6th EUROPEAN CHEMICAL BIOLOGY SYMPOSIUM

ECBS/LS EuChemS

www.ecbs2019euchems.eu

3-5 APRIL
2019
MADRID-SPAIN

ABSTRACTS BOOK

ORGANIZED BY:

EuChemS
European Chemical Society

RSEQ
Real Sociedad Española de Química

eu openscreen

Abstracts Book

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

Design, layout and correction: Ronda Vázquez Martí.

c/ Imagen, 1 y 3
28801 Alcalá de Henares (Madrid). SPAIN
Tel. +34 91 879 74 30 – Email: congresos@fgua.es
Website: www.fgua.es

Welcome

On behalf of the Scientific and Organizing Committees, it is our pleasure to welcome you to Madrid for the 6th ECBS/LS-EuChemS meeting. This occasion brings together the 6th European Chemical Biology Symposium and the meeting of the EuChemS Division "Chemistry in Life Sciences" (LS-EuChemS) and it is jointly organized by EuChemS and EU-OPENSCREEN. This meeting builds on the success of 4th ICBS/ECBS joint meeting in Berlin (2015) and 5th ECBS in Budapest (2017).

Chemical biologists from all parts of Europe and overseas come together to present and discuss the latest achievements in cutting edge chemical sciences. This present joint meeting gathers researchers from organic & biological chemistry, proteins biotechnology, computational biology, protocells, machine learning, bacterial resistance, drug discovery, synthetic biology, biomedical materials and chemical glycobiology.

We encourage young researchers to present their work at the meeting (oral & flash presentations will be selected from abstracts), discuss with the speakers and embrace the exciting developments currently happening in chemical biology.

The venue for the 6th ECBS/LS-EuChemS meeting will be at CSIC headquarters beautifully located near to Madrid city center. It offers a perfect base for exploring the historic centre of Madrid.

We are looking forward to meeting you in Madrid!

Dr. Sonsoles Martín-Santamaría
EuCheMS Division of "Chemistry in Life Sciences Division", Chair

Dr. Philip Gribbon
EU-OPENSCREEN Coordinator

General index

Organizing Committees

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

ORGANIZING COMMITEE

- Sonsoles Martin-Santamaria
  Center for Biological Research, CIB-CSIC, Spain
- Philip Gribbon
  EU-OPENSCREEN
- Valle Palomo
  Center for Biological Research, CIB-CSIC, Spain
- Joan Guzman-Caldentey
  Center for Biological Research, CIB-CSIC, Spain
- Bahne Stechmann
  EU-OPENSCREEN, Deutschland
- Jordi Quintana
  University Pompeu Fabra, Spain
- Katja Herzog
  EU-OPENSCREEN, Deutschland
- Elena Gomez-Rubio
  Center for Biological Research, CIB-CSIC, Spain

SCIENTIFIC COMMITTEE

- Sonsoles Martin-Santamaria (Chair)
  Center for Biological Research, CIB-CSIC, Spain
- Philip Gribbon (Chair)
  EU-OPENSCREEN, Deutschland
- José Luis Mascareñas
  CIQUS, University of Santiago de Compostela, Spain
- Bahne Stechmann
  EU-OPENSCREEN, Deutschland
- Jordi Quintana
  University Pompeu Fabra, Spain
- Marko D. Mihovilovic
  Vienna University of Technology, Austria
- María Jesús Vicent
  CIPF, Spain

- Krister Wennerberg
  FIMM-EMBL, FI

- Petr Bartunek
  Institute of Molecular Genetics, Czech Republic

- Alleyn Plowright
  Sanofi, Deutschland (EFMC representative)

- Haian Fu
  Emory University, USA (ICBS representative)

Symposium Programme

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

PROGRAMME

WEDNESDAY, 3 APRIL

09:30 Registration

10:50 OPENING
Elena Domínguez – CSIC Vice-President
Enrique de la Rosa – CIB-CSIC Director
Wolfgang Fecke – Director General EU-Openscreen
Sonsoles Martín-Santamaría – Chair
Philip Gribbon – Chair

11:00 **SESSION I. ICBS Session**
Chair: Haian Fu
PLENARY LECTURE
Synthetic Protobiology
Stephen Mann – University of Bristol, UK

11:45 INVITED LECTURE
Illuminating the Biochemical Activity Architecture of the Cell
Jin Zhang – UCSD, USA

12:15 ORAL COMMUNICATIONS
OC1: Jacek Kolanowski – IBCH-PAS, PL
Fluorescent probes for multiparametric investigation of biochemical analytes in cellular models
OC2: Salvador Tomás – Birbeck-University of London, UK
Protocells promote complexity within: self-assembly and condensation

12:45 Lunch break & Poster Session

14:00 **SESSION II**
Chair: Francesco Peri
PLENARY LECTURE
Lectins from Bacteria and Fungi: Therapeutical Targets and Research Tools
Anne Imberty – CNRS, FR

14:45 ORAL COMMUNICATIONS
OC3: Antoni Planas – IQS, ES
Dual N-acetylglucosamine and N-acetylmuramic peptidoglycan deacetylation. Specificity and biological functions
OC4: Derek S. Tan - Memorial Sloan Kettering Cancer Center, USA
Diversity and design: new chemical probes for biology and medicine

15:15 FLASH COMMUNICATIONS
FC1: Benedikt Heinrich – University of Marburg, ES
Far-red fluorescent DNA binder allows host-pathogen interaction studies of multidrug-resistant bacteria
FC2: Diana Rodríguez – CIQUS-USC, ES
Novel covalent inhibitors of carbapenem-hydrolyzing class D β-lactamases enzymes for disabling antibiotic resistance in superbugs
FC3: Alba Vázquez Arias – University of Vigo, ES
Exploring bacterial surface display as a platform for developing engineered glycan-binding proteins

15:30 INVITED LECTURE
Effects of Antibiotics on Cell Cycle Progression of Staphylococcus aureus
Mariana Gomes de Pinho – ITQB, PT

16:00 Coffee break & Poster Session

16:30 **SESSION III. EU Openscreen ERIC Session**
Chair: Jordi Quintana
INVITED LECTURE
Applying Established Concepts of Pharmaceutical Development to Industrialization of Cellular Cancer Immunotherapy
Margit Jeschke – Novartis, CH

17:00 ORAL COMMUNICATIONS
OC5: Bahne Stechmann – EU-OPENSCREEN, DE
Access models for eu-openscreen eric
OC6: Albert A. Antolin – ICR, UK
Probe miner v2: updated objective data-driven assessment of chemical probes

17:30 ORAL COMMUNICATIONS
OC7: Marie Louise Jung – Prestwick Chemical, FR
Smart screening libraries over the past 20 years and success stories in medicinal chemistry
OC8: Bernhard Ellinger – Fraunhofer IME, DE
Validation of a high content assay to identify DNAJB6 agonists
OC9: Francisca Vicente – MEDINA, ES
A novel natural product for pancreatic cancer

18:15 FLASH COMMUNICATIONS
FC4: José García-Tenorio – Molport, LV
Screening compounds & building blocks sourcing and procurement solution in a customized way
FC5: Mª Angélica Luque-González – GENYO-UGR, ES
Trypanosomatids identification by chem-NAT technology
FC6: Bruno Cuevas Zuviría – CBGP-UPM, ES
aAMDlib: testing transferability of electron density models on chemical libraries with biological interest

20:15 Speakers & Committees dinner

THURSDAY, 4 APRIL

08:30 **SESSION IV. RSEQ-GEQB Session**
Chair: Concepción González-Bello
PLENARY LECTURE
DNA Simulations from the Electron to the Chromosome... and Beyond
Modesto Orozco – IRB Barcelona, ES

09:15 ORAL COMMUNICATIONS
OC10: Gonzalo Jiménez Osés – University of La Rioja, ES
Computer design and directed evolution of a highly active and enantioselective metallo-hetero-diels-alderase
OC11: Macarena Sánchez Navarro – IRB Barcelona, ES
Paving the way towards the brain delivery of biotherapeutics: Modification of proteins with blood-brain barrier peptide shuttles

09:45 INVITED LECTURE
What Do We Know about Ion Conduction in Ion Channels?
Carmen Domene – University of Bath, UK

10:15 FLASH COMMUNICATIONS
FC7: Ángela **Rodríguez-Costa** – CIQUES-UCS, ES
Hydroxylamine derivatives for specific lysine covalent modification of bacterial type I dehydroquinase
FC8: Andrea Canal Martín – CIB-CSIC, ES
Thiol-disulfide reversible chemistry in non-conventional conditions
FC9: Nuria Lafuente-Gómez – IMDEA nanoscience, ES
Functionalization of magnetic nanoparticles with gemcitabine via disulfide bonds: a new approach for the pancreatic cancer treatment

10:30 Coffee Break & Poster Session

11:00 **SESSION V**
Chair: Philip Gribbon
INVITED LECTURE
Combining Cheminformatics and Bioinformatics in Drug Discovery - Lessons Learned and New Opportunities
Gerard JP van Westen – University of Leiden, NL

11:30 ORAL COMMUNICATIONS
OC12: María Duca – CNRS, FR
Synthetic small-molecule RNA ligands: scope and applications
OC13: Olalla Vazquez – Philipps / Universität Marburg, DE
Optochemical Tools for Biological Processes

12:00 ORAL COMMUNICATIONS
OC14: José Brea – UCS – Innopharma, ES
Therapeutic potential of blood glutamate grabbing in stroke: a proof of concept
OC15: David Albesa-Jové – CIC bioGUNE, ES
Deciphering the mechanism of action of toxins delivered by the Type-VI Secretion System (T6SS)n in *Pseudomonas aeruginosa*

12:30 FLASH COMMUNICATIONS
FC10: Ferrán Feixas – IQCC, ES
Accelerating (bio)molecular recognition and assembly
FC11: Davide Avagliano – ITC, AT
Drastic effect of the electrostatic energy on the binding mode of spiropyran photoprobe to dsDNA
FC12: María J. Matos – USC, ES
Neurodegenerative diseases: from small molecules to targeted therapies

