverage *Shigella* vaccine. Emphasis is on hapten selection, glycovaccine design and production of a GMP batch. Safety and immunogenicity data following first use in human are exposed and the next steps towards establishing efficacy in human are discussed. Moreover, the presentation reports progress on a synthetic carbohydrate-based vaccine designed to provide broad coverage against *S. flexneri*.

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[3]https://clinicaltrials.gov/ct2/show/NCT02797236.

Acknowledgements
This work was supported by the European Commission Seventh Framework Program (FP7/2007-2013) under Grant agreement No. 261472-STOPENTERICS and the Institut Pasteur Vaccinology Program (GPF Vaccinologie) under Grant agreement FlexBiVac.

THE CHEMISTRY-MEDICINE CONTINUUM: APPROACHES TO HIV/AIDS ERADICATION AND TO ENHANCED IMMUNO-THERAPY

Paul A. Wender
Department of Chemistry, Department of Chemical and Systems Biology
Stanford University, Stanford, CA 94305 USA
https://web.stanford.edu/group/pawender/

Our research involves studies in chemistry, synthesis, materials science, biology and medicine. Our program seeks to create function through synthesis-informed design, often inspired by natural products and directed at unsolved medical problems including a cure for HIV/AIDS, enhanced immuno-therapy, overcoming cancer resistance, a platform strategy to treat infectious disease, gene delivery, a universal flu vaccine, multiple sclerosis and neurological disorders. This lecture will focus on the as yet unachieved goal of eradicating HIV/AIDS and the use of our own cells to make agents that elicit an immune response to prevent or cure cancer, two seemingly disparate goals that represent a merging of virology and immunology. Our function-oriented synthesis studies (*Accts.* 2015, 2169) provide a unifying strategy for the use of small molecules or mRNA delivery to modulate protein expression. For the HIV/AIDS project, this provides a mechanism to eliminate latently infected cells that re-supply the active virus and thus require lifelong treatment to control the disease with associated cost, compliance and chemo-exposure issues. To address this problem, epi-genetic modulators are used to induce activation of HIV infected cells, leading to their clearance by immunotoxin or immune responses and a possible cure. We have reported the design, synthesis and evaluation of small molecules, including prostratin (*Science* 2008), bryostatin (*Science* 2018) and their analogs (e.g. *ACS Central Sci.* 2018), that serve to activate HIV positive cells through a PKC pathway (e.g., PLoS Pathogens 2017, Virology 2018). Computer (*Nature Commun.* 2017) and REDOR solid state NMR studies (*JACS* 2015) on the structure of PKC and PKC modulatory ligands, the design and synthesis of new PKC modulators, and their latency reversing activities (*Nature Chem.* 2012; *PNAS*, 2013) will be presented. Significantly and in a similar mode of action to HIV latency reversal, bryostatin is also proposed to activate cancers cells leading to the enhanced presentation of CAR T cell targetable antigens. This platform strategy is proposed for clinical evaluation in 2020 for treating acute lymphoblastic leukemia. While epi-genetic modulators can be used to induce protein expression through transcription and translation, we have also developed a gene delivery platform that allows for direct translation of genes in cells and animals. This new drug/gene delivery platform (CARTs: PNAS 2017, 2018, 2018; *Cancer Res.* 2019) enables delivery of mRNA (as well as DNA, CRISPR/Cas, etc) into cells and animals thus controlling protein expression. Remarkably, we have found that this CART-RNA technology can be organ targeted and can be used to prevent or cure cancer in mice.

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•Paul A. Wender, Jung-Min Kee, and Jeffrey M. Warrington "*Practical Synthesis of Prostratin, DPP, and Their Analogs, Adjuvant Leads Against Latent HIV*" *Science* **2008**, 649-652
•Ryckbosch, Wender, Pande "Molecular Dynamics Simulations Reveal Ligand-controlled Positioning of a Peripheral Protein Complex in Membranes" *Nature Comm.* **2017**, **8**, 6.
•Yang, Staveness, Ryckbosch, Axtman, Loy, Barnes, Pande, Schaefer, Wender, Cegelski "REDOR NMR Reveals Multiple Conformers for a Protein Kinase C Ligand in a Membrane Environment" *ACS Central Science*, **2018**, 89
•Marsden, Wu, Mottahedan, Loy, Schrier, DeChristopher, Shimizu, Hardman, Ho, Ramirez, Wender, Zack "Characterization of designed, synthetically accessible bryostatin analog HIV latency reversing agents" *Virology* **2018**, 83
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•Benner, N.; Near, K.; Bachmann, M.; Contag, C.; Waymouth, R.; Wender, P. "Functional DNA Delivery Enabled by Lipid-Modified Charge-Altering Releasable Transporters (CARTs)" *Biomacromolecules* **2018** 2812
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•Benner, McClellan, Turlington, Haabeth, Waymouth, Wender "Synthesis of Poly(serine esters) by Organocatalytic Ring Opening Polymerization and their use as Charge-Altering Releasable Transporters for mRNA Delivery in vitro and in vivo" *Journal of the American Chemical Society* **2019**, 8416

Tttle A niche for every cell – a closer look at the importance of biophysical cues title title

W. T. S. Huck

Institute for Molecules and Materials,Radboud University, Nijmegen, the Netherlands
w.huck@science.ru.nl

The mechanical properties of the extracellular matrix (ECM) have emerged as an important microenvironmental cue regulating cell spreading and cell fate decisions. In this talk, I will show results of epidermal stem cell differentiation on a range of (micro-patterned) substrates, and dissect the different chemical, mechanical and topological parameters involved in dictating cell phenotype. A crucial aspect that has received little attention thus far is the dynamic nature of how complex systems such as cells adapt to their environment. I will discuss experiments where we correlate energy consumption and cell spreading on different substrates with stem cell fate. Finally, much of what we know about the influence of biophysical cues on stem cell fate comes from the cell culture studies on 2D micropatterned substrates. Our recent work shows that cell volume is a key parameters in controlling cell behaviour in well-defined 3D microniches. I will also discuss preliminary experiments on the organization of embryonic stem cells in multicellular assemblies within large 3D gel microniches.

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Breaking the limits in understanding glycan recognition by NMR

J. Jiménez-Barbero

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Molecular recognition by specific targets is at the heart of the life processes. The interactions between proteins (lectins, enzymes, antibodies) and carbohydrates mediate a broad range of biological activities, from fertilization and tissue maturation, to pathological processes. The elucidation of the mechanisms that govern how sugars are accommodated in the binding sites is a topic of interest. Although X-ray diffraction has widely been used to deduce structures of many sugar/lectin complexes, the inherently flexible sugars are handled poorly in X-ray structural biology and they are often reported with stereo- and regio-chemical errors that reflect incorrect refinement of the deposited sugar that could have huge implications when interpreted in a bio-context. Moreover, even in those cases where glycan/protein complexes can be crystallized, it is difficult to observe electron density for the flexible sugars and therefore the interaction modes of these regions cannot be clearly inferred. Therefore, the key tool for studying at atomic resolution the recognition processes in which glycans are involved is NMR. Solution NMR is unique in providing stereochemical and conformational information.

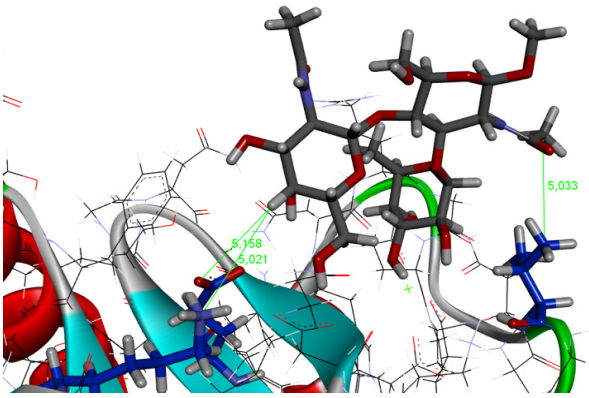

Figure. View of the recognition of one of the histo blood group antigens by a C-type lectin as deduced by NMR

Thus, I herein focus on the application of state-of-the-art NMR methods both from the ligand and receptor's perspective to study recognition processes between lectins of biomedical interest and glycans. Different lectins have been used to evaluate the relative importance of polar (hydrogen bonding, electrostatic) and non-polar (van der Waals, CH-π) forces in the recognition event, with especial emphasis in the application of novel NMR methods, pescially paramagnetic NMR, and on the analysis of the kinetics and thermodynamics.[1-7]

Acknowledgements

I thank the European Research Council for financial support (ERC-2017- AdG, 788143-RECGLYC-ANMR). I also thank AEI for grant RTI2018-094751-B-C21 and the Severo Ochoa Excellence Accreditation (SEV-2016-0644).

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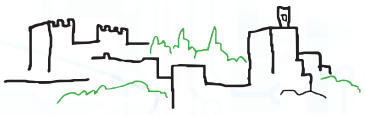

February 19-21, 2020

V GEQB ChemBio Group Meeting

Invited Lecture

Impact of biophysical techniques on Drug Discovery

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Biophysics technologies provide valuable data for binding confirmation, removal of false positives from functional assays and understanding mechanism of action of ligands, and they have become a key component of drug discovery workflows.[1,2] This presentation reviews the application of biophysical techniques to screening, hit confirmation, affinity measurements and characterization of protein-ligand interactions, with special focus on the value of ^{19}FNMR screening for fragment-based approaches.

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Quadruplexes are everywhere... but where exactly?

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[2]G-quadruplexes and i-DNA are unusual nucleic acid structures which can find applications in biology, medicine, as well as biotech- and nano-technologies [1]. We are developping tools to understand their folding and polymorphism [2]. In parallel, we proposed a new algorithm for prediction of G4 propensity of unprecedented accuracy [3]. We are now applying this G4-Hunter prediction tool to a number of genomes. In humans, we showed an association of G4-prone motifs with origins of replication [4]. We also became interested in quadruplexes quadruplex-prone regions conserved in the genome of a number of prokaryotes (incl. [5]), Archaea, viruses (HIV, HCV [6], Ebola…) and in "G4-poor" model organism such as *Dictyosteliumdiscoideum* or *Plasmodium falciparum* to confirm the importance of G-quadruplexes in biology[7]. In addition, we are investigating new families of G4 ligands (G4L), either as fluorescent light-up probes [8], anti-allergic [9] or anti-parasitic [10] drugs.

Regarding cancer, our team developed a series of novel 2,4,6-triarylpyridines G4 ligands with fair to excellent selectivity towards G-quadruplexes. Using a wide set of analysis methods such as transcriptomic and proteomic analyses as well as imaging and biochemical studies, we showed that one of these triarylpyridines, **20A,** elicits a significant activationofbiological pathways related to DNA damage response (DDR), autophagy and cell growth arrest [11]. These observations prompted us to investigate the relationship between these three processes in response to **20A**. Our results revealed that **20A** causes growth arrest in *in vitro* and *in vivo* cancer models. Notably, **20A** promotes a p53-independent induction of senescence and apoptotic cell death. We found that **20A** treatment leads to induction of DNA damage with subsequent activation of two DDR pathways, ATM/CHK2 and ATR/CHK1. Taken together, our study brings evidence that the activation of the ATM/autophagy pathway by the G4L **20A** plays a pivotal role in regulating cell fate choice between senescence and apoptosis. From a therapeutic of view, inhibiting the ATM/autophagy pathway impairs senescence and drives cells to apoptotic cell death, which offers a promising strategy to optimize the potential therapeutic efficacy of the G4L **20A** in cancers.

Acknowledgements

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Computational prediction of activity-enhancing mutationsbyallostery networks optimization

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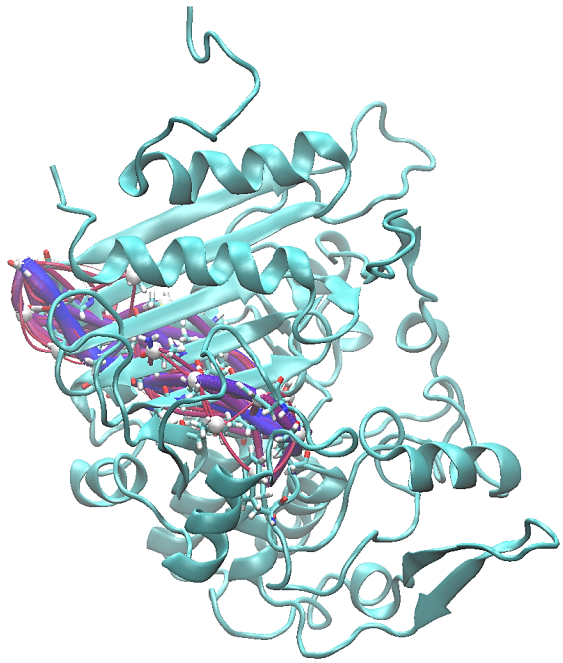
An artificialvariant of acyltransferase LovD has been discovered to be an efficient catalyst to obtain an unnatural product of high pharmaceutical value as a lipid-lowering medication – simvastatin – at industrial scale as a result of a directed evolution study.[1] This variant is 1000-fold more efficient in the synthesis of the drug than wild-type LovD and is able accept an unnatural acyl donor (DMB-SMMP) instead of the natural acyl carrier protein (ACP) partner. Through microsecondmolecular dynamicssimulationsperformed on the different variants evolved through laboratory engineering, it was proposed that one of the main roles of the mutations scattered throughout the entire enzyme was to maintain the catalytic triad in an optimal conformation in the absence of the ACP partner.

Traditionally, proteins structures have been studied by their secondary structure and folding patterns; alternatively, they can be treated as a highly dimensional network of dynamically interacting amino acid residues.



By combining this emerging paradigm in enzyme catalysis (i.e. activity regulation by distant, dynamically coupled residues) and our previous knowledge in the directed evolution of LovD, a new purely *in silico* approachmerging primary sequence alteration and molecular dynamics relaxation has been developed. Mutationspotentially able to improve the catalytic activity of the wild-type enzyme have been predicted though a dynamical network analysis. Such analysisprogram identifies not only the optimal but also the suboptimal pathways of correlated motions between pairs of residues that are not directly linked and may play important roles in the transmission of allosteric signals.[2]

Of note, our protocol has predicted influential mutations in both novel and previously engineered positions by directed evolution; moreover, specific mutations generated by laboratory evoluation have been found. More importantly, all the mutational hotspots discovered by our protocol are located in very remote regions from the active site, which differs from traditional enzyme design protocols based on active site remodelling.The necessary experimental evaluation of the highest ranked computational variants is currently ongoing.

Acknowledgements

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Sustainable Synthesis of Muramic and Glucuronic acid Glycodendrimers as Dengue Virus Antagonists

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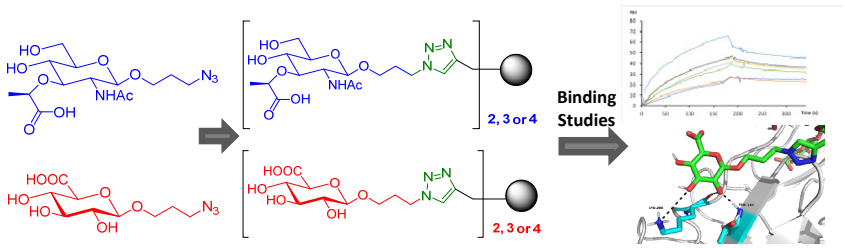
Carbohydrates are involved in many importantpathological processes, such as bacterial and viral infections,by means of carbohydrate-protein interactions. Glycoconjugateswith multiple carbohydrates are involved in multivalentinteractions, thus increasing their binding strengths to proteins. *N*-acetylmuramic(MurA) and glucuronic (GlcA) acids are both monosaccharides that play a key role in biological processes such as bacterial or viral infection.It is well known that Dengue virus binds to glycosaminoglycans (GAG) throughthe putative GAG binding sites within their envelope proteins(DENV) to gain access to the surface of host cells. These interactionsare predominantly ionic where positively charged, basic aminoacidresidues in the GAG binding site of DENV, interact withnegatively charged residues in the cell receptor, a highly sulphated form of the GAG heparan sulfate. Since heparan sulfateGAG is a putative receptor for DENV, one can envisage that agood strategy to develop viral infectivity inhibitors is the use soluble multivalent highly charged glycodendrimers. Therefore, conjugation of several copies ofMurA or GlcA to a multivalent scaffold will certainly result innew multivalent glycostructures with good binding affinities totheir receptors.In this communication, we present the efficient and sustainable synthesis ofnovel MurA and GlcA glycodendrimers as potential Dengue virus antagonists. Due to the complexity of the synthetic route leading to MurA and GlcA acid derivatives, new strategies have been developed.A series of O-linked MurA and GlcA β-glycosides functionalizedwith a suitable linker for further conjugation withalkyne-terminated scaffolds were prepared.For this purpose, we have explored two approaches, chemical[1] and enzymatic.[2] Aromatic scaffolds were coupled to MurA and GlcA derivatives by click chemistrythrough sustainable and optimized synthetic strategies to afford the desiredglycodendrimers with high yields.
Surface Plasmon Resonancestudies have demonstrated that the compounds reportedbind efficiently to the DENV.Molecular modelling studies were carried out to simulateand explain the binding observed. These studies confirmthat sustainable and efficient synthesis of glycodendrimers bearing a simple monosaccharide (GlcA or MurA) as ligand can bebrought about easily offering a versatile strategy to findnew active compounds against Dengue virus.



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Biopolymers/stem cells hybrids for bottom-up tissue engineering applications

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Tissue Engineering has been integrating principles of engineering, chemistry, materials science, biology and health sciences in order to develop regenerative-based therapeutic strategies combining stem cells and biomaterials. From the different sources of biomaterials, natural-based polymers have been proposed to produce matrices able to interact favourably with cells. Due to their hydrophilic nature and richness in chemically active groups, such polymers can be used to produce a variety of structures fabricated using aqueous-based or other environmental-favourable procedures. Examples are shown on the precise chemical modification of polysaccharides and human-derived proteins and processing of devices into different sizes and shapes with structural and functional characteristics suitable to be used in tissue engineering and regenerative medicine applications. The macromolecular design of the systems may be used to produce special hydrogels with improved mechanical properties. The selection of the right biomaterials/cells combinations can be aided by the use of high-throughput screening. Distinct strategies involving bioinspired approaches and nano/micro-technologies will be shown to prepare hybrid soft systems containing cells, in the form of microparticles, microgels or liquified capsules, that could give rise to biomedical devices using bottom-up strategies.

Discovery of Novel Spirocycles as Inhibitors of the Mitochondrial Permeability Transition Pore Opening for the Treatment of Myocardial Reperfusion Injury

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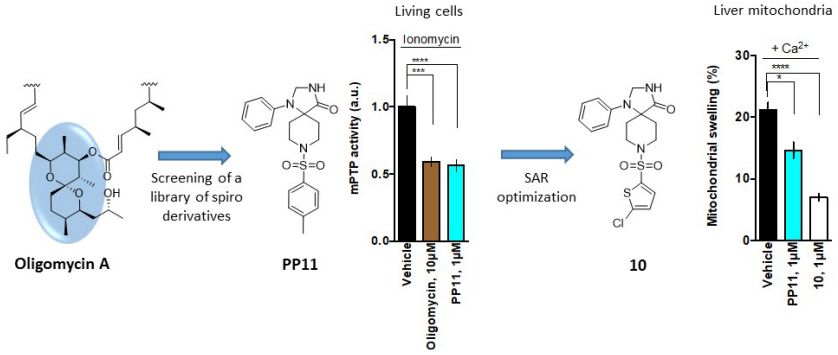
Acute myocardial infarction (MI) is a major cause of death worldwide and experimental studies have shown that nearly 50% of the final infarct size is due to the so-called reperfusion injury (RI), an elusive phenomenon that remains resistant to treatment.[1]Recent cardiology research investigations have demonstratedthat the opening of a large pore in the mitochondrial membrane, namely the mitochondrial permeability transition pore (mPTP), plays a key contributionin the final step of RI and is responsible for mitochondrial and cardiomyocyte death.[2,3]We recently reported the discovery,optimization, and structure−activity relationship (SAR) studiesof the first small-molecules able to target the c subunit of the F_1/F_O-ATP synthase complex thus inhibitingmPTP opening.[4]This first series of molecules is characterized by a1,3,8-triazaspiro[4.5]decane scaffold that has been designed starting from the more complex structure of oligomycin A. The approach led to three potentialcompounds exhibiting good mPTP inhibitory activity in vitro. Treatment during reperfusion with one of the mostpromising inhibitor (**10**) showed beneficial effectsin an ex vivo model of MI, with a reduction of apoptotic cell death upon RI.In addition, selected compounds did not show off-target effects at the cellular andmitochondrial levels and, of note, preserved the mitochondrial ATP content despite interacting with the ATPsynthase complex.



Besed on these preliminary results we have explored new spirocycles and synthetic strategies to identify novel, clinically useful mPTP inhibitors. These results validated a new pharmacological approach for the treatment of MI. Future perspectives will be oriented toward the understanding of the exact binding site between the developed inhibitorsand mitochondrial protein(s). Photoaffinity labeling of themost promising compounds identified here may lead to usefultools that will facilitate future binding site deconvolutionstudies advancing structure-based drug design approaches inthis field.

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Turning stem cells in a medicine

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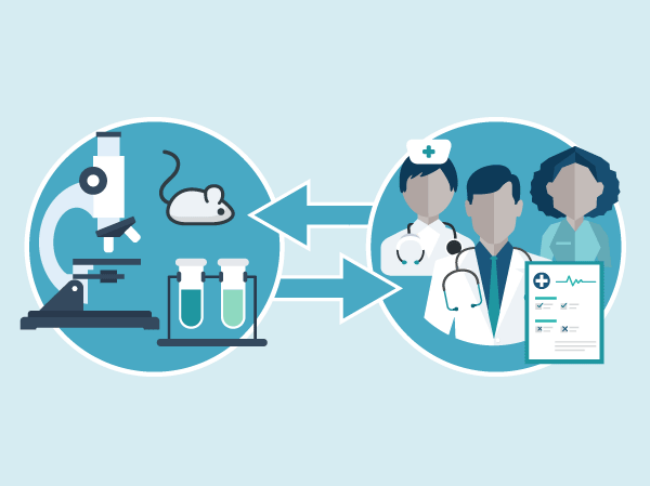
The current management of autoimmune disorders involves the treatment with immunosuppressive drugs coupled to symptomatic and functional interventions such as anti-inflammatory therapies and hormone replacement. However, because autoimmunity is chronic by nature and it is the result of a loss of self-tolerance by immune cells, the ideal therapeutic strategy would be to reinduce self-tolerance before significant tissue damage has accrued. This strategy involves the rebalance of inflammatory-mediated autoimmune response and the generation of tolerogenic immune cell populations (i.e. regulatory T cells, tolerogenic dendritic cells or alternatively activated macrophages).Drug discovery against common regulatory factors and pathways in this multitarget scenario has concentrated a tremendous effort in the last two decades by pharmaceutical companies, with some success cases. In parallel, several potentially tolerogenic cellular therapies have been developed in recent years. Among them, therapies based in the use of adult stem cells, mainly mesenchymal stromal cells (MSCs), have probably shown the best safety and efficacy profiles in a variety of clinical scenarios related with inflammatory and autoimmune disorders. By using in vitro cell culture and animal studies, we and others initially demonstrated that MSCs isolated from different adult tissues, mainly bone marrow and fat, have the capacity to modify immune responses and to enhance tissue repair, by producing a plethora of immuneregulatory factors and educating immune cells to be tolerant against self at different levels. Due to their immunemodulatory properties, we found that MSCs could be used in an allogeneic system, opening the possibility of design an “off-the-shelf” treatment. This property turned a cell-based therapy into a medicine and exponentially increased the interest of the pharmaceutical industry for tolerogenic cellular interventiorns. I will explain our experience in the development of adipose-derived MSCs in the first allogeneic cell-based treatment authorized by the European Medicines Agency for treating an autoimmune complication, such as Crohn disease, and as one of the most promising therapies in immune-mediated disorders.



Structural Studies of Weak Protein-Ligand Complexes by NMR: Novel Saturation Transfer Difference (STD) NMR Approaches

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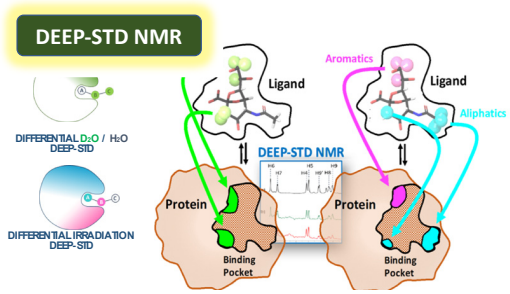
Saturation Transfer Difference (STD) NMR has demonstrated to be a powerful ligand-based NMR technique for weak ligand screening of protein targets and for gaining some structural information from biologically relevant protein-ligand complexes.[1] The approach is appropriate for small/medium-sized molecule binders of medium/weak affinity (K_D from high nM to low mM), there is no upper limit for protein size, and labelling is not required. Although technique is popular in the context of hit identification in drug discovery, its translation into a quantitative technique to generateNMR-validated 3D molecular models of the weak complexes is still to come.



In this talk we will show our recent steps towards a quantitative 3D structural view of STD NMR, and the investigation of biologically relevant systems will be presented.[2-5] Although broadly applicable to study many different protein-ligand systems, e.g. in Fragment Based Drug Design, the talk will keep a focus on its application to specific cases of recognition of glycans by proteins. Protein-glycan interactions are very relevant protein-ligand interactions in Nature and are processes typically falling within the range of fast chemical exchange (weak affinity) but still showing high specificity.[6]These protein-glycan systems will allow to introduce along the talk novel methodological developments in STD NMR produced in our lab, as the identification about how the fast ligand rebinding process can affect the determination of accurate dissociation constants by STD NMR,[7] as well as the development of the recent method "*DiffErential EPitope mapping STD NMR* (DEEP-STD NMR)"that allows for the first time to identify the nature of the protein-ligand contacts in the bound state from STD NMR approaches.[8]

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Bio-inspired Materials Based on Simple Peptidic Derivatives

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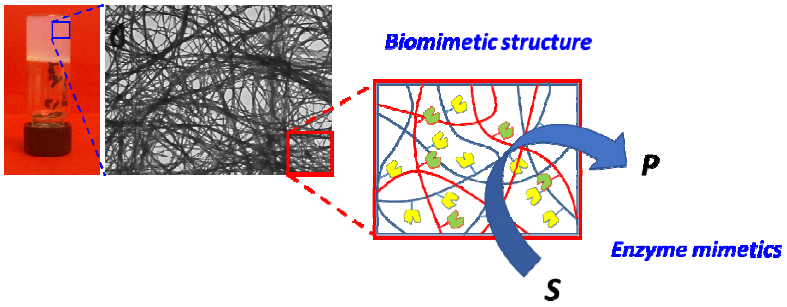
Nature has been a source of inspiration for a wide variety of functional supramolecular materials. For instance, protein structure and function has been emulated by simple synthetic systems with the concurrence of non-covalent interactions.[1]Among them, peptide-based self-assembled fibrillar materials (hydrogels) are particularly attractive because of structural as well as functional reasons (i.e. rigidity of the amide bond, rich chemical space provided by the amino acid side chain functionality, ability to form specific H-bonding patterns leading to hierarchical self-assembled structures).[2]
Here we will discuss on low molecular weight peptide-based materials as protein structure mimetics as well as materials with emergent catalytic properties.[3]



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DNA-based nanodevices for diagnostic and drug-delivery applications

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DNA nanotechnology uses synthetic DNA (or nucleic acids) as a versatile material to rationally engineer tools and molecular devices that can find a multitude of different applications (e.g., in-vivo and in-vitro diagnostics, drug delivery, genetic circuits etc.).

During this presentation I will introduce the field of DNA nanotechnology and I will show how to exploit the "designability" of DNA to fabricate nature-inspired DNA-based nanoswitches and nanodevices that are specifically designed to undergo a conformational change (switch) upon binding to a specific input (i.e. target). This input-triggered conformational change can be used for diagnostic, drug-delivery or synthetic-biology applications.

I will demonstrate how to characterize and recreate in-vitro several mechanisms to control the response of DNA-based nanodevices and how to regulate their activity with different chemical and environmental stimuli including pH, antibodies, enzymes, small molecules and redox inputs.

Development of synthetic Toxin-Antitoxin-based therapeutic systems for cancer treatment

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Prokaryotic organisms and many of their resident plasmids have evolved toxin-antitoxin (TA) systems that function as stress response elements. These encode intracellular toxins that target vital cellular processes, but that pass unnoticed to bearing cells most of the time, because they are neutralized by co-expressed antitoxins. However, the latter are rapidly degraded in response to stress, which unleashes the toxic activity of their cognate partners. Many of these toxins are selective endoribonucleases, and their activation alters gene expression profiles precisely and specifically, enabling affected bacterial cells (or plasmids) to respond effectively to the inducing stress.[1,2]

Although not evolved to function in eukaryotes, one of these toxins, Kid, killshuman cells, an effect that is neutralized by its cognate antitoxin, Kis[3]. This led us to propose that these two proteins could be used to engineer synthetic devices capable of achieving the elimination of predetermined populations of human cells without harming non-targeted cells, which could find applications in cancer therapy. Yet, successful implementation of this approach required that TA pair were conferred the ability to operate autonomously in human cells, not only to distinguish targeted from nontargeted cells, but also to modulate Kid/Kis ratios appropriately in response to that distinction, so that the former cells were killed whilst the latter cells remain protected by Kis.

We have successfully engineered this TA pair to create synthetic systems that only become active in human cells exposed to predetermined oncogenic insults, and induce their selective elimination.[4,5] In this presentation I will introduce these synthetic TA systems, and will discuss ongoing work aiming at exploiting this approach to producedifferent types of personalized anticancer strategies that avoid off-target effects, a challenge hardly tractable so far.

Acknowledgements

We acknowledge support from the Medical Research Council (UK) and theConsejería de Salud de la Junta de Andalucía.

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Supramolecular Chemistry Synthesizing Artificial Biology

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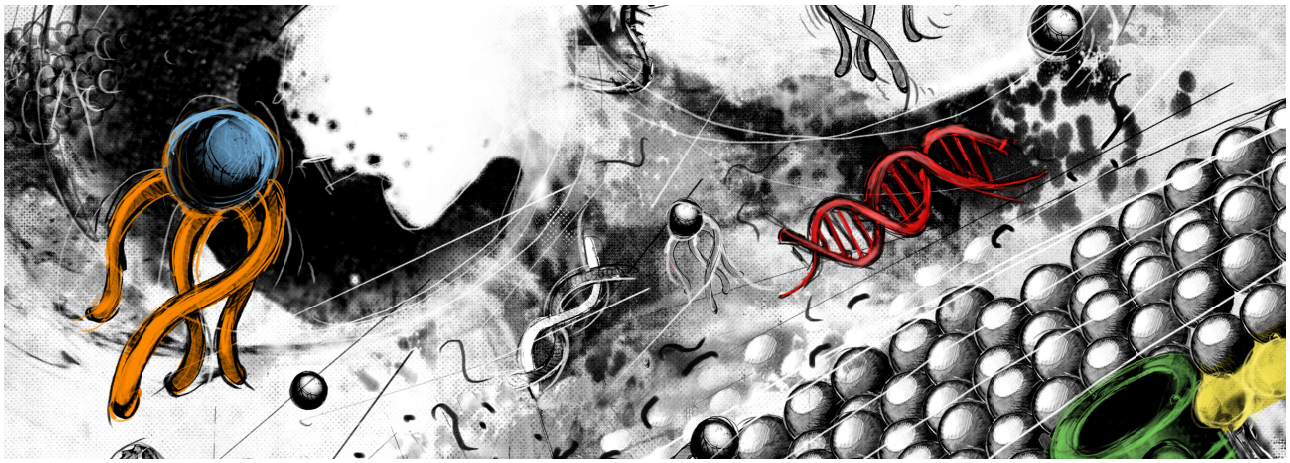
Our research group is interested in the application of supramolecular chemistry to understand and manipulate biology.[1,2]Ourwork philosophy is based in the importance of weak and non-covalent forces to control the shape and the topology of biomolecules, which are governed by the principles described by supramolecular chemistry. This topology is ultimately responsible of properties and function of biomoleculesand organelles and thus we believe that by modulating the shape we can control and improve functional behaviour. With focus in supramolecular interactions for artificial membranes and tubular composites, we investigate the construction of synthetic systems for controlling and emulating biology and life-like soft systems.[3-5]



Acknowledgements

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The design of mGlu2 NAMs for neuropsychiatric disorders and studies towards potential tracers for PET imaging

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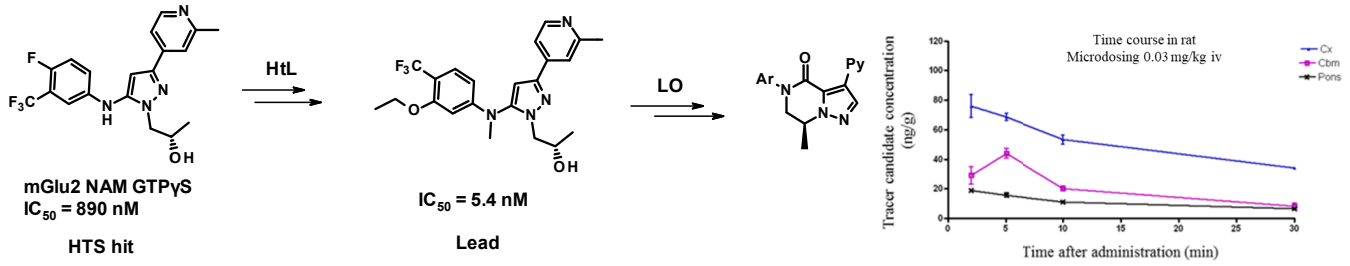
Glutamate, the main excitatory neurotransmitter in the brain, acts on two distinct classes of receptors: the ionotropic (NMDA, AMPA, Kainate) and metabotropic glutamate (mGlu) receptors. The mGlu receptors play an important modulatory role in neurotransmission and are closely involved in a variety of physiological functions. Preclinical data support the therapeutic potential of negative allosteric modulation of the mGlu2 receptor in neuropsychiatric disorders such as depression and improvement in cognitive function in disorders like Alzheimer Disease.