12:45 Lunch break & Poster Session

14:00 **SESSION VI**
Chair: María J. Vicent
PLENARY LECTURE
Multiplexing and Multivalency in Nanomedicine Design
Giuseppe Battaglia – University College of London, UK

14:45 ORAL COMMUNICATIONS
OC16: Gustavo Bodelón – University of Vigo, ES
Plasmonic ZIF8 metal-organic frameworks (MOFs) with self-assembled proteins as optical probes for imaging cell surface receptors
OC17: Luca Laraia – Technical University of Denmark, DK
New tool compounds for studying autophagy

15:15 FLASH COMMUNICATIONS
FC13: Carlota Tosat – CIB-CSIC, ES
Specific Biosensors in Drug Discovery for Amyotrophic Lateral Sclerosis
FC14: Marisa Juanes – CIQUS-UCS, ES
Amphiphilic peptide/Cas9 nanocomplexes for the delivery of gene editing nucleases
FC15: Andreia Mónico – CIB-CSIC, ES
Computational modeling and cysteine crosslinking studies of vimentin

15:30 INVITED LECTURE
New Peptides Designs for Gene Delivery, Genetic Edition and the Preparation of Cytoskeleton Mimics
Javier Montenegro – CIQUS-USC, ES

16:00 Coffee Break & Poster Session

16:30 **SESSION VII**
Chair: Juan Carlos Morales
INVITED LECTURE
Merging Magnetic Hyperthermia with other Anticancer Treatments for Tackling Cancer
Teresa Pellegrino – IIT, IT

17:00 ORAL COMMUNICATIONS
OC18: Amadeu Llebaria – IQAC-CSIC, ES
Precise control of GPCR receptors with light and allosteric photoswitchable ligands in cells and living animals
OC19: Luís Pinto da Silva – CIQUP, PT
Preparation and Evaluation of Self-Illuminating Photosensitizers for Photodynamic Therapy of Cancer

17:30 INVITED LECTURE
Perturbation of mitochondrial genome stability with OGG1 inhibitors as a novel strategy to treat cancer
Torkild Visnes – SINTEF Insdustry/ Karolinska Institutet, NO

18:00 FLASH COMMUNICATIONS
FC16: Paula Milán Rois – IMDEA nanoscience, ES
Reprograming Uveal Melanoma Cells: A Combination Therapy Based on Gold Nanoparticles
FC17: Antonio Aires – CIC biomaGUNE, ES
Green synthesis of designed protein-stabilized CdS quantum dots for bio-imaging applications
FC18: Carmen Yuste-Calvo – GBGP-UPM-INIA, ES
Functionalization of elongated viral nanoparticles through chemical conjugation to molecules of different kinds
FC19: Giulia Caron – University of Torino, IT
Ro5 vs bRo5: the winner is …
FC20: Marta Sanz-Murillo – CIB-CSIC, ES
Structural basis of RNA polymerase I stalling at UV light-induced DNA damage
FC21: Fabio Alessandro Facchini – Università degli studi di Milano-Bicocca, IT
FP7, a synthetic TLR4 antagonist, reduces inflammation in the Gastrointestinal Tract

20:30 Conference Dinner

FRIDAY, 5 APRIL

08:30 **SESSION VIII. RSEQ Session**
Chair: Itziar Alkorta
PLENARY LECTURE
Beyond Watson and Crick: Chemical Modifications in G-Quadruplexes Regulate Gene Expression
Cynthia J. Burrows – University of Utah, USA

09:15 ORAL COMMUNICATION
OC20: Ruth Pérez Fernández – CIB-CSIC, ES
Protein-directed Dynamic Systems: Insights into Real Time Chemical Processes

09:30 FLASH COMMUNICATIONS
FC22: Iris Alicia Bermejo – University of La Rioja, ES
Insights into the molecular recognition and conformational preferences of the STn antigen derived glycopeptides as tools in cancer vaccination and diagnosis.
FC23: Beatriz Fernández del Toro – CIB-CSIC, ES
Resolving extended N-glycans by NMR: New insights into influenza hemagglutinin N-glycan interactions
FC24: Ana Díaz-Fernández – University of Oviedo, ES
Selection of aptamers recognizing the glycosylation site of PSA: aptasensors for detection of cancer-related glycosylation changes

09:45 INVITED LECTURE
Artificial Metalloenzymes: Challenges and Opportunities
Thomas R. Ward – University of Basel, CH

10:15 ORAL COMMUNICATION
OC21: Marc García-Borrás – IQCC, ES
Computational characterization of enzymatic reactive intermediates for the discovery and design of new biocatalytic activities

10:30 Coffee break & Poster Session

11:00 **SESSION IX. EFMC Session**
Chair: Yves P. Auberson (EFMC President)
INVITED LECTURE *Sponsored by ChemPubSoc Europe*
Discovery and Development of Antibiotics from (Myxo)bacterial Secondary Metabolites
Rolf Müller – Helmholtz-HZI, DE

11:30 ORAL COMMUNICATIONS
OC22: Mariapina D'Onofrio – University of Verona, IT
Introduction of post-translational modifications in vitro modulates TAU protein fibrillation
OC23: Manfred Jung – University of Freiburg, DE
Targeted release of epigenetic histone demethylase inhibitors via enzymatic bioreductive activation from prodrugs

12:00 INVITED LECTURE
Repurposing Ciclopirox as a Pharmacological Chaperone
Oscar Millet – CIC bioGUNE/ATLAS, ES

12:30 ORAL COMMUNICATION
OC24: M. Eugenio Vázquez – University of Santiago, ES
Dynamic stereoselection of kinetically-inert DNA-binding metallopeptide cylinders

12:45 Lunch break & Poster Session

14:00 **SESSION X. EuChemS Session**
Chair: Sonsoles Martín-Santamaría
INVITED LECTURE
Mechanistic insights into the Function of HSP90 Co-chaperones by cryo-EM
Oscar Llorca – CNIO, ES

14:30 ORAL COMMUNICATION
OC25: Marc Nazare – FMP, DE
Selective Tankyrase Inhibitors Through a Structure-guided Deconstruction and Hybridization Approach

14:45 INVITED LECTURE
Stabilization of 14-3-3 Protein-Protein Interactions
Christian Ottman – Eindhoven University of Technology, NL

15:15 CLOSING PLENARY LECTURE (IYPT)
The Biological Chemistry of Selected Elements from the Periodic Table
Federico Gago – University of Alcalá, ES

16:00 CLOSING, AWARDS & FINAL REMARKS.
Best Poster and Flash Communications awards. PhD Tesis 2018 Award of the RSEQ Chemical Biology Division to Dr. Elsa Hernando.
Pilar Goya – EuChemS President
Sonsoles Martín-Santamaría – Chair
Philip Gribbon – Chair.

Communications Index

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

Plenary Lectures

Invited Lectures

Oral Communications

Flash Communications

Poster Communications I

Poster Communications II

Plenary Lectures

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

Synthetic protobiology: the chemistry of life-like objects

Stephen MANN

Centre for Protolife Research and Centre for Organized Matter Chemistry, School of Chemistry, University of Bristol, Bristol BS8 1TS, UK.

Recent progress in the chemical construction of micro-compartmentalized semipermeable colloidal objects comprising integrated biomimetic functions is paving the way towards rudimentary forms of artificial cell-like entities (protocells) for modelling complex biological systems, exploring the origin of life, and advancing future proto-living technologies. Although several new types of protocells are currently available, the design of synthetic protocell *communities* and investigation of their collective properties has received little attention. In this talk, I review some recent experiments undertaken in my laboratory that demonstrate simple forms of higher-order dynamic behaviour in synthetic protocells. I will discuss four new areas of investigation: (i) enzyme-powered motility and collective migration in buoyant organoclay/DNA protocells,[1] (ii) artificial predatory, phagocytosis and endosymbiosis behaviour in mixed populations of synthetic protocells,[2,3,4] (iii) chemical communication and DNA computing in ordered protocell communities,[5] and (iv) the chemical construction of beating prototissues.[6] I will use these new model systems to discuss pathways towards chemical cognition, modulated reactivity, basic signalling pathways and non-equilibrium activation in compartmentalized artificial micro-ensembles.

References:

[1] Kumar P B V V S, Patil A J and Mann S. *Enzyme-powered motility in buoyant organoclay/DNA protocells.* Nature Chemistry **10**, 1154-1163 (2018).

[2[Qiao Y, Li M, Booth R and Mann S. *Predatory behaviour in synthetic protocell communities.* Nature Chemistry **9**,110-119 (2017).

[3] Rodríguez-Arco L, Li M and Mann S. *Artificial phagocytosis in synthetic protocell communities of compartmentalized colloidal objects.* Nature Materials, **16**, 857-863 (2017).

[4] Martin N, Douliez J-P, Qiao Y, Booth R, Li M and Mann S. *Antagonistic chemical coupling in self-reconfigurable host-guest protocells.* Nature Communications **9**, 3652 (2018).

[5] Joesaar A, *et al*; *Distributed DNA-based communication in populations of synthetic protocells.* Nature Nanotechnology DOI: 1038/s41565-019-0399-9 (2019).

[6] Gobbo P, Patil A J, Li M and Mann S. *Programmed assembly of synthetic protocells into contractile prototissues.* Nature Materials, **17**, 1145-1153 (2018).

Stephen Mann is Professor of Chemistry at the University of Bristol UK and distinguished for contributions to biomineralization, bioinspired materials chemistry and protocell research. Prof Mann was elected Fellow of the Royal Society UK (2003), and awarded the RSC de Gennes Prize (2011), SCF French-British Prize (2011), RSC Nyholm Medal (2018) and Royal Society Davy Medal (2016). He was visiting professor at the College de France (2009) and Harvard University (2011). He has published over 500 scientific papers with a *h* index of 120.