A high throughput screening (HTS) campaign resulted in an attractive pyrazole hit with moderate potency as negative allosteric modulator of the mGlu2 receptor. A focused medicinal chemistry optimization effort led to a lead compound with single digit nanomolar potency.[1] Further evaluation of this lead, focused on reducing lipophilicity by means of a drastic change in the central scaffold, led to a pyrazolo-dihydropyrazinone bicycle with improved drug-like properties. Further optimization towards the ideal substitution pattern resulted in the selection of a candidate for clinical evaluation.

In parallel to our Medicinal Chemistry efforts, we started a research program to discover a mGlu2 NAM-based PET radiotracer for in vivo imaging of this receptor in brain, applying our reported strategy.[2] Several differently substituted derivatives framed within the pyrazolo-dihydropyrazinone bicycle were synthesized, containing motifs that could be eventually radiolabeled either with ^{11}C or ^{18}F. We will present the synthesis and biological evaluation of a set of compounds belonging to this new series of mGlu2 NAMs, as well as the radiolabeling and preliminary assessment as potential PET ligands of the most promising candidates.



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Conformational regulation in enzyme evolution

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Enzymes exist as an ensemble of conformational states, whose populations can be shifted by substrate binding, allosteric interactions, but also by introducing mutations to their sequence. Tuning the populations of the enzyme conformational states through mutation enables evolution towards novel activity.[1]A common feature observed in many laboratory-evolvedenzymes, is the introduction of remote mutations from the catalytic center, which often have a profound effect in the enzyme catalytic activity.[1,2]As it happens in allosterically regulated enzymes, distal mutations regulate the enzyme activity by stabilizing pre-existing catalytically important conformational states. In this talk, ournew computational tools based on inter-residue correlations from microsecond time-scale Molecular Dynamics (MD) simulations and enhanced sampling techniques are applied in two different cases: the allosterically-regulatedTryptophan synthase (TrpS) and its laboratory-evolved stand-alone variants, and Alcohol Dehydrogenases (ADH) for the production of steroidal medicines.
We show how the distal mutations introduced inTryptophan synthase (TrpS) resuscitate the allosterically-driven conformational regulation and alter the populations and rates of exchange between these multiple conformational states, which are essential for the multistep reaction pathway of the enzyme.[3]Our computational analysis on evolved Alcohol Dehydrogenases (ADH)demonstrates that by altering the active site conformation populations (particularly the position of an α-helix) bulky industrially-relevant substrates can be properly accommodated for catalysis.[4] Both studieshighlight the importance of studying the conformational dynamics of enzymes and lays foundation for the development of computational protocols based on MD simulations for the design of new enzyme variants for biosynthethic applications.

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Bioorthogonal turn-on photosensitizers

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Our young group pursues the design and synthesis of novel photosensitive molecules capable of sensing biological processes,[1] and remotely controlling the sophisticated cellular machinery behind relevant disease-related processes.

Herein, we will focuson our recent work towards combating the current limitations of photodynamic therapy. Thus, despite that photodynamic therapy is a medical treatment in use, its widespread applicability is still hampered by the lack of selectivity. Inspired by the powerful advances in bioorthogonal chemistry, our young group has taken an interdisciplinary approach to first achieve organelle-targeted selectivity via novel halogenated BODIPY-tetrazinephotosensitisers, where tetrazine unit in situ mediates the conditional singlet oxygen generation causing the death of cancer cells. Our novel bioorthogonal-activatable probes increase selectivity via external small-molecule activation as well as organelle-specific localisation. Thus, as a proof of concept, we directed the photodynamic effect to the cellular nuclei of cancer cells.[2]

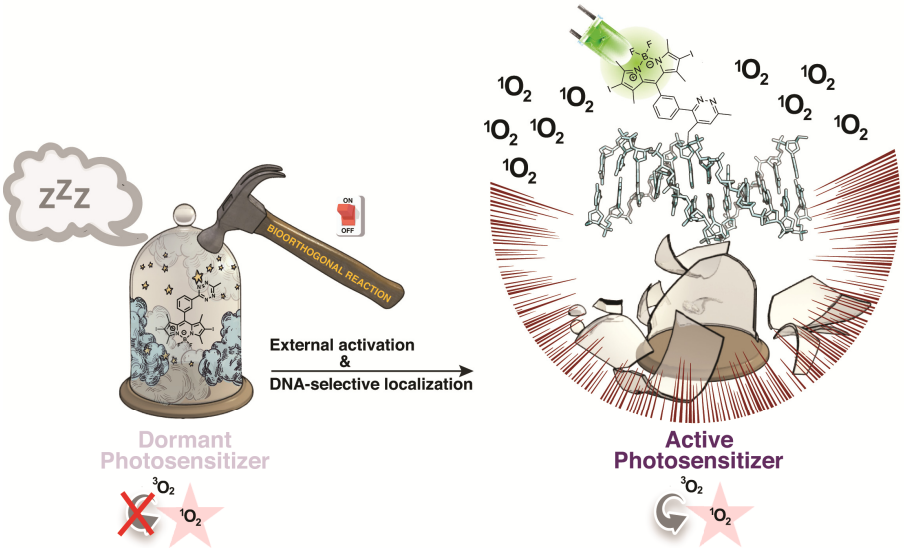

Figure 1. Outline our bioorthogonal turn-on probes for photodynamic therapy.

Acknowledgements

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Combined NMR and biosynthetic gene cluster analysis strategies for the determination of the structure and full absolute configuration of complex microbial natural products

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Natural products frequently possess complex structures, including multiple chiral centers, that make their structuralelucidation a challenging task requiring the use of many different spectroscopic approaches and computational techniques. Different structural classes may need the use of different strategies and in general a combination of several spectroscopic and chiroptical techniques, chemical derivatization and degradation reactions, and molecular modelling are used to solve the full structure and absolute configuration of complex molecules.

With the development of next generation sequencing technologies, whole genome sequencing of microbial strains has experienced an enormous breakthrough in the last decade and is nowadays a fast and affordable process. Bioinformatics tools have also been developed in parallel to mine the biosynthetic potential of the sequenced genomes and, among others, they allow to predict the absolute configuration of most chiral centers in a wide range of natural products encoded by the different biosynthetic gene clusters (BGCs). The combined use of those predictions with NMR analysis has become a very efficient and powerful approach to establish the full absolute configuration of complex natural products, specially polyketides and non-ribosomal peptides. In these structural classes, the configuration of chiral centers predicted by BGC analysis is usually confirmed and complemented with that determined by NMRfor other chiral centers that might be present in the molecule,originated from post-PKS or post-NRPS tailoring steps.

This combination of NMR and BGC analysis has been successfully applied in our laboratory to solve the structure of two complex families of actinomycete natural products: caniferolides A-D,[1]a family of complex antifungal and cytotoxic polyketide macrolides isolated from the marine derived actinomycete *Streptomyces caniferus*, and the krisynomycins,[2]a family of non-ribosomal depsipeptides isolated from*Streptomyces* sp. that act as potentiators of the antibacterial drug imipenem versus methicillin-resistant *Staphylococcus aureus*, whose structural elucidation was additionally complemented by Marfey´s analysis. Details on the structural elucidationof these two families of bioactive natural products, including the determination of the absolute configuration of all their chiral centers, will be commented.

Acknowledgements

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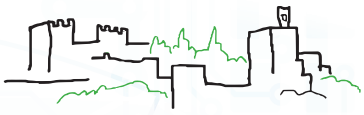

February 19-21, 2020

V GEQB ChemBio Group Meeting

Oral Communications

OC7

Glycodendropeptides as potential synthetic vaccines against allergy

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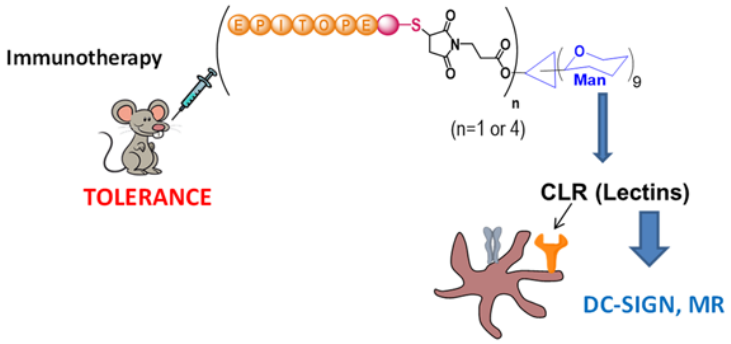
Immunotherapy (IT) is considered as a new therapeutic strategy for the treatment of diseases such as cancer or allergy. In the case of allergy, IT combines the use of specific allergens together with adjuvants capable to activate Dendritic Cells (DCs). After the processing of the antigen and the maturation of DCs, these cells stimulate naïve T-cells to produce a specific immune response with the aim to induce allergen tolerance.
DCs are one of the major players of APCs (Antigen Presenting Cells) which function is the surveillance for the detection of antigents to trigger a specific immune response. This function is achieved through the recognition of these antigens using specific receptors such as Toll-like Receptors (TLRs) and C-type Lectins (CTLs). Among CTLs, DC-SIGN (Dendritic Cells Specific ICAM-3 Grabbing Non-integrin) is one of the most important lectin involved in the detection of glycan antigens, leading to the maturation of DCs and inducing the activation of T-cells. In fact, mannosylation of peptides and proteins has been used as a succesful strategy to facilitate the internalization and presentation of peptides by APCs.[1]



We have conjugated a nonavalent dendron of mannoses with two different allergens, one from peach (Pru p 3) and another one from olive tree pollen (Ole e 1). These conjugates has been tested in cellular models using samples from tolerant and allergic donors. Also, in the case of Pru p 3, the corresponding conjugate has been evaluated *in vivo* using an anaphylactic mouse model. The preliminary results are very promising and suggest that this type of conjugates can be considered as potential new synthetic vaccine for allergy inducing tolerance.[2]

Acknowledgements

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OC27

Development of new streamlined saponin vaccine adjuvants: molecular-level insights into saponin conformation correlating with adjuvant activity

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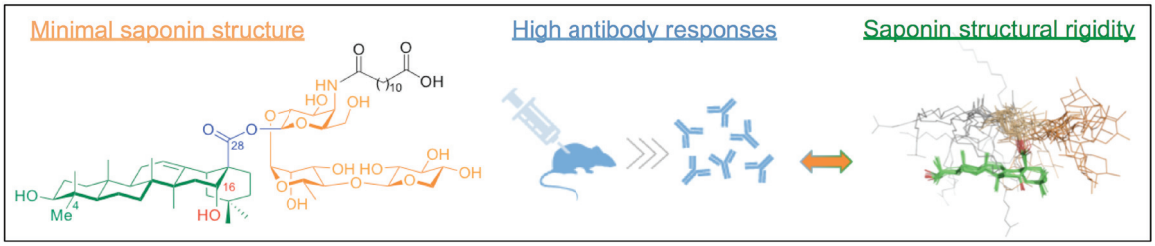
Current subunit vaccines based on well-defined molecular antigens offer improved safety and more precise targeting than whole-pathogen vaccines. However, they are inherentely less immunogenic and require coadministration with an adjuvant to increase antigen immunogenicity and potentiate the immune response. QS-21 is a saponin natural product adjuvant extracted from the *Quillaja* saponaria (QS) tree that has been recently approved as part of the malaria and shingles vaccines. In my previous postdoctoral studies at MSKCC (NY, USA), we identified critical structure-activity relationships of the QS saponin class[1] that have now been exploited in the design and synthesis of streamlined saponin vaccine adjuvants.[2] In this talk, I will present the latest studies in my group at CIC bioGUNE (Bilbao, Spain) on the development of new saponin variants that feature minimal and optimal structural elements for adjuvant activity. These minimal saponins have been accessed in reduced synthetic steps (22 total steps) compared to previously reported QS saponin candidates, and elicited potent antibody responses in mice without appreciable toxicity. The obtained immunological results emphasize the dispensability of the native triterpene C4-aldehyde substituent for adjuvant activity and the importance of the triterpene C16-hydroxyl group in these variants, since the corresponding congeners lacking the C16-hydroxyl showed attenuated antibody responses. To seek a molecular rationale for this finding, we carried out the first combined experimental (NMR) and computational (molecular dynamics simulations) conformational analysis on such synthetic saponins. The conformational study revealed that active variants exhibited higher structural rigidity and adopted a main, folded conformation in contrast to the attenuated congener, which displayed a more flexible conformational behaviour with the presence of an additional conformation associated to the lack of the C16-hydroxyl group.



In summary, this presentation will cover our most recent multidisciplinary studies at the Chemical Immunology Lab at CIC bioGUNE, which have provided expedient synthetic access to streamlined saponin vaccine adjuvants and molecular-level insights into saponin conformation that correlate with *in vivo* adjuvant activity.

Acknowledgements

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OC13

sp^2-Iminosugar glycolipid mimetics as immunoregulatory agents

E. M. Sánchez-Fernández,[1] M. I. García-Moreno,[1] A. I. Arroba,[2] M. Aguilar-Diosdado,[2] J. M. Padrón,[3] R. García-Hernández,[4] F. Gamarro,[4] J. M. García Fernández,[5] J.-E. Sánchez-Aparicio,[6] L. Masgrau,[6] C. Ortiz Mellet[1]

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Monosaccharide analogs of the sp^2-minosugar family offer unprecedented opportunities to access enzymatically and chemically stable glycoconjugate mimetics amenable to drug discovery programs. In this context, the 1-dodecylsulfonyl-5*N*,6*O*-oxomethylidenenojirimycin (DSO_2-ONJ) (Figure 1), a sp^2-iminoglycolipid (IGL) representative, has shown outstandinganti-inflammatory activity in microglia, reducing gliosis in diabetic *db*/*db* mice.[1]DSO_2-ONJ further inhibited the activation of human monocyte-derived dendritic cells (DCs) by lipopolysaccharide (LPS) in vitro and in vivo in amouse model of acute inflammation.[2]Biochemical studies and computational docking experiments endorsed a mechanism of action that implies attachment to the allosteric lipid binding pocket of p38αmitogen-activated protein kinase (MAPK), a key protein in the regulation of the innate immuneresponse. This binding mode has also been demonstrated with sp^2-IGLs incorporating at the pseudoanomeric position different polyfluoroalkyl segments of varied lengths that facilitate passage through cell membranes. This modification resulted in a 10-fold higher effectiveness at eliciting p38 (Figure 1).[3]In this communication we will present the synthesis of differentfluorinated sp^2-IGLs and their biological evaluation in the context of three pathologiesthat share common signaling pathways: cancer, Leishmania infection and inflammation.

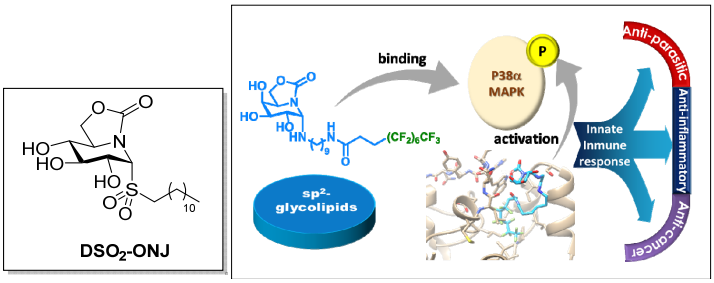

Figure 1.DSO_2-ONJ and polyfluoroalkylsp^2-iminosugar glycolipids as immunomodulatory agents.

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OC12

Interaction of amyloid-β with functionalised gold nanoparticles

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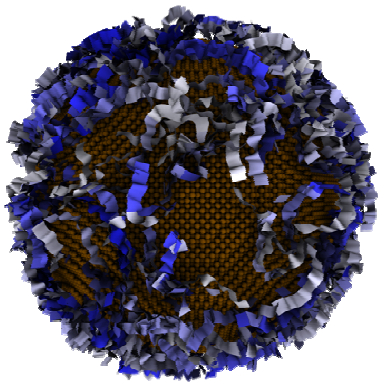
Alzheimer's disease (AD) is ranked as the 5th leading cause of death worldwideby the WHO. One of the hallmarks of the pathogenesis of this neurodisorder is the toxicity of amyloid-β (Aβ). Aβ aggregation and the formation of senile plaques are responsible of neuroinflammation,whilesoluble oligomers induce synaptic disruption, hence triggering cell death. The potential use of functionalised gold nanoparticles (AuNPs) as a mean to lessen Aβ-induced toxicity in AD is discussed in this presentation. High surface-to-volume ratio and loading capacity, low cytotoxicity andblood-brain-barrier permeability make gold nanoparticles as greatsystems for biomedicinal applications.



We haveobserved that polyethylene glycol-coated gold nanoparticles dramatically affect the aggregation (mechanism) of Aβ(1–40). The colloid concentration, surface charge and effective surface area appear to be crucial factors for rationalizing this effect. Nanoparticlesfunctionalisedwith particular peptides,namely (i) copper chelators[1]to sequester this metal ion from toxic copper-stabilised Aβ oligomers and (ii) β-sheet breakers to avoid the formation of protein aggregates, have been investigated as well.

Acknowledgements

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OC6

Chemo-Enzymatic Total Synthesis of Sorbicillinoid Natural Products

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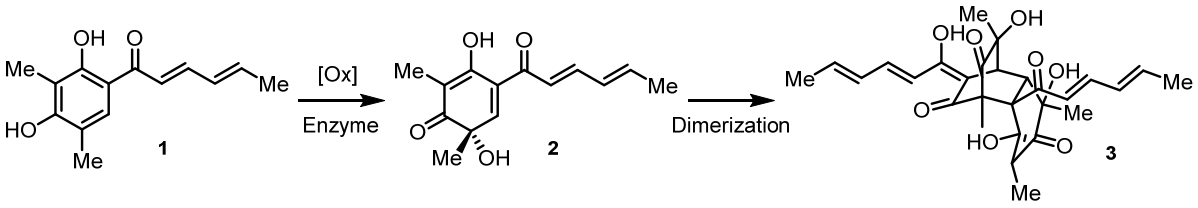
Natural products are one of the main sources of lead structures in drug design. A class of natural products with significant structural and biomedical interest are the fungal sorbicillinoids.[1] Their biological activities range from anti-infective to radical-scavenging and cytotoxic properties. However, classical chemical total synthesis of members of the sorbicillinoid family is difficult to achieve because of their remarkable structural complexity.



To avoid long and inefficient synthetic routes towards this natural product family, we established a chemo-enzymatic total synthesis of monomeric, dimeric and further functionalized sorbicillinoids.[2][3][4] The key step of our approach is an enzymatic oxidative dearomatization of the central precursor sorbicillin (**1**) to the highly reactive sorbicillinol (**2**), the key biosynthetic intermediate of all sorbicillinoids.[5] This biocatalytic transformation proceeds with perfect regio- and stereocontrol and thus proofs superior over all purely synthetic counterparts developed so far, particularly the oxidative dearomatisation with lead(IV) acetate.[6] Starting with sorbicillinol (**2**), a multitude of sorbicillinoids is available by fast and controlled dimerization- or derivatization reactions. An interesting example is the assembly of bisorbicillinol (**3**) by *in-situ* dimerization by a Diels-Alder cycloaddition. This presentation will provide insights into the heterologous expression and purification of the oxidative enzyme employed in the formation of **2** and present the broad range of complex products accessible from this simple precursor.

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OC53

Applying dynamic chemistry for miR-21 direct detection and quantification

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miR-21 is anoncomiRupregulated in a lot of pathological conditions, being often associated with poor prognosis in cancer.[1] miR-21 upregulation is directly associated to cell proliferation, cell migration, and invasion, aside from the generation of chemoresistance.[2]To date, the gold standard technique to identify and quantify miRNAs is RT-qPCR; nevertheless, it is not particularly suitable for small RNA species.[3]The need of isolating small RNA population from biological specimens during sample prep joined to the elongation and conversion of target molecules into cDNA to then perform, PCR amplification steps,represents substantial drawbacks.Aiming to overcome these issues, a chemical and PCR-free approach for the detection and quantification of miRNAs, especially miR-21, directly from cells, in a rapid, robust, and cost-effective way, has been developed.[4]

For that, peptide nucleic acids (PNA) probes fully complementary to miR-21 and containing an abasic position in front of a nucleotide of study (**DGL probes**) were coupled through EDC chemistry to magnetic Dynabeads® to give rise to **Magbeads-21**. Pellets obtained from tumour cell lines MDA-MB-231 (breast cancer) and H1975 (lung cancer) overexpressing miR-21, and from PBMCs, which lack miR-21 expression, were used as biological sources. Thereupon cell lysis,**Magbeads-21** were incubatedwith cell lysates to capture miR-21 molecules which hybridise in an antiparallel orientation with the DGL probes, forming a perfect duplex. After a magnetic purification, **Magbeads-21** capturing miR-21 were added to a solution containing a biotinylated reactive aldehyde-modified non-natural adenine (**Smart-2dA-biotin**) and a reducing agent ($NaBH_3CN$). The perfect duplex formed between the DGL probes and miR-21molecules acts as a template for a thermodinamically controlled reaction that takes placebetween the aldehyde of the **Smart-2dA-biotin** and the secondary amine of the abasic position, giving rise to a reversible specie iminium, which is immediately reduced to a stable tertiary amine, producing a dynamic chemistrylabelling (**DCL**). In order to detect the **Smart-2dA-biotin** specific incorporation, a biolabelling step is performed through a fluorogenic enzymatic assisted assay: Streptavidin-β-Galactosidase(SbG) binds to biotinylated**Smart-2dA** and Resorufin-β-D-Galactopyranoside (RGP) reacts with SbG, releasing the fluorescent compound resorufin upon enzymatic hydrolisis. Given thatresorufin fluorescence is directly proportional to the amount of SbGpresented in **Magbeads-21**, the number of molecules of miR-21 presented in the tumour cells could be determined using a calibration curve.[4] This methodology is not limited only to cells and can be extended to other biological sources such as plasma or serum.

Acknowledgements

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OC14

Dendrimers as a powerfull tool inbiomedicine. Applications in tissue regeneration processes and as biomarkers.

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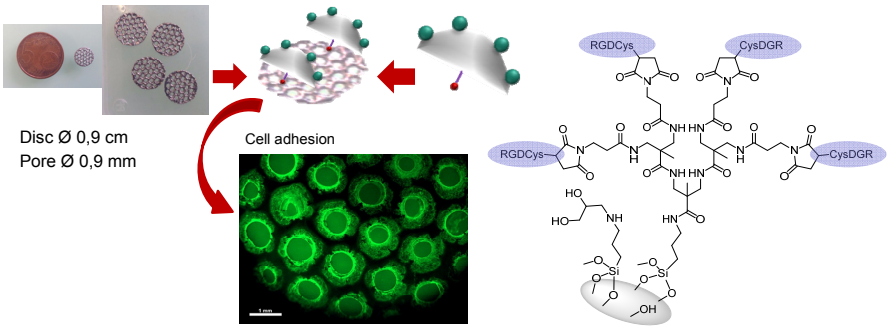
Since their conception, dendrimers have drawn considerable interest due to their potential applications in many fields of science. These well-defined branched macromolecules have been studied for a wide variety of applications ranging from material science to biomedicine. The possibility to add multiple functionalities in precise locations of the dendritic structure and their structural perfection, generated great expectations for the application of dendrimers in nanomedicine. Moreover, as non-biological macromolecules, it is crucial to develop multifunctional dendrimers with well-defined structures at the same level of precision as biological molecules. A key requirement for producing new dendrimeric material is the development of novel synthetic schemes that can deliver the required degree of structural accuracy.

Herein, we present the synthesis ofofarginine-glycine-aspartic acid (RGD) conjugated dendrimers and their application in tissue regeneratrionproseccess. We demonstrate that dendrimer-presented tripeptides efficiently increase surface cell adhesion and proliferation ofcells, and that their effectiveness is related to how they are presented by the dendrimer to the cell.[1,2]ComertialPAMAM dendrimers has been firstlyused. However, new families of stable amino-terminal dendrimersbased on aliphatic- amidescafolshas been prepared. The use of dendrimers designed and synthetized by ourself opens new possibilities as the insertion in the structure of luminescent units. These new dendrimers results excellent candidates for using as biomarkers.[3-6]



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OC34

Ruthenium-catalyzed redox isomerization inside living cells

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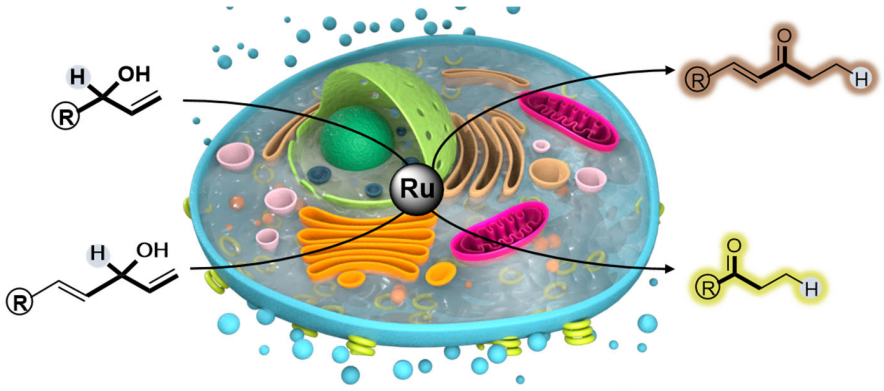
Under the pressure of evolution, enzymes have learned to be extremely efficient and highly selective; however, they tend to lack versatility in terms of substrate scope. Along the years, chemists have invented many catalytic processes that do not occur in nature, with reactions involving metal catalysis being specially appealing.[1] However, translating the potential of metal catalysis to "natural" environments, such as water, presents numerous challenges associated to their biocompatibility, stability and reactivity in crowded aqueous environments. In the last years, there has been an increasing number of reports on the application of transition metal-catalyzed reactions in biological settings and, in some cases, even in intracellular environments.[2]

In this context, Sadler and Do have independently reported the use of osmium or iridium complex to alter NADH/NAD$^+$ cellular equilibriums, or to promote reductions of specific aldehydes or ketones via metal-hydride species.[3] Taking into account these observations, and considering previous contributions on ruthenium-mediated isomerization of allylic alcohols to ketones in water,[4] we wondered if this type of redox-neutral processes could be achieved in biorelevant media or even in intracellular media.

Here we report metal-catalyzed isomerization reactions that can be carried out in complex aqueous habitats, and even in living mammalian cells. This transformation could be formally viewed as a non-natural isomerase capable of working in the native milieu of enzymes. The redox-neutral isomerization allows to transform non-functional, abiotic allyl alcohols into fluorescent or bioactive ketones in the interior of cells. These results open new avenues in this emerging research field at the boundary of metal catalysis and cellular biology,[4] and promises to yield important applications in biomedicine.



Acknowledgements
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OC62

Fighting Resistances: Photoswitchable Antimicrobials Fully Operated under Visible Light

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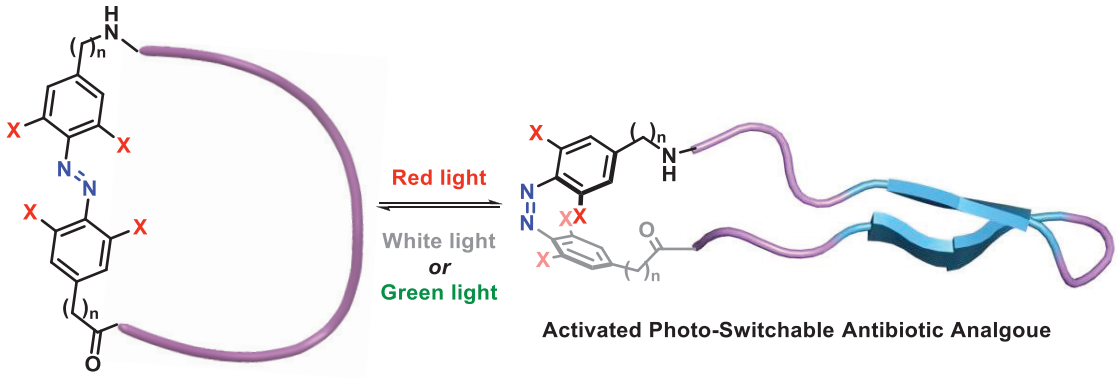
Antibiotic resistance is one of the major healthcare issues that humankind is facing at present and is becoming a growing concern as current therapies become inefficient against resistant strains.[1] Discovering new classes of antibiotics that aim at new molecular targets can be helpful; however, these will eventually face the same fate as microbes become resistant to them due to their accumulation in the environment. Thus, a novel approach that changes the paradigm on how we fight against bacterial infections is on high demand.

Switching antibiotics off after their therapeutic use renders them inactive to highly diminish the chances of resistance appearing as their accumulation in the environment does not increase the evolutionary pressure on bacteria. Most approaches to photo-switchable drugs rely on the use of azobenzenes, which require the use of harmful UV light for their activation. However, these can be modified to cause a red-shift that allows activation with visible light that does not harm tissues and can penetrate deeper than shorter wavelengths.[2]



We are currently developing new azobenzene-based photo-switches that can be prepared efficiently on gram scale and are fully operated under visible light wavelengths to avoid harmful light sources. Moreover, the new photo-switch design is compatible with solid phase peptide synthesis methods to speed up the synthesis and discovery of new photo-switchable antibiotics.

Acknowledgements

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OC36

Dynamic stereoselection of kinetically-inert DNA-binding metallopeptide cylinders

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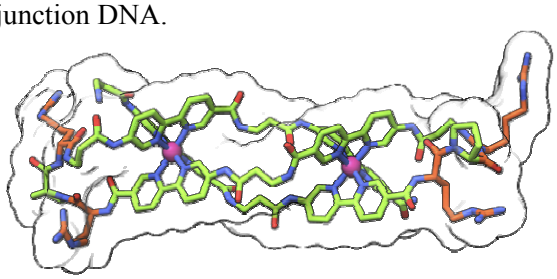
Helicates are discrete metal complexes in which one or more organic ligands are wound around and coordinated to two or more metal ions.[1] Helicates are inherently chiral species that can appear as right-handed or left-handed isomers, according to the orientation in which the ligands coil around the helical axis defined by the metal centers. Indeed, the supramolecular chirality of helicates represents an additional challenge for the synthesis of these complexes and, despite some noteworthy examples,[2] no general approach for the efficient and versatile stereoselective synthesis of helicates is yet available.

We propose that peptides are ideal platforms for the programmed assembly of helicates.[3] In this context, we present the synthesis and study of peptide helicate precursors featuring 2,2´-bipyridine ligands that predictably fold into complexes with defined chirality in the presence of metal ions. The folding of the peptide ligands into bimetallic helicates is directed by a particular heterochiral combination residues in the loops connecting the coordinating bipyridines that allow the thermodynamic control over the supramolecular chirality of the final three-stranded metallocylinders after the coordination of Co (II) or Fe (II) ions. Furthermore, the Co(II) peptide helicates can be locked into defined configurations by *in situ* oxidation to Co(III), thus providing a straightforward method for stereoselective selection of kinetically inert helical metallopeptides in aqueous media.

The DNA-binding properties of these labile Co(II), Fe(II), and kinetically-inert Co(III) peptide helicates were studied by fluorescence titrations and electroforetic mobility shift assays with three-way junctions and double-stranded DNAs. Both the labile and the kinetically-inert complexes display good DNA-binding properties and selectivity towards three-way junction DNA.



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OC63

GaudiMM: Opening New Horizons in Molecular Modeling of Chemobiological Systems

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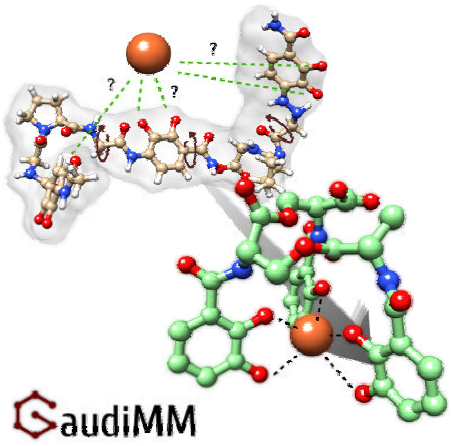
Molecular modelling has become a major ally for many chemical biology subjects. The increaseof computational power, userfriendliness of graphical interfaces or the improvement on how to combine several methodologies together are some factors that contribute to make molecular modelling increasingly popular.