Lectins from bacteria and fungi: therapeutical targets and research tools

Anne IMBERTY

CERMAV, CNRS and Université Grenoble Alpes, 601 rue de la Chimie, 38041 Grenoble, France.

Contact e-mail: Anne.imberty@cermav.cnrs.fr

A large number of pathogenic microorganisms display receptors for specific recognition and adhesion to the glycoconjugates present on human tissues. In addition to membrane-bound adhesins, soluble lectins are involved in infections caused by the bacteria *Pseudomonas aeruginosa* and *Burkholderia cepacia* and by the fungus *Aspergillus fumigatus* that are responsible for hospital-acquired diseases. Accumulated knowledge about the structures of the lectins and the interactions with host glycoconjugates has led to the design of powerful glyco-derived inhibitors that can serve as antimicrobial therapeutic agents, as a complement to or an alternative to antibiotic therapy. Design of glycosylated chips, liposomes, fullerenes and other nano-glycoparticles have provided information on multivalent interaction between receptors and cell surfaces. This also result in development of nanomaterials that can be used in diagnostic applications.

Furthermore, the multivalency of lectin is proposed to play a role in their strong avidity for glycosylated cell surfaces and also in their ability to affect membrane dynamics by clustering glycosphingolipids. Bacterial lectins are able to bind to glycoconjugates on human tissues and are therefore thought to be involved in the first step of infection. The role of lectins in membrane invagination indicates that they could also play a role in internalization of intracellular pathogens. Lectins as multivalent and specific compounds are also powerful tools for labeling tumor cells that present altered glycosylation. Engineering of lectins allows the creation of novel supramolecular tools, superlectins, neolectins and Janus lectins with modified architecture, valency and specificity.

DNA Simulations from the Electron to the Chromosome... and Beyond

Modesto OROZCO

IRB Barcelona, Spain.

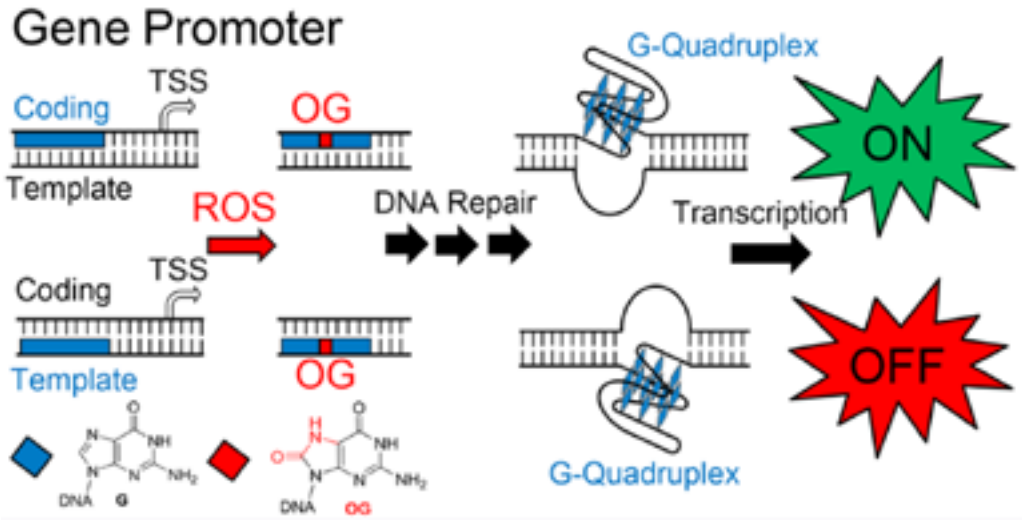
DNA is a large and polymoprhic structure of paramount biological importance and which constitutes the best example of a multiscale system whose treatment and theoretical analysis should be tackled from a multi-physic approach. I will summarize during my talk the approaches we are developing in the group to treat DNA from the subatomic level to the entire chromatin. The challenges on implementing experimental data into the simulations will be discussed.

Beyond Watson and Crick: Chemical Modifications in G-Quadruplexes Regulate Gene Expression

Cynthia BURROWS[(1)], Aaron FLEMING[(1)], Yun DING[(1)], Judy ZHU[(1)]

[1] Department of Chemistry, University of Utah, Salt Lake City, UT 84112-0850, USA.

Contact e-mail: burrows@chem.utah.edu



Studies with the regulatory *VEGF* promoter G-quadruplex (G4)-forming sequence have identified this G-rich region is prone to oxidation yielding 8-oxoguanine (OG),[1] and we postulated that oxidation of the regulatory sequence could alter gene expression. We used synthetic chemistry to install the *VEGF* G4 with OG at oxidation-prone sites in the promoter of a luciferase gene that was transfected in mammalian cells to understand how gene expression changed. The modified sequences were installed in either the native coding strand or the non-native template strand of the promoter.[1,2] Monitoring the time dependency in gene expression found a nearly 300% increase in expression when OG was in the coding strand and a nearly 400% decrease in expression for OG in the template strand. Studies in base excision repair (BER) proficient or deficient cell lines support BER as the process for gene activation for OG in the coding strand, and template strand OG turned gene expression off in a BER-independent process. Furthermore, we found that human DNA repair gene promoters are favorably biased with potential G4-forming sequences that can alter gene expression when OG is present. Thus, we hypothesize that certain potential G4-forming sequences are switches for gene expression during oxidative stress. To determine whether OG is prevalent in gene regulatory regions we developed a sequencing protocol and found enrichment of OG in gene promoters and 5'-UTRs.[3]

Acknowledgements: This work was supported by National Cancer Institute grant no. R01 CA090689.

References:

[1] Fleming, A.M.; Ding, Y.; Burrows, C.J. *Proc. Nat. Acad. Sci. U.S.A.* **2017**, *114*, 2604.

[2] Fleming, A.M.; Zhu, J.; Ding, Y.; Burrows, C.J. *ACS Chem. Biol.* **2017**, *12*, 2417.

[3] Ding, Y.; Fleming, A.M.; Burrows, C.J. *J. Am. Chem. Soc.* **2017**, *139,* 2569.

The Biological Chemistry of Selected Elements from the Periodic Table

Federico GAGO

Department of Pharmacology, Universidad de Alcalá de Henraes, Madrid, Spain.

Contact e-mail: federico.gago@uah.es

Every kind of material around us, living and nonliving, is related to the properties of the chemical elements of the periodic table. The Earth's changing environment allowed elements to mix, react, and exchange. Over the eons different elements were naturally selected to shape the chemical capabilities and functions of living organisms. The simple association of organic chemistry with life and inorganic chemistry with the inanimate world only is quite misleading because life was built and is maintained on both organic and inorganic compounds. Emergence and persistence of life required multiple chemical mechanisms to draw materials from the environment and build the three main classes of mutually interdependent biomolecules – peptides, lipids and nucleic acids – that define life in molecular terms. In this International Year of the Periodic Table of Chemical Elements (IYPT2019), I will provide a brief overview of how and why the electronic configuration of the atoms making up «the biological periodic system of the elements» allows biochemistry to be mapped onto the underlying quantum-mechanical logic of chemistry and provides functional value to living systems in general and human beings in particular.

Invited Lectures

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

Illuminating the Biochemical Activity Architecture of the Cell

Jin ZHANG

University of California, San Diego.

The complexity and specificity of many forms of signal transduction are widely suspected to require spatial microcompartmentation and dynamic modulation of the activities of signaling molecules, such as protein kinases, phosphatases and second messengers. We have developed a series of fluorescent biosensors to probe the compartmentalized signaling activities in living cells. In this talk, I will present several new fluorescent biosensors that we recently developed; I will then focus on cAMP/PKA and PI3K/Akt/mTORC1 signaling pathways and present studies where we combined genetically encoded fluorescent biosensors, superresolution imaging, targeted biochemical perturbations and mathematical modeling to probe the biochemical activity architecture of the cell.

Effects of Antibiotics on Cell Cycle Progression of Staphylococcus aureus

Mariana PINHO

Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Portugal.

The study of the bacterial cell cycle is an underexplored area in the context of antibiotic discovery. We have used super-resolution microscopy techniques to characterize the cell cycle of the gram-positive bacterial pathogen *Staphylococcus aureus*. *S. aureus* cells spend approximately half of the cell cycle before initiation of septum synthesis, one quarter of the cell cycle undergoing synthesis of the septum, and one quarter of the cell cycle undergoing maturation of the septum, prior to daughter cell separation. Different antibiotics impair cell cycle progression in different stages. I will describe the effect of an inhibitor of the tubulin homologue FtsZ on cell cycle progression. The recruitment FtsZ to the future division site initiates the assembly of the divisome, a multi-molecular machinery that carries out cytokinesis. The origin for the force required to drive cytokinesis has been a matter of debate, with the two main hypotheses being FtsZ itself or the synthesis of peptidoglycan, the main component of the bacterial cell wall. Our results using an FtsZ inhibitor reconcile these two hypotheses.

Applying Established Concepts of Pharmaceutical Development to Industrialization of Cellular Cancer Immunotherapy

Margit JESCHKE

Novartis, Switzerland.

The potential for cellular immunotherapy as a curative treatment of cancers has been demonstrated by the unprecedented clinical responses in patients with B cell malignancies using autologous T cells genetically modified to express chimeric antigen receptors (CARs) specific for CD19. Tisagenlecleucel (Kymriah) is a personalized immunotherapy approved for the treatment of children and young adults with relapsed/refractory (r/r) B cell acute lymphoblastic leukaemia (B-ALL) in several countries, including EU and USA. This success has encouraged a large number of clinical trials worldwide in various indications and it is assumed that CAR T products may soon complement other biological and chemical therapies. However, inherent heterogeneity and complexity of this living API and lower stability when compared to recombinant proteins impede successful industrialization of autologous cell therapies.

This presentation will describe challenges in the development of cell-based therapies and will highlight differences with respect to classical medicines using the experience made during commercialization of Kymriah.

What Do We Know about Ion Conduction in Ion Channels?

Carmen DOMENE

Department of Chemistry 1, South Building. University of Bath. Bath BA2 7A. United Kingdom.