While most of the challenges that the computational community work on consist inreducing calculations times to reach systems of higher dimensionalities or improve energetic functions, some others are barely explored. One of those consists in fast hands-off molecular builders to identifyphysically sound models on systems with scarce structural information. Such methods would avoid modellers the initial molecular puzzling exercise, frequently "manual", that makes difficult or even unfeasible simulation of higher accuracy.

GaudiMM[1] is a computational platform thought and developed to solve this problem. Based on a multi-objective genetic algorithm, GaudiMM allows complex molecular building by rapidly searching physically sound structures in a vast biochemical and structural space that satisfya series of specificgeometric and energetic descriptors.

GaudiMM has already showed very encouraging results in fields like enzyme design, peptide folding or molecular docking. Here, we present the our lattest advances and steps forwards that includes metal-mediated molecular docking, binding site prediction, ligand diffusion[2] and mutagenesis prediction.



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OC66

Quinolimide-based probes: Newtoolsto study protein-protein interactions

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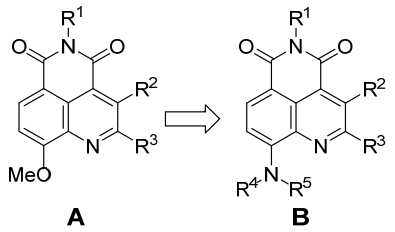
Solvatochromicfluorophores have demonstrated high potential as tools for the study of protein dynamics and protein-binding interactions. In this field, we have recently described the design and synthesis of 9-methoxy-4,5-quinolimide derivatives (**A**) as novel highly solvatochromic fluorophores, which showed higher solvatochromic behavior, red-shifted emission and higher water solubility than wellknown naphthalimide analoguefluorophores.[2] Now, we have improved the photophysical properties of quinolimides **A**, by replacing their 9-methoxy group by diverse amino groups (**B**). These 9-amino-quinolimide-based fluorophores have been used to study the CDK5/p25 protein interacion and the β-amyloid aggregation. In this communication we will present the most significant results.



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OC68

Non-coding RNAs delivered by Magnetic and Gold Nanoparticles for the treatment of Uveal Melanoma

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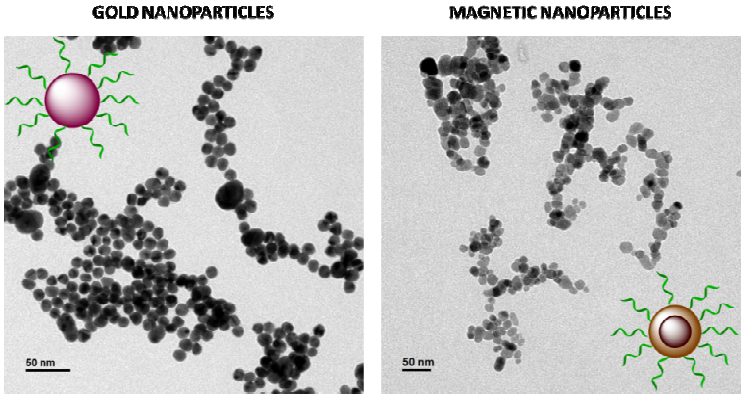
Uveal melanoma (UM) is the most common primary intraocular malignant tumor in adults with a high metastatic risk.[1] For this reason, new therapeutic approaches for this disease are needed. In this regard, microRNAs have shown to play a crucial role in neoplasia progression and might be used as a potent therapeutic tool. It has been observed in cancers, including UM, that microRNAs implicated in tumor suppression are downregulated, whereas elements involved in tumorigenesis are upregulated. Thus, restoring the normal levels of selected microRNAs can restore the normal behavior of cells, or increase their sensitivity to drugs. However, oligonucleotide-based therapies present an array of challenges, such as reduced tissue or cell selectivity, poor cell internalization, and low stability. In this research, we have studied the use of gold and magnetic nanoparticles in the delivery of drugs and oligonucleotides. Particularly, we have evaluated a microRNAs cocktail (34a, 144, 137 and 182) combined with chemotherapy, SN38, the active compound of Irinotecan. In this case, the combination therapy led to a 40% reduction in cell viability.[2]



In the case of magnetic nanoparticles, we have also assessed the potential use of magnetic hyperthermia to treat UM. In this case, Mel202 cells were treated with a non-toxic amount of MNPs (0.1 mg Fe/ml) for 24h. Then, an electromagnetic field of 202 kHz and 30 mT was applied for 5 min, leading to a 40 % reduction in cell viability. Currently, we are assessing the effect of hyperthermia with acocktail of non-coding RNAs. In summary, gold and magnetic nanoparticles can be used to deliver non-coding RNAs and reduce cell viability in the cancer cells. In the case of magnetic nanoparticles, the use of magnetic hyperthermia can be used to enhance the therapeutic effect of the microRNAs.

Acknowledgements

This research was funded by the Spanish Ministry of Economy and Competitiveness (SAF2017-87305-R), Comunidad de Madrid (S2013/MIT-2850), Asociación Española Contra el Cáncer,the European Union's Horizon 2020 research and innovation programme(GA No 685795, NoCanther) and IMDEA Nanociencia IMDEA Nanociencia acknowledges support from the 'Severo Ochoa' Programme for Centres of Excellence in R&D (MINECO, Grant SEV-2016-0686). We thank the Materials for Medicine and Biotechnology Group (ICMM-CSIC) for allowing us to use their facilities for the hyperthermia experiments.

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OC86

G-quadruplex DNA Structures as Drug Targets for Small Molecules

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Over the last years, different nucleic acids structures have been documented a part from B-type duplex such as A-/Z-type duplex, triplexes, three-way junctions, i-motifs or G-quadruplexes (G4). In particular, the later structure formed by certain guanine-rich DNA or RNA sequences have attracted much attention during the past few years since they have been found in numerous regions of human and virus genomes.[1] A G-quadruplex is a four-stranded structure where two or more stacked coplanar guanines are stabilized by hydrogen bonds and coordinated to alkali metal cations.

There have been an increase number of evidences that suggest the pivotal role of G4 structures in key biological processes and their druggable targeting for therapeutic intervention.[2] Of particular importance is their role in the chromosome ends, named telomeres that play an important role in cell proliferation in cancer and aging processes.[3] Furthermore, they have been found in promoter regions of oncogens, with the possibility of regulating the biological processes derived from them.

Thus far, a large number of G4 binders have been reported with a strong interaction with G4 structure and, usually, sligh selectivity over duplex. Nevertheless, there is still a lack of G4 binders in clinical trials due to the little selectivity over canonical DNA, which comprises the most genomic DNA, and the large costs of potential therapeutic molecules for pharma industry. Therefore, we present a series of new G4 binders charactherized by a triphenylamine central core and polyamine-based chains and studied the binding abilities to G4s and duplex by means of FRET melting, fluorescence and CD experiments.[4a] The first generation of new G4 binders has high affinity for G4 structures but poor selectivity over duplex. Having this in mind, the second generation of G4 binders preserved the high affinity for G4s and increased the selectivity over duplex by using low-cost reactants and a simple synthetic protocol.[4b] All the results point out the new series of molecules as leading drugs to study the biological effect and give insights about the importance of the molecular design in the development of G4 drugs.

Acknowledgements

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OC71

Studying the Reaction Mechanism of Orotate Phosphoribosyltransferase by means of X-ray Crystallography and Computational Simulations

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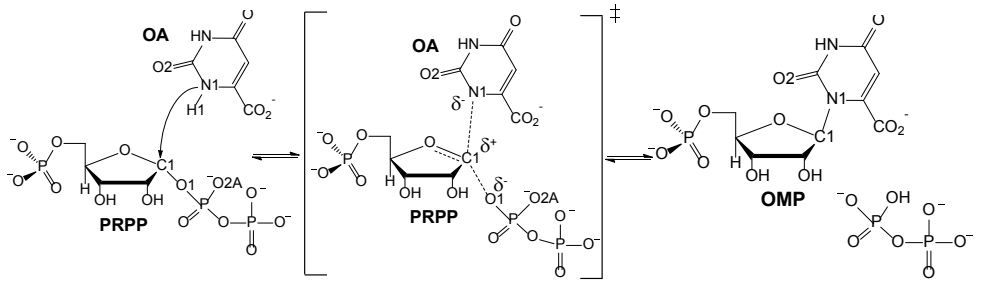
Orotate phosphoribosyltransferase (OPRTase) catalyzes the reaction between the ribose donor α-D-5-phosphoribosyl-1-pyrophosphate (PRPP) and orotate (OA) in presence of Mg^{2+} ion to obtain pyrophosphate and pyrimidine nucleotide orotidine 5'-monophosphate (OMP), a key precursor in *de novo* biosynthesis of pyrimidine nucleotides. OPRTases are widely distributed among different organisms such as *Plasmodium falciparum* (*Pf*OPRTase), *Mycobacterium tuberculosis* (*Mt*OPRTase), *Salmonella typhimurium* (*St*OPRTase) and humans (*Hs*OPRTase) to mention a few. Thus, OPRTase is an attractive target for the rational design of antimalarial, antitubercular and anticancer drugs.



In this work, several structures of the dimeric *Escherichia coli* orotate phosphoribosyltransferase (*Ec*OPRTase) have been crystallized at high resolution. Molecular dynamics (MD) simulations have been carried out and structural analysis from the X-ray and MD simulation structures reveals conformational changes related to the flexible catalytic loop that establish hydrogen bond interactions with the pyrophosphoryl group of PRPP. It is proposed that the OA substrate can be in equilibrium in its tautomeric forms. Starting from the most stable tautomeric form, all the plausible mechanisms have been explored by means of quantum mechanics/molecular mechanics (QM/MM) molecular dynamics (MD) simulations using the adaptive string method.[1] The free energy barrier obtained for the most feasible mechanism is in very good agreement with the experimental data reported. Analysis of some relevant distances between key residues and the substrates (OA and PRPP) at the reactant state and transition state (TS) of the rate-limiting step allow us to understand the role of some conserved residues electrostatically stabilizing the TS and preserving the flexible catalytic loop in a closed conformation during the enzymatic reaction.

Acknowledgements

Spanish Ministerio de Economía y Competitividad (2015) and Ministerio de Ciencia, Innovación y Universidades (2018) through grants co-financed with European Union ERDF funds PGC2018-094852-B-C22, CTQ2015-66206-C2-2-R, RTI2018-1018-102242-B-I00, 'Ramón y Cajal' contract (RYC-2014-16592). Generalitat Valenciana AICO/2018/238 project. Universitat Jaume I UJI-B2016-28 project.
Computational facilities of Universitat Jaume I and Universitat de València (Tirant Supercomputer) and the access to the ALBA Synchrotron Light Source, Barcelona.

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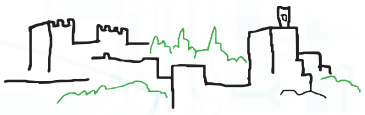

February 19-21, 2020

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Poster / Flash Communications

P1 / C2

Binding site plasticity in the third WW domain of human NEDD4 modulatesviral late domain recognition

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Budding of multiple viruses such as Ebola, Marburg, HTLV1 or Rabies is mediated by viral late domains through a PPXY motif that is recognized by the third WW domain of the HECT-E3 ubiquitin ligase NEDD4 (hNEDD4-WW3)[1]. Based on this evidence, several studies have validated hNEDD4-WW3 as an adequate target for the development of broad-spectrum antivirals[2], since the target is located at the host instead of the virus. However, in the search of inhibitors blocking hNEDD4-WW3, we lacked the key determinants for binding and specificity of the domain. In this work, we present a detailed thermodynamic and structural study showing the interaction of hNEDD4-WW3 with peptides derived from viral late domainscombining several techniques including isothermal titration calorimetry, NMR structural determination and molecular dynamics simulations. The results show a conformational selection mechanism[3], which hinders a straightforward rational design of plausible high-affinity ligands. Thus, the combination with other non-rational or high-throughput methodologies, such as peptide phage display, may offer a complementary way of finding potent inhibitors of therapeutic interest.

Acknowledgements

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Supramolecular hydrogels based on different coated iron magnetic particles

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Short-peptide supramolecular hydrogels have recently found several biomedical applications due to their properties, such as high porosity, high water content, biocompatibility or biodegradability. Therefore, this hydrogels able to self-assemble by non-covalent interactions, have turned into excellent candidates for the construction of smart and functional materials[1].However, they still present some drawbacks, such as their weak mechanical properties or lack of proper internal structure. A strategy usually followed to control the mechanical properties and internal structure of these hydrogels is to add magnetic particles to the peptide network, creating controllable magnetic supramolecular hydrogels by the application of external magnetic fields. In addition, these particles can be functionalized using peptides that promote cell growth and differentiation, such as arginine-glycine-aspartic (RGD) ligands[2].

Based on the previous premises, and since the properties of these materials depend on the type of interaction between the magnetic particles and the peptides that form the hydrogel, in this work we studied the effect of the coating of the magnetic particles on the mechanical properties and the internal structure of magnetic supramolecular hydrogels. For this aim, Fmoc-diphenylalanine hydrogels (Fmoc-FF) were prepared following an adaptation of the protocol described in[3] and iron microparticles with three different types of coatingswere used: (i) uncoated, (ii) silica coated and (iii) coated with polyethylene glycol (PEG) and Fmoc-FF. Firstly, the stability and corrosion of the magnetic particles were characterized, as well as the type of inclusion within the peptide network. Then, their rheological properties were measured at different concentrations of particles, obtaining different trends depending on the type of coating thereof. Finally, it has been studied how the shape of magnetic hydrogels changes in the presence of external magnetic fields of different intensities.

Acknowledgements

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P3 / C4

Hybrid supramolecular hydrogels for regenerative medicine

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Supramolecular hydrogels are a particular type of gels formed by a low molecular weight solid (amino acids or peptides). These hidrogels are capable of self- assembly through non-covalent interactions, forming a three-dimensional reticular structure that immobilizes the macroscopic flow of water (up to 99% water).[1] These hydrogels are of special importance because they possess biocompatibility, biodegradability and a high porosity, important characteristics for cell growth. In addition, thanks to its great versatility, they allow the introduction of different peptides, some of which, such as arginylglycyl aspartic acid, promote cell growth.[2] They also allow the introduction of magnetic nanoparticles (MNP), which stimulate the adhesion, proliferation and differentiation of cells.[3]

Despite the clear advantages of peptide hydrogels, they have the disadvantage of their low mechanical strength, which limits the applications in clinical situations. This work focuses on overcoming this limitation; in order to do that, the peptide hydrogels have been combined with high molecular weight polymers, which allows benefiting from their advantages, avoiding their inconveniences. Furthermore, combining these hybrid hydrogels with MNP, their mechanical properties can also be modified.

To achieve the formation of these hybrid hydrogels, the peptide solution is prepared by the pH-switch method and then, the polymer solution (alginate or agarose) is added. Figure 1 shows an environmental electron microscope image of one of these hybrid hydrogels. The next step is the introduction of MNP. The final goal is to have hydrogels with physiological pH, for this, cell culture medium is diffused once the gel is formed.

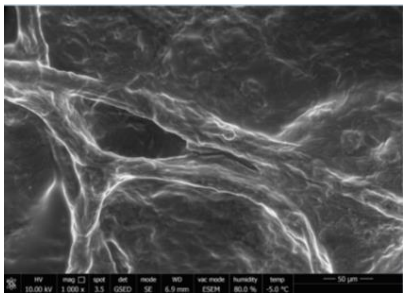

Figure 1: ESEM image of peptide hybrid hydrogel (Fmoc-Phe-Phe-OH) with alginate.

Once prepared, hybrid hydrogels are studied as artificial matrices for cell growth both on the surface and in three dimensions. Its cytotoxicity, viability and cell proliferation are evaluated by several standard tests: analysis of cell morphology; permeability of the nuclear membrane by quantification of DNA release. The mechanical properties of hybrid hydrogels are studied by rheological measurements.

Acknowledgements

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NMR STUDIES OF THE SPECIFIC INTERACTION OF DC-SIGN WITH THE BLOOD GROUP A/B ANTIGENS

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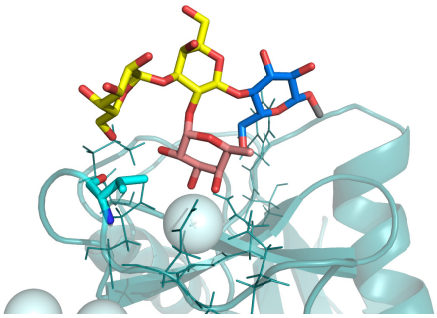
The DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin) protein is a key receptor of the C-type lectin receptor (CLR) family. This human lectin acts as a pathogen recognition receptor (PRR) through the specific interaction with complex oligosaccharides distributed on the pathogen surface, via its calcium-dependent binding site. At the same time, its role in the immune modulation and homeostasis has been also described by means of specific targeting of endogenous glycoproteins like ICAM-3.[1] The broad specificity in binding is one particular feature of this lectin. Previous glycan array data[2,3] agree with the existence of two main ligands for DCSIGN: L-fucose (Fuc) and D-mannose (Man). Fuc is commonly found at the terminal positions of Lewis-type and ABH-type antigens in mammalian cells, while Man is the major component of the highly branched oligosaccharides attached to membrane glycoproteins in different pathogens, as gp120 in HIV. In addition, these studies also speculated on how glycan presentation and subtle structural differences between similar Fuc- or Man-containing motifs might tune or modify the binding affinities[4]. In this regard, the interaction details at a molecular level, especially for the endogenous Fuc-containing partners, remain rather unexplored. We have unraveled the key structural details of the molecular recognition event of the A- and B- histo blood group antigens by DC-SIGN. We have employed a variety of NMR methods assisted by molecular modeling protocols to define the binding features of these antigens to the carbohydrate recognition domain of DC-SIGN. This methodology has allowed us to propose a well-defined binding model for both blood groups. These results provide the initial milestones for forthcoming studies with other ligands, including longer and branched saccharides.



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'e thank Agencia Estatal de Investigacion of Spain and the European Research Council, the Industrial Biotechnology Catalyst nnovate UK, BBSRC, EPSRC, BB/M028941/1), the EPSRC (core capability EP/K039466/1) and J. F. Espinosa (Lilly esearch Laboratories, Alcobendas, Spain).

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P5 / FC2 / C8

Avenues to characterize the interactions of extended N-glycans with proteins by NMR spectroscopy: the influenza hemagglutinin case.

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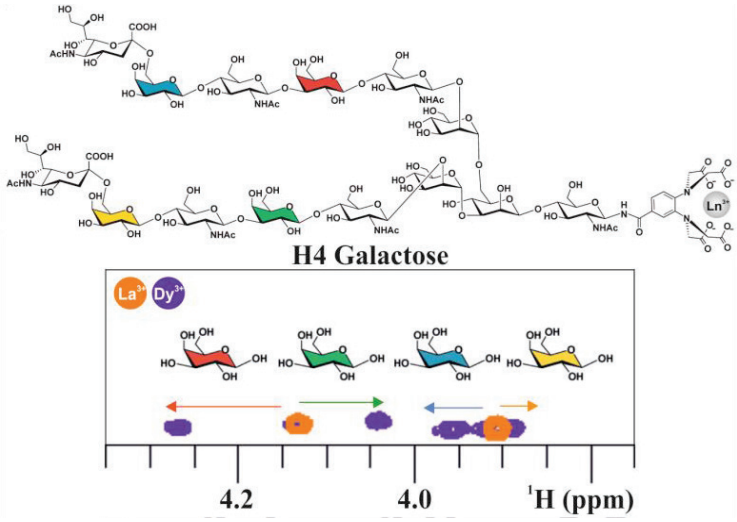
Seasonal flu (influenza virus infection) is one of the major threats to human health, causing up to 500 thousand deaths worldwide annually.[1] Hemagglutinin, a glycoprotein presented in the surface of the influenza virus, binds sialylated N-glycans located on the surface of host epithelial cells and provides the initial step for infection.

The complexity, heterogeneity and flexibility of long-chain multiantenna N-glycans have hampered their structural elucidation and conformational studies for years. Moreover, from the NMR perspective, the isochronous chemical shifts presented for the same units located at different branches make impossible the distinction of their signals.

However, taking advantage of the recently developed paramagnetic-based lanthanide approach, an unambiguous assignment of each carbohydrate unit has been achieved for the target biantennary N-glycans containing one and two LacNAc units, permitting their conformational elucidation and the detailed analysis of their recognition features by the HK/68 hemagglutinin strain.[2] The key aspects of the process and the major conclusions at the conformational, interaction and biological level will be discussed.



Acknowledgements

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Target Synthesis via Innovative C-H Amination

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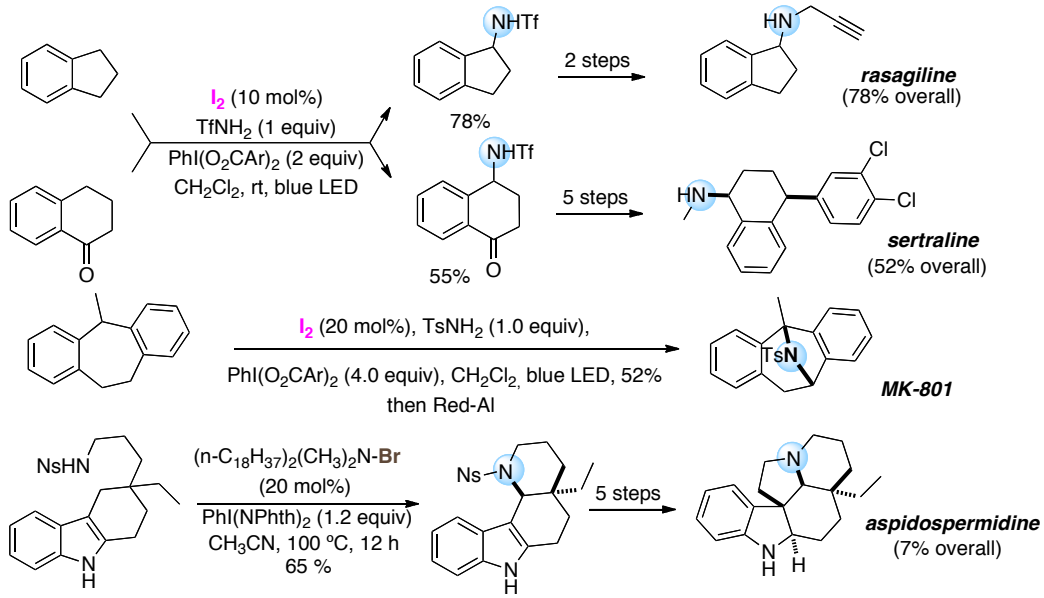
The C-N bond constitutes a key functional entity in molecules of defined biological and pharmaceutical activity. In opposition to Nature's preferred strategy of reductive amination, the direct amination of ubiquitous hydrocarbon bonds constitutes an attractive alternative. This advanced synthetic tool allows for an expedious installment of C-N bonds and for broad diversification of existing structures.

Over recent years, we have devised various synthetic protocols for oxidative amination reactions, which rely on redox-active halides as the sole promoters. These innovative redox protocols allow for a number of innovative C-N bond formation reactions, which are characterised by their high productivity and complete selectivity, together with any absence of environmental and toxicological issues.

Examples to this end include an iodine(III)-promoted aromatic C-N bond formation for an advanced step-economic synthesis of representative family members of binding inhibitors of the chemoattractant peptide chemerin to the G-protein coupled receptor ChemR23.[1] In the area of intramolecular aliphatic C(sp^3)-H amination, the use of an iodine-redox manifold enabled an enantioselective total synthesis of the parasympathomimetic alkaloid nicotine and several derivatives containing substituted pyridine and bipyridine subunits, respectively.[2]

Unprecedented halide redox catalyses can also promoted intermolecular C(sp^3)-H amination reactions. For the cases of iodine catalyses under light-induced radical processes, such reactions proceed with excellent chemo- and regiocontrol. Their synthetic utility has been demonstrated through uniquely short syntheses of pharmaceuticals such as rasagline, sertraline and MK-801.[3]



An alternative redox manifold is based on bromine catalysis and proceeds under ionic reaction conditions. It enables unprecedented regioselectivity in the C(sp^3)-H amination alpha to indoles. This reactivity pattern was successfully employed in a synthesis of the complex alkaloid aspidospermidine.[4]

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P7 / C10

Novel nNOS inhibitors based in imidamide.

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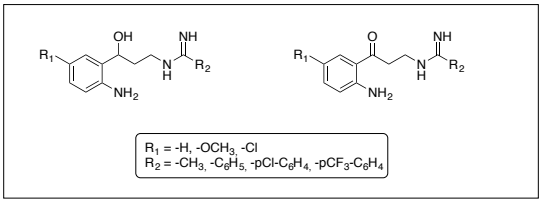
Nitric oxide (NO) is an important signalling molecule produced in the organism by the Nitric Oxide Synthase (NOS). In humans there are three main isoforms: iNOS (inducible isoform present in inflammation and immune response), eNOS (endothelial isoform involved in vasoregulation) and nNOS (neuronal isoform which takes part in neurotransmission)[1].

Several studies show that neurodegenerative diseases, such as Alzheimer, Parkinson or Huntington, present high levels of NO on neurons[2]. Due to the cytotoxic behaviour of some NO metabolic derivatives, it overproduction in nNOS could be one of the main etiological factors for these illnesses[3].

Consequently, many researchers have chosen the selective inhibition of nNOS in order to slow down the pace of neuronal damage[4].

By combining the structure of two already known inhibitors with different mechanism of action, our research group has developed a full new family of compounds able to inhibit nNOS *in vitro* at μM scale.



The imidamide moiety resembles the NOS substrate, while the lateral chains could interfere with calmodulin, one of the indispensable cofactors for nNOS activation.

Acknowledgements

Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Universidad de Granada.

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Purine derivatives linked to benzoxazines as new antitumoural agents

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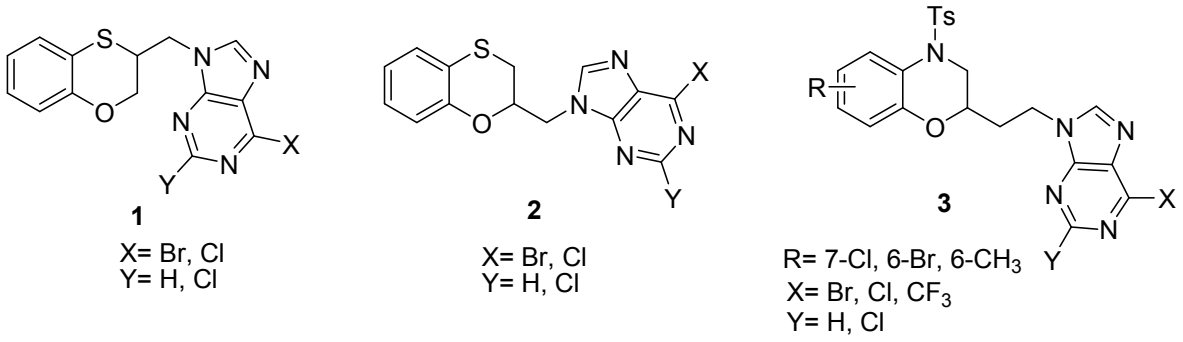
Nowadays, cancer has become a serious health problem and its incidence increases every year. Drug therapy is an essential method for the clinical treatment of tumours.[1] However, one of the main problems is the lack of selectivity of drugs by the tumoural cells that leads to side effects on healthy cells. For this reason, the development of novel anticancer agents with lower toxicity to normal cells is needed.[2]

Several benzo-fused six-membered rings linked to purines have been described previously as compounds with interesting antiproliferative activities: Substituted 9-(2,3-dihydro-1,4-benzo[*b*][1,4]oxathiin-3-ylmethyl)-9*H*-purines **1**[3] and their isomers, 9-(2,3-dihydro-1,4-benzo[*b*][1,4]oxathiin-2-ylmethyl)-9*H*-purines **2**[4] have shown high apoptosis levels on the human breast adenocarcinoma MCF-7 cells.

Thus, taking as prototypes derivatives **2**, our research group has developed a new family of benzoxazines **3** with diferent electron-withdrawing and electron-donating groups in the benzene ring to explore this new chemical space. In addition, the influence of the distance between the heterocycle and the purine has been also evaluated by introducing a two-carbon linker.



Acknowledgements

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P9 / C15

Design of Hybrid Structures for Bioorthogonal Drug Photoactivation and Photocatalysis

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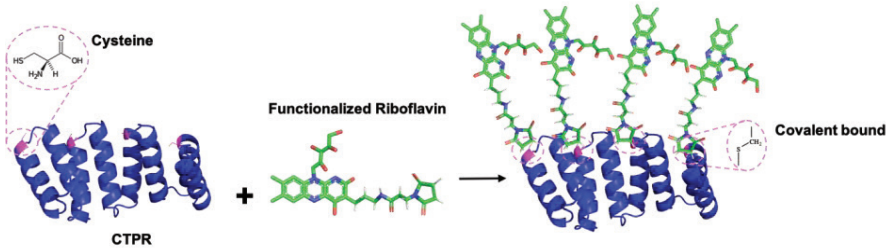
Advances in biorthogonal catalysis are creating new opportunities to perform chemoselective transformations in complex biological environments with applications in biomedicine and bioimaging. Recently, riboflavin photocatalysis has been used to activate Pt^{IV} anticancer prodrugs *in vitro* under light irradiation.[1] In this work, we propose to expand this strategy by using engineered repeat proteins as template to generate integrated assemblies[2] where the positions of the riboflavin and Pt^{IV} prodrug are determined by protein structure. This system is envisioned to have more efficient drug activation and will facilitate controlled cell internalization.

Specifically, we use tetratricopeptide repeat protein (CTPR) as template. The CTPR module is a 34 amino acid sequence that folds in a helix-turn-helix structure defined by few conserved residues, which permit the rational manipulation of the protein scaffold while retaining the structure. The CTPR module combined in tandem form superhelical arrays with different number of repeats (from 2 to 20, i.e. CTPR2 to CTPR20). Previous works showed that CTPRs are robust scaffolds for organizing optical and electro-active elements with defined distance and orientation.[2]

In this contribution, I will discuss the conjugation between CTPR4 and a N3-functionalized riboflavin by exploiting cysteine (Cys) residues of the protein scaffold and maleimide coupling. We have designed protein mutants with cysteine groups at different distances to achieve the maximum riboflavin loading while avoiding the undesired self-quenching of the riboflavin excited state. Currently, we have achieved a distribution in the number of functionalized riboflavins linked to the CTPR. Optimization of the protocol is on-going to obtain a CTPR4 platform where each Cys holds a riboflavin unit.



Light-irradiation studies at 460 nm indicated that riboflavin loaded on CTPR4 has increased photostability, compared to free riboflavin, likely due to the ability of the protein scaffold to protect the modified riboflavin from the photodegradation of its ribityl chain.
Catalysis studies performed on two different functionalized CTPR4 mutants (2 Cys and 4 Cys, both in position 17 of the protein loop) show that riboflavin preserves its photocatalytic activity upon conjugation to the protein and displays a higher efficiency in the reduction of model Pt^{IV} substrate into cisplatin.

Acknowledgements

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Preparation of Dendrimeric antigen-silica particle composites

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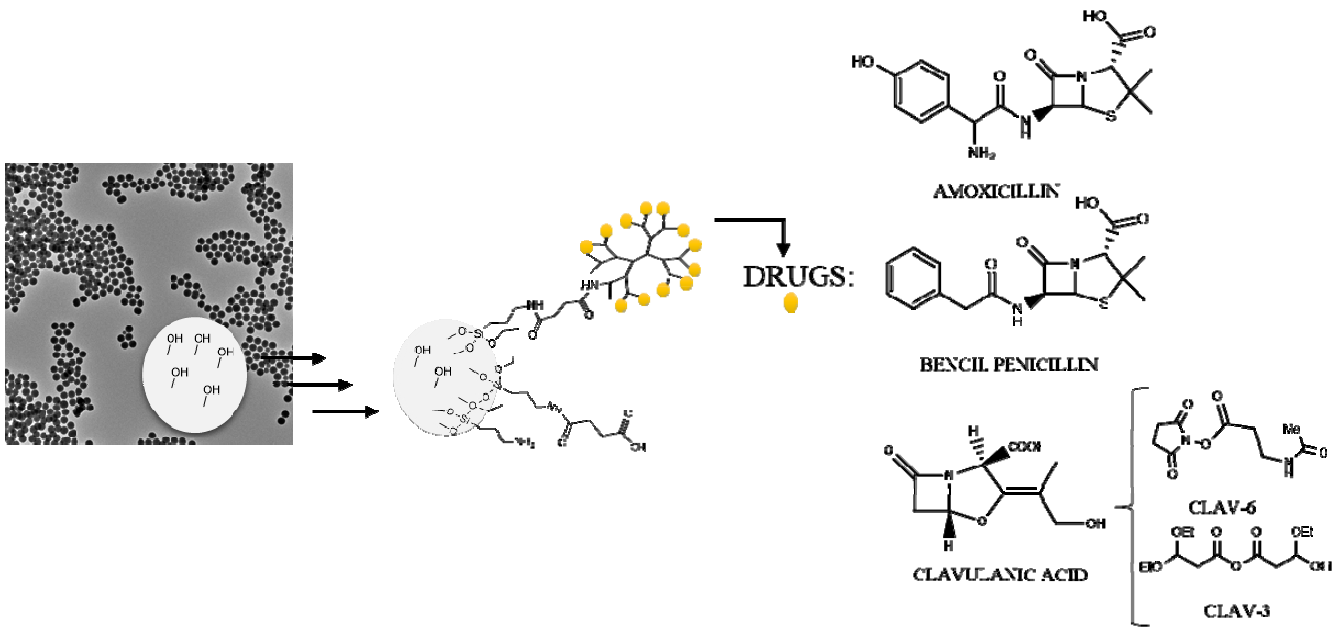
The desing and synthesis of new materials for biomedical applications is a high-priority research topic in a great number of biomedical areas. Moreover, advances in the fabrication of these materials are of growing interest in antibody-based diagnostic techniques.These materials consist in a solid support anchored with the desired bioactive molecules. Such solid supports need to be robust enough and possessurrounding reactive groups that enables the chemical bonding of the active components.Furthermore, these materialsneed tomeet certain conditions to ensure biocompatibility and non-toxicity. In this sense, silica nanoparticles have been widely used.