Contact e-mail: C.Domene@bath.ac.uk
Phone: +441225386172

Transient receptor potential (TRP) ion channels constitute a notable family of cation channels involved in the ability of organisms to detect noxious mechanical, thermal and chemical stimuli that gives rise to the perception of pain. One of the most experimentally studied agonist of TRP channels is capsaicin, which is responsible for the burning sensation produced when chili pepper is in contact with organic tissues. Understanding how TRP channels are regulated by capsaicin and other natural products is essential to high impact pharmacological applications, particularly those related to pain treatment. By selected examples from the work we have carried out, I will provide an overview of the current knowledge we have about activation, permeation and selectivity of one of these human molecular thermometers.

Combining Cheminformatics and Bioinformatics in Drug Discovery Lessons Learned and New Opportunities

Gerard J. P. VAN WESTEN

Computational Drug Discovery, Drug Discovery & Safety, Leiden Academic Centre for Drug Discovery, Leiden University.

Pharmaceutical science is changing the influence and catalytic effect of data science on drug discovery cannot be denied. Yet, history dictates this new development will likely be a synergistic addition to drug discovery rather than a revolutionary replacement of existing methods (like the history of HTS or combichem, a new tool in the toolbox). Moreover, more and more scientific data is becoming public and even open access. Better computing capabilities and more data make it easier to successfully learn from more data.

Central to drug discovery in the public domain is the ChEMBL database which provides literature obtained activity data for a large group of (protein) targets and chemical structures.[1,2] Machine learning can leverage this data to obtain predictive models able to predict the activity probability of untested chemical structures contained within the large collections of chemical vendors on the basis of the chemical similarity principle.[3] However, the relative sparseness and large experimental error in the data contained in ChEMBL can hamper the applicability and reliability of these methods.[4,5]

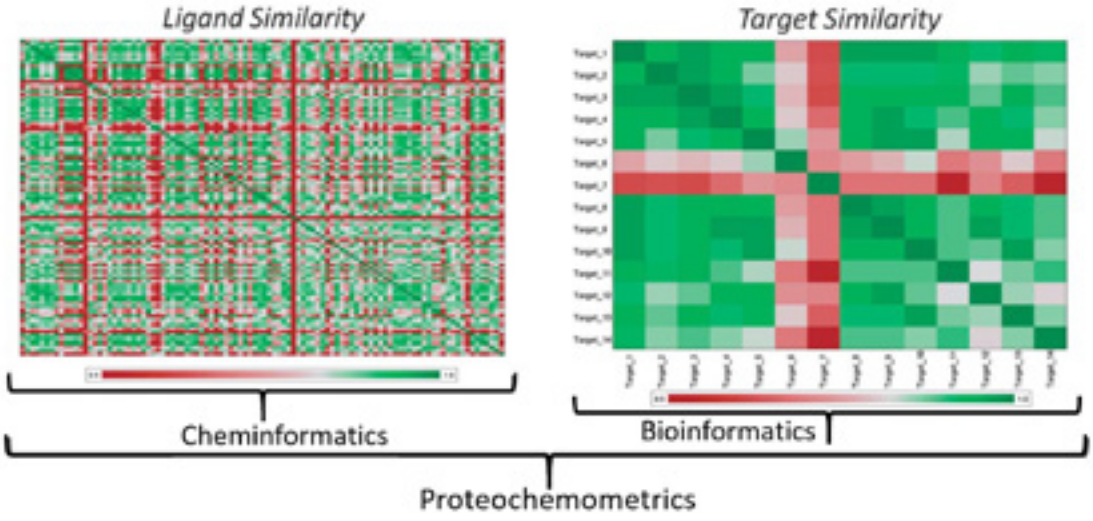


Figure 1: Concept of proteochemometrics, adapted from van Westen et al.[6]

At the turn of the century in Uppsala a novel approach was introduced, deemed proteochemometrics (PCM, **Figure 1**).[7] PCM is able to learn not only from the chemical structures but also includes an explicit protein descriptor that quantifies the protein (binding site) similarity. Hence, PCM combines knowledge from cheminformatics and bioinformatics. In this way PCM can both increase the confidence of model predictions (by combining more data), provide new applications such as target extrapolation (not possible in the absence of a protein descriptor), and even estimate the effect of point mutations on binding.[6,8]

In this talk I will give an overview of PCM and its applications. I will highlight some examples we have published previously and finish with the application of PCM using deep learning,[9,10] a machine learning technique which has proven invaluable in speech and image recognition.

References:

[1] Sun, J., Jeliazkova, N. *et al. J. Cheminf.* (**2017**) 9, 17, doi:10.1186/s13321-017-0203-5.
[2] Gaulton, A., Bellis, L. J. *et al. Nucleic Acids Res.* (**2012**) 40, D1100-D1107, doi:10.1093/nar/gkr777.
[3] Bender, A. & Glen, R. C. *Org. Biomol. Chem.* (**2004**) 2, doi:10.1039/b409813g.
[4] Kalliokoski, T., Kramer, C. *et al. PLoS ONE* (**2013**) 8, e61007, doi:10.1371/journal.pone.0061007.
[5] de la Vega de León, A., Chen, B. *et al. J. Cheminf.* (**2018**) 10, 26, doi:10.1186/s13321-018-0281-z.
[6] Van Westen, G. J. P., Wegner, J. K. *et al. Med. Chem. Commun.* (**2011**) 2, 16-30.
[7] Lapinsh, M., Prusis, P. *et al. Biochim. Biophys. Acta* (**2001**) 1525, 180-190.
[8] Van Westen, G. J. P., Van den Hoven, O. O. *et al. J. Med. Chem.* (**2012**) 55, 7010-7020, doi:10.1021/jm3003069.
[9] Cortés-Ciriano, I., van Westen, G. J. P. *et al. Bioinformatics* (**2016**) 32, 85-95, doi:10.1093/bioinformatics/btv529.
[10] Lenselink, E. B., ten Dijke, N. *et al. J. Cheminf.* (**2017**) 9, 45, doi:10.1186/s13321-017-0232-0.

New Peptides Designs for Gene Delivery, Genetic Edition and the Preparation of Cytoskeleton Mimics

Javier MONTENEGRO

Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostel, 15782, Spain.

Contact e-mail: www.javiermontenegrochemistry.com

Supramolecular Chemistry allows us to understand the interactions that regulate the processes accomplished by living organisms.[1-3] The intracellular transport of biomacromolecules is of fundamental important in fields such as chemical biology, molecular biology, medicine and beyond.[3] The non-covalent complexation between cationic amphiphiles and anionic nucleic acids and proteins constitutes powerful strategy for gene delivery and gene therapy.[3] We apply the synthetic potential of dynamic covalent bonds (i.e. hydrazone) to tune the properties of polymers and peptides and trigger the delivery of biomacromolecules with biological relevance such as siRNA or DNA and functional proteins including Cas9 for gene edition by CRISPR/Cas9.[4,5]

We are also interested in applying supramolecular chemistry for the fabrication of artificial tubular networks as cytoskeleton mimics. We use the self-assembly of robust peptide structures in confined spaces constitute as an excellent synthetic tool for the development of bottom up approaches for minimal cell-like entities in synthetic biology.[5]

Conceptual drawing of amphiphilic vehicles and nucleotide cargos for membrane translocation and cell delivery.

We acknowledge the support from MINECO (CTQ2014-59646-R, SAF2017-89890-R) and the Xunta de Galicia (ED431G/09, ED431C 2017/25 and 2016-AD031), the ERDF, the ERC Starting Grant (DYNAP-677786) and the Human Frontier Science Research Program (RGY0066/2017).

References:

[1] Lostalé-Seijo, I.; Montenegro, J. Synthetic Materials at the Forefront of Gene Delivery. *Nat. Rev. Chem.* **2018**, *2*, 258-277.

[2] Fuertes, A.; Marisa, J.; Granja, J. R.; Montenegro, J. Supramolecular Functional Assemblies: Dynamic Membrane Transporters and Peptide Nanotubular Composites. *Chem. Commun.* **2017**, *53*, 7861-7871.

[3] Gasparini, G.; Bang, E.-K.; Montenegro, J.; Matile, S. Cellular Uptake: Lessons From Supramolecular Organic Chemistry. *Chem. Commun.* **2015**, *51*, 10389-10402.

[4] Louzao, I.; García-Fandiño, R.; Montenegro, J. Hydrazone-Modulated Peptides for Efficient Gene Transfection. *J. Mat. Chem. B* **2017**, *5*, 4426-4434.

[5] Lostalé-Seijo, I.; Louzao, I.; Juanes, M.; Montenegro, J. Peptide/Cas9 Nanostructures for Ribonucleoprotein Cell Membrane Transport and Gene Edition. *Chem. Sci.* **2017**, *13*, doi:10.1039–C7SC03918B.

Perturbation of mitochondrial genome stability with OGG1 inhibitors as a novel strategy to treat cancer

Torkild VISNES[1,2,†], Armando CAZARES-KÖRNER[2], Carlos BENITEZ-BUELGA[2], Bishoy Magdi Fekry HANNA[2], Tove BEKKHUS[2], Saeed ESHTAD[2], Kumar SANJIV[2], Oliver MORTUSEWICZ[2], Olov WALLNER[2], Therese PHAM[2], Camilla GOKTURK[2], Azita RASTI[2], Sharda SUMAN[2], Ulrika WARPMAN BERGLUND[2], Sandra RODRÍGUEZ-PERALES[3], Raúl TORRES-RUIZ[3,4], Juan Miguel BAQUERO[5], Antonio SARNO[6,7], Elisee WIITA[2], Ingrid ALMLÖF[2], Judith Edda UNTERLASS[2], Aleksandra PETTKE[2], Monica PANDEY[2], Patrick HERR[2,9], Christina KALDEREN[2], Javier BENÍTEZ[5,8], Hans E. KROKAN[6,7], Mikael ALTUN[2], Thomas HELLEDAY[2,9,†]

Contact e-mail: Torkild.visnes@sintef.no

1 Department Biotechnology and Nanomedicine, SINTEF Industry, N-7465 Trondheim, Norway.

2 Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institutet, S-171 76 Stockholm, Sweden.

3 Molecular Cytogenetics Group, Human Cancer Genetics Program, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid 28029 Spain.

4 Josep Carreras Leukemia Research Institute and Department of Biomedicine, School of Medicine, University of Barcelona, Barcelona 08036 Spain.

5 Human Genetics Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain.

6 Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

7 The Liaison Committee for Education, Research and Innovation in Central Norway, Trondheim, Norway.

8 Spanish Network on Rare Diseases (CIBERER), Madrid, Spain.

9 Sheffield Cancer Centre, Department of Oncology and Metabolism, University of Sheffield, Sheffield S10 2RX, UK.

Oxidative damage to macromolecules such as DNA is important in the aetiology of many diseases such as inflammation and cancer. The altered oncogene expression in cancer cells causes genomic instability and lost redox homeostasis, causing a high level of damage to macromolecules such as DNA. Pathways for preventing accumulation of oxidative DNA lesions raise the threshold of how much oxidative stress a replicating cell can handle and are therefore promising targets for cancer therapy[1]. Here, we investigate the effect of inhibiting oxidative DNA repair pathways with our recently developed OGG1 inhibitor TH5487[2]. We find the compound to be toxic to a range of human cancer cell lines, but well tolerated by non-transformed lines, thus demonstrating cancer phenotype lethality. Treated cells accumulate DNA damage markers and experience severe S-phase problems, leading to growth inhibition and cell death through the accumulation of oxidative DNA damage specifically in mitochondrial DNA. This study validates OGG1 as a potential novel therapeutic cancer target and exemplifies targeting of cancer phenotypic lethality.

References:

[1] Visnes, T.; Grube, M.; Hanna, B. M. F.; Benitez-Buelga, C.; Cázares-Körner, A.; Helleday, T. Targeting BER Enzymes in Cancer Therapy. *DNA Repair* **2018**, *71*, 118-126. https://doi.org/10.1016/j.dnarep.2018.08.015.

[2] Visnes, T.; Cazares-Körner, A.; Hao, W.; Wallner, O.; Masuyer, G.; Loseva, O.; Mortusewicz, O.; Wiita, E.; Sarno, A.; Manoilov, A.; *et al*. Small-Molecule Inhibitor of OGG1 Suppresses pro-Inflammatory Gene Expression and Inflammation. *Science* **2018**, *362 (in press)*.

Artificial Metalloenzymes: Challenges and Opportunities

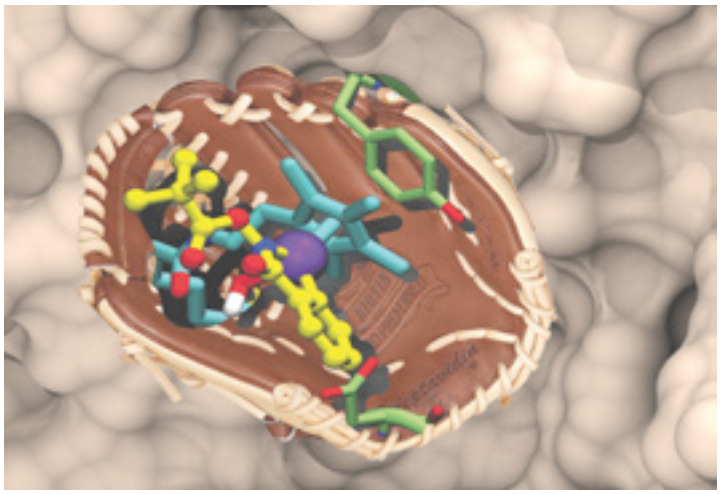
Thomas R. WARD(1)

[1] Department of Chemistry, University of Basel, Mattenstrasse 24a, 4002-Basel, Switzerland.

Contact e-mail: thomas.ward@unibas.ch

Artificial metalloenzymes (ArMs) result from the incorportation of a catalyst precursor within a host protein, see Scheme. The resulting hybrid catalysts display features that are reminiscent of both homogeneous catalysts and enzymes. The optimization of the catalytic performance of ArMs is achieved by combining both chemical- and genetic means. The versatility of this chemo-genetic optimization strategy will be illustrated with selected examples including: transfer-hydrogenation, C–H activation, olefin metathesis, hydroamination etc.[1]

With the aim of integrating artificial metalloenzymes *in vivo*, the second part of the talk will present our efforts to combine ArMs with natural enzymes to mimic essential features of the metabolism including: cascade reactions as well as up- and cross-regulation. Having identified the critical metabolites leading to ArM's inhibition, our efforts towards engineering enzyme cascades *in vivo* will be summarized[2].



Reaction implemented
Hydrogenation
Transfer hydrogenation
Hydroamination
Allylic substitution
Suzuki cross-coupling
Dihydroxylation
Sulfoxidation
Alcohol oxidation
Peroxidation
Olefin metathesis
C–H activation
Cyclopropanation
Michael addition
Enzyme cascades

Scheme. Anchoring a catalyst precursor (ball & stick representation) within a host protein (baseball glove) affords an artificial metalloenzyme. The catalytic performance of the resulting hybrid catalyst can be optimized by chemo-genetic means: variation of the nature and position of the cofactor (turquoise stick representation) and mutation of aminoacid residues (green stick representation).

References:

[1] A. D. Liang, J. Serrano-Plana, R. L. Peterson, T. R. Ward, *Acc. Chem. Res.* **2019**, *asap*, DOI: 10.1021/acs.accounts.8b00618.

[2] M. Jeschek, R. Reuter, T. Heinisch, C. Trindler, J. Klehr, S. Panke, T. R. Ward, *Nature* **2016**, *537*, 661.

Discovery and Development of Antibiotics from Myxobacterial Secondary Metabolites

Rolf MÜLLER

Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research, Department Microbial Natural Products and Department of Pharmacy at Saarland University, University Campus E8 1, 66123 Saarbrücken, Germany.

Amongst the well-established bacterial producers myxobacteria have a great track record for the discovery of entirely new natural product scaffolds exhibiting promsing bioactivities(1). This is at least in part due to the fact that they have been much less studied in the past in comparison to other traditional sources such as actinomycetes and bacilli. Nevertheless, the issue of rediscovery is a major hurdle for myxobacterial extracts as well. I will disucss recent results from our efforts to culture previously uncultured myxobacteria and to connect phylogentically distant clades to novel metabolites by metabolome and genome mining(2). Examples of novel and genetically engineered natural products in preclinical development as broad spectrum antibiotics exhibiting novel mode of action(s) will be shown[3,4,5,6]. In addition, I will show examples of heterologous expression of myxobacterial compounds yielding producer strains making production of lead compounds for pharmaceutical development feasible(7).

References:

[1] 1Herrmann, J., Abou Fayad, A. and Müller R. (2017) Natural products from myxobacteria: novel metabolites and bioactivities, Nat. Prod. Rep., 34 (2):135-160.

[2] Hoffmann, T., Krug, D., Bozkurt, N., Duddela, S., Jansen, R. Garcia, R., Gerth, K., Steinmetz, H. and Müller, R. (2018) Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria, Nat. Commun., 9 (1): 803.

[3] Baumann, S., Herrmann, J., Raju, R., Steinmetz, H., Mohr, K.I., Hüttel, S., Harmrolfs, K., Stadler, M. and Müller, R. (2014) Cystobactamids: myxobacterial topoisomerase inhibitors exhibiting potent antibacterial activity, Angew. Chem. Int. Ed.,53 (52): 14605-14609.

[4] Kling, A., Lukat, P., Almeida, D.V., Bauer, A., Fontaine, E, Sordello, S., Zaburannyi, N., Herrmann, J., Wenzel, S.C., König, C., Ammerman, N.C., Barrio, M.B., Borchers, K., Bordon-Pallier, F., Brönstrup, M., Courtemanche, G., Gerlitz, M., Geslin, M., Hammann, P., Heinz, D.W., Hoffmann, H., Klieber, S., Kohlmann, M., Kurz, M., Lair, C., Matter, H., Nuermberger, E., Sandeep T., Fraisse, L., Grosset, J.H., Lagrange, S. and Müller, R. (2015) Targeting DnaN for tuberculosis therapy using novel griselimycins, Science, 348 (6239): 1106-12.

[5] Lesnik, U., Lukezic, T., Podgorsek, A., Horvat, J., Polak, T., Sala, M., Jenko, B., Harmrolfs, K., Ocampo-Sosa, A., Martinez Martinez, L., Herron, P.R., Fujs, S., Kosec, G., Hunter, I.S., Müller, R. and Petkovic, H. (2015) Construction of a new class of tetracycline lead structures with potent antibacterial activity through biosynthetic engineering, Angew. Chem. Int. Ed., 54 (13): 3937-40.

[6] Hüttel, S., Giambattista, T., Herrmann, J., Planke, T., Gille, F., Moreno, M., Stadler, M., Brönstrup, M., Kirschning, A. and Müller, R. (2017) Discovery and total synthesis of natural cystobactamid derivatives with superior activity against Gram-negative pathogens, Angew. Chem. Int. Ed., 56 (41): 12760-64.

[7] Sucipto, H., Pogorevc, D., Luxenburger, E., Wenzel, S.C. and Müller, R. (2017) Heterologous production of myxobacterial α-pyrone antibiotics in Myxococcus xanthus, Metab. Eng., 44:160-170.

Repurposing Ciclopirox as a Pharmacological Chaperone

Oscar MILLET

Protein Stability and Inherited Disease Laboratory, CIC bioGUNE.