Our research involves the study of hybrid materials that combine the high functionality of silica nanoparticleswith well defined size and controlled peripheral multivalence components like dendrimers. Dendrimer antigens, which are synthetic antigens where the role of the carrier protein is performed by a dendrimer, were supported on silica particles.
These organic-inorganic hybrid materials were carefully characterized and the preparation methodology was confirmed to be highly reproducible. Such hybrid materials were used for the *in vitro* diagnosis of patien allergic to amoxicillin.[1]Herein, we present the preparation of novel nano-materials containing newantigenicsdeterminants of antibiotics. Amoxicillin, bencyl penicillin, clavulanic acid and its derivateswere used to prepares diferentdendrimeric antigens supported on silca particles. These particles will be used tospecifically and selectivelydetect and quantify IgEin sera from allergic patients. Thesenew materialsarea promising candidate to improve the practice of *in vitro* clinical diagnosis.

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P11 / C17

Small anionophores display *in vitro* antimicrobial activity against clinically relevant bacterial strains

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One of the major threats modern medicine faces is the emergence of nosocomial infections caused by multidrug-resistant (MDR) bacteria. Resistance to almost all antibiotics employed in the treatment of infectious diseases has been reported, so the development of new drugs capable of fighting the bacteria provoking such infections is urgent. Among those bacteria, the ones included in the ESKAPE group (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* spp.) are usually involved in hospital-acquired infections.[1] This is especially worrying in intensivecare units, where their presence compromises the life of criticallyill patients.[2]

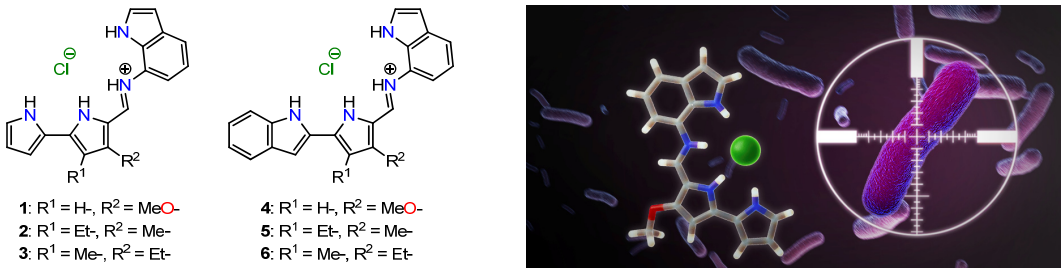

Fig. 1. Left: studied compounds. Right: artistic representation of compound**1** fighting an ESKAPE bacteria.

Herein we present a series of highly active small-molecule anion transporters that display activity against some of the bacteria forming the ESKAPE group (Fig. 1).[3]From a structural point of view, these are tambjamine-like compounds; they possess a 1*H*,1'*H*-2,2'-bipyrrole or 2-(1*H*-pyrrol-2-yl)-1*H*-indole core substituted with either a methoxy group or two alkyl chains (R^1 and R^2) and an indol-7-yl fragment (Fig. 1). Transmembrane anion transport experiments, conducted in model liposomes (POPC) with a chloride-selective electrode, reveal that the six compounds are able to exchange chloride and nitrate and chloride and bicarbonate across the liposomes' membrane, the EC_{50} values being well below the micromolar range. Their antibacterial activity was screened against two Gram-positive (*E. faecium* and *S. aureus*) and two Gram-negative (*A. baumannii* and *P. aeruginosa*) bacterial strains, with all of them inhibiting the growth of the former ones and compound **1** displaying a remarkable activity against *A. baumannii*. **1** was also screened against a panel of Gram-positive and Gram-negative drug-resistant clinical isolates, confirming these results. Further assays indicate that **1**exhibits a good hemocompatibility at concentrations showing significant antibacterial activity.Hence, this class of compounds offers promise as a strategy to develop novel antibacterial agents effective against antibiotic-resistant bacteria.

Acknowledgements

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Isophthalamide derivatives as active transmembrane anion carriers

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The development of low molecular weight organic molecules that function as anionophores is an area of growing interest in supramolecular chemistry.[1]The vast majority of this class of compounds use hydrogen bond interactions to coordinate the anion, forming a lipophilic supramolecular complex which is able to diffuse across the lipid bilayer.[2] Among them, isophthalamides are a classical example of anion binding motifs (Fig. 1).Although simple isophthalamides have proven to be ineffective as transmembrane anion transporters,[3] the presence of electron-withdrawing groups in their structures is commonly employed to increase their anion transport properties,[4] lipophilicity being a key parameter to tune their activity as anion carriers.

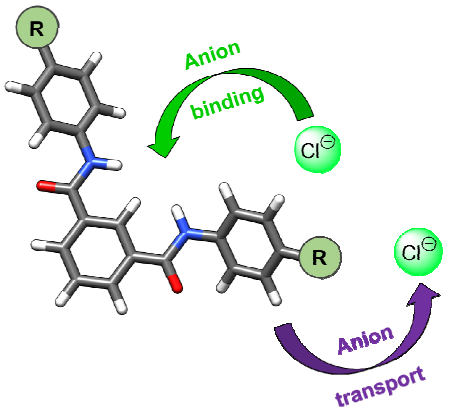

Fig. 1. Chloride binding and transport by an isophthalamide derivative.

Herein we present a study on how the different substitution pattern of a series of isophthalamide-like compounds influences their anion binding and transport activity. Seven of these compounds containing either electron-donating (methyl, *tert*-butyl) or electron-withdrawing (halide, trifluoromethyl, perfluorophenyl, pentafluorosulfanyl) groups were synthesized and properly characterized. Their anion transport properties were studied in model liposomes (POPC) with a chloride-selective electrode and by emission spectroscopy. The carboxyfluorescein-based assay confirmed the lack of detergent effect exerted by these compounds. ISE experiments, conducted both for the chloride/nitrate and chloride/bicarbonate exchanges, allow to conclude that those containing electron-withdrawing groups are the most active of the series, whereas the HPTS-driven assay shows that the compounds are able to dissipate pH gradients in a trend similar to that observed for the ISE experiments.

Acknowledgements

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P13 / C19

Complement system alternative pathway proteins: molecular recognition studies by computational approaches.

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Complement system (CS) plays a critical role in innate immunity and its three pathways converge in the production of C3b, an opsonin that unspecifically binds self and non-self surfaces. Further, the alternative pathway has a basal activation and therefore it requires regulators to avoid damage in host tissues. Factor H (FH) is the main regulator of the alternative pathway upon distinguishing between host and non-host cells via sialic acid recognition.[1] However, there are other proteins like Factor H-related protein 1 (FHR-1) that competes with FH for C3d binding,[2] and dysregulation in these processes can cause chronic disease like atypical hemolitic uremic syndrome, C3 glomerulophaty or age-related macular degeneration.[3] The understanding of sialyl derivatives recognition and the identification of selective modulators for FHR-1 and mutants can be helpful to identify modulators useful for the treatment of those pathologies.

By computational methods we have addressed the study of the molecular recognition process of sialyl derivatives by FH, FHR-1 and mutants, their binding to C3d (Fig. 1), and thesearch of small molecules able to selectively modulate FHR-1 andmutants. We have identified conformational differences that affect the sialic acid recognition and C3d binding, and we have also identified 25 possible selective modulators by means of virtual screening. Our findings may shed light into the mechanism of CS related pathologies at atomic and molecular level, and be of help for the finding of modulators with therapeutic application.

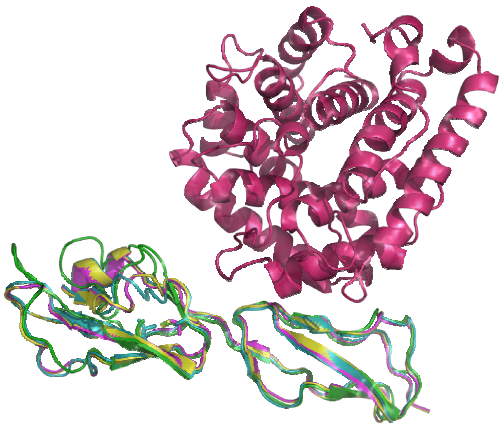

Figure 1. Superimposition of FH/C3d (cyan/dark pink), FHR-1 (yellow), S1191L (green) and L290V (magenta).

Acknowledgements

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Design and synthesis of new dendrons for biomedical applications

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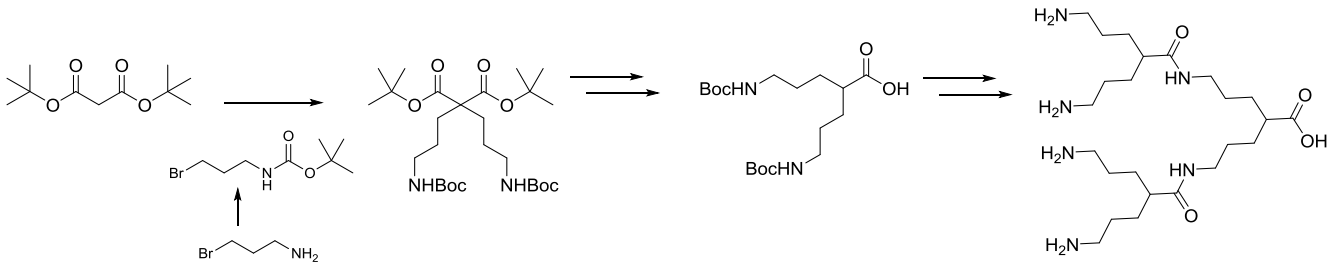
Dendrons are excellent multifunctional platforms for many different applications. In particular, the development of multivalent molecular nanostructures, with a well-defined size and shape, has an enormous interest in the field of biomedicine[1].

Great efforts are being made in the design of new dendritic structures since their precise structure, multivalency and the possibility of functionalizing their terminal groups makes them a useful tool for certain applications.

Recently, a new type of dendrimer (dendron) was designed and synthesized based on the iterative coupling of 2,2-Bis (AminoAlkyl) Propanamide units (BAPAD)[2,3]. This represents a versatile model when incorporating certain functionalities in its structure. However, the synthesis of these new dendritics structures was addressed using 3,3'-dichloropivalic acid as the starting substrate, which translated in the superficial amino groups of these new macromolecule being in relative positions 1,3. For certain applications, where the functionalization of the dendritics structures on its surface requires the introduction of relatively bulky groups, this may result in a problem of reactivity given the steric congestion.

Here, we present the design and synthesis of new dendrons where the surface amino groups are in relative positions 1,7. Formally, it involves the preparation of dendrons with longer arms that can minimize the problems of steric congestion.



These dendrons can be designed to modify surfaces superficially thanks to the versatility of the carboxylic acid which can be modified to introtoduce in the focal poin diferent functional groups (azido, amido, thiol, etc…).

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P15 / FC3 / C21

Enzyme regulators with self-delivery capabilities

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The discovery that multivalent presentations of glycosides can display cross-talk behaviours between lectins and glycosidases offers the possibility of developing multi-specific glycodevices capable of or participating on different recognition phenomena [1]. In this communication we provide a proof of concept of this idea by reporting multitargeted multimannosides that exhibit high affinity towards the macrophage mannose receptor (MMR) and behave as potent inhibitors of the lysosomal enzymes β-glucocerebrosidase (GCase; e.g. **1**) or α-mannosidase (LAMAN; e.g. **2**), the glycosidases that are dysfunctional in Gaucher disease and α-mannosidosis patients, respectively (Figure 1). The motivation of this work is the possibility of exploiting the multiconjugates in pharmacological chaperone therapies, overcoming the anticipated delivery issues, given that (a) macrophages are especially affected in such pathologies and (b) both disease-causative misfolded mutant GCase and LAMAN have been found to be responsive to rescuing by multivalent ligands [2, 3]. We believe that our results represent a new paradigm in multivalent glycoligand design, where the multiconjugation of a glycoside motif serves to assemble glycosidase modulators that become their own delivery agents [4].

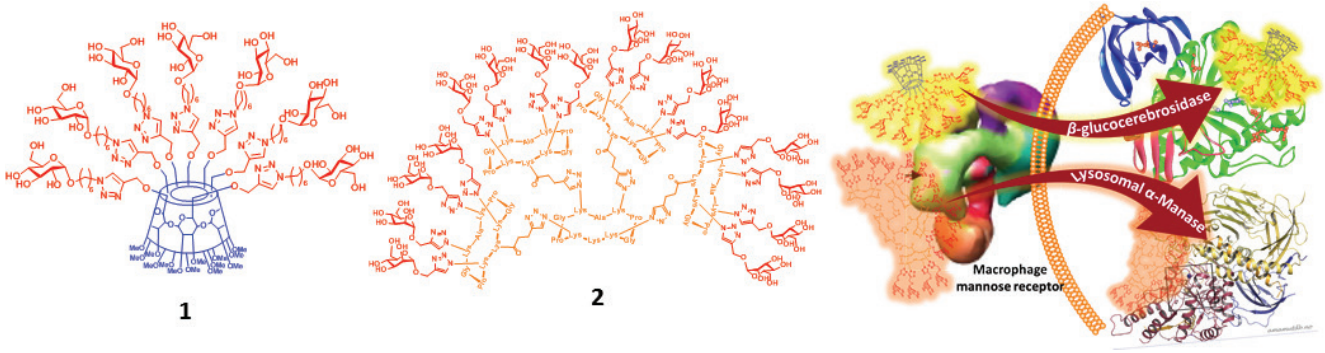

Figure 1. Structure of glycoclusters with dual MMR/lysosomal glycosidase (GCase or LAMAN) selectivity and schematic representations of the the self-deliverable glycosidase regulator concept.

Acknowledgements
The authors thank MINECO and MCIU/AEI (SAF2016-76083-R and RTI2018-097609-B-C21), the Junta de Andalucía (FQM2012-1467), the European Regional Development Funds (FEDER and FSE, UE), CNRS, Université Grenoble Alpes, ICMG FR 2607, the French ANR project Glyco@Alps (ANR-15-IDEX-02), Labex ARCANE, CBH-EUR-GS (ANR-17-EURE-0003), the COST action GLYCONanoPROBES (CM18132) and the JSPS KAKENHI Grant 17K10051 for financial support. O.R. acknowledges the European Research Council Consolidator Grant "LEGO" (647938) for D.G. M.G.-C. is a FPI fellow.

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Molecular determinants in binding affinity of the UEV domain of TSG101 protein.

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Viral late domains, present in multiple viruses such as Ebola, HIV or human leukemia, mediate virus budding through the interaction with the UEV domain of the human TSG101 protein (TSG101-UEV)[1]. Blocking TSG101-UEV has been proven as an efficient way of stoping viral egressing of viruses like HIV[2], and therefore has been proposed as a novel way of generating broad-spectrum antivirals. Two basic approaches may be used to develop inhibitors of TSG101-UEV. On the one hand, knowledge of the key determinants in binding affinity and specificity may offer information for the rational design of candidates. Alternatively, high-throughput methodologies may provide extra information that is unlikely to find just with rational design. The present work shows both approaches, which are complementary to each other. We have performed a detailed structural and thermodynamic analysis of the binding of TSG101-UEV with the peptides derived from the late domains of HIV, Ebola and human leukemia. Additionally, we have found molecules from two compounds libraries and active extracts that bind to TSG101-UEV by sorting natural product libraries from marine actinomycetes and fungi, proving effective our high-throughput screening protocol via thermal shif assay in these mostly unexplored kind of samples. The combination of these two approaches produced valuable information about the molecular determinants of binding affinity in this system, of interest for the development of broad-spectrum antivirals.

Acknowledgements

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P17 / C23

Design of consensus tetratricopeptide repeat (CTPR)-based orthogonal scaffolding units for the ordered assembly of enzymes

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Nanotechnology is a growing field of increasing interest and emerging applications in many areas. Some of the most outstanding applications are found in protein engineering and biocatalysts, since biomolecular nanoengineering approaches can be applied for the development of nanostructured biocatalysts. In this context, rational protein design can be applied to generate scaffolds onto which different cascade enzymatic reactions can be assembled to obtain controlled multi-enzymatic systems. In this work, we explore the use of repeat proteins, in particular the consensus tetratricopeptide repeat (CTPR) module for the design of a variety of scaffolds towards the assembly of multi-enzymatic pathways. We selected two strategies based on supramolecular assembly of repeated modules[1] (SCAB units) and on the biomolecular recognition of orthogonal protein-peptide pairs using the designed tetratricopeptide repeat affinity (TRAP) proteins[2]. In the first approach, the enzymes are fused to the SCAB units and the assembly is driven by the SCAB modules. In the second approach, the enzymes are fused to tag peptides and assembled onto the TRAP scaffolds by specific binding to the scaffold. We aim to obtain nanometric arrangement of the multi-enzymatic systems that will result in optimized biocatalytic cascade reactions.

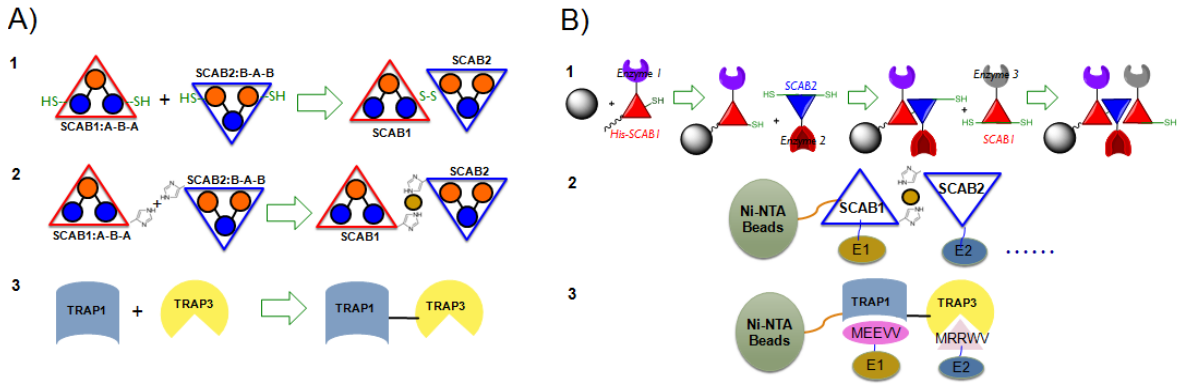

Figure 1. A) Scaffolding strategies. 1st and 2nd strategies are based on SCABS modules and the 3rd strategy is based on TRAP modules. B) Assembly of enzymes by the different scaffolding strategies.

Acknowledgements

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STRUCTURAL ELUCIDATION OF LARGE AND STEREOCHEMICALLY RICH POLYKETIDE MACROLIDES COMBINING NMR SPECTROSCOPY AND BIOINFORMATIC GENE CLUSTER ANALYSIS

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Complex polyketide macrolides are an important class of secondary metabolites primarily produced by actinomycetes.[1] These natural products are characterized by their rich structural diversity and potent bioactivities.[1,2] Structural elucidation of this class of compounds is a challenging task where NMR spectroscopy is essential for determining the polyketide connectivity and the relative configuration within each stereocluster.[3] However, establishing the configurational relationship between stereoclusters by NMR is not trivial, specially in the case of large macrolides.[3] Fortunately, the remarkable advances in microbial genetics during the last decades have uncovered the biosynthetic gene clusters and the formidable enzymatic machinery, the multi-modular type I polyketide synthases (PKS), involved in the assembly of many polyketides.[4] Structural, biochemical and bioinformatic studies have considerably advanced our understanding of the logic of polyketide biosynthesis up to a level of detail that nowadays, in most cases, the substituents and stereochemistry of a polyketide chain can be predicted by detailed sequence analysis of the corresponding multi-modular type I PKS genes within the cluster.[5] In this work we report a multidisciplinary approach combining NMR spectroscopy and bioinformatic gene cluster analysis for the complete structural elucidation of caniferolides A-D, a novel family of antifungal glycosylated 36-membered polyketide macrolides isolated from the marine-derived *Streptomyces caniferus* CA-271066.[6] The two methods complement each other in a synergic way. It will be shown on the one hand how the bioinformatic analysis helps to relate the configuration of the stereoclusters determined by NMR and to establish the absolute configuration of the polyketide chain, and on the other hand how NMR is essential to determine the position and nature of post-polyketide synthase modifications such as cyclizations, epoxidations, hydroxylations, sulfonations or glycosylations which cannot be predicted *in silico*. Aditionally, NMR will be shown to provide the absolute configuration of the monosaccharide units based on their configurational relationship with the absolute stereochemistry of the polyketide aglycon which had been previously determined by bioinformatic analysis.

The example of caniferolides A-D demostrates that this approach, based on the integration of genomic data with NMR analysis to assign the full stereostructure of complex and stereochemically rich polyol macrolides,[7] is much more efficient than traditional methods based on chemical degradation and derivatization, especially considering the current affordability of genome sequencing and the extraordinary power of state-of-the-art bioinformatics tools

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P19 / C25

"Click" Synthesis and Pharmacological Evaluation of sp^2-Iminosugars *S*- and *C*-Glycosides

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sp^2-Iminosugarsare carbohydrate mimics with unique properties in terms of chemical and structural versatility. The presence of a pseudoamide nitrogen at the position of the ring oxygen in mosaccharidesenables glycosidationreacions and strongly favors the α-orientation of glycosidic substituents by the convergence of the anomeric and steric effects. In the last years, several approaches have been explored to access sp^2-iminosugar *O*-, *N*-, *S*-, and *C*-glycosides asmimetics of bioactive glycoconjugates.Thus, sp^2-iminoglycolipid (sp^2-IGL) analogsof immunoregulatoryglycolipids were synthesized and found to exhibit a variety of pharmacological activities against cancer,[1]parasitic diseases[2]and inflammatory disorders.[3] In this context, we now present a molecular diversity-oriented strategy to access α-*S* or α-*C*sp^2-IGLs in high yield and with total stereocontrol that exploit several "click" methodologies. A series of derivatives have been prepared in this manner and their glycosidase inhibitory, antiproliferative and antileishmanial activitieswere screened in parallel in the frame of structure-activity relationship studies.

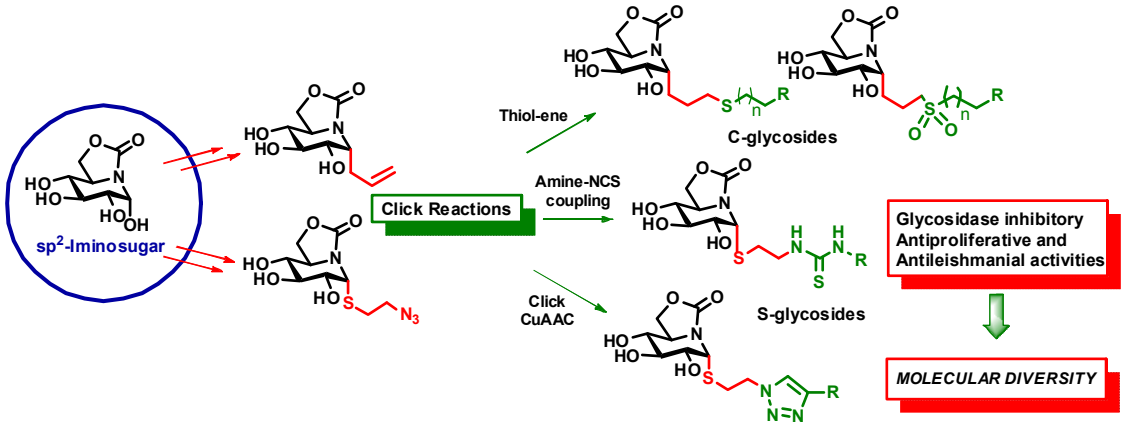

Figure 1. Chemical structures "click" strategies implemented for accessingsp^2-iminosugar α-*S*and α-*C*-glycosides.

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Computational approaches to the structure and activation mechanism of Toll-like receptor 4

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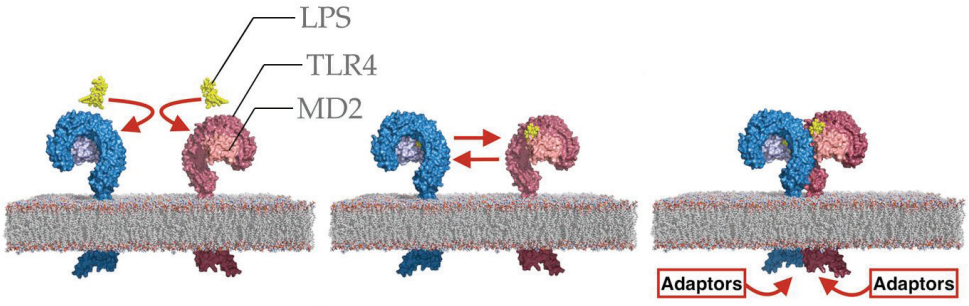
Toll-like receptors (TLRs) are pattern recognition receptors involved in the innate immunity. In particular, TLR4 binds to lipopolysaccharides (LPS), a membrane constituent of Gram-negative bacteria, and together with MD-2 protein, forms a heterodimeric complex which leads to the activation of the innate immune system response. TLR4 activation has been associated with certain autoimmune diseases, noninfectious inflammatory disorders, and neuropathic pain.[1] Therefore, TLR4 has risen as a promising therapeutic target, and design of TLR4 modulating drugs constitutes a highly relevant and active research area.[2, 3]

Specific molecular features of extracellular, transmembrane and cytoplasmic domains of TLR4 are crucial for coordinating the complex innate immune signaling pathway. Although X-ray, NMR and biological structural data is currently available for the independent TLR4 domains, the structure fragments only provide a partial view, because full-length proteins are flexible entities and dynamics play a key role in their functionality. Therefore, many structural and dynamical features of the TLR4 mode of action remain largely unknown.

Computational studies of the different independent domains composing the TLR4 are undertaken, using homology modeling, protein-protein docking, and molecular dynamics simulations, to understand the differential domain organization of TLR4 in a wide range of membrane-aqueous environments, including liquid-disorder and liquid-order membrane models, to account for the TLR4 recruitment in lipid-rafts over activation.

Our final goal is, from the information gathered in our studies, to propose a full TLR4/MD-2 dimer model that explain most of the molecular information known to date increasing the current knowledge of the complex mechanism of receptor activation, and adaptor recruitment in the innate immune signaling pathway, and make use of this new knowledge to design novel TLR4 modulators and probes.



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P21 / C29

Carbohydrate recognition by complement Factor H and related proteins viewed by NMR

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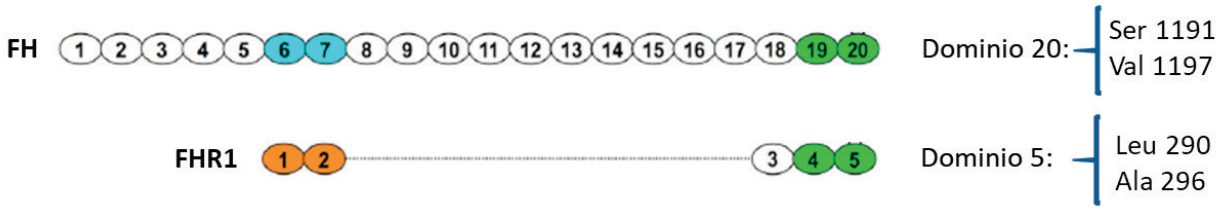
The complement system consists of a tightly regulated network of proteins that play an important role in host defense and inflammation.[1] The complement system has an intrinsic capacity to distinguish host cells from pathogens. The Factor H (FH), an abundant plasma glycoprotein with contains 20 sushi domains, has a major role in protecting host cells from complement-mediated damage and elimination.[2] Factor H-related proteins (FHR) are plasma glycoproteins related in structure and antigenicity to each other and to the complement inhibitory protein FH. In contrast to FH, they have no strong complement inhibitory activity. A common feature of CFHRs is that they bind to the C3b component of complement.[3]



Cell surface carbohydrates play an essential role in self/non-self recognition by FH and FHR. The most C-ter domain works as a cell-surface sensor through the recognition of sialylated glycans.[4] In recent years nucleotide variants in FH domain 20 and homologous domain in FHR, have been described associated with various diseases.[5] We propose that the related pathogenicity to these FH/FHR molecules with altered C-ter domains is a consequence of changes in its carbohydrate recognition properties.

For studying carbohydrate-FH and FHR proteins molecular recognition processes we have applied NMR strategies based on ligand observation,[6] saturation transfer difference (STD). This NMR experiments confirms that FH bind 2-3 linked sialic acid glycans un its C-terminal domain,[4] while native FHR-1 does not. Whereas the binding capacity to sialic acid can be restored due to the mutations of the FHR-1 protein, like FHR-1::FH. In case of the mutations of the FH, the FH(V1197A) reduces binding to 2-3 linked sialic acid glycans, while FH(S1191L) completely abolishes the binding. Of the two common SIAs, the 2,6 is not bound by any of the tested FH and FHR-1, only FH(L290V).

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^{19}F-NMR SCREENING AND CHEMICAL MAPPING OF CARBOHYDRATE-LECTIN COMPLEXES

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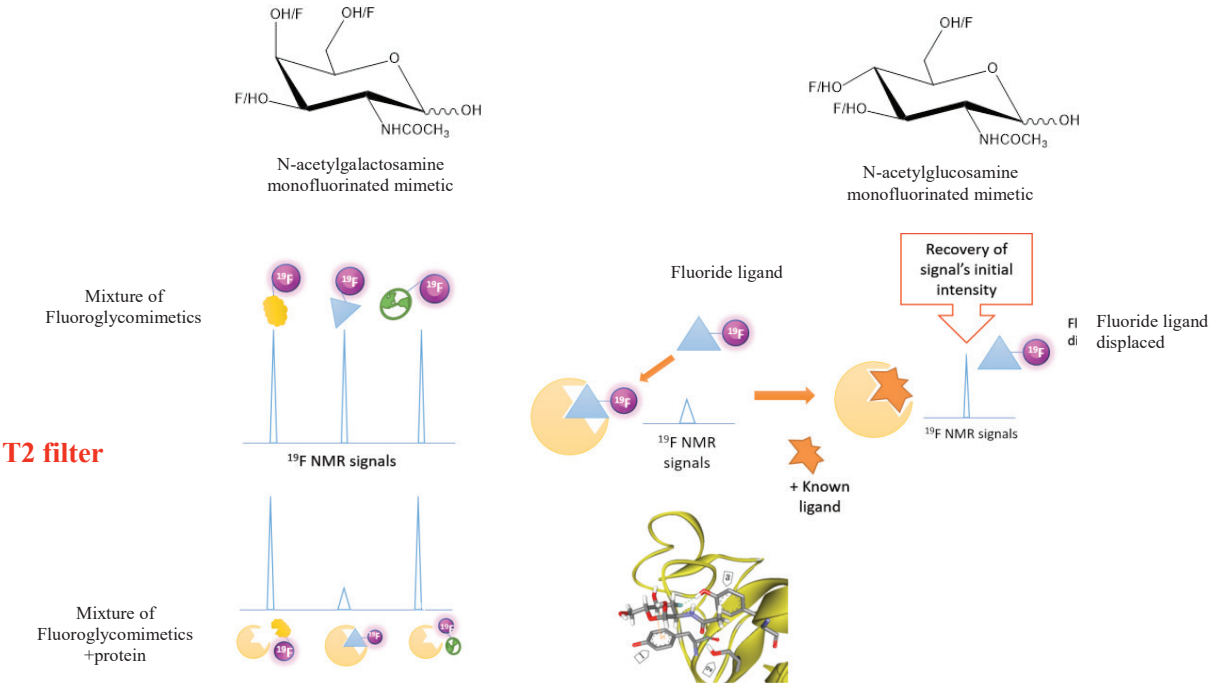
The protein-carbohydrate interaction activates intracellular modulation functions and intra- and interspecies recognition. The lectin proteins are readers of this 'sugar code', being able to identify different types of sugars[1]. There is a great interrelated complexity between types of glycans and types of lectins[2]. Due to the great diversity of lectins, a screening method is needed that provides information on the ligands of these proteins, as well as the characteristics of this possible interaction.

We will examine a molecular screening method based on synthetic 3F-, 4F-, and 6F- monofluorinated substituted glycomimetics derived from N-acetylated sugars, such as *N*-acetylgalactosamine and *N*-acetylglucosamine by employing the ^{19}F-Nuclear Magnetic Resonance (NMR) approach using the T2 relaxation filter strategy[3]. With this approach it is posible to identify in one simple experiment, on a mixture of fluoroglycomimetics, which compounds behave as a lectin ligand and at the same time, the fluorine-hydroxyl substitution reveals their crucial hydroxyl groups for the interaction with the lectin. Using this method, ligands from Wheat Germ Agglutin lectin (WGA) and *Helix pomatia* lectin (HPA) were identified.



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P23 / C31

Fluorinated and deoxy-monosaccharides by high-resolution spectroscopies

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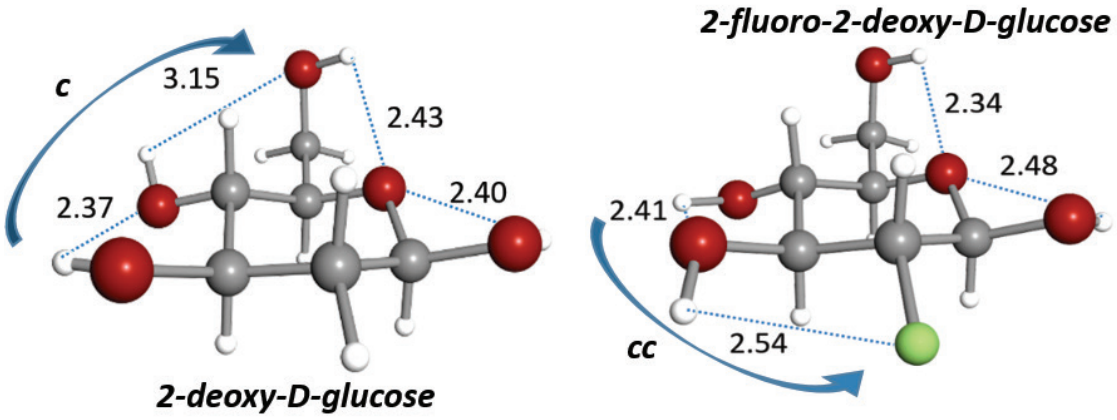
Bioisosteric OH-to-F replacements, particularly those produced in saccharides, have become a cornerstone in the development of novel drugs and tracers for the non-invasive imaging modalities, like (^{19}F-MR/MRI) and (^{18}F-PET). F and OH groups are isoelectronic and possess similar polarity and steric impact. Despite its remarkable purposes, there is a lack of knowledge about the structural non-covalent interactions involving F in their native context, which are key determinants of the biophysical properties of the biomolecule to which F is attached.



We present several studies on carbohydrates exploiting an experimental strategy which combines synthesis, microwave and laser high-resolution spectroscopies, solid-state and solution NMR experiments. Laser spectroscopy offers high sensitivity and selectivity, making it ideal for studying biochemical systems of medium-large size.[1-2] Moreover, microwave spectroscopy provides higher resolution and direct access to the molecular structure.[3-4] This combined approach[5] affords not only accurate chemical insight on conformation, structure and molecular properties, but also benchmarking standards, guiding the development of theoretical calculations. In order to illustrate these possibilities, we present the results on the conformational landscape of several fluorionated and deoxy monosaccharides with different biological roles.

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Intracellular catalysis by palladium-containing miniproteins

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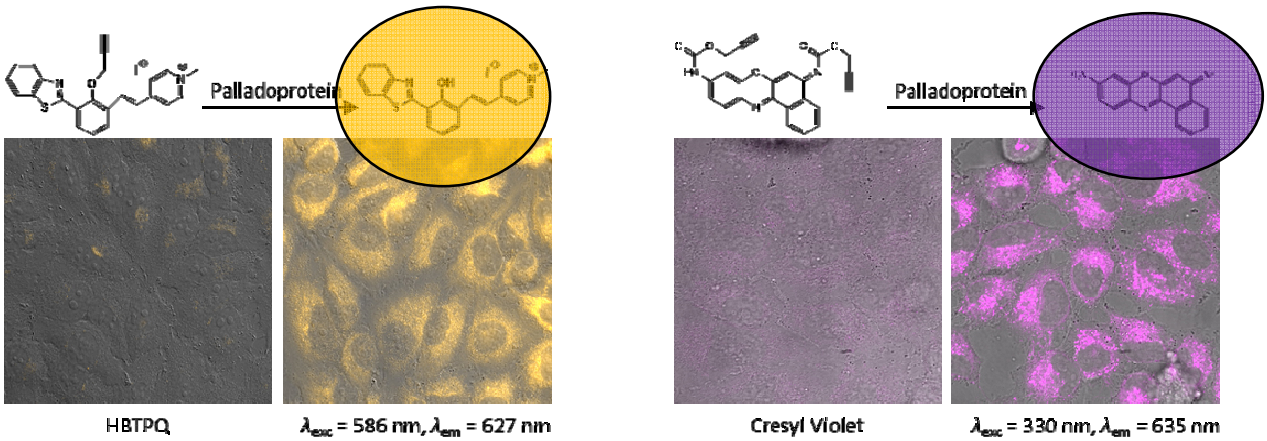
A fragment of the DNA basic region (br) of the GCN4 bZIP transcription factor has been modified to include two His residues at designed i and i+4 positions of its N-terminus. The resulting monomeric peptide ($brHis_2$) does not bind to the target DNA, but addition of cis-$Pd(en)Cl_2$ promotes its alpha-helical folding and DNA binding. The peptide-DNA complex is disassembled by addition of a slight excess of a palladium chelator, and the interaction can be reversibly switched multiple times by playing with controlled amounts of either the metal complex or the chelator. Importantly, while the peptide $brHis_2$ fails to translocate across cell membranes on its own, addition of the palladium reagent induces an efficient cell internalization. [1]

To obtain more information on the molecular mechanism of the cell internalization process, we have carried out different studies which include modelling, study of uptake pathways, mutation of specific residues to uncover structure/activity relationships, and localization of palladium inside cells.

The intracellular delivery ofametallopeptideraised the question of whetherthe conjugate couldpromote in *cellulae* reactions. Therefore, we made different type of palladium/peptide complexes exhibiting a variety of ligandsand tested their biorthogonal catalytic activity. The *in vitro* reactions performed in PBS, demonstrated thatthe palladium-clipped peptide complexis able to promote the removal of propargylic appendages in designed caged probes.Gratifyingly, the reactions can be carried outinside mammalians cells, as shown by the generarion of a strong fluorescence when we incubate the metallopeptide with non-fluorescent propargylatedprecursors (Figure 1). Several control experiments confirmed that the activity is associated to the palladoprotein.



Acknowledgements

This work has received financial support from Spanish grants (SAF2016-76689-R, ORFEO-CINQAnetwork CTQ2016-81797-REDC), the Consellería de Cultura, Educación e OrdenaciónUniversitaria (2015-CP082, ED431C-2017/19 and Centro Singular de Investigación de Galiciaaccreditation 2016-2019, ED431G/09), the European Union (European Regional DevelopmentFund-ERDF corresponding to the multiannual financial framework 2014-2020), and theEuropean Research Council (Advanced Grant No. 340055).Soraya Learte-Aymamí thanks the MINECO for a PhD fellowship.

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P25 / C37

Identification of Benzo[*cd*]indol-2(1*H*)-ones as novel Atg4B inhibitors via a structure-based virtual screening and a novel AlphaScreen assay

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Targeting autophagy is a promising therapeutic strategy for cancer treatment. As a result, the identification of novel autophagy inhibitors is an emerging field of research. Herein, we report the development of a novel high-throughput screening (HTS) assay that combined with a structure-based virtual screening have enabled the identification of novel autophagy inhibitors.

Correct autophagy requires the association of the autophagy marker LC3 to the autophagosome membrane. To achieve this, LC3 needs to be C-terminally cleaved by the cysteine protease Atg4B and subsequently conjugated to a phosphatidylethanolamine. With the final aim of identifying Atg4B inhibitors, we established a mass spectrometry (MS)-based assay to detect the cleavage of LC3 and the release of the corresponding pentapeptide, as well as a novel HTS assay based on the Alphascreen technology that uses a doubly labelled LC3 protein as a substrate. A structure-based high-throughput virtual screening was next performed. With this aim, theNational Cancer Institute Open Database (NCIOD) library, containing more than 260.000 compounds, was screened against two Atg4B targets site, the catalytic center and an allosteric binding pocket. Two different docking softwares, Glide and Autodock Vina, were employed thereby providing four potential list of inhibitors.

The biological screening of 250 selected compoundsled to the identification of novel scaffolds targeting Atg4B such as **NSC611216**(Figure 1). Structural-activity relationship analysis of the initial hit provided an optimized lead compound bearing a7-aminobenzo[*cd*]indol-2-[1*H*]-one scaffold and a propyl group replacing the chlorine. Their activity and specificity could be further validated using additional biochemical and cellular studies. We are convinced that the developed AlphaScreen and MS-based assays can be key tools enabling the high-throughput identification of novel Atg4B inhibitors. Moreover, the aminobenzo[*cd*]indol-2-[1*H*]-one scaffold represents a novel chemotype for the further development of small molecule inhibitors of Atg4B.

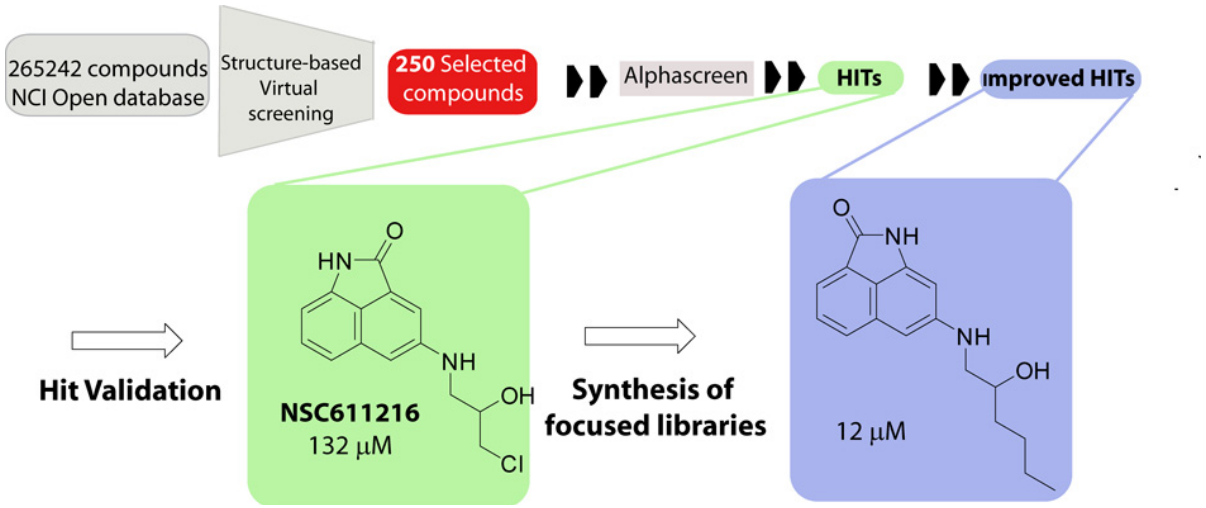

Figure 1. Workflow for the identification of Atg4B inhibitors.

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Computer-accelerated discovery of new site-selective protein modification strategies

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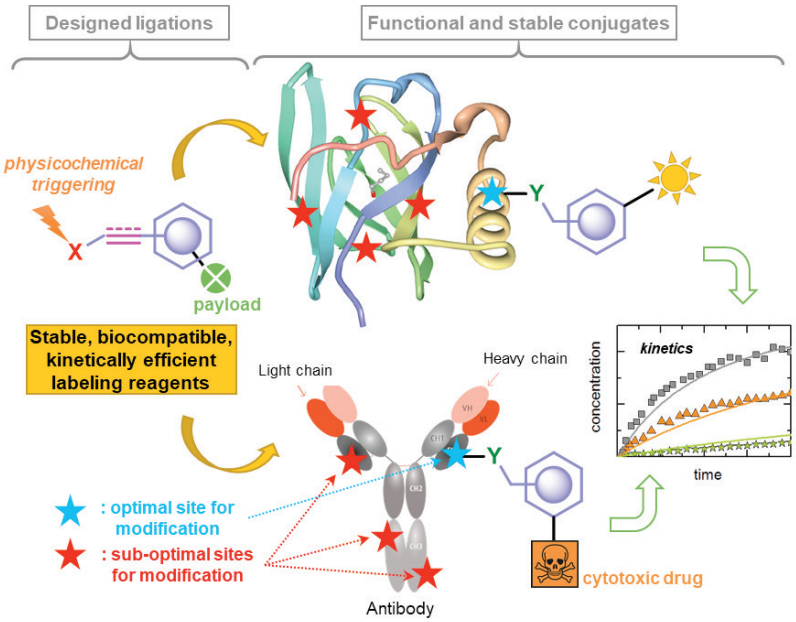
Site-selective chemical conjugation of synthetic molecules to proteins expands their functional and therapeutic capacity. For instance, it is possible to bind covalently a very potent drug to an antibody, namely antibody–drug conjugates (ADCs), by means of a precise chemical reaction to shuttle this drug to a specific tissue. Proteinogenic amino acids, e.g. lysine, cysteine, tryptophan or methionine side chains, are particularly suitable to modify native proteins. However, cysteine is probably the most commonly used due to the high nucleophilicity of the sulfhydryl side chain and the low abundance of free cysteine residues. Current protein modification methods, based on synthetic and biochemical technologies, can achieve site selectivity, but these techniques often require extensive sequence engineering. Computational chemistry allows rapid screening of large compound libraries and determining potential site-selective reagents.[1] Based on this technique, we discovered that quaternization of the nitrogen of vinyl- and aklynyl pyridines transforms these simple molecules into very reactive electrophiles for cysteine-selective bioconjugation.[2]



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P27 / C40

NMR insights on the recognition mechanism of histo-blood group antigens by human galectin-1

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The recognition of glycans by lectins at the surface of most cells is at the heart of important living processes. Indeed, this kind of interactions are key events in biological processes such as signal transduction, cell-cell adhesion and migration. Protein-glycan interactions are also linked with several diseases, including infection, cancer or autoimmune disorders. In the last decades, many families of lectins have emerged as important biomedical target studied for the development of therapeutic agents. The real effort is to achieve the creation of molecules able to target only a specific lectin, despite the sequence identity among members of the same family is very high. To achieve this goal it is essential to dissect, at molecular level, the protein-glycan recognition event.

In this context, we herein focus onhow human galectin-1 (*h*Gal-1) recognizes the blood group antigens. *h*Gal-1 is a β-galactoside binding lectin, involved in a large variety of relevant biological events such as inflammatory responses, differentiation trafficking, survival of immune cells and establishment and maintenance of T-cell tolerance and homeostasis *in vivo*[1]. Furthermore, *h*Gal-1 is implicated in cancer progression[2] and its overexpression in tumours positively correlates with a metastatic phenotype[3].

The binding of *h*Gal1 to natural oligosaccharides containing LacNAcdisaccharide motif (its 3'-O-α-Gal derivative and the H type II, B type II and A type II) antigens has been investigated using a multidisciplinary approach with ligand-, receptor-based and relaxation NMR techniques, ITC and molecular dynamics simulations.
Thus, the binding features have been analysed and the molecular basis of the recognition events have been deduced from the thermodynamic and kinetics perspectives. The effect of protein and ligand dynamics has highlighted. Interestingly, the terminal galactose residue modulates binding in an opposite way to that reported for human galectin-3[4].
Overall, our results provide a comprehensive analysis of the glycan-lectin binding process, necessary to guide the design and development of human galectin 1-based therapeutic tools.

Acknowledgements

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Deciphering the N-glycan profile and interactions of FcεRIα by NMR

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N-linked glycosylation is a protein post-trasductional modification that results in the covalent attachment of an oligosaccharide onto a given asparagine residue at the polypeptide chains [1]. It is involved in protein folding, trafficking, and stability, and regulates many cellular activities, especially of extracellular nature [2]. The α subunit of the IgE high affinity receptor (FcεRI α) is a transmembrane protein with 7 N-glycosylation sites on the extracellular domain. The glycosylation is key for secretion and stability of the protein [3].

Herein, we have analysed the glycoprofile of the FcεRI α expressed in human HEK 293T cells, identifying the presence and the relative abundance of specific glycan epitopes, using NMR Spectroscopy. The anomeric region of the ^{1}H,^{13}C-HSQC spectrum shows a high degree of heterogeneity in terms of glycosylation. The deduced structures include high-mannose (23%), hybrid (35%), and bi- tri- and tetra-antennary complex type (32%) N-glycans with different degrees of fucosylation and sialylation.

The estimation of the solvent accessible surface area (SASA) of FcεRI α revealed that Asn132 is the most protected residue. For that reason, we guessed that the high mannose glycan chain is attached to this site. [4] Molecular modelling protocols permitted to suggest that the glycan is packed within the two immunoglobulin domains, thus contributing to the stability and structure of the glycoprotein. This hypothesis has been evaluated by expressing the Asn132Ala mutant. The analysis of the secondary structure of the mutant protein using circular dichroism along with the quantification of the HSQC spectrum has permitted to describe the existing differences in glycan composition between the WT and mutant proteins.

The 7 glycans on FcεRI α are important for secretion and stabilization of the protein [5], as well as for recognition processes through binding with lectins [6]. Herein, we have analyzed the binding between FcεRI α with human Galectin-3 and Siglec-8.

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P29 / FC7 / C42

Computational prediction of activity-enhancing mutations by allostery networks optimization

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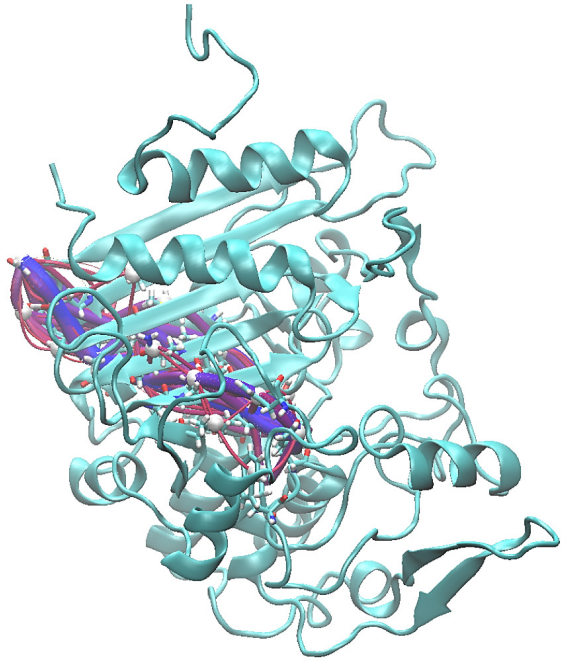
An artificial variant of acyltransferase LovD has been discovered to be an efficient catalyst to obtain an unnatural product of high pharmaceutical value as a lipid-lowering medication – simvastatin – at industrial scale as a result of a directed evolution study.[1] This variant is 1000-fold more efficient in the synthesis of the drug than wild-type LovD and is able accept an unnatural acyl donor (DMB-SMMP) instead of the natural acyl carrier protein (ACP) partner. Through microsecond molecular dynamics simulations performed on the different variants evolved through laboratory engineering, it was proposed that one of the main roles of the mutations scattered throughout the entire enzyme was to maintain the catalytic triad in an optimal conformation in the absence of the ACP partner.

Traditionally, proteins structures have been studied by their secondary structure and folding patterns; alternatively, they can be treated as a highly dimensional network of dynamically interacting amino acid residues.



By combining this emerging paradigm in enzyme catalysis (i.e. activity regulation by distant, dynamically coupled residues) and our previous knowledge in the directed evolution of LovD, a new purely *in silico* approach merging primary sequence alteration and molecular dynamics relaxation has been developed. Mutations potentially able to improve the catalytic activity of the wild-type enzyme have been predicted though a dynamical network analysis. Such analysis program identifies not only the optimal but also the suboptimal pathways of correlated motions between pairs of residues that are not directly linked and may play important roles in the transmission of allosteric signals.[2]

Of note, our protocol has predicted influential mutations in both novel and previously engineered positions by directed evolution; moreover, specific mutations generated by laboratory evoluation have been found. More importantly, all the mutational hotspots discovered by our protocol are located in very remote regions from the active site, which differs from traditional enzyme design protocols based on active site remodelling. The necessary experimental evaluation of the highest ranked computational variants is currently ongoing.

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Stereoselection of three-standedhelicates with trimeric peptide structures

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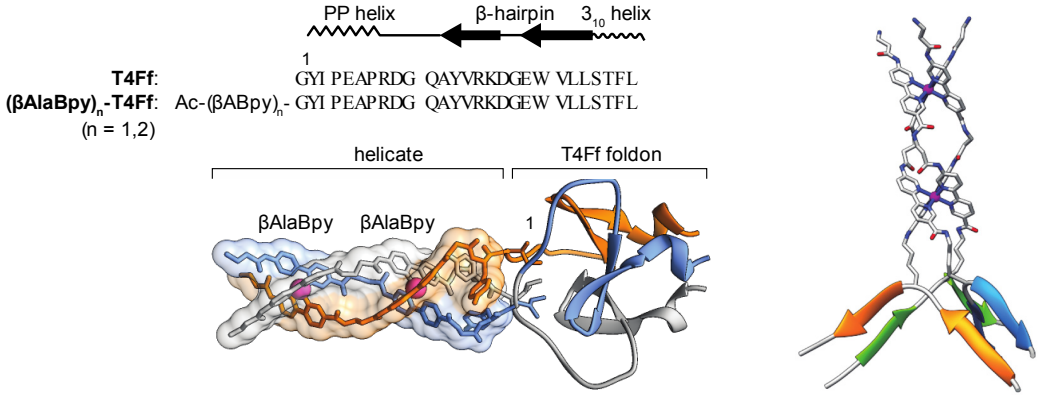
Helicates, first described by Prof. Jean-Marie Lehn more than twenty years ago, are discrete metal complexes in which one or more organic ligands are wound around and coordinate two or more metal ions.[1]Helicates are inherently chiral species that can appear as right-handed or left-handed (*M*, or ΛΛ) helicity, according to the orientation in which the ligands coil around the helical axis defined by the metal centers.These compounds display promising anticancer and antibacterial activity, probably due to its DNA-binding properties,[2]buttheir supramolecular chirality represents a challenge for their synthesis that has hampered their development, and despite some noteworthy examples,[3] no general approach for the efficient and versatile stereoselective synthesis of helicates is yet available. Furthermore, current methods do not allow the precise control of the relative orientation of the helicate ligands, which has led to the study of symmetric helicates

To overcome these barriers, we have proposed the synthesis of peptide-based helicates, in which the chelating ligands can be easily accessed through solid-phase peptide synthesis protocols. In this communication we describe two examples of the implementation of that concept. In the first one we show that a small trimeric protein domain, the bacteriophage T4 Fibritinfoldon, or the β-annulus motif derived from the Sesbania Mosaic virus,[4,5]can be engineered with metal-chelating 2,2'-bipyridine ligands to direct the formation of a three-stranded parallel helicate in a stereoselective manner. In both cases, the resulting metallopeptides canselective recognize three-way DNA junctions over double-stranded DNA.



Acknowledgements

FinancialsupportfromtheSpanishgrant CTQ2015-70698-R, theConsellería de Cultura, Educación e Ordenación Universitaria GRC2013-041, and Centro singular de investigación de Galicia accreditation 2016-2019, ED431G/09, and theEuropean Regional DevelopmentFund (ERDF), isgratefullyacknowledged. J. G.-G. thankstheSpanish MINECO forhis FPI fellowship.

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P31 / C44

A nanoparticle-based ligand for CuAAC fluorogenic "click" reactions in water.

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The most widely used bioorthogonal "click" reaction is the Cu (I) catalyzed azide-alkyne cycloaddition (CuAAC)[1], in which 1,2,3-triazole is formed. It has been used for the modification of a variety of biomolecules, such as proteins, nucleic acids and enzyme inhibitors, and even cells and viral particles. Additionally, this process is also used in organic synthesis for the preparation of many derivatives in a straightforward manner. However, this reaction usually requires the use of ligands to reduce its side products or effects (e.g., regioselectivity, toxicity, degradation of molecules), such as TBTA, THPTA or BTTAA.
In this work, we are developing a ligand based on gold nanoparticles (AuNPs) and oligonucleotides, which is being evaluated for the generation of a fluorescent product through a fluorogenic CuAAC reaction[2] in aqueous media. (**Figure 1**).

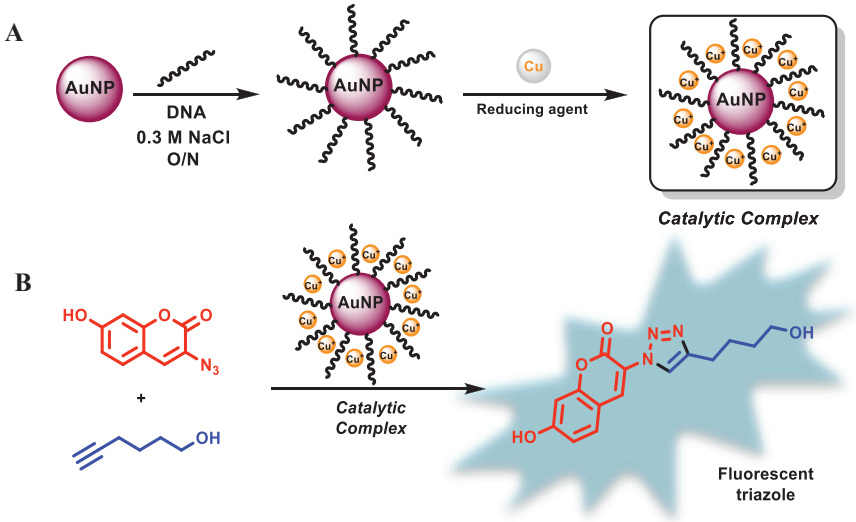

Figure 1. Representation of the ligand-catalyst complex employed in fluorogenic Cu (I) catalyzed azide-alkyne cycloaddition (**A**) and reaction scheme (**B**).

The synthesis of the ligand involves the functionalization of AuNP with different oligonucleotides. Interestingly, the activity of the system depends on the sequence employed, and the best construction presents better efficiency than traditional ligands.

Acknowledgements

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A ruthenium-based DNA-binding complex selectively inhibits cancer stem cell growth

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Since the discovery of the antitumorigenic activity of metal-based DNA binders such as cis-platinum, there has been a great interest in the development of other efficient antitumoral metal complexes with improved selectivity and reduced secondary effects. Several ruthenium complexes that form covalent DNA adducts have been studied as promising alternatives. Recently, we have described that the Ru complex $[Ru(tpy)(bpy)(H_2O)]^{+2}$ [**1**] can selectively metalate unpaired guanines present in DNA structures called parallel G-quadruplexes (GQs) while exhibiting very low toxicity[1]. Nevertheless, the disturbances in the structure of the G-quadruplexes caused by this compound can perturb cellular transcriptional programs. We reasoned that the combination of low toxicity and selectivity of this compound could offer excellent opportunities for the modification of specific biological responses.

[1]

In our presentation, we will show that **1** reduces the self-renewal and tumorigenic capacity of cancer stem cells (CSCs) isolated from primary patient tumours. We will also provide evidence that this effect is mediated primarily through the perturbation of mitochondrial homeostasis. On one hand, mechanistic studies confirmed the ability of this compound to reach and associate to mitochondria both *in vitro* and in intact cells. Functional assays, on the other hand, demonstrated the impairment of the mitochondrial function by showing both decreased oxidative phosphorylation and generation of reactive oxygen species. To understand the mechanism of action of complex **1** it is essential to highlight that the substitution of its aquo position for a pyridine or a thioether ligand rendered kinetically stable complexes that didn't react with GQs and failed to promote any biological response on CSCs. This result provides further evidence for a mechanism of action where the metalating activity of the complex is responsible for its biological activity.

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P33 / C46

NMR study of pH-dependentchloridetransportby pseudopeptidiccages in lipid dodecylphosphocholinemicelles

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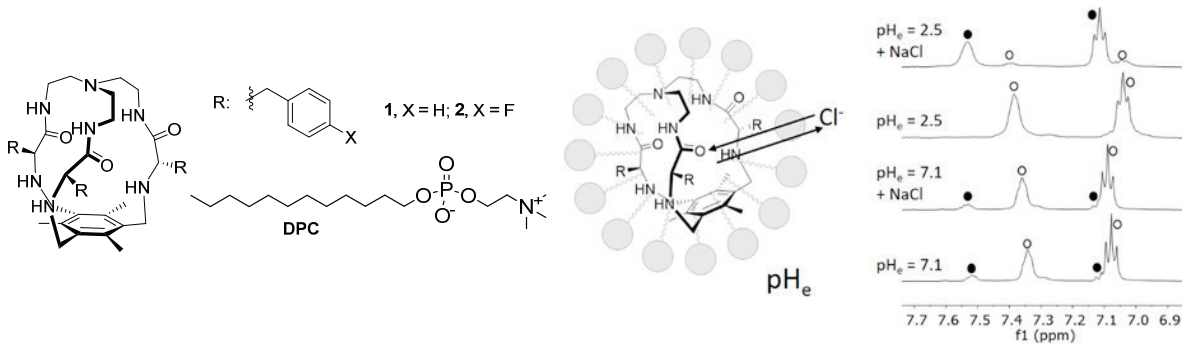
Synthetic anionophores are increasingly used in Chemical Biology as research tools and potential therapeutic agents.[1]Within this field, we have been studying pseudopeptidic cages as chloride receptors.[2] Very recently, the biological activity of the cages in living cells was tested in a human lung adenocarcinoma cell line (A549) and the cytotoxicity was determined using the MTT assay. A fluorinated Phe derivative (see Figure) showed an increased HCl transport ability and a higher cytotoxicity towards cancer cells in the presence of pH gradients resembling those characteristic of tumor microenvironments.[3]In this work, we usedT_2proton NMR relaxation times and translational self-diffusion measurementsto assess the incorporation of pseudopeptidic cage□like anionophores to deuterated DPC-micelles as a model of anaqueous-lipid phase. Also, we evaluatedthe possibility to study and characterizethe relationshipbetween structure/dynamics and anion binding in membrane mimetic media using these NMR methodologies. Different conditions of the external aqueous phase were studied ranging from neutral to acidic pH, and also in the absence and presence of salt. Diffusion ordered NMR spectroscopy (DOSY) performed with two of the cages showed self□diffusion rates compatible with the practically totalincorporation of the cages within the micelles, but we observed smaller diffusion coefficients for 4FPhe cage aromatic protons incomparison with Phe cage protons at acid pH, suggesting a higher proportion of 4FPhe cage inside micelles at this pH.Also, we used paramagnetic doxyl lipid radicals (5-DSA and 16-DSA)to qualitatively evaluate the cages location inside the DPC-micelles.We observed different NMR time-scale dynamic behavior for aromatic protons when comparing cage 1 (Ar = Ph) and cage 2 (Ar = 4-F-Ph) in micellar aqueous media in the presence of an excess of chloride anion: slow exchange between free and chloride-bound cage in the chemical shift (and T_2 relaxation) time scale for 4-F-Phe cage and fast exchange in the chemical shift time-scale (but slow exchange in T_2 relaxation) for Phe cage.The detailed NMR study gives some clues about the diferential behavior of these otherwise very similar receptors.



Acknowledgements
Financial Support from MCIU/AEI/FEDER(EU) (RTI2018□096182□B□I00,), AGAUR (2017 SGR 208) and personal support for L. Tapia (BES□2016□076863) are gratefully acknowledged.The 500MHz spectrometer was funded by MINECO□FEDER (CSIC13□4E□2076).

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Controlling Oncogenic KRAS Signalling Pathways in Living Cells with a Designed Metallopeptide

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RAS proteins are molecular switches associated with critical signaling pathways that regulate cell growth, proliferation, and differentiation.[1] It is known that up to 30% of all cancers, including 95% of pancreatic and 45% of colorectal cancers are driven by mutations in the *RAS* family of genes. More specifically, KRAS is the most frequently mutated isoform—responsible for 85% of all cancers associated with RAS—that generates the more aggressive phenotypes with the worse prognosis.[2] In this context, our laboratory has started a comprehensive research program aimed at the development of new chemical tools to study the activity, localization, and signaling of KRAS in cells, as well a the adaptation of these tools for the identification of new chemotherapeutic agents.