Congenital erythropoietic porphyria (CEP) is a rare genetic disease produced by deleterious mutations in *UROS* gene reducing the activity and stability of uroporphyrinogen III synthase enzyme (UROS), increasing the accumulation of toxic porphyrins; uroporphyrinogen I (UROI) and coproporphyrinogen I (COPROI). In previous studies, we demonstrate that the catalytic activity of UROS can be restored by incorporating residues prone to interact with the hotspot R73. Based on this proof-of-concept we propose a new therapeutic approach based on the use of pharmacological chaperones to intracellularly modulate and increase the kinetic stability of the enzyme. We have screened a library of compounds and some hit compounds stabilize the hostspot C73R *in vitro*, as monitored by circular dichroism (CD) and nuclear magnetic resonance (NMR). Additionally, the intracellular activity for a set of hit compounds was monitored through the analysis of EGFP-tagged version of UROS(C73R) mutant in M1 Fibroblast human cells. The results obtained at HC automated fluorescent microscope suggest that the compounds are able to interact with the protein UROS(C73R). Using the novel CRISPR-Cas9 system, we obtained HEK UROIIIS(C73R) mutant stable cell line and checked compounds selected previously like pharmacological chaperones. We found a molecule that decreases the accumulation of porphyrins by Cytometry and drastically decrease the accumulation of UROI by HPLC. All results together indicate that the molecule can be effectively used as a novel therapeutic intervention line against CEP.

Mechanistic insights into the Function of HSP90 Co-chaperones by cryo-EM

Oscar LLORCA

Structural Biology Programme. CNIO, Spanish National Cancer Research Centre. Madrid, Spain.

Phosphatidylinositol-3-kinase-like kinases (PIKKs) are a family of large protein kinases comprising several members (ATM, ATR, DNA-PKcs, TRAPP, SMG1 and mTOR), with essential roles in variety of cellular functions, such as DNA repair, DNA damage signalling, cell growth and nonsense-mediated mRNA decay. Assembly, cellular stability and activation of PI3-kinase-like kinases (PIKKs) require the action of HSP90 together with a large multi-subunit HSP90 co-chaperone complex made of multiple subunits, including the RUVBL1 and RUVBL2 ATPases. Interestingly, other complexes such as RNA Pol II also require of this chaperone system for assembly, and the emerging view is that this is a specialised machinery for the assembly and stability of several large and complex macromolecular assemblies.

How HSP90 and the RUVBL1-RUVBL2 containing co-chaperone complex work in concert for the assembly and maturation of PIKKs and other complexes is poorly understood. As part of a joint collaboration with the group of Laurence H. Pearl (University of Sussex, UK), we are addressing these questions using cryo-electron microscopy (cryo-EM). We are using cryo-EM to describe and understand flexible complexes and transient conformations of these HSP90 co-chaperones.

Stabilization of 14-3-3 Protein-Protein Interactions

Christian OTTMANN

Eindhoven University of Technology.

Contact e-mail: c.ottmann@tue.nl

Modulation of protein-protein interactions (PPIs) is one of the most promising fields in drug discovery and chemical biology. However, the focus of both academia and industry lies heavily on **inhibition** of PPIs, despite the fact that many natural products like rapamycin, forskolin and brefeldin are **stabilizers** of PPIs. In this lecture, I will advertise the strategy of small-molecule stabilization of PPIs by presenting a number of examples where we have used natural products, semi-synthetic derivatives, and supramolecular ligands to stabilize the interaction of the adapter protein 14-3-3 with partner proteins like C-Raf, TASK3, CFTR, ERα, and Cdc25.

Oral Communications

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

Fluorescent probes for multiparametric investigation of biochemical analytes in cellular models

Jacek L. KOLANOWSKI[(1)(2)]

[1] Department of Molecular Probes and Prodrugs, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland.

[2] Centre for High Throughput Screening Studies, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland.

Contact e-mail: jacek.kolanowski@ibch.poznan.pl

Small molecule responsive probes that change their fluorescence in response to changes in the biochemical environment are key to understanding chemistry in cells. This is, in turn, is crucial in explaining most important questions concerning human health.

Through a combination of expertise in chemical synthesis, bio-analytics and imaging (Figure 1), we have initially developed fluorescent responsive probes to monitor real-time dynamics of oxidative capacity and labile pools of biologically-relevant and therapeutic metal ions in live cells.[1,2] Currently we move from single-analyte to multiple analyte small molecule sensing tools in order to investigate simultaneously several parameters in biological model (aka multiparametric imaging – Figure 2).[3]

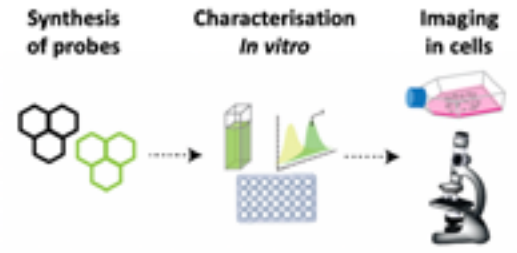


Figure 1. Probe development in our

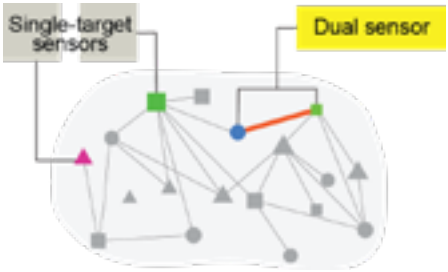


Figure 2. Single vs dual-analyte probes

Such probes are capable of uncovering biological complexity and more reliably visualise interactions between biochemical partners in living systems. We will initially use them to investigate the interplay between oxidative stress and levels of labile Fe(II) ions, to evaluate biochemical heterogeneity of prostate and lung cancer at different stages of disease development. In addition, by combining multiple-analyte probes and robot-operated high throughput screening infrastructure, developed in our Institute, we also aim at more reliable identification of biologically-active small molecules and search of optimal composition of mixtures of potential drugs

These tools and newly-developed assays can also be adapted to other disease models and significantly contribute to basic and preclinical research, promoting a progress of personalised medicine.

Acknowledgements: Financial support from the Foundation for Polish Science (project no. Homing/2017-4/33) and Polish Ministry of Science and Higher Education (the KNOW programme and POL-Openscreen project) is kindly appreciated.

References:

[1] Kaur, A.; Kolanowski, J.L.; New, E.J. *Angew. Chem. Int. Ed.* 2016, 55, 1602.

[2] Kolanowski, J.L., Shen, C.; New, E.J. *Fluorescent Probes for the Analysis of Labile Metals in Brain Cells;* White, A.R. (Ed.); Metals in the Brain; Springer Protocols, 2017.

[3] Kolanowski, J.L.; Liu, F.; New, E.J. *Chem. Soc. Rev.* 2018, 47, 195.

Protocells promote complexity within: self-assembly and condensation

Salvador TOMAS

Department of Biological Sciences and Institute of Structural and Molecular Biology (ISMB).
Birkbeck, University of London. Malet Street, WC1E 7HX, London, UK.

Contact e-mail: s.tomas@bbk.ac.uk

An optimal understanding of the chemistry inside protocel cavities is essential to fully understand abiogenesis, while at the same time will provide tools for the design of artificial protocells, based on alternative chemistries, with functions (such as sensing or drug delivery) programed within their chemical composition. In our lab we have been studying in detail self-assembly and reactivity in the cavity of lipid vesicles as models of protocell. Our studies have demonstrated that both chemical reactivity and molecular self-assembly are strongly influenced by confinement within the bounds of a lipid membrane. We have shown, on the one hand, that hydrolysis reactions are suppressed for vesicle-confined molecules, while their condensation with membrane-permeable building blocks is favored.[1] On the other hand, we have shown that confinement of one building block of a bi-component supramolecular polymer encourages the protocell to incorporate the second building block, which is membrane permeable, whereupon intra-protocell polymeres are formed. These polymers are then stabilized by a confinement effect that is akin to effective molarity for multivalent systems.[2] Overall, we show that the cavity of a protocell becomes a focal point for the accumulation and assembly of complex chemical species, once the initial building blocks have been incorporated. To use a macroscopic analogy, lipid vesicles are like ship-in-a bottle constructs capable of directing the assembly of the confined ship following the confinement of a few key wooden planks (Fig. 1). Therefore, we believe that this confinement effect would have played an important role in shaping the increase of chemical complexity within protocells during the first stages of abiogenesis. Additionally, we argue that this effect can be exploited to design increasingly efficient functional devices based on comparatively simple vesicles for applications in biosensing, nanoreactors and drug delivery vehicles.

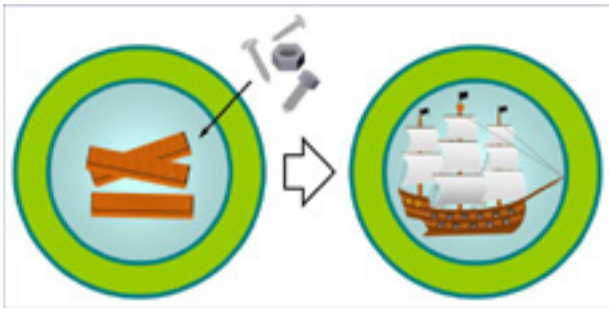


Figure 1. Cartoon representation of a lipid vesicle as ship-in-a-bottle construct directing the assembly of the ship after confinement of key planks

References:

[1] Grochmal, A.; Prout, L.; Makin-Taylor, R.; Prohens, R.; Tomas, S. *J. Am. Chem. Soc*. **2015**, *137*, 12269.

[2] Lopez-Fontal, E.; Grochmal, A.; Foran, T.; Milanesi, L.; Tomas, S. *Chem. Sci.* **2018**, *9*, 1760.

Dual N-acetylglucosamine and N-acetylmuramic peptidoglycan deacetylation. Specificity and biological functions

Laia GRIFOLL-ROMERO, Xevi BIARNÉS, Antoni PLANAS*

Laboratory of Biochemistry, Institut Químic de Sarrià, University Ramon Llull, Via Auguts 390, 08017 Barcelona, Spain.