Herein we present preliminary results of our work that demonstrate, for the first time, that the activity of KRAS can be effectively modulated in living cells using a designed metallopeptide cofactor (**αH[Pd]**, Fig. 1a). This artificial modulator, which was obtained by introducing appropriately positioned His residues in a peptide fragment derived from a natural KRAS cofactor, acts as competitive inhibitor of the GDP/GTP exchange process required for KRAS activation. Experiments with living cells show that incubation with this metallopeptide cofactors inhibits KRAS signaling pathways. In a related project, we show that introduction of the environment-sensitive fluorophore in a KRAS-binding peptide yields a selective fluorescent sensor with low μM affinity for KRAS that lights-up in the presence of this protein (Fig. 1b).[3]

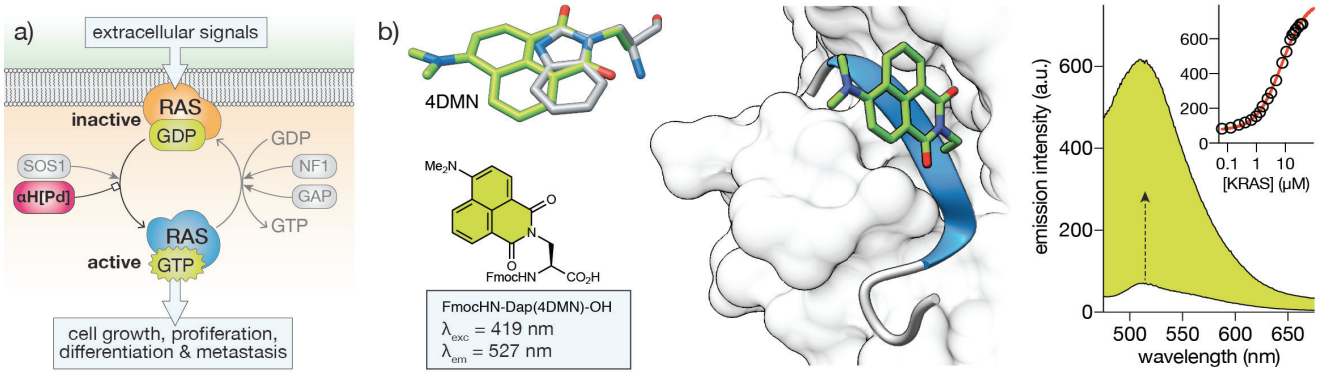

Figure 1. a) KRAS molecular switch and effect of αH[Pd] that acts as inhibitor of the GDP/GTP exchange, modulating downstream signaling pathways; 1b) Structure of the environment-sensitive 4DMN and comparison of this fluorophore with the indole side chain of Trp residues; cartoon of the interaction of the fluorogenic peptide with KRAS, and observed increase in emission and titration profile of the sensor.

Acknowledgements

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P35 / C48

Helical Oligoalanines for Protein Delivery

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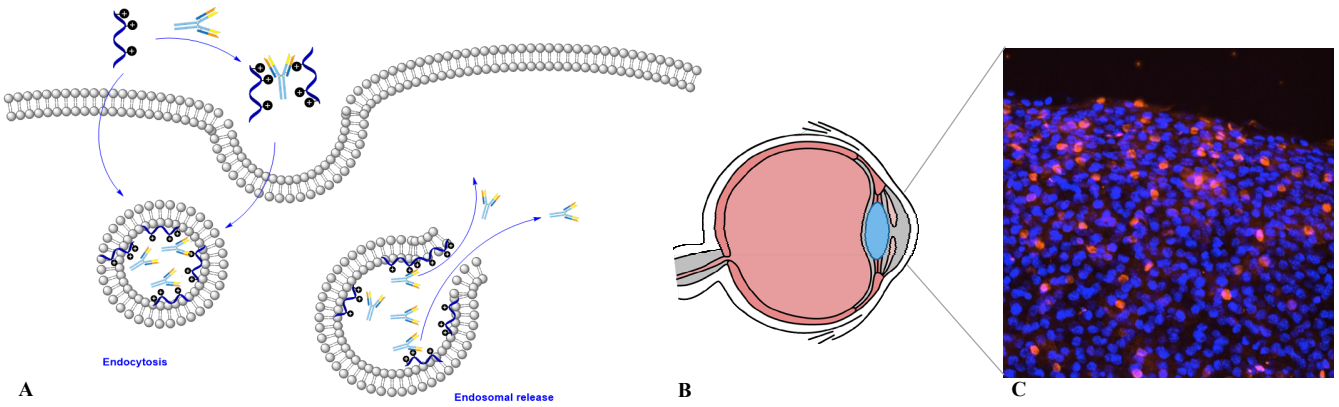
Protein therapeutics have emerged as a way to correct a variety of pathologies using innate processes. However, despite the commercial availability of protein drugs, their formulation and delivery still pose substantial challenges. Proteins typically use the endocytic pathway as a route of cellular entry, but the majority of them typically remain trapped inside the endosomes. Therefore, the amount of protein that reaches the cytosol of cells is low and the biological response and therapeutic effect are reduced. A possible solution to this problem is to increase the ability of proteins to escape from the endocytic pathway. Ideally, a delivery strategy should combine efficient endosomal escape and low cellular toxicity. In the past several years, the potential of a variety of peptides, called cell penetrating peptides (CPPs), to cross cell membranes and deliver a variety of cargos have been demonstrated.[1] However, the endosomal escape of macromolecules and proteins still remains a difficult task for the typical peptides developed so far. In this study we propose a peptide based on alanines and leucines, with only three positive charges due to arginine residues, that undergoes a transition from random coil conformation in water to strong helical character in membranes, presenting also selectivity for anionic membranes. We investigated the use of this new CPP as an endosomolytic agent to deliver protein cargos into cells.[2] The delivery and endosomolytic properties of this CPP were studied using a variety of proteins, which differ in terms of function, size and intracellular localization/site of action, and in different cell lines and tissues. The obtained results confirmed this peptide as a non-toxic and efficient agent to deliver functional proteins, also with therapeutic potential, into cells and tissues.



A. Scheme of the cell penetrating peptide helping the endosomal release of the antibody. **B.** Scheme of the murine eye. **C.** Distribution of the Anti-Nuclear Pore Complex antibody (0,15 mg/ml) incubated with 250 µM CPP for 1 hour on murine cornea.

Acknowledgements

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Lipid responsive helical oligoalanines for cell penetration

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Positively charged molecules and macromolecules with a certain degree of hydrophobicity can interact with anionic membranes and be taken up by cells.[1] Cell penetrating peptides are good vectors that translocate the cell membrane and introduce inside cells a wide variety of different cargoes.[2] The limitation of a lot of drugs is the release from endosomes into the cytosol.[3]

Our hypothesis is that if we controlled the helical structure of peptides in different media we can control de endosomal release. We have prepared simple peptides based on alanines, arginines and leucines.[4] Penetrating efficiency studies indicated that peptide MP1 is able to deliver proteins and antibodies in the cytosol cells. The supramolecular structures and interactions of these peptides with the membrane has been studied. The application of different techniques such as circular dichroism, fluorescence, FRET or BLM allowed us to identify a hitherto not described self-assembled nanopore as the delivery mechanism.

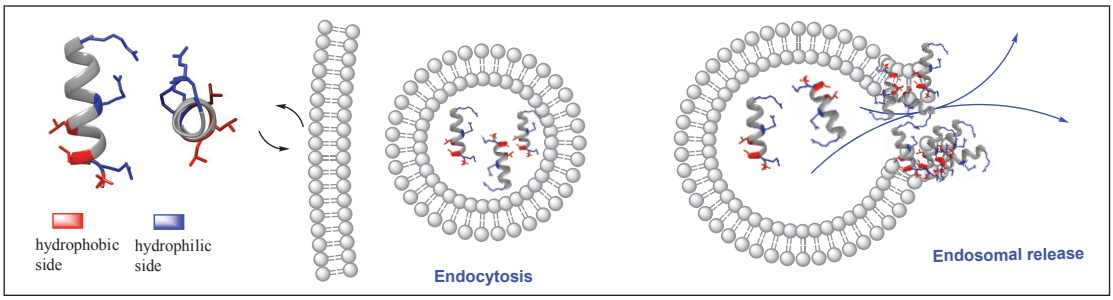

Figure 1: Scheme showing the switchable system between aquos and membrane media and pensomal release.

Acknowledgements

We acknowledge the support from MINECO (CTQ2014-59646-R) and the Xunta de Galicia (ED431G/09, 2016-AD031 and ED481A-2017/142*), the ERDF and the ERC (Starting Grant DYNAP-677786), Young Investigator Grant from the Human Frontier Science Research Program (RGY0066/2017).*

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P37 / C50

Bioorthogonal carbene transfer reactions in biological media

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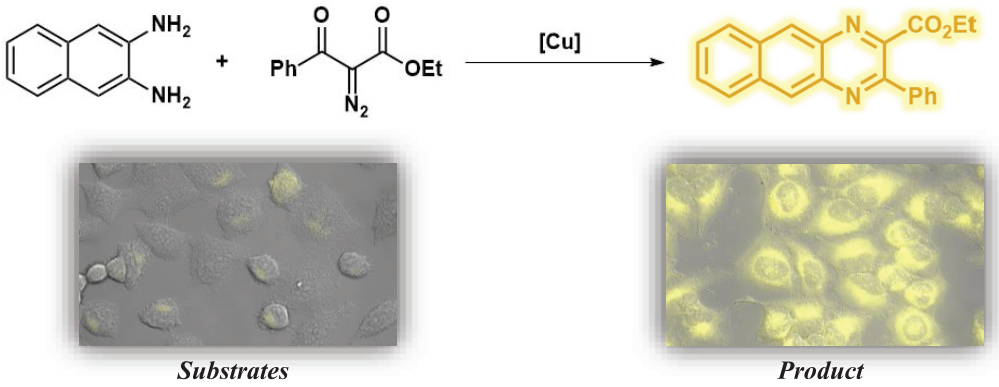
Bioorthogonal chemistry has revolutioned the way in which chemical biologists conceive chemical reactions. Recent work has shown that many organic transformations are compatible with water; however, chemoselectivity, bioorthogonality and biocompatibility remain as critical challenges when trying to export these reactions to biological environments.[1] In the last few years, our research group has developed a series of biocompatible reactions mediated by transition metals that can take place in biological media and even in cellular environments.[2] Most of them involve uncaging or isomerization processes. Whereas several carbene transfer reactions mediated by transition metals have been shown to be compatible with water, especially when using artificial metalloenzymes,[3] to the best of our knowledge, they have never been carried out in complex biological settings.

Herein, we demonstrate the viability of achieving intermolecular metal-promoted carbene transfer reactions, not only in biological mixtures, but also in mammalian cells. The reaction involves a one-pot domino N-H insertion, cyclization, and oxidation in a single step, and proceeds under mild reaction conditions, in presence of copper salts. Moreover, the fluorescent properties of the product facilitate its use as a probe to monitor the reaction in biological settings.



Preliminary experiments using HeLa cells allowed to observe a clear yellow intracellular fluorescence signal distributed across the cytoplasm and in vesicles, without altering the morphology of the cells. Control experiments in absence of the metal catalyst, using the same threshold observation parameters, confirmed that the signal must necessarily come from the expected metal-promoted process.

Acknowledgements

Financial support from the Fondo Europeo de Desarrollo Regional (FEDER) from the European Union under the Interreg V-A POCTEP España-Portugal program is gratefully acknowledged.

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Development of salicylate derivatives with double GO/LDHA inhibitory activity as pharmacological approach for the treatment of primary hyperoxaluria type 1

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Primary hyperoxaluria type 1 (PH1) is a rare genetic disease related to disorders in glyoxylate metabolism. This inborn error of liver metabolism is caused by a deficit in the enzyme alanine-glyoxylate aminotransferase (AGT), which is in charge of glyoxylate detoxification.Under these circumstances, glyoxylate is oxidized into oxalate by enzymes like glycolate oxidase (GO) and lactate dehydrogenase A (LDHA).[1]Since oxalate constitutes an end-product of metabolism, it precipitates as insoluble calcium oxalate crystals inthe renal system, causing kidney malfunctioning and deterioration, and combined kidney-liver transplantation is the only healing treatment for this disease.

Substrate reduction therapy (SRT) is currently being studied as a strategy focused on preventing glyoxylate accumulation, which can be achieved with the inhibition of those enzymes involved in its formation using small drugs or *si*RNA. Regarding the small molecule approach, GO inhibition has been validated as a safe and efficient target for SRT in a PH1 mouse model. Thus, with the aim of developing a useful pharmacological approach for the treatment of PH1, and following SRT as our main strategy, we identified furylsalicylates as moderate GO inhibitors and efficient agents reducing oxalate output on *Agxt1*$^{-/-}$ mouse hepatocytes culture.[2]

However, for some of our furylsalicylates behaving as modest GO inhibitors, we found a significant reduction in oxalate production in culture, and therefore, the possibility of additional targets for these compounds was considered. Both GO and LDHA are able to metabolize the same substrate, glyoxylate. In fact, it has been recently published that *si*RNA-mediated LDHA and GO inhibition achieve efficient oxalate reduction and prevents the deposition of calcium oxalate crystals in animal models.[1] Thus, we contemplated that our inhibitors could also be able to bind the active site of LDHA, which would lead to dual GO/LDHA inhibition.

In order to verify this hypothesis, those compounds suspected to have additional biological targets were tested for LDHA inhibition. The results obtained confirm that some of our GO inhibitors, which resulted excellent agents decreasing oxalate production on *Agxt1*$^{-/-}$ hepatocytes, also present a notable inhibitory activity against LDHA. Hence, for the first time, LDHA inhibitory activity has been attributed to salicylates, which constitutes an unprecedented feature for these compounds. This represents a step forward in understanding the mechanism of action of these molecules and also shows the potential use of multi-target drugs for the treatment of PH1, an unexplored field of research so far. This, along with the drug-like structure and easy synthesis of our active compounds, makes them promising candidates for future drug development.

Additionally, docking studies on GO and LDHA are also being conducted to study the interactions between our inhibitors and these enzymes.

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P39 / FC10 / C52

A fluorescence ICT sensor for aminopeptidase detection in live cells

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The fluorescence microscopy imaging technique is a powerful tool in biological analysis and visualization of dynamic events within living organisms, so the synthesis and application of new fluorescent probes has achieved great relevance in the current research of intracellular and intercellular processes. However, the resolution of the image decreases depending on the depth at which the fluorescent probe is located. It is well-known that low energy radiation has a deeper penetration in tissues, so fluorescent probes that are excited by two-photon absorption (TPA) and emit in the near infrared region are searched.[1] Internal charge transfer (ICT) based probes have been broadly used for sensing of a extensive type of analytes.[2] Within this kind of probes, dicyanomethylene derivatives (DCM) are small molecules chromophores that show one broad absorption band resulting from an ultra-fast process of ICT, along with emission wavelength in the NIR region and high photostability over a wide biological pH range.

Although some fluorescent probes have been used to detect protease activity[3], to our knowledge, there are very few studies on probes excited by two-photons for the detection of peptidases in cells and living tissues. We have focused our attention on the detection of the enzyme alanine aminopeptidase because its overexpression is associated with pro-inflammatory disorders and with tumor progression of breast cancer.

In this communication we present the synthesis and photophysical study of the derivative of benzopyran, dicyanomethylene-4H-pyran, with alanine as side group (DCM-NH-Ala), and its use in the study of the enzymatic activity of alanine aminopeptidase in cells.

Acknowledgements

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Anion-selective transmembrane carriers at work: studies in airway epithelium models

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Supramolecular medicinal chemistry, the development of supramolecular systems to function in biological systems with therapeutic benefit, is still at an early stage.[1] We are investigating the biological activity of synthetic anion transporters capable of exchanging anions across lipid bilayers.[2] Small molecules which show anion transport activity represent minimalist mimics of transmembraneproteins and could have potential application in the treatment of diseases caused by the defective regulation of chloride and bicarbonate transport. Moreover the effect of anion transporters in cell homeostasis and pH regulation offer possibilities to modulate cellular processes.[3]
Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, resulting in the lack of functional CFTR protein expressed in epithelial cells and thus defective chloride and bicarbonate secretion affecting the upper and lower airway, intestine, endocrine and reproductive organs. Loss of CFTR protein function imbalance the transepithelial homeostasis, generating a reduced and acidic ASL and impairing ciliary beating. This environment leads to accumulation of sticky mucus, inhibiting the antimicrobial activity and favoring the survival and proliferation of bacteria thus contributing to the genesis of CF lung disease. We have demonstrated that synthetic anionophores are able to correct mucus viscosity, air surface liquid (ASL) pH and fluid transport in epithelium cultures derived from CF and non-CF patients to values comparable to non-CF epithelia and therefore they may represent a promising starting point for the development of new drug candidates for CF therapy.

Acknowledgements

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P41 / FC11 / C55

Transition Metal-Promoted Cycloadditions in Biologic Media

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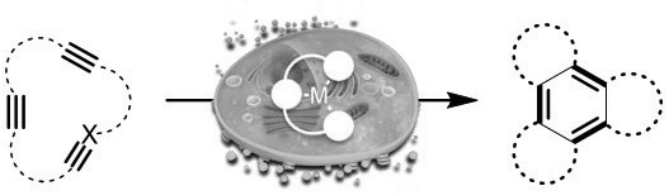
As the smallest unit of life, cell has been widely explored since his discovery in the sixties. Like chemists, chemical biology allows us the application of synthetic small molecules for the study of life. Since the recent incorporation of bioorthogonal reactions as new tools for cell research, chemical biology has been boosted, revolutionizing closely related fields like imaging, drug development or biotechnology due to its ability to manipulate small exogenous compounds inside living systems without disturbing the host metabolism. However, albeit their success, the discovery of novel bioorthogonal transformations is still a challenge due to their nature: the components must be innocuous, soluble, easily internalized, reactive at physiological conditions and must interact in a pool of complex biological molecules inside living systems, reacting as fast and specific as possible. Thus, general and versatile intracellular processes are still needed.[1]

In this sense, our group has gained experience through the years, reporting for the first time new metal-mediated bioorthogonal reactions inside living systems:[2] deprotection of alloc amines or allyl alcohols promoted by Ru, hydroarylations catalysed by Au or, more recently, redox isomerizations mediated by Ru.

In the pool of plausible bioorthogonal reactions, the cycloadditions captured our attention due to the posibility of perfoming interesting aromatic structures in an intermolecular manner. Our first incursion in the field led us to develop the Ru promoted Alkyne Cycloaddition with thioalkynes in complex biological media and the Cu catalysed Azide Alkyne Cycloaddition inside living cells with impressive applications in bioconjugation.[3]

Herein we report intra-and intermolecular formal [2+2+2] ruthenium-mediated cycloadditions that can be performed inside living cells. Importantly, these examples represent the first example of multicomponent annulations to be achieved in living settings. The process cleanly merges alkynes into aromatic structures, involving the formation of several C-C bonds, such as heliquats, anthraquinones or phthalans. Moreover, the last two examples constitute important secondary metabolites that can not be synthesized by mammalian cells. Thus, this bioorthogonal transformation represents *"a non-natural metabolic pathway"* built up artificially inside living cells.



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Halogenated reagents in the Ugi reaction

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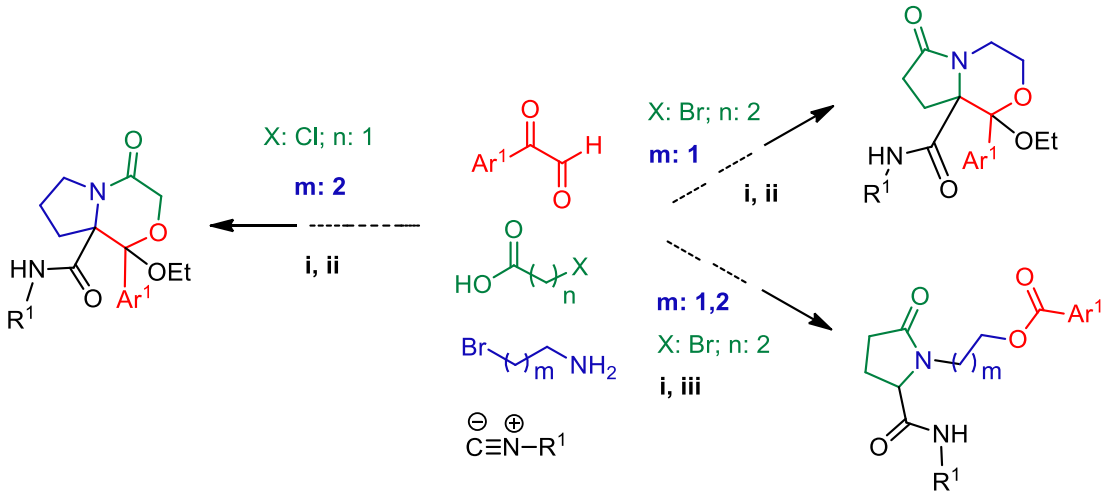
The Ugi reaction is one of the most important multicomponent reactions since it generates peptidic systems in a simple way with high atom economic. Moreover, this reaction is highly tolerant to different functional groups, making possible the use of functionalized starting reagents. This fact increases the synthetic possibilities of the reaction, so it is possible to carry out post-condensation reactions, obtaining different types of compounds, including nitrogen heterocyclic compounds, systems with high potential biological activity.[1]

Our research group has reported different Ugi/post-condensation sequences using halogenated carboxylic acids, such as 3-bromopropionic and chloroacetic acid, [2] in combination with arylglyoxals. These methodologies have allowed the synthesis of different heterocyclic compounds, as azetidinones, oxazinones, pyrrolidinones, pyrrolobenzodiazepines or pyrroloquinazolines.

Based in these results, we decided to introduce a second halogenated starting reagent, the amine, choosing commercial amines as 3-bromopropanamine and 2-bromoethylamine. Surprisingly, the post-condensation reactions carried out on the Ugi adducts generated unexpected compounds, such as cyclic ketals or rearrangement products, depending on the reaction conditions (Figure 1). These results can be explained by the intramolecular reaction between the acyl group (derived from the arylglyoxal) and the second halogenated position introduced.



i) MeOH, r.t. 24 h. ii) Cs_2CO_3 (2 eq), EtOH, ultrasonic, 3h. iii) Cs_2CO_3 (2 eq), MeCN, heat, 1 h.

Fig 1. Synthesis of cyclic ketals and rearrangement compound by an Ugi/post-condesation sequence.

Acknowledgements

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P43 / C57

Measuring pH in liposomes using HPTS: the importance of calibration

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Anion transport across biological membranes is a fundamental process, as it maintainsion and pH homeostasis in cells.[1]Model liposomes are widely employed to assess the transmembrane transport activity ofion carriers, as well as their ability to dissipate pH gradients.[2]The sodium salt of the 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) has been frequently used as fluorescent indicator to monitorpH changes taking place inside the liposomes when performing these experiments.[3] This fluorophore possesses a hydroxyl group (Fig. 1) which, depending on the pH of the medium, may undergo deprotonation. The protonated and deprotonated forms of this moleculeexhibit different absorptionmaxima (403 and 460 nm, respectively) and identical emission wavelength (510 nm). Plotting the ratio of the fluorescence intensities at 510 nm for the basic (λ_{exc} = 460 nm) and the acidic species (λ_{exc} = 403 nm) against time results in a curve. This ratio can be transformed into pH values, but in order to do that a calibration curveinvolving the pH-sensitive dye HPTS is required. However, this calibration is not universal, as it highly depends on the experimental conditions.

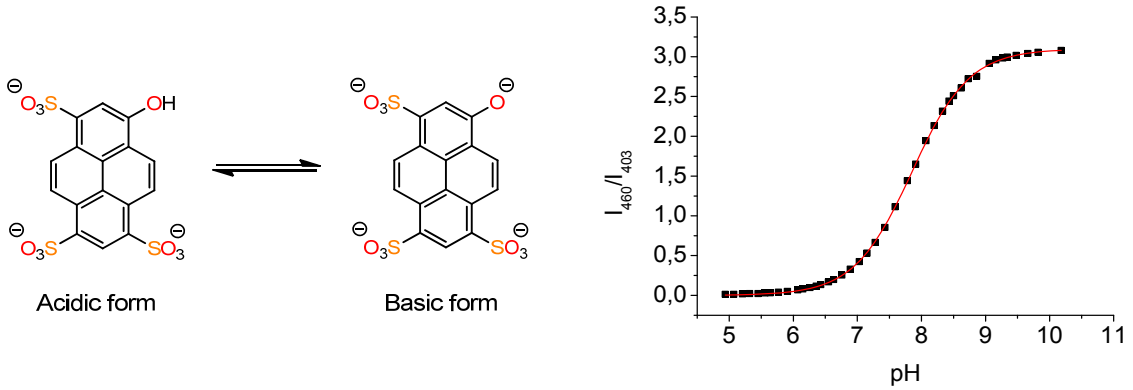

Fig. 1. Left: HPTS' protonated and deprotonated forms. Right: a typical calibration curve of free HPTS.

Herein we present the results of our calibration experiments with HPTS, both free and encapsulated in model (POPC) liposomes.From the experimental point of view, the assay consists of a titration of a buffered aqueous solution containing either HPTS or HPTS-loaded vesicles with sodium hydroxide. In all cases a pH increase leads to a rise of the emission intensities ratio, yielding a sigmoidal curve, typical of an acid-base titration (Fig. 1). However, the parameters obtained from the fitting changedepending on several variables, especially on whether the fluorophore is encapsulated or not, but also on the concentration of HPTS, on the ionic strength of the medium and even on the salt with which the ionic strength is controlled. The equation used to fit the curve is also relevant. Hence, the transformation of the fluorescence intensities ratio into pH values is not trivial and must be performed carefully.

Acknowledgements

Co-funding from Consejería de Educación (Junta de Castilla y León) and Programa Operativo de Empleo Juvenil(Fondo Social Europeoand Iniciativa de Empleo Juvenil) is gratefully acknowledged by V. A.-L.Funding from Junta de Castilla y León (project BU075G19) is equally acknowledged.

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miR-122 direct detection in human serum by time-gated fluorescence imaging

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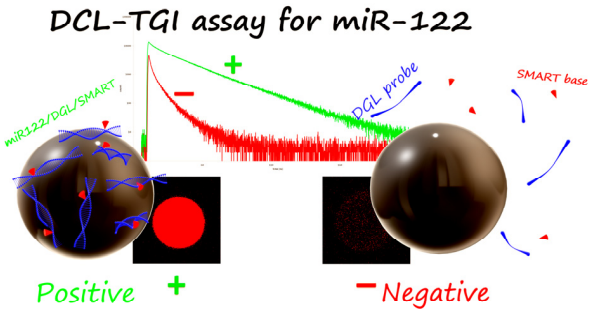
A simple method for direct detection of microRNAs (miRs) in human serum without the use of polymerase amplification is presented, achieving low miR-122 concentrations and importantly, discerning effectively single-base sequence mutations. The method is based on the capture of target miRs with synthetic peptide nucleic acid oligomers, dynamic chemical labelling, separation with quaternary amine microplatforms and detection using time-gated fluorescence imaging.[1]



Acknowledgements

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P45 / FC13 / C59

Target-based *de novo* design of heterochiral cyclic peptides against the PD-1/PD-L1 interaction

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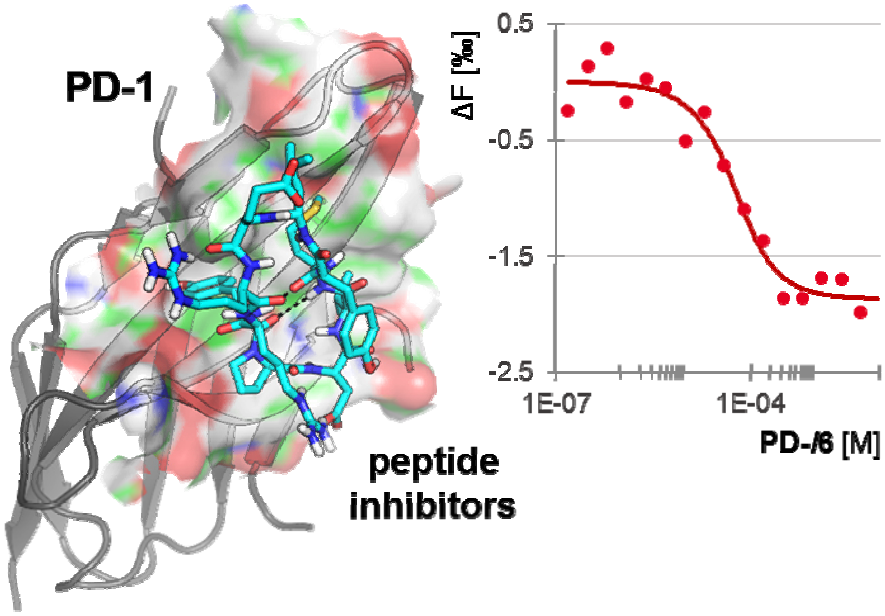
Cancer cells can block and escape immune responses through activation of inhibitory immune checkpoint proteins, such as PD-1 and PD-L1, and CTLA-4.[1] Therapeutic targeting of these proteins with monoclonal antibodies restores normal T-cell function and has represented a major breakthrough in the fight against melanoma, lung cancer, and other types of cancer.[2] In this work, we have applied a novel computational framework to design *de novo* small heterochiral cyclic peptides that work as PD-1 ligand decoys. The best designs were selected for PD-1 binding activity using complementary biophysical tools such as surface plasmon resonance (SPR), calorimetry and microscale thermophoresis (MST),[3] and their solution structure was solved using high-field NMR. Altogether, we showcase the combined potential of computational methods and biophysical tools to design new ligands against challenging protein-protein interactions.



Acknowledgements

This study was funded by MINECO-FEDER (BIO 2016-75327-R) and Generalitat de Catalunya (XRB and 2017SGR-998). IRB Barcelona is the recipient of a Severo Ochoa Award of Excellence from MINECO (Government of Spain).

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Specific Reverse Transcriptase Inhibitors for Mammalian LINE-1 Retrotransposons

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Long interspersed element class 1 (LINE-1 or L1) is the only active autonomous transposable element in humans. It comprises 17% of our genomic mass, although most L1 copies are inactive due to mutations and rearrangements. However, an average human genome contains 80-100 active L1s, and their mobilization continue to impacts our genome. L1s move by a copy and paste mechanism that involves the reverse transcription of an intermediate L1 RNA. In humans, L1 mobilization during early embryogenesis led to the accumulation of heritable insertions that sporadically can be mutagenic and generate a genetic disorder. Surprisingly, L1s are also active in cancer cells and the human brain, impacting our somatic genome. L1 elements are frequently overexpressed in human tumors, and the accumulation of new insertions can potentially increase the malignancy and metastasis potential of tumor cells. Thus, although the role of somatic retrotransposition is currently unknown, it has the potential to influence cancer origin and progression, as well as brain biology[1–3]. To learn more on the role of L1 activity in cancer cells and the brain, Loss of Function (LOF) strategies will be very informative. Here, and to explore whether inhibition of the Reverse Transcriptase of L1 can be used as an effective LOF strategy, we tested currently available and de novo synthesized nucleoside related compounds as selective L1 inhibitors. After analysing more than 25 nucleoside structures using an L1 retrotransposition assay, we identified three non-toxic selective L1 inhibitors that exhibit no activity against other retroelements (LTR-containing retrotransposons)[4]. Notably, these compounds can efficiently inhibit human and mouse LINE-1s, and could be used to determine the impact of L1 activity in mouse models, in a LOF approach. Importantly, these compounds also have the potential to be effective in the treatment of those cancers characterized by a high L1 expression.

Acknowledgements

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P47 / C61

Design of peptides able to modulate Protein-Protein Interactions (PPIs)

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p53 is a tumor suppressor protein that is able to bind DNA and activate other proteins in order to repair it. This protein, also known as 'genome guardian', is biologically active only in its tetrameric form. However, it is known that the single mutation, R337H, can significantly disrupt the tetramer, especially if the pH is above 6,5.[1] This disruption further leads to development of non-curable pediatric adrenocarcinoma.
The aim of this project consists in the design and synthesis of peptides able to rescue the tetrameric form of p53R337H. The tetramerisation domain (TD) presents two hydrophobic pockets surrounded by negative charges from glutamate residues (**Figure 1**). Previuos results from our group[2,3] showed the ability of polyguanidinium ligands to bind the mutated TD. A calix[4]arene (**Figure 2**) was designed to fit the pocket with its hydrophobic lower rim and to interact with the glutamate of the TD with four guanidinium groups in the upper rim. The experimental results showed the ability of this ligand to bind and stabilize the TD of p53R337H. However, it was cytotoxic.[2] A library of linear polyarginine peptides was studied as well.[3] Those peptides were designed to interact with glutamate residues spaced in a path of *i,i+3*. It was studied the binding of the library with several proteins having this characteristic. It was found that those peptides were able to bind the p53TD. Based on those results, our idea is to design new polyguanidinium cyclic peptides and study their binding with the TD.
Several biophysics methods will be explored to study the binding, such as NMR, fluorescence, MS and CD among others.

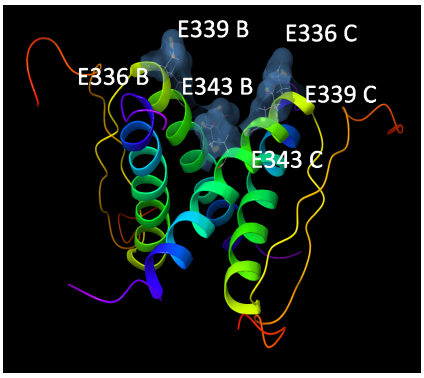

Figure 1. Pocket of p53TD

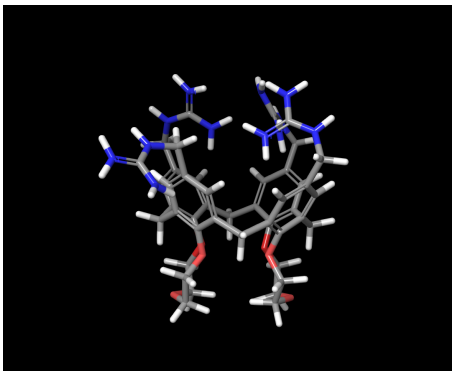

Figure 2. Calix[4]arene

Acknowledgements
This work is financed by MINECO-FEDER (BIO2016-75327-R).

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Unraveling miniAp4 internalization and transport mechanism

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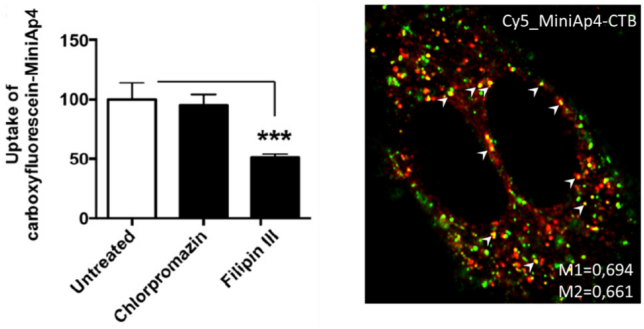
Venoms are an outstanding source of bioactive compound, being some of them able to reach the central nervous system without causing a toxic effect.[3] In our lab we have developed two families of blood-brain barrier peptide shuttles derived from venoms, MiniAp-4 and miniCTX-3.[1,2] These peptides are highly protease resistant and able to cross the BBB and to increase the transport of various substances ranking from small molecules to proteins and nanoparticles. Understanding the internalization and transport mechanism of these compounds would help in the design of efficient brain delivery agents.

In this work, we describe the various approaches used to elucidate miniAp-4 route of entry. First, we have developed internalization experiments in endothelial cells in combination with high resolution confocal microscopy to compare miniAp-4 with two well stablished proteins, transferrin and cholera toxin.
Second we photoreactive miniAp-4 analogue was designed and synthetized in order to map the interaction within the selected cell line, combining photo-crosslinking with target affinity purification, trytic digestion and top down mass-spectrometry-based analysis.

By combining these two strategies, we can conclude that MiniAp-4 uses dynamin-dependent endocytosis route to internalize in b.End3 cells.



Acknowledgements

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P49 / C65

Ruthenium tools for Chemical Biology: activation of pi bonds as new tool for carbon-carbon bonds formation

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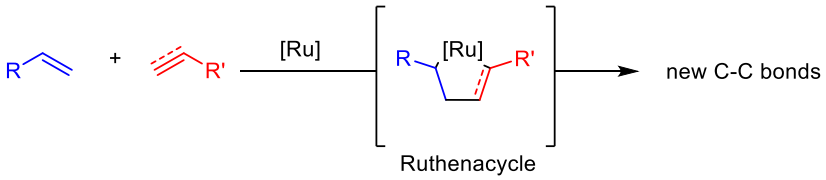
Metal complexes have attracted attention in the scientific community since its very first discover. Transition metal complexes are not only great partners in organic synthesis, but also in biology, because they are in the core of the metabolism, many transition metals are part of the active site in a wide variety of essential enzymes.

However, the use of the potential of non-natural occurring metal complex to perform new to nature transformations remains still in its infancy. Apart from the pioneer examples of Megger,[1a] back in 2009, and examples by others such as: Bradley,[1b] Cheng[1c] or our group,[2] among others, the scope and tools of metal based bioorthogonal transformations remains scarce. Evenmore, if we want to create new carbon-carbon bonds we just have either cross-coupling reactions, mainly Suzuki,[1b] and cross metathesis.[3]

We envisioned that the ability of metal complexes to activate relative inert bonds, particularly, pi bond in organic compounds, may lead to a straightforward manner to create new carbon-carbon bonds and expand the chemical toolbox of metal based bioorthogonal transformations.

In this work we have studied the use of Ruthenium complexes to activate pi bonds, forming metallocycle intermediates that can act as new way to form carbon-carbon bond under biologically relevant conditions.



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Antiparasitic acivity of triazolopyrimidine and nicotinate metal complexes of aluminium and gallium

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The search of new drug candidates for potent, selective and less toxic antiparasitic therapeutic agents is considered as one of the biggest social and scientific challenges at present day. In recent years, our research group has been working with triazolopyrimidine derivatives to form new leishmanicidal metal complexes [1] and with nicotinate metal complexes of tin and titanium to give several anticancer compounds [2] with interesting biological properties. In this context, and bearing in mind the demonstrated biological activity of triazolopyrimidine and nicotinate derivatives we dediced to explore the antiparasitic activity of gallium and aluminium compounds using both triazolopyrimidine and nicotinate ligands as gallium complexes have been recently revealed as promising candidates in antiparasitic studies [3].
Thus, this communication will be focused on the synthesis and characterization of a series of triazolopyrimidine and nicotinate complexes of Ga(III) and Al(III) (Figure 1) and the presentation of a preliminary antiparasitic study describing the activity *in vitro* of the new complexes against different strains of *Leishmania spp*. and *Trypanosoma cruzi*.

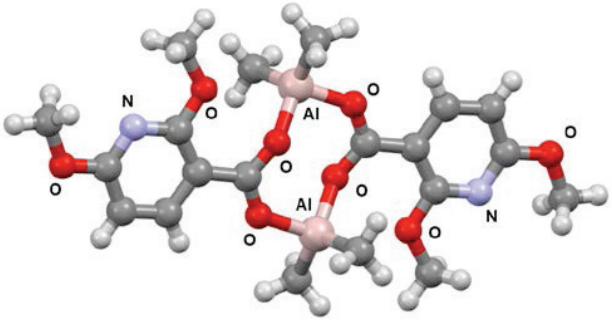

Figure 1. Al(III) complex with a nicotinate ligand

Acknowledgements

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P51 / C69

Solid-phase peptide synthesis vs in solution: a diketopiperazine synthesis

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Diketopiperazines (DKPs) are natural compounds with several applications. DKPs are cyclopeptides composed by two aminoacids head-to-tail cyclised. We have previously described a family of DKPs which can be used as BBB-shuttles, compounds able to cross the Blood Brain Barrier (BBB) and to increase the transport of compounds that cannot cross unaided.

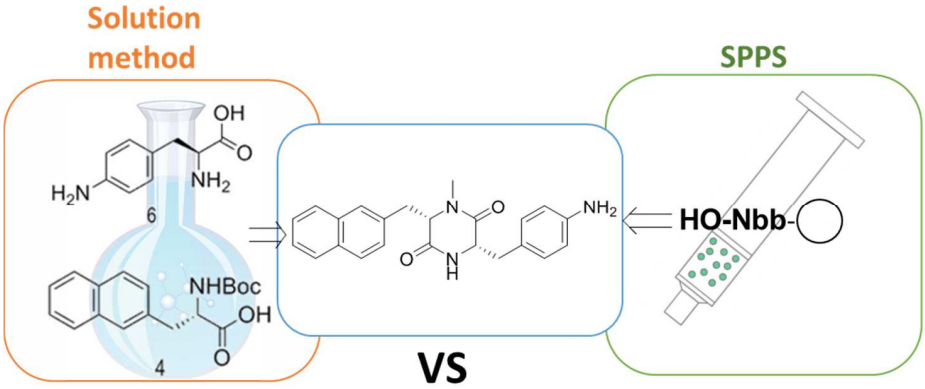
The potential applications of DKPs make the development of an efficient synthetic method highly desirable.[1]

Traditionally, these DKPs have been prepared by SPPS. However, this synthesis is difficult to scale-up and has low reproducibility. For this reason, a protocol in solution protocol was designed in order to develop a robust synthetic method.

The synthesis of DKP Phe-*N*-Me-2-Nal in solid-phase peptide synthesis (SPPS) was performed using p-MBHA resin and 4-bromomethyl-3-nitrobenzamido-benzylpolystyren (Nbb linker).[1] The good electron withdrawing properties of this linker enabled the nucleophilic attack of the first aminoacid, 3-(2-naphthyl)-L-alanine (2-Nal), facilitating its incorporation.[2] Afterwards a Mitsunobu reaction was performed in order to methylate the amine. Then, the second aminoacid, (p-NH_2)Phe, was coupled and the peptide was cleaved from the resin, promoting the cyclization of the dipeptide.[1]

The synthesis in solution was done in 5 steps. The *N*-methylation over 2-Nal, using methylsulfate as methylating agent, was done in parallel with the ester methylation and the Fmoc protection of (p-NH_2)Phe. Afterwards both building blocks were coupled using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and hydroxybenzotriazole (HOBt).[3] After the removal of all protecting groups, the cyclization was performed using morpholine to form the final DKP.[3]

Although this method needs further optimization, it provides an easy and feasible route to prepare DKPs.



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V GEQB ChemBio
Granada (Spain), February 19-21, 2020
P52 / FC15 / C70

Towards the chemo-enzymatic synthesis of a N-glycomimetic library for the targeting of human DC-SIGN

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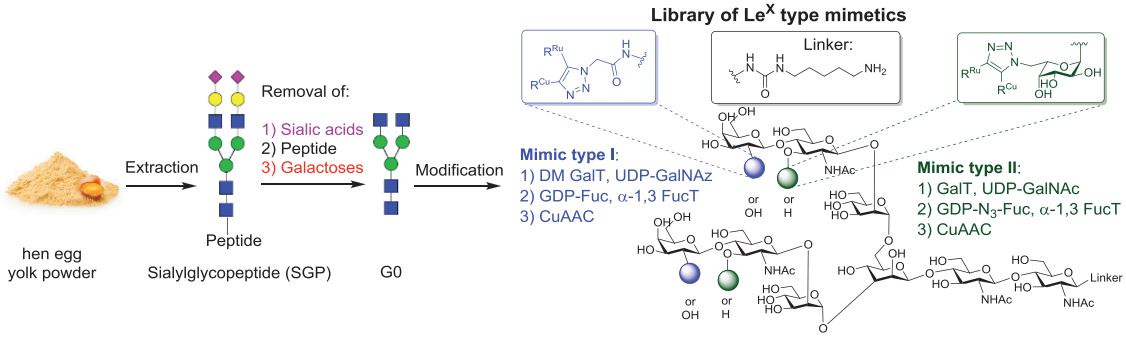
Keywords: Glycomimetics - DC-SIGN - LewisX - Inhibitors - Antagonis

DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin) is a C-type lectin located on the surface of dendritic cells and a key player in the initial immune response against various pathogens. It displays a carbohydrate recognition domain which importance is reflected by high specificity for high-mannose carbohydrates and fucose containing Lewis type antigens (Le^X, Le^Y, Le^b and Le^a).[1] Pathogens such as HIV, Dengue virus, Ebola virus and *S. mansoni* express surface exposed glycan structures that allow them to hijack DC-SIGN and infiltrate the host. Blocking this entry point is of great interest by means of the synthesis of multivalent mimetics that result in high affinity towards DC-SIGN.
Here, we present the synthesis of an N-glycomimetic library based on the modification of a biantennary complex N-glycan with terminal GlcNAc residues (G0, Error! Reference source not found.) to obtain Le^X mimetics for improved DC-SIGN binding. For this purpose, sialylglycopeptide (SGP) was extracted from hen eggs[2] and truncated to G0 which was further modified by chemoenzymatic approaches. The attachment of an urea type linker to the reducing end is aimed to increase the stability towards enzymatic hydrolysis and allows the immobilisation on selected surfaces. Elongation at the non-reducing end resulted in two different types of Le^X glycomimetics: Type I was accessible by elongating with GalNAz and Fuc whereas Type II was obtained by elongating with Gal and N_3-Fuc. The introduction of the azido functionality at the respective position allowed the modification by CuAAC (or RuAAC) with various alkynes resulting in a large library of glycomimetics.[3] Evaluation by screening of the resulting mimetics against human DC-SIGN is envisioned to reveal lead compounds for binding studies and further ligand optimisation.



Scheme 1. Overview of the synthesis towards an N-glycomimetic library.

Acknowledgements

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P53 / C72

A 3D view by NMR into the interaction between human galectin-4 and the histo blood group antigens

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Galectins are a family of β-galactoside-binding proteins that mediate in a broad variety of physiological functions as result of their interaction with carbohydrates, participating in cell-cell interactions, immune response, and intracellular signaling, among others[1].

Human galectin-4 (hGal-4) is a tandem-repeat protein with two carbohydrate recognition domains (CRD) united through a linker-peptide. This lectin is mainly expressed in healthy epithelial cells of the intestinal tract, playing a crucial role on the stabilization of lipid rafts and participating in apical trafficking[2]. Both up- and down-regulation of hGal-4 expression levels have been found in cancerous tissues, marking this lectin an interesting drug target[3].

The relevance of the molecular recognition of human blood group antigens by hGal-4 in biological processes has been already reported[4]. However, the essential features of the interaction event, including the knowledge of the dynamic properties of both the receptor and the ligand are yet to be ascertained.

To gain insight into the key elements of the recognition process, the interactions between hGal4 CRD1 and the blood group A- and B-antigens have been studied by NMR from the ligand and receptor's perspectives. In particular, the analysis of STD-NMR, tr-NOESY, EXSY and HSQC experiments have permitted shedding light into the fine details of the glycan-lectin interaction.

Finally, the combination of the NMR and ITC experimental data with those deduced from computational protocols have allowed explaining the selectivity of the lectin towards the distinct ligands.

Acknowledgements

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Efficient Tyrosine trapping by the supramolecular protection from Src phosphorylation

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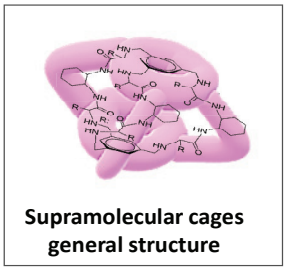
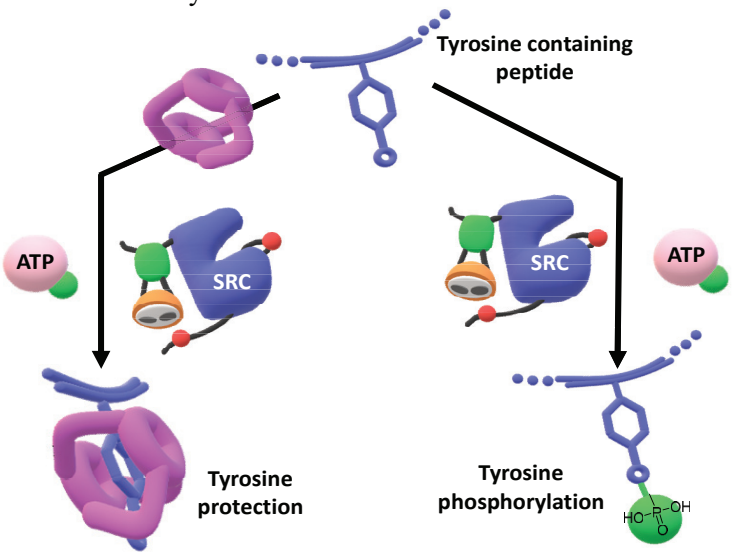
Molecular recognition of peptides is a challenging strategy for the modulation of biological processes. Nature has brightly surpassed this request using enzymes for many processes regulation in living organisms. Protein tyrosine kinases (PTKs) are a family of enzymes that catalyse the transfer of a phosphoryl group from ATP to a Tyr hydroxyl group. This phosphorylation is closely connected with cell regulation, signalling and growth. Therefore the abnormal function of PTKs has been associated to serious diseases including diabetes, cancer and neurodegenerative disease which pushes researchers to constantly look for new strategies to regulate PTKs activity[1]. One of the families of Kinases with high medical interest are Src kinases, a family of non receptor PTKs whose dysregulation is closely related to different cancer types[2]. Dr. I. Alfonso and co-workers reported two molecular cages that bind the EYE peptide epitope and show modulation of the Src activity by competitive substrate caging[3]. Following this strategy we have extended this family of compounds aiming to modulate their Tyr binding properties. Different aminoacids with different side-chain functionalitines (R groups) have been selected to synthetize new cages with different binding affinities for different Tyr containing peptide sequences that can be phosphorylated by SRC. Binding assays using fluorescence titrations show that changes in the R substituents of the cage leads to different affinity for different Tyr containing substrates due to the interactions of the R group with the aminoacids nearby the Tyr. In vitro kinase assays using synthetic peptides and purified Src demonstrate that these compounds can compete with Src for the substrate, protecting it from phosphorylation. Moreover, it has been observed that the inhibition capacity of ech cage depends on the aminoacids sequence of the tyrosine containing peptide, especially in the presence or absence of charged residues. The obtained positive in vitro results highlight the potential of these compounds as Src activity modulators.





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P55 / C74

Targeting Tumor Microenvironment via Inflammosome Modulation in Breast Cancer with Rationally designed Polypeptide-based Nanoconjugates.

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The invasion and metastasis of cancer is a complex pathological process[1].Plenty of studies show that the inflammation participates in both cancer progression and immune escape and is related to the possibility of cancer cell's migration in the regional lymph nodes and lymphoid organs. Recently, lots of studies find that microenvironment, in particular tumor associated macrophages, plays an indispensable role in cancerous progression, and it might be crucial in the invasion and metastasis[2]. The related research findings were reviewed to discuss the relationship between macrophages and the behavior of cancerous invasion and metastasis so that it might offer a new method to explore the hypostasis of carcinoma. Emerging data suggested that inflammation is a potent promoter of tumor metastasis, but this role depends on tumor type. In breast cancer, the elimination of the inflammasome components and the associated reduction in proinflammatory cytokine release decrease tumor size and metastasis, and, therefore, may represent a target for patient treatment[3]. However, the use of direct inflammasome inhibitors in breast cancer models remains relatively unexplored[3,4]. We have identified a new inflammasome inhibitor, QM-378, that inhibits inflammasome assembly, thereby preventing the release of proinflammatory cytokines[5].

However, its use, at its full potential, is limited by an adequate solubility and poor pharmacological profile, limitations that can be overcome by means of rationally designed nanomedicines[6], in particular Polymer-drug conjugates. The use of TME targeted nanoconjugates has been already studied in our group[7] and demonstrated that drug conjugation to a polymer not only enhances its aqueous solubility but also changes drug pharmacokinetics (PK) at the whole organism and even subcellular level with the possibility to clearly enhance drug therapeutic value. Our lab has wide expertise in the development of well-defined biodegradable polypeptide-based architectures prepared using controlled polymerization techniques[8,9] .That has been combined with the design of bioresponsive linkers for drug(s) and/or imaging probes by post-polymerization techniques. Importantly, by following a bottom-up strategy we have been able to obtain star-based PGAs with the capacity to self-assemble yielding supramolecular nanostructures with interesting properties after stabilization by means of a bioresponsive crosslinking strategy[10]. A remarkable feature of these constructs (CSS-PGA) is their lymphotropic character[10] offering the best drug delivery strategy for QM-378. Therefore, we have performed the chemical conjugation of QM-378 to our polyglutamate through a bioresponsive linker. To achieve this a family of QM-378 derivatives had to be developed bearing different polymer-drug spacers and those will be presented herein. The obtained QM-378 polyglutamate conjugates have been fully characterized and currently we are performing drug release kinetics assays. It is planned also to evaluate these systems in vitro in differentiated THP1 macrophages (M1 vs M2) and in co-culture with Triple negative breast cancer cells (TNBC) MDA-MB231 always comparing with the free parent compound in order to evaluate their therapeutic performance as inflammosome modulators.

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Catalytic mechanism of Leukotriene A4 Hydrolase: QM/MM calculations

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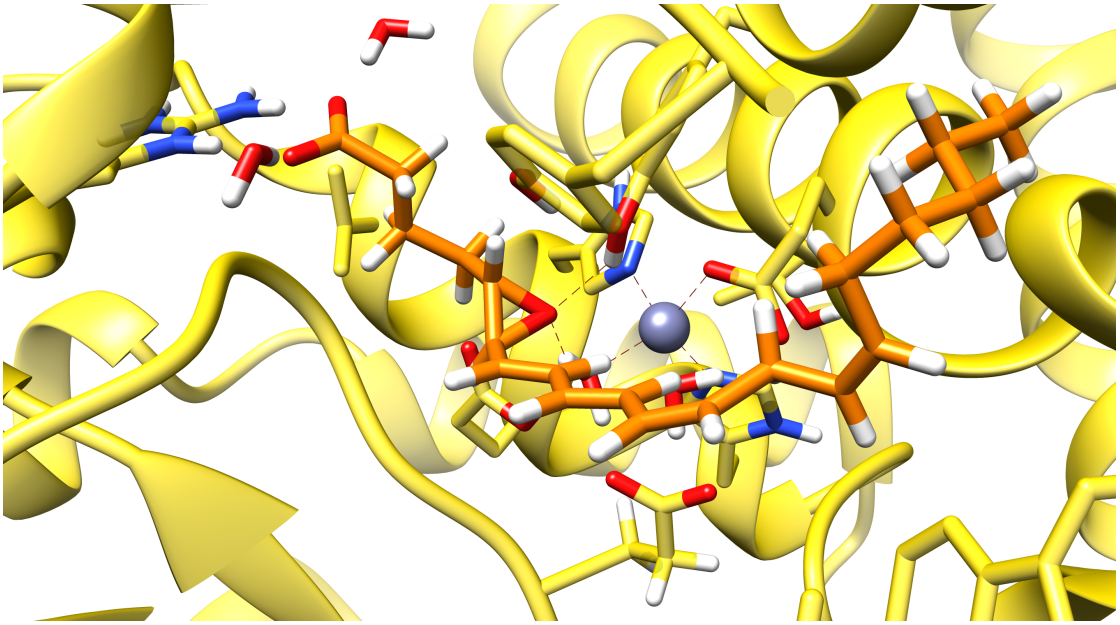
Leukotriene A4 hydrolase (LTA_4H) is a zinc containing metalloenzyme with a bifunctional activity: it can act as an epoxide hydrolase or as an aminopeptidase. The present study focuses on revealing the molecular insights of the LTA_4H catalytic mechanism as a hydrolase. For this purpose, leukotriene A4 (LTA_4) has been selected as the substrate since it is the natural substrate for this enzyme. LTA_4 is an inflammatory mediator obtained from the oxidation of arachidonic acid catalyzed by the lipoxygenase ALOX5, which epoxidates the fatty acid at the 5,6 positions. LTA_4H goes further in the biosynthetic pathway catalyzing the conversion of LTA_4 to leukotriene B4 (LTB_4) by following a mechanism that consists in the epoxide ring opening to generate an alcohol at the C5 position, and in the addition of a water molecule to the C12 position.



This theoretical study has been performed using a QM/MM method to build up the potential energy surface (PES) of the catalytic mechanism. The QM region has been described at the B3LYP/6-31G* level except for the Zn ion that has been described using the STRLC ECP. The AMBER force field has been used for the MM part. A bidimensional PES has been calculated for the epoxide ring opening reaction and for the water addition process. The Minimum Energy Path (MEP) of the reaction along with the minimum energy structures and transition states show that both chemical reactions are not concerted, and it is clear that the water addition only takes place once the epoxide ring is opened, so the global mechanism is an S_N1-like reaction. Moreover, the protonation of the oxygen initially forming the epoxide ring, which is stabilized by the zinc ion along the global reaction, is the third and last step of the mechanism. As a product of the global reaction, a diol is obtained from an epoxidated PUFA.

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P57 / C76

A versatile theranostic nanodevice based on an orthogonal bioconjugation strategy for efficient targeted treatment and monitoring of triple negative breast cancer

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A novel chemical-based orthogonal bioconjugation strategy to produce tri-functionalised nanoparticles (NPs) carrying doxorubicin (DOX), near-infrared cyanine dye (Cy7) and a homing peptide CRGDK, a peptide specifically binds to neuropilin-1 (Nrp-1) overexpressed on triple negative breast cancer (TNBC) cells, has been validated. These theranostic NPs have been evaluated in vitro and in vivo using an orthotopic xenotransplant mouse model using TNBC cells. *In vitro* assays show that theranostic NPs improve the therapeutic index in comparison with free DOX. Remarkably, in vivo studies showed the preferable location of theranostic NPs in the tumor area reducing the tumor volume in a similar grade than free DOX while presenting lower side effects. This multifunctionalized theranostic nanodevice based on orthogonal conjugation strategies could be a good candidate for the treatment and monitoring of Nrp-1 overexpressing tumors. Moreover, this versatile nanodevice can be easily adapted to treat and monitor different cancer types by adapting the conjugation strategy[1].

Acknowledgements

This work was supported by grants from the Ministry of Economy and Competitiveness (MINECO, National Programme for Research Aimed at the Challenges of Society, project reference BIO2016-80519-R&FEDER funds, grant number MAT2015-62644.C2.2.R), the Health Insitute Carlos III (ISCIII) (Projects of technological development in health - ref DTS18/00121) and the Chair "Doctors Galera-Requena in cancer stem cell research". This research was additionally supported by the University of Granada - PLAN OF RESEARCH AND TRANSFER 2016 –PSETC- PSE/16/003). The authors thank the Research Results Transfer Office (OTRI) of the University of Granada for their support for the technological development of this project. S.A.N-M. acknowledges the MINECO for providing a PhD fellowship (FPI) through the project MAT2015-62644.C2.2.R. Conflicts of interest: There are no conflicts to declare.

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1.María Victoria Cano-Cortés; Saúl Abenhamar Navarro-Marchal; María Paz Ruiz-Blas; Juan José Díaz-Mochón; Juan Antonio Marchal; Rosario M. Sánchez-Martín. *A versatile theranostic nanodevice based on an orthogonal bioconjugation strategy for efficient targeted treatment and monitoring of triple negative breast cancer*.Nanomedicine: Nanotechnology, Biology and Medicine. pp. 102120 - 102120. 2019.

Modular Artificial Light Harvesting Systems

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A modular light harvesting system for the study of the fundamental principles of biological light capture is designed. The model system is constructed on idealised modular tetratricopeptide repeat (TPR) protein-based scaffold, that provides a regular ordered surface for homogenous display of functional elements. Components of the individual light capture (e.g. porphyrin, flavin dyes),[1] energy transduction (e.g. iron-sulfur clusters)[2] and conversion (e.g. model hydrogenase active sites) systems are constructed separately as individual modules for subsequent controlled assembly, allowing to assess the contribution of the individual components on the efficiency of the final assembly. The model system will enable the study of fundamental processes of biological light harvesting, independent from unique biological adaptaions of existing systems.

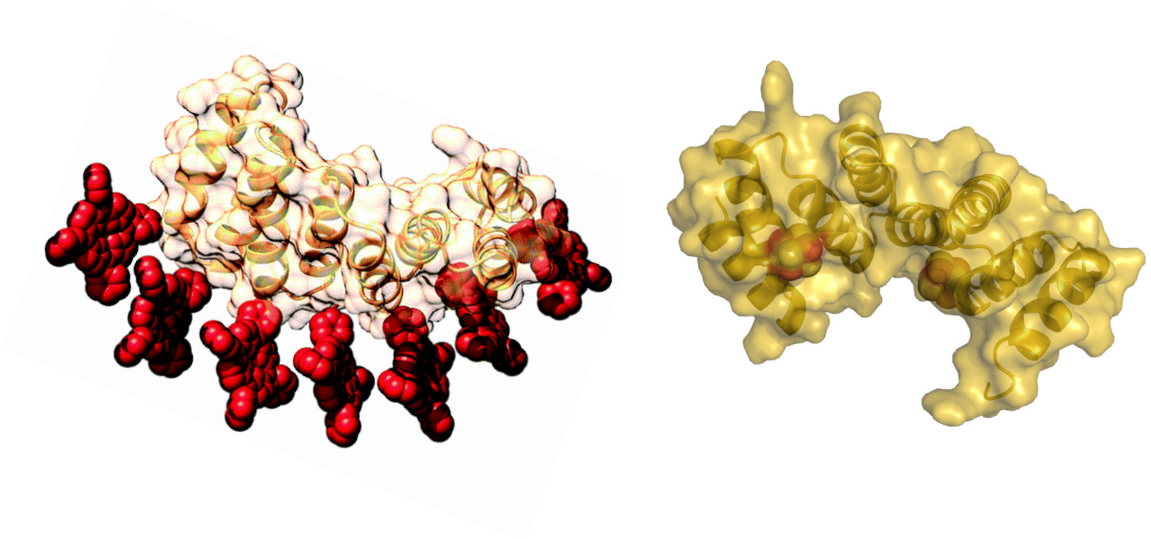

Figure 1. Light harvesting (left) and energy transduction (right) modules of the artificial light harvesting system.

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P59 / C78

SYNTHESIS,DNA INTERACTION AND BIOLOGICAL ACTIVITY OF GLYCOMIMETIC LIGANDS DERIVED FROM 1H-IMIDAZO[4,5-f]1,10-PHENANTHROLINE

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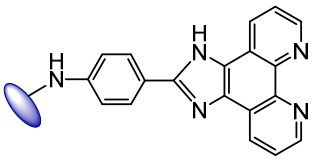
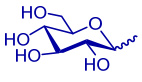
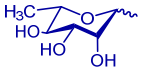
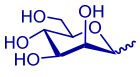
The importance of the telomere/telomerase system as a feasible therapeutic target in fighting against cancer is widely recognized. In approximately 85% of human tumor cells, telomerase activity is very significant, preventing telomere shortening and, therefore, abnormally lengthening the life of the cell.[1]One strategy to inhibit telomerase is based on the stabilization of the quadruplex DNA structures formed at the telomeres. However, G-quadruplexes are not limited to telomeric sequences, as they can be found in many other parts of the human genome including several oncogene promoters. Thus, a wide variety of compounds capable of selectively binding and stabilizingquadruplex DNA or RNA structures formed have been developed.[2]

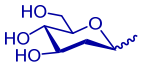
In our research group we focus on the development of novel compounds that can act as efficient G-quadruplex DNA binders. Some of the target compounds are based on the use of heterocycles with extended aromatic surface tethered to carbohydrate units, which confer them interesting properties regarding solubility, cell permeability, and quadruplex binding selectivity, among others. One example is a family of glycopyranosyl amines designed as DNA ligands and efficient metal chelators.[3]Moreover, their Cu(II) complexes have provided good G-DNA ligands with relevant antitumor biological activity.[4]

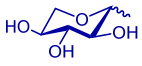












With the aim of identifying novel carbohydrate-based heteroaromatic ligands as scaffolds for potential bioactive compounds, we report hereinthe synthesis, DNA recognition and biological activity of a new familyglycomimetic ligands derived from 1*H*-imidazo[4,5-f]1,10-phenanthroline. These derivatives and their Cu(II) complexes interact with telomeric quadruplex DNA with good affinity and selectivity. Moreover, they showsignificant antitumor activity in cultured cells.

Acknowledgements

Financial support from MINECO-AEI (CTQ2015-72625-EXP/AEI), from Comunidad de Madrid (PEJ-2017-AI/SAL-6160) and from UAH(CCG2017-EXP019, CCG2018/EXP-024) is grateful acknowledged.

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Design and development of novel nanodevices for biomedical applications

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Polymeric nanoparticles offer a great flexibility adapting its chemistry composition, size, stability, morphology and surface functionality[1]. As a result, they are used in Biomedicine as transporters of drugs and diagnostic agents for a wide range of applications in diagnosis, therapy and theranostics[2,3]. The main objective of this project is to develop and to validate multifunctional nanosystems for different biomedical applications. To this end, we have synthetized aminefunctionalized monodisperse polystyrene nanoparticles of different sizes. They have been characterized by size (using DLS, confocal microscopy, SEM, TEM and AFM), by its surface (zeta potential and amine-groups quantification), by its stability and batches' consistency. The synthetized nanoparticles also showed their favourable nanofection in different cell lines with no toxicity. Thanks to the stability of these nanoparticles in organic solvents provided by the cross-linking structure, numerous chemical reactions have been carried out easily on these materials and the general standard solid-phase multistep for the generation of multifunctional nanoparticles.

Acknowledgements

This work was supported by grants from the Ministry of Economy and Competitiveness (MINECO, National Programme for Research Aimed at the Challenges of Society, project reference BIO2016-80519-R; FEDER funds, grant number MAT2015-62644.C2.2.R), the Health Institute Carlos III (ISCIII) (Projects of technological development in health - DTS18/00121) and the Chair "Doctors Galera-Requena in cancer stem cell research". We thank the technical support of Microscopy and Molecular Image Unit and Flow Cytometry from GENYO, TEM Service and Preparation of Bilogical Samples Laboratory from C.I.C. of UGR. J.A.L.R. thanks to the Fundación Benéfica Anticáncer San Francisco Javier y Santa Cándida for PhD funding.

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P61 / C80

In situ ChemNAT: Genomic sequences detection in colon cancer cell lines.