Contact e-mail: antoni.planas@iqs.edu

The bacterial cell wall peptidoglycan (PG) is an elaborate polymeric mesh composed of a glycan chain of alternating β1,4-linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) units cross-linked via peptidyl bridges attached to the 3-*O*-lactoyl group of MurNAc residues. Pathogenic bacteria utilize acetylation (6-*O*-acetylation of MurNAc) and deacetylation (2-*N*-deacetylation of GlcNAc and/or MurNAc residues) of their cell wall PG to evade detection by the innate immune system. Deacetylation of MurNAc residues of PG is also involved in endospore formation and germination.

Peptidogycan deacetylases are members of family 4 carbohydrate esterases (CE4 enzymes) which operate by a metal-assisted general acid/base catalytic mechanism[1]. A number of PG GlcNAc deacetylases have been characterized and their 3D structures determined by X-ray crystallography. They are specific for GlcNAc residues in the PG chain but they have also shown to deacetylate chitooligosaccharides, homo-oligomers of GlcNAc residues. In contrast, few PG MurNAc deaceylases have been biochemically characterized. The *B. subtilis* PdaA deacetylates MurNAc residues of peptidoglycan devoid of the peptide linked to the muramic acid 3-*O*-lactoyl group of MurNAc residues, which is consistent with its function during sporulation to form muramic acid δ-lactam residues in the spore cortex peptidoglycan. It is specific for MurNAc residues and it is not active on chitooligosaccharides. Only the 3D structure of two PG MurNAc deacetylases, *Bs*PdaA and *Ba*CE4, have been determined by X-ray crystallography, revealing a modified $(\beta/\alpha)_8$ fold characteristic of CE4 enzymes, but lacking the otherwise conserved Asp residue of the metal binding catalytic triad. Kobayashi *et al*.[2] have recently identified a novel peptidoglycan MurNAc deacetylase, *Bs*PdaC, which deacetylates MurNAc residues of peptidoglycan, it is inactive on the glycan backbone devoid of peptidyl substitutions and, more strikingly, it also deacetylates chitooligosaccharides, an activity that was thought to be restricted to GlcNAc deacetylases.

To understand the molecular bases of such dual activity, we here report the biochemical characterization of *Bs*PdaC, its mode of action on chitooligosaccharide substrates, the X-ray 3D structure of the CE4 catalytic domain, and structural comparison with canonical MurNAc deacetylases[3]. We propose that PdaC is the first member of a new subclass of peptidoglycan MurNAc deacetylases based on these differential functional and structural characteristics.

Acknowledgements: Work supported by grant BFU2016-77427-C2-1-R from MINECO and AGAUR grant 2017SGR-727 from the Generalitat de Catalunya.

References:

[1] H.Aragunde, *et al*. (2018) Substrate recognition and specificity of chitin deacetylases and related family 4 carbohydrate esterases. *Int. J. Mol. Sci.* **19**, 412.

[2] Kobayashi, K., *et al*.. (2012) Identification and characterization of a novel polysaccharide deacetylase C (PdaC) from *Bacillus subtilis*. *J. Biol. Chem.* **287**, 9765-9776.

[3] L.Grifoll-Romero, *et al*. Structure-function relationships underlying the dual N-acetylmuramic and N-acetylglucosamine specificities of the peptidoglycan deacetylase PdaC from *Bacillus subtilis.* Submitted

Diversity and Design: New Chemical Probes for Biology and Medicine

Derek S. TAN

Memorial Sloan Kettering Cancer Center. 1275 York Ave., Box 422, New York, NY 10065.
http://www.mskcc.org/tan

Contact e-mail: tand@mskcc.org.de

Existing small-molecule drugs address only a small set of ≈550 protein targets that are encoded in the human genome. Efforts to expand beyond this narrow range of biological targets have been thwarted by a heavy focus on a correspondingly narrow range of complementary chemical structures in modern drug discovery. To address this problem, we leverage insights from natural products in both diversity-oriented synthesis and rational drug design to identify novel small-molecule ligands for a variety of targets in infectious diseases and cancer. In the area of diversity-oriented synthesis, we use structural motifs found in biologically active natural products as attractive starting points for library design. At the core of these efforts is the development of new synthetic routes to provide flexible, efficient, systematic access to these structures. Along these lines, we have developed syntheses of spiroketals, polyketides, alkaloids, macrocycles, medium rings, and oxygen heterocycles having stereochemical and skeletal diversity. These libraries access distinct regions of chemical space compared to conventional drug-like libraries and are being screened against a wide range of biological targets. In the area of rational drug design, we are engaged in complementary efforts to design natural product-based sulfonyladenosine inhibitors of adenylation enzymes, a mechanistic superfamily that is implicated in a wide range of biological processes and includes promising new antibacterial and anticancer targets. Leveraging structural and mechanistic information about individual targets of interest, we have developed new antibiotic lead compounds that inhibit biosynthetic enzymes required for virulence of pathogenic bacteria, including *Mycobacterium tuberculosis*. In related work, we are developing a general platform to assess small-molecule permeability in bacteria and correlations with physicochemical properties in an effort to address this major obstacle in antibiotic drug development. We have also designed semisynthetic protein inhibitors of enzymes in the ubiquitin conjugation cascade to probe active site remodeling during catalysis. We leverage multidisciplinary collaborations with biologists to evaluate the molecules we synthesize with the long-term goals of probing complex biological processes and pursuing new therapeutic opportunities in cancer and infectious diseases.

Access Models for EU-OPENSCREEN ERIC

Bahne STECHMANN

[1] EU-OPENSCREEN ERIC, Robert-Rössle-Str. 10, 13125 Berlin, Germany.

Contact e-mail: bahne.stechmann@eu-openscreen.eu

The European research infrastructure EU-OPENSCREEN (www.eu-openscreen.eu) was founded in April 2018 with the aim to support projects in early drug discovery. It offers complementary expertise and instrumentation in the field of chemical biology through its 20 partner institutes across Europe and its open-access operational model ensures that researchers from academia and industry can readily access EU-OPENSCREEN´s collection of chemical compounds, equipment and associated screening data. During my talk, I will outlined how biologists and chemists in Europe and beyond can use the compound collection, open-access database, as well as high-throughput screening and medicinal chemistry capabilities of EU-OPENSCREEN to identify novel chemical tool compounds.

Acknowledgements: EU-OPENSCREEN receives funding from the EU Horizon 2020 Research and Innovation programme under grant agreement No 654248, 823893, 823798, 654008, 824063 and 824087.

References:

[1] Brennecke P *et al*., *SLAS Discov.* ***2019*** *Jan 7:2472555218816276.*

Probe Miner V2: Updated objective data-driven assessment of chemical probes

Albert A. ANTOLÍN[1,2], Christos KANNAS[1], Joe E. TYM[1], Angeliki KOMIANOU[1], Ian COLLNS[1], Paul WORKMAN[1], Bissan AL-LAZIKANI[1,2]

[1] Cancer Research UK Cancer Therapeutics Unit, Division of Cancer Therapeutics.

[2] Division of Data Science, The Institute of Cancer Research, London, UK.

Contact e-mail: Albert.Antolin@icr.ac.uk

Chemical probes are important widely-used reagents in chemical biology for understanding biological systems and for target validation. However, selection of chemical probes is largely subjective and prone to historical and commercial biases. Moreover, scientists often select probes through web-based searchers or previous literature that are heavily biased towards older and often flawed probes our use vendor catalogues that do not discriminate between probes.[1]

In 2018 we developed the Probe Miner resource (http://probeminer.icr.ac.uk) capitalising on the plethora of public pharmacological data to enable quantitative, unbiased, objective, data-driven assessment of chemical probes and complement expert-curated approaches.[2] Despite demonstrating that large-scale public data can contribute to improve chemical probe assessment and prioritization, we also identified that many chemical probes were not available in public medicinal chemistry databases.[2]

Here, we present an updated version of Probe Miner, where we assess >1.9m compounds for their suitability as chemical tools against >2,300 human targets. Moreover, we also present progress towards developing an improved user interface and a better linkage with The Chemical Probes Portal. Importantly, the main advancement of the new version of Probe Miner is a new dataset of chemical probe bioactivity data curated from publications that are not available in public medicinal chemistry databases, demonstrating the importance of data availability to empower researchers in the selection of chemical tools for biomedical research and target validation.

Figure 1. Overview page of the Probe Miner resource where chemical tools can be assessed objectively using publicly available data.

References:

[1] Blagg, J.; Workman, P. *Cancer Cell* **2017**, *32*, 9.

[2] Antolin, AA. *et al., Cell Chemical Biology* **2018** 25, 194.

Smart screening libraries over the past 20 years and success stories in medicinal chemistry

Marie-Louise JUNG

Prestwick Chemical 220 boulevard Gonthier d'Andernach 67400 Illkirch – France.

Contact e-mail: marielouise.jung@prestwickchemical.com

Prestwick Chemical, created 1999 as a spin-off from the University of Strasbourg has a worldwide recognition for providing smart screening libraries, whereof the Prestwick Chemical Library®, made of drugs out of patent is the flagship. Numerous publications mention this Library. Examples of repositioned drugs into various new applications will be presented [1, 2, 3]. Other libraries, including a recent Fragment collection, are reported for their high interest in various research fields in academic labs & institutions and industry worldwide.

Once screening is performed, Prestwick is considering hit analysis, classification into chemical families and analog search. First steps of SAR are therefore tackled.

Prestwick has as well an impressive track record of compounds designed and prepared in-house which reached clinical phase. Currently, 10 molecules are developed in the clinic (phase I to phase III), coming out of our lead optimization work. One is on the market in oncology.

A collaborative project between Prestwick & biotech & academic lab will be presented, which led to a CNS compound currently in clinical phase II.

References:

[1] Gaud, A.; Simon, JM.; Witzel, T.; Carre-Pierrata, M.; Wermuth, CG.; Segalat, L. *Neuromuscul. Disord.* **2004,** *14,* 365.

[2] Tribouillard-Tanvier, D.; Béringue, V.; Desban, N.; Gug, F.; Bach, S.; Voisset, C.; Galons, H.; Laude, H.; Vilette, D.; Blondel, L. *PLoS ONE,* **2008**, *3,* e1981.

[3] Corbel, C.; Zhang, B.; Le Parc, A.; Baratte, B.; Colas, P.; Couturier, C.; Kosik, KS.; Landrieu, I.; Le Tilly, V.; Bach, S. *Chemistry & Biology,* **2015***, 22*, 472.

Validation of a high content assay to identify DNAJB6 agonists

Bernhard ELLINGER[(1)], Ebru YURTSEVEN[(1)], Joanna JENSEN[(1)], Ole PLESS[(1)], Abhi PANDEY[(2)], Steven BERGINK[(2)], Harm H. KAMPINGA[(2)]

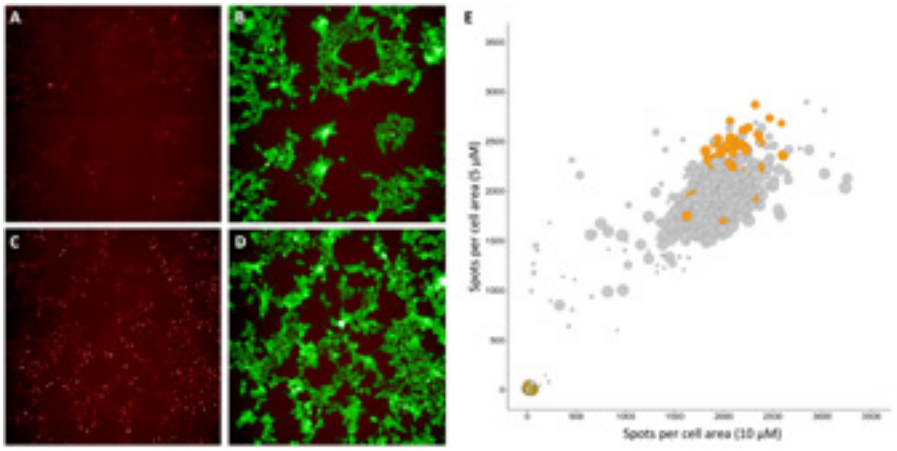
[1] Branch Lab ScreeningPort of the Fraunhofer IME, Schnackenburgallee 114, 22525 Hamburg, Germany.

[2] University Medical Center Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.

Contact e-mail: Bernhard.Ellinger@ime.fraunhofer.de

The DNAJ protein family are co-chaperones of the HSP70 molecular chaperones and crucial for protein quality control. They are very potent suppressors of aggregation-related toxicity of polyglutamine proteins and modulation of the cellular heat shock response can affect both the onset of protein folding diseases and the aging process.

We therefore aimed at identifying agonist of DNAJB6, a chaperone especially relevant in the context of Huntingtons disease, by using a cellular model based on aggregation of RFP-tagged version of exon-1 of the *HTT* gene with 119 CAG repeats[1].



Screening results of the LOPAC library. A, B) Active compound at 10 µM. C, D) Untreated control. A, C) RFP-tagged protein aggregates, B, D) Overlay including phase contrast in green. E) Comparison of activity at 5 µM and 10 µM, viability indicated as point size (strong impact on viability –small data point; weak impact on viability – large data point).

We first screened the 1280 compounds containing LOPAC library as part of our assay development. As seen in the figure, we were able to identify active compounds, which reduced the aggregation of polyglutamine marker protein, while retaining cellular vitality. These compounds were active in a concentration dependent manner (E). The parameters obtained were used for screening of a 100,000 compound containing diverse library. We were able to identify a number of hit molecules, which we followed up further.

References:

[1] Hageman, J.; Rujano, M.A.; van Waarde M.A.W.H., Kakkar V.; Dirks, R.P.; Govorukhina, N.; Oosterveld-Hut, H.M.J.; Lubsen N.H.; Kampinga H.H. *Molecular Cell* **2010**, *37*, 355-369.

A Novel Natural Product for Pancreatic Cancer

Francisca VICENTE, Carmen RAMOS, Bastien CAUTAIN, Nuria DE PEDRO, Víctor GONZÁLEZ-MENÉNDEZ, Gloria CRESPO, Lorena RODRÍGUEZ, Caridad DÍAZ, José PÉREZ-PALACIO, Mercedes DE LA CRUZ, Fernando REYES, Olga GENILLOUD

Fundación MEDINA, Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía. Avda. Del Conocimiento, 34. Edif. Centro de Desarrollo Farmacéutico. Parque Tecnológico de Ciencias de la Salud. 18016 Granada, Spain.

Contact e-mail: francisca.vicente@medinaandalucia.es

Natural Products (NP) have been extensively exploited for medicinal purposes since ancient times. Recent technological advances for the rapid identification and structure elucidation of novel bioactive NPs have opened a new era and greatly improved the NP discovery process.

In the recent years, drug discovery efforts in Pancreatic Cancer (PC) have been directed to new chemotherapeutic agents directed to novel targets that could become a novel approach for prevention and treatment. The MAPK/ERK pathway is involved in the regulation of normal cell proliferation, survival and differentiation and its aberrant regulation contributes to cancer. MAPK/ERK pathway has been the subject of intense research scrutiny leading to the development of pharmacological inhibitors for the treatment of cancer. Since constitutively active MEK1/2 and ERK1/2 proteins are present in high number of pancreatic tumors, new and safe inhibitors of this pathway represent both a great therapeutic opportunity and a tremendous challenge for cancer therapy.

In this context, MEDINA has discovered the MAPK/ERK pathway inhibitor MDN-0090, a new natural product small molecule of microbial origin, with effect on pancreatic tumors both in *in vitro* and *in vivo* primary studies. MDN-0090 presents specific and highly cytotoxic effect in pancreatic tumor cell lines that has been confirmed in pancreatic cancer stem cells. Additionally, MDN-0090 shows synergistic effects with gemcitabine (first-line therapy) and selumetinib (experimental drug) *in vitro*. MDN-0090 has been shown to determine a decrease of active ERK1/2 by western blot confirmed by AlphaLisa, suggesting the mode of action of the compound. The compound has acceptable drug-like physicochemical properties and no cardiotoxicity *in vitro.* Furthermore, it has shown *in vivo* effect in subcutaneous tumor models with human pancreatic tumor cell lines, inhibiting the tumor development, and in pancreatic cancer stem cells, inhibiting the tumor initiation. The compound can be obtained via microbial fermentation and its *de novo* chemical synthesis is currently being explored.

Computer Design and Directed Evolution of a Highly Active and Enantioselective Metallo-Hetero-Diels-Alderase

Sophie BASLER(1), Sabine STUDER(1), K. N. HOUK(2), Donald HILVERT(1), Gonzalo JIMÉNEZ OSÉS(3)

1 Laboratory of Organic Chemistry, ETH Zurich, 8093 Zurich, Switzerland.

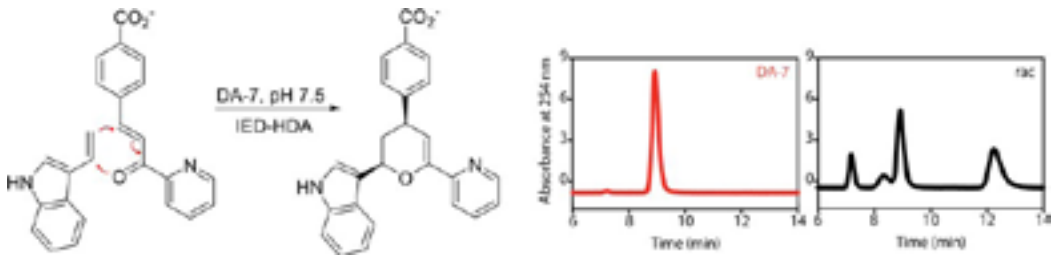
2 Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095-1569, UnitedStates.

3 Departamento de Química, Centro de Investigación en Síntesis Química, Universidad de La Rioja, 26006 Logroño, Spain.

Contact e-mail: gonzalo.jimenez@unirioja.es

Enzymes are highly efficient, selective, and biodegradable catalysts that can be synthesized in large amounts by microorganisms. The ability to design functional biocatalysts that can operate under varying conditions to perform chemical reactions not found in Nature, and which can be used for the large-scale productions of chemicals, would revolutionize synthetic and materials chemistry.[1]Several research groups have made notable advances in this highly multidisciplinary research area, and have demonstrated that design of enzymes for unnatural reactions is feasible.[2]Computer simulations based on quantum mechanics, Newtonian molecular mechanics and molecular dynamics principles are at the core of many enzyme design strategies, and have demonstrated superior performance for creating new types of catalysis with respect to random mutagenesis. On the other hand, computer enzyme design combined with Directed Evolution has been demonstrated to be a very powerful and successful strategies to discover enzymes for unnatural reactions with catalytic efficiencies comparable to the natural ones, as reported in many examples from our labs.[3]

In this work, we present the Computer-Aided Design and Directed Evolution of an artificial Zn^{2+}-based metalloenzyme able to catalyze the inverse electronic demand hetero-Diels Alder (IED-HDA) reaction between a 3-vinylindole and an aza-chalcone, a reaction that is not catalyzed by any natural biocatalyst. After several rounds of laboratory evolution starting from the initial computational design, the engineered metalloenzyme catalyzed the reaction with complete regio-, diastereo- and enantioselectivity, and with reaction rates superior to previously reported catalytic antibodies and to any natural diels-alderase known to date.



Acknowledgements: MINECO (projects CTQ2015-70524-R and RYC-2013-14706 to G.J.O.).

References:

[1] Kiss, G.; Çelebi-Ölçüm, N.; Moretti, R.; Baker, D.; Houk, K. N. *Angew. Chem. Int. Ed.* **2013**, **52**, 5700-5725.

[2] Jiang, L.; Althoff, E. A.; Clemente, F. R.; Doyle, L.; Rothlisberger, D.; Zanghellini, A.; Gallaher, J. L.; Betker, J. L.; Tanaka, F.; Barbas, C. F., III; Hilvert, D.; Houk, K. N.; Stoddard