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Most current molecular techniques allow massive analysis of key nucleic acids from tissues affected by different pathologies. Micro-RNAs, long non-coding RNAs as well as key transcripts are analyzed primarily by standard RT-qPCR methods. But most of these methods consist on whole nucleic acid extractions, where the heterogeneous spatial context is lost. For diseases such as cancer, the knowledge of spatial distribution and intratumoral heterogeneity gain special importance in the context of therapeutic treatments [1]. The only way to identify and detect these different molecular states is to do single cell and histology ISH analyses, being able to associate measurements and quantification of abundance of nucleic acids with spatial information [2].

Herein, we present a proof of concept in which we combine dynamic chemistry with ISH techniques to be able not only to detect the spatial distribution of these nucleic acids, but also the analysis of SNPs or punctual mutations of interest in these nucleic acids. Dynamic chemistry is based on the use of PNA probes with an abasic position which is used to interrogate the target nucleic acid. Then, a dynamic chemical reaction takes place to identify which nucleotide is located in the target nucleic acid. This is possible through the use of labelled reactive nucleobases (SMART-Nucleobases), that allow the correct detection of this key nucleotide [3].

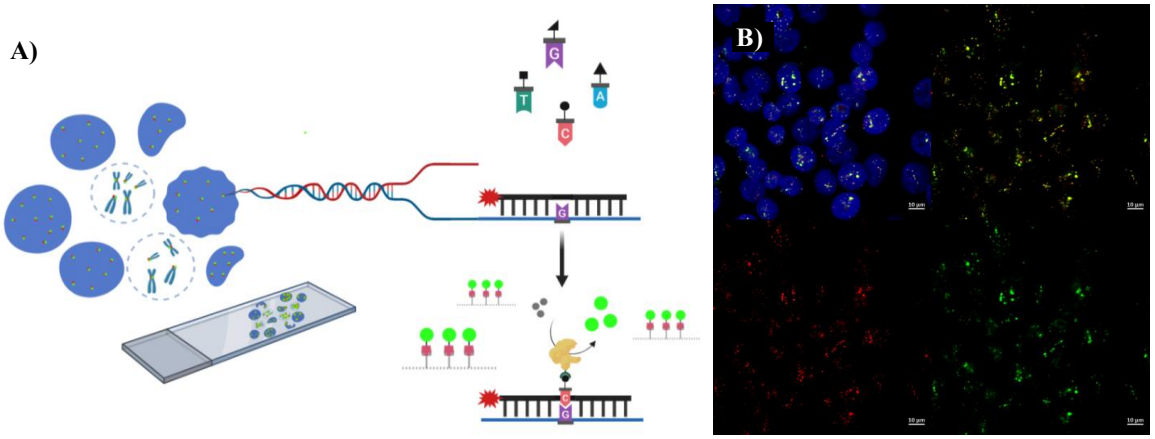

Figure 1. A) *in situ* ChemNAT reaction: The co-localization of the probe signal (red) and the SNP signal (green) allow the spatial location of the sequence as well as the SNP of interest in this sequence. B) Co-localization of the abasic PNA probe labelled with Cy3 and the SNP of interest detected Alexa Fluor 488 in de nuclei in blue (DAPI).

During the V GEQB ChemBio we will present the first results of the detection of *in situ* ChemNAT technology in a comprehensive protocol optimization process using targets genomic sequences such as Alpha DNA Satellite and telomers in nuclei from colorectal colon cancer cell lines.

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Unraveling sugar binding modes to DC-SIGN by employing a ligand-based ^{19}F-NMR approach

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Over the last decades, ^{19}F-NMR has become an invaluable tool for screening libraries of compounds against a receptor of interest in drug discovery projects[1]. ^{19}F nucleus is 100 % natural abundant and possess a large gyromagnetic ratio that makes it a serious competitor to ^{1}H in sensitivity. Moreover, ^{19}F-NMR presentsenormous advantages over ^{1}H-NMR for ligand-based screening purposes, namely: a) does not suffer from the presence of background signals neither arising from biomolecules nor solution buffers, b) displays a large range of chemical shifts, and c) is a very fine probe to detect even a minuscule fraction of bound molecule. All together, these properties allow using large libraries of compounds (30-50) at the same time for the screening, without the overlapping problem that would otherwise make difficult^{1}H-NMR spectra interpretation.

Herein it is describeda strategy based on ^{19}F-NMR relaxation filter experiments that permits, in one shot, to screen a mixture of fluorinated sugars and to infer the chemical groups of the ligands that are key to the binding process (Figure 1). A recent application of this methodology to the study of DC-SIGN[2], an important C-type lectin that specifically recognizes highly-glycosylated patterns at the surface of pathogens and host glycoproteins, will bediscussed.Supported by molecular modelling analysis and STD experiments, the ^{19}F-NMR strategy lead to the discovery of a new binding mode of Man moieties to DC-SIGN lectin, and revealed very subtle details of the binding interactions of other monosaccharides[3].

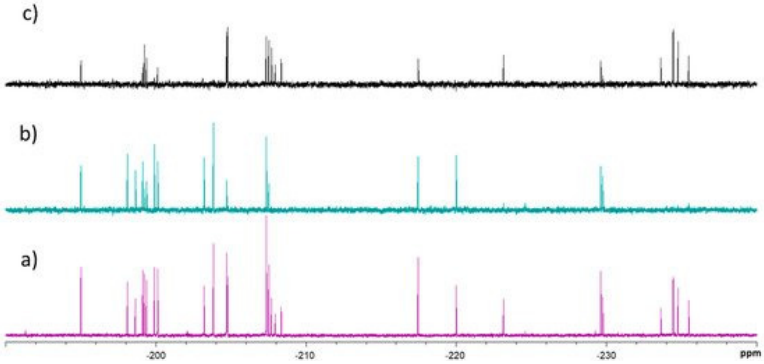

Figure 1. ^{19}F-NMR relaxation filter experiments performed for the fluorine-containing monosaccharide library in the presence of tetrameric dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN). The F-sugar/lectin ratio was 47:1. (**a**) Spectrum acquired without T_2 relaxation filter, (**b**) spectrum acquired after 2.4 s of T_2 relaxation filter, and (**c**) difference spectrum. Only the peaks of those molecules that display a significant decrease in their T_2 are present. These peaks correspond to DC-SIGN binders.

Acknowledgements

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P63/ C83

Stabilizing telomeric G-quadruplex DNA by a novel ligand: Synthesis, DNA interaction and biological activity

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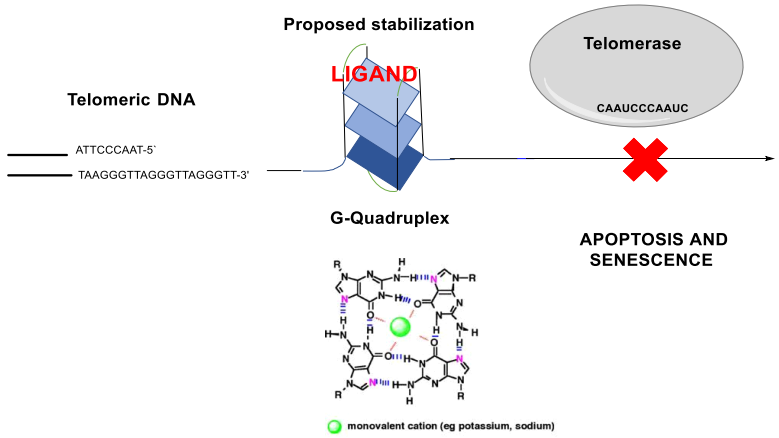
DNA, responsible for the storage of genetic information, has a fundamental role in its replication (essential in cell growth and division) and in protein synthesis. The high rate of replication of cancer cells makes DNA an important therapeutic target, and the "attack" can be made directly on the structure of the DNA. In recent years a novel therapeutic DNA target has emerged: the G-quadruplex.[1] The biological role of this non-canonical DNA sequence has been demonstrated by its participation in different processes related to oncogenes and telomeres.[2] Based on this discovery, a wide variety of compounds developed to selectively interact with this structure have been developed.[3,4]

The aim of this work is the design and synthesis of a novel G-quadruplex ligand based on 1,10-phenanthroline as the heterocyclic core, with guanidine groups integrated in the structure. On the other hand, we have focused on the study of its DNA binding interactions, determining its preferential capacity to selectively recognize G-quadruplex against double stranded DNA.



Thus, we report herein the synthesis of this novel quadruplex ligand and its preliminary biological testing. Specifically, we have evaluated the interactions with telomeric G-quadruplex DNA and duplex DNA by different techniques (DNA FRET melting, without and with a dsDNA competitor sequence, CD, equilibrium dialysis, etc.) and its cytotoxic activity in tumor cultured cells.

Acknowledgements

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Potential Processive Catalysis by an Exo-Hydrolase Identified by Experiment and Computation

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José-Emilio Sánchez-Aparicio,[1] Jaime Rodríguez-Guerra,[1] José M. Lluch,[1] Jean-Didier Maréchal,[1]
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HvExoI glycosidase supports seed germination in barely by hydrolysing β-D-glucans (composed of β-1,3 and β-1,4 glycosidic linkages). A vital conclusion drawn from the structural studies carried out by Prof. M. Hrmova (University of Adelaide) with the native enzyme isolated from barley seedlings, was that the glucose (Glc) product remains entrapped in the enzyme active site until an incoming substrate (or substrate analogue) binds. Here we present a combined structural and molecular modelling study of the product exit pathway of this plant exo β-glucosidases.[1] The results suggest a potential processive mechanism for this polysaccharide hydrolysing enzyme thanks to the identification of a putative transient lateral cavity next to the -1/+1 subsites that would allow the exit of the hydrolyzed glucose product (Fig. 1). To the best of our kowledge, and contrary to the case of endoglucanases or cellobiohydrolases, this is a mechanism that has not been proposed for any exo-acting hydrolase. The finding could have implication for the biotechnological use of such enzymes in biomass degradation.

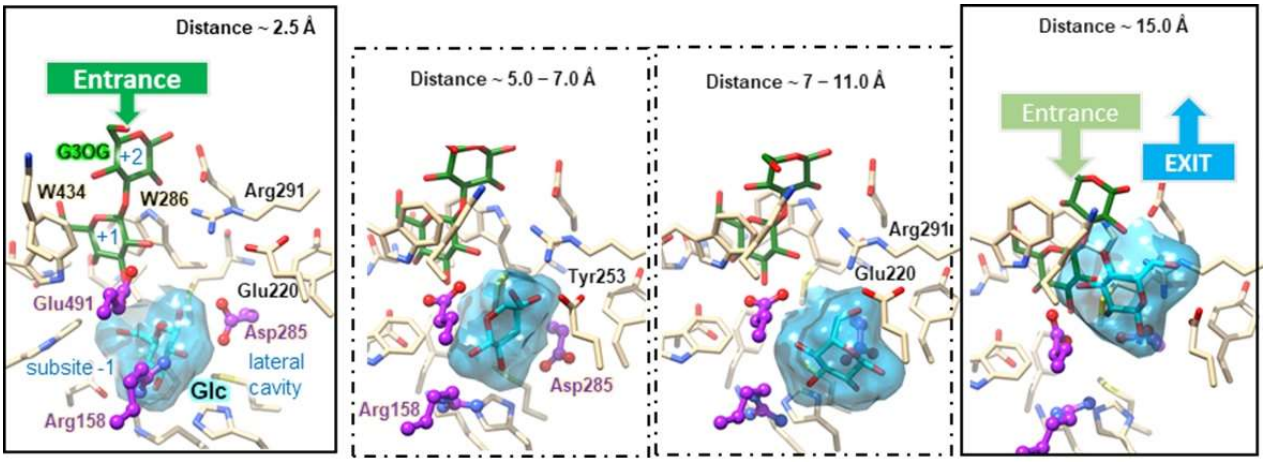

Fig 1. Schematic view of the Glc (in blue) exit pathway from HvExoI active site when a new substrate (in green) binds.

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P65/ C85

New THIQs derivatives as CD44 inhibitors: design, synthesis and biological evaluation

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Hyaluronic acid (HA), initially perceived only as an inert component of connective tissue, it is now known to be involved in multiple signaling pathways, including those implicated in cancer pathogenesis and progression. High HA levels in tumorous tissues are correlated with the malignancies of tumours.[1] HA functions are mediated by molecular interactions with the cluster of differentiation 44 (CD44) and other hyaladherins. CD44 is a transmembrane protein encoded by a single gene which is expressed on the majority of cells and is the main HA-binding receptor. Pathological conditions promote alternate splicing and post-translational modifications to produce diversified CD44 molecules with enhanced HA binding, which leads to increased tumorigenicity.

HA-CD44 interaction initiates signal transduction pathways leading to cancer cell growth, adhesion, migration, invasion and metastasis. Therefore, targeting hyaladherins presents a very promising approach against HA-induced tumourigenesis.[2] Small HA oligosaccharides (HAos) have shown to interrupt HA-CD44 interaction. Anti-CD44 antibodies reduce HA induced vascular smooth muscle cell migration and promote the production of inflammatory cytokines and reactive oxygen species in vascular injured mice. However, a clinical trial initiated to study the utility of CD44 antibodies in cancer chemotherapy was halted due to toxicity.[3]

The first nonglycosidic small structure inhibitors of HA binding were reported in 2014. Using fragment screening, biophysical binding assays and crystallographic characterization of complexes with the HA-CD44 binding domain, an inducible pocket adjacent to the HA binding groove was discovered. Structure-driven design and iterations of fragment combination have established the tetrahydroisoquinoline (THIQ) scaffold as an attractive starting point for lead optimization. Also, is reported that bulky groups like aromatic rings can block the interaction HA-CD44 by steric hindrance effect.[4]

Herein, we present the design, synthesis, characterization and biological evaluation of a new series of CD44 inhibitors structurally based in the THIQ scaffold substituted in 5 or 8 position with an amine group and linked to an aromatic moeity though different linkers in position 2. Exocyclic amine is introduced to interact by H-bonding with the HA binding domain of CD44 and the aromatic ring is added to extend farther into the HA binding groove and thus directly compete for HA binding. Biological activity of compounds was evaluated against A549 lung adenocarcinoma and MDA-MB-231 breast cancer cell lines with antiproliferative effects at the low micromolar range. Currently, we are optimizing a tool to evaluate these small molecules as HA-CD44 interaction inhibitors using HA fluorescently labeled (HA-FITC) by flow cytometry.

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THIO SUGARS AS VECTORS FOR G-QUADRUPLEX LIGANDS: POTENT ANTICANCER AND ANTIPARASITIC DRUGS

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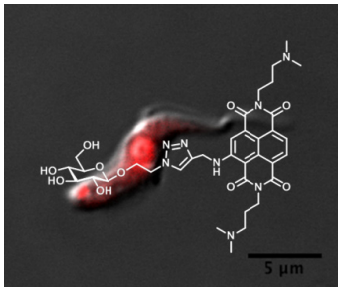
G-quadruplexes (G4) are DNA secondary structures which play important roles in the regulation of gene expression in human cells and have been proposed as therapeutic targets in cancer. At the same time, putative G-quadruplex forming sequences have also been found on the genome of parasites *T. brucei*, *L. major* and *P. falciparum* suggesting they could also be explored as therapeutic targets.[1]

G-quadruplex ligands based on sugar conjugated naphthalene diimide scaffold (carb-NDI), resultedon notable antiproliferativeactivity[2] and showed very relevant antiparasitic activity.[1] These G-quadruplex ligands presented IC50 values in the nanomolar range against *T.brucei* and they displayed high selectivity against MRC-5 human cells. Moreover, confocal microscopy experiments confirmed that the carb-NDIs localize in the nucleus and kinetoplast of T. brucei suggesting they can reach their potential quadruplex targets.
Recently, we have designed and prepared thio sugar-NDIs aiming to improve ligand stability and efficacy. Comparative results on cancer cells and different parasites will be presented.



Acknowledgements

This work was supported by the Spanish Ministerio de Economía y Competitividad (CTQ2012-35360, CTQ2015-64275-P, and SAF2016-80228-R), Junta de Andalucía (BIO1786), Worldwide Cancer Research Foundation (16-0290), Italian Association for Cancer Research (AIRC, IG2013-14708), AgenceNationale de la Recherche (ANR Quarpdiem, ANR-12-BSV8-0008-01), the SYMBIT project (reg. no. CZ.02.1.01/0.0/0.0/15_003/0000477) financed by the ERDF.

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P67/ C88

Accessing Fluoroalkyl Glycomimetics and Nucleosides Using "Ligandless" CuR_f Complexes

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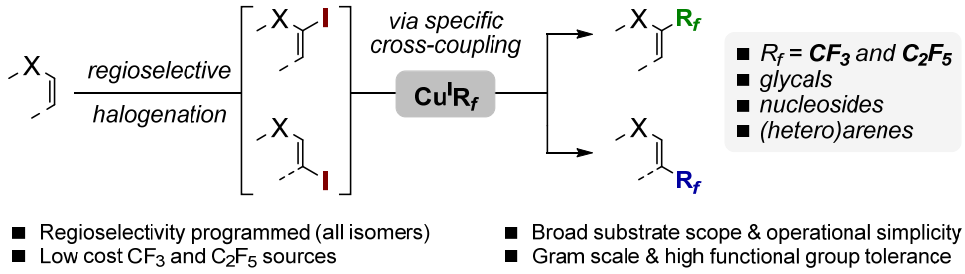
Chemical (glyco)biology has experienced an impressive growth in the last decade as a result of the discovery of the role of carbohydrates in relevant recognition processes, yet the use of fluoro glycoconjugates to study such events is still in its infancy. Selective incorporation of fluorine into biomolecules allows simultaneous modulation of their electronic, lipophilic and steric parameters, all of which can influence their biological function. Moreover, these elements have been widely employed as structural, functional and mechanistic probes for the study of biological processes by several cutting-edge non-invasive molecular imaging techniques.



In this abstract we present a survey of synthetic methods developed to access novel fluoroalkyl glycomimetics and nucleosides using well-defined "ligandless" CuR_f complexes.[1,2] Our flexible, sequential approach exploits the regiocontrolled introduction of an iodine handle and the subsequent cross-coupling of the C(sp^2)–I bond using highly active (1) fluoroform-derived "ligandless" $CuCF_3$ and (2) "ligandless" CuC_2F_5 reagent obtained by the controlled self-condensation of ready available $TMSCF_3$-derived $CuCF_3$. These methods gave access to valuable fluoroalkyl-derived glycals and nucleosides with potential applications in the chemical (glyco)biology field.

Acknowledgements

We thank the Spanish Government-MCIU and the national agency of investigation-AEI (CTQ2017-89750-R, CTQ2017-90088-R, and RYC-2015-17705), the European Regional Development Fund, and the Universitat Rovira i Virgili (2012BPURV-67) for financial support.

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TOWARDS TWO-PHOTON ABSORPTION BIOIMAGING THROUGHNEW FLUOROPHORES BASED ON INDOLIUM DERIVATIVES

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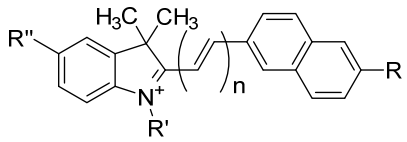
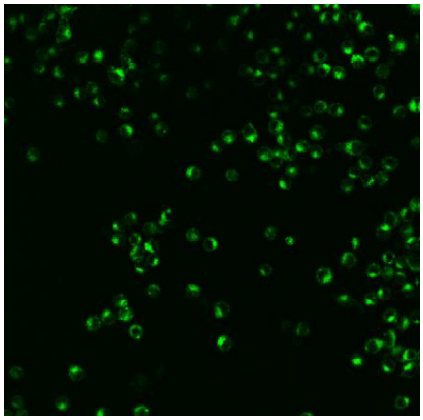
Fluorescence based methods,as consequence of the non-invasive nature of the light, have became essential in biomaging field. The use of light has been demonstrated as one of most prolific modes tounderstand biological processes, ranging from the monitoring of cellular processes to the recognition of biomacromolecular structures in tissues or the visualization of cellular organelles.[1]

In the last decades, two-photon (TP) microscopy is unseating classical one-photon (OP) microscopy. The use of lower energy excitation wavelengths or the possibility of focus in depth, are probably the more highligth characteristics of this technique.[2]Thus, the design and the developmen of new probes for TP microscopybioimaging is an interesting research challenge nowadays. Fluorophores based on indolenines are a family of compounds with promising properties in this sense,[3] as it is exposed in our recent work.[4]

With the aim of offering a general view about the potential application of this new scaffold, we present the design, synthesis and characterization of some of these indolium derivatives. They exhibit promising properties to be used in bioimaging applications in living cells with different purposes.





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Spanish Ministry of Economy,Industry, and Competitiveness (grant CTQ2016-75870-P) and Universidad de Málaga. Campus de Excelencia Internacional Andalucía Tech.

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P69/ C90

Selectins Ligands to Target Metastatic Tumors

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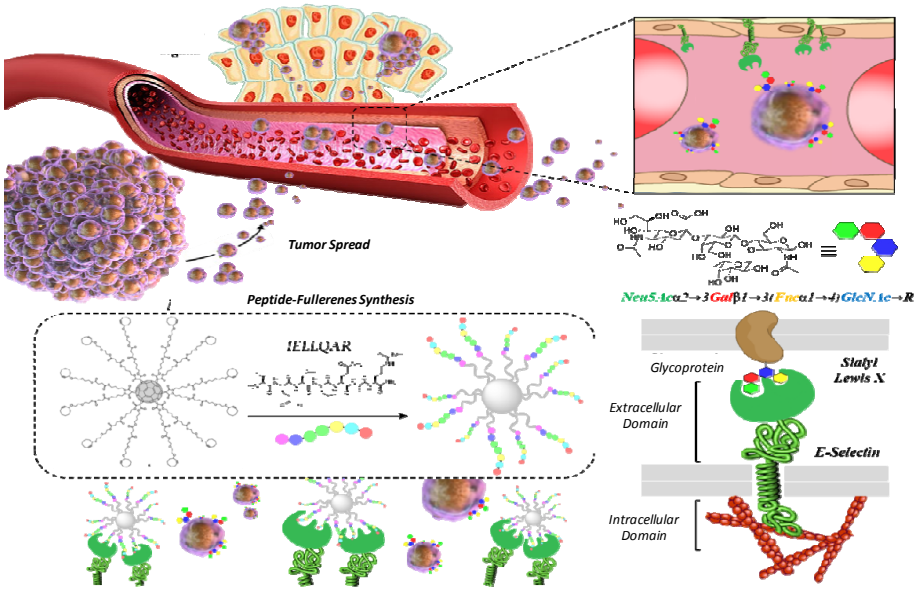
The molecular recognition through ligand-receptor interactions is a very important mechanism for metastatic processes. Among all of them, multivalent protein-carbohydrate interactions play a fundamental role in tumor cell-endothelial cell recognition processes. In particular, the interaction between SLex and SLea expressed in circulating tumor cells and proteins of the family of selectins, overexpressed in endothelial cells.Unlike tumor cells, vascular endothelial cells are a target with stable membrane receptors and not subjected to genetic modifications. In addition, the elimination of a single endothelial cell involves the death of hundreds of tumor cells.Targeting receptors of endothelial cells offers a high potential in tumor diagnosis and therapy.



SLex and SLea are the natural ligands of selectins, buttheir complicated chemical synthesis and their low affinity for selectins type endothelial receptors made impossible to continue with their development. Recently, through "phase display" it was identified a simple peptide with a linear sequence of seven amino acids (Ile-Glu-Leu-Leu-Gln-Ala-Arg) called IELLQAR that interacts specifically with selectins. IELLQAR is a very simple compound, which can be synthesized using SPPS. IELLQAR can be functionalized and easily conjugated to fullerenes for its multivalent presentation. In addition, it can be combined with diagnostic and therapeutic agents.

Acknowledgements

We acknowledge funding fromERC Starting Grant (DYNAP-677786) andBeatriz Galindo Program (BEAGAL18/00051)

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INTERACTION OF A NEW AZA-MACROCYCLIC PYRAZOLE COMPOUND WITH G-QUADRUPLEX DNA

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G-quadruplexes are secondary structures that can be found in guanine rich DNA sequences.[1] They can be naturally formed in telomeric and in transcriptional regulatory regions of multiple genes and oncogenes. Therefore, these structures have attracted attention as supramolecular targets for developing new antitumoral drugs.[2] Herein, we present the design, synthesis and characterization of a novel tripodal polyamine derivative, which includes an aromatic core and three pending arms. The pending arms are based on polyamine macrocycles containing an 1*H*-pyrazole moiety as aromatic spacer that may provide an alternative interaction. The acid-base behavior in aqueous medium has been studied by means of potentiometric titrations and fluorescence emission spectroscopy. The interaction with canonical and G-quadruplex DNA models has been assessed by FID and FRET melting methods. As a preliminary result, a highly selective interaction for G-quadruplex over duplex B-DNA structures has been observed.

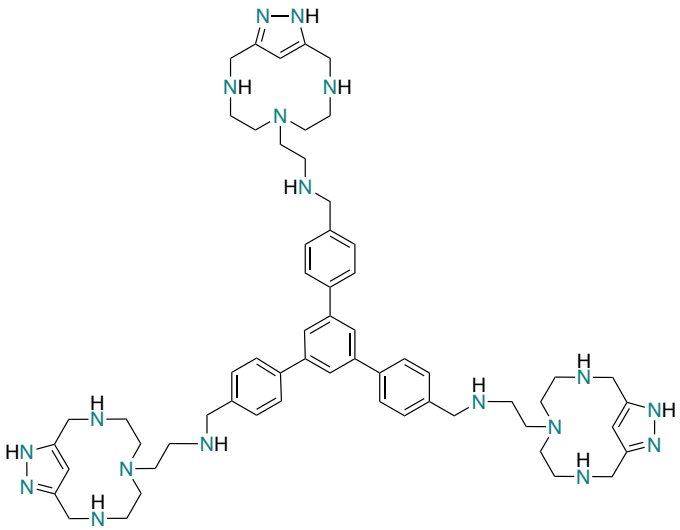

Figure 1. Studied ligand.

Acknowledgements

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P71 / FC12 / C92

ACETALS AS ACID-SENSITIVE CLEAVABLE LINKERS FOR THE DESIGN OF ANTIBODY-DRUG CONJUGATES

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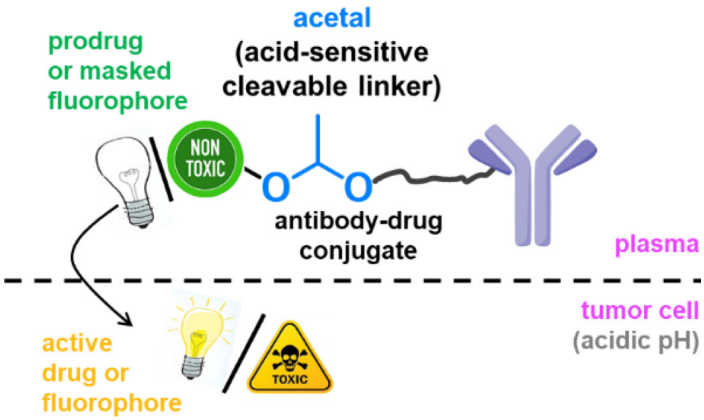

Several acid-cleavable linkers, based on an acetal group, that feature either a deactivated coumarin or a prodrug derived from the potent anti-cancer agent duocarmycin, have been designed. The linkers are stable in plasma and can rapidly break down in acidic pH to generate the free fluorophore or the toxic drug in situ. These scaffolds were conjugated to a Trastuzumab antibody that is specific for Her2, a receptor that is overexpressed in breast cancer cells. Interestingly, although the Trastuzumab-coumarin conjugate is stable in plasma, the analogue that carries the duocarmycin derivative slowly decomposes under similar conditions. Molecular dynamics (MD) simulations performed on these antibody-drug conjugates (ADCs) suggest that a lysine residue of the antibody nearby the conjugation site can act as an acid, which promotes hydrolysis of the acetal bearing the duocarmycin derivative. These data demonstrate that both the conjugation site of the antibody and the combination linker-payload can modulate the stability of the conjugate. Finally, we show that both ADCs retain their specificity to cancerous cell lines expressing Her2 and that the ADC bearing the prodrug efficiently kills antigen-positive cancer cells. Therefore, the straightforward synthesis combined with the possibility of incorporating different payloads, makes the use of acetals an innovative and competitive strategy for the design of targeted drug-delivery systems.

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Site-selective protein modification withnovel dehydroalanine derivatives

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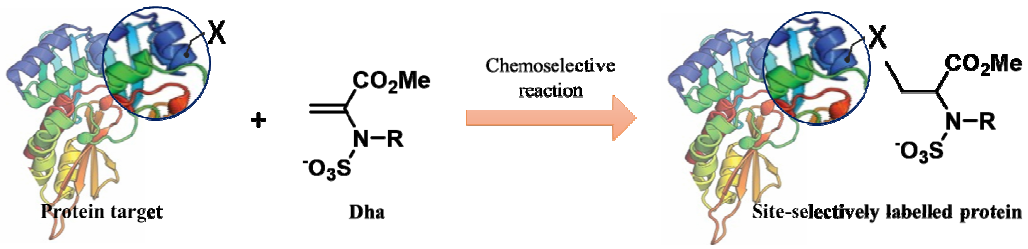
Bioconjugation is a very potent tool to chemically modify proteins in order to install new functionalities such as fluorescent probes, cytotoxic payloads, etc.[1]When exploring the natural reactivity of the amino acid side chains, for example, cysteine[2] and lysine[3] ubiqutuous biological nucleophiles may competewith electrophilic reagents, depending on the working conditions. Despite those issues, several methods based on selective reactions of certain amino acids have been developed in recent years.

α,β-Dehydroamino acids are well-known electrophiles occasionally used for protein modification, leading to a range of natural and unnatural post-translational modifications (PTM) such as lanthionines and lysinoalanines. However, the low reactivity of these functionalities, which require the concurrence of enzymes for natural PTM, or the use of large electrophile excess for chemical modification, has limited their use and scope.

This work presents the design, synthesis and evaluation of new water-soluble dehydroalanine derivatives able to react with amino acids different from commonly targeted cysteine and lysine. The reactivity and chemoselectivity of these reagents as Michael acceptors observed with both small-molecule nucleophiles and in protein bioconjugation, is described.



In parallel, an innovative non-destructive, reagent-freeassaybased in 2D NMR spectroscopy to unequivocaly determine the extent and identity of the protein modifications directly in aqueous solution, is presented.

Acknowledgements

Ministerio de Ciencia, Innovación y Universidades (RTI2018-099592-B-C21 and RTI2018-099592-B-C22 projects)

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P73/ C94

Exploring the use of unnatural MUC1 derivatives fordetection of antibodies in cancer patients

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MUC1 is one of the most studied mucins because it is a glycoprotein overexpressed in around 80% of human cancers.[1]In general, although MUC1 exhibits complex *O*-glycans in healthy cells, in tumor tissues, it is decorated with short carbohydrates. Consequently, different antigens that are masked in healthy cells, such as the Tn antigen (GalNAc-α-1-O-Thr/Ser), are exposed in cancer cells and can trigger an immune response. In support of this general concept, it has been reported that some cancer patients can induce natural auto-antibodies directed against native MUC1 and with an improved survival rate.

The main goal of this work is to develop a new assay that allows the detection of low concentrations of these auto-antibodies in the serum of cancer patients by using unnatural MUC1 glycopeptides as potent antigens.[3] To increase the sensibility of the method, we will functionalize gold nanoparticle with the unnatural MUC1 glycopeptides. Finally, the efficacy of our methodology will be tested with several real samples.

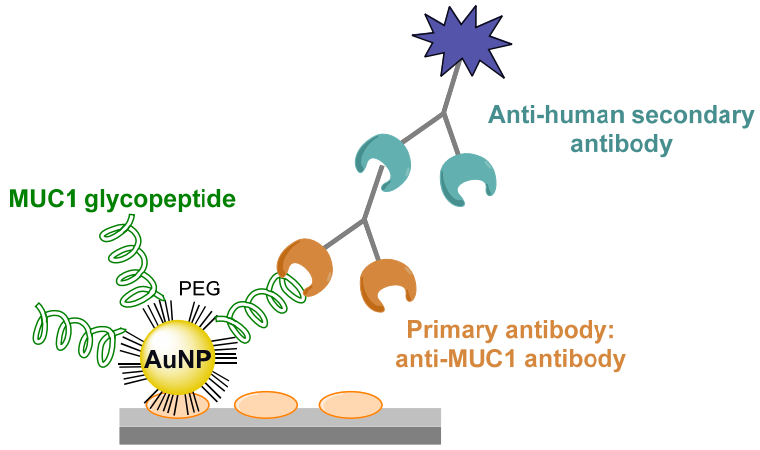

Figure 1: Proposed assay to detect anti-MUC1 antobodies in cancer patients.

Acknowledgements

We thank the *Ministerio de Economía y Competitividad* (project: RTI2018-099592-B-C21). A. A. thanks the *Asociación Española contra el Cáncer* (AECC-La Rioja) for a fellowship.

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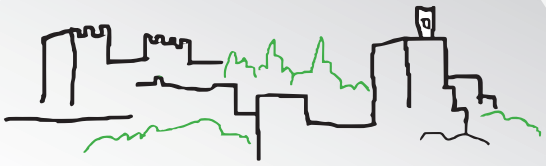

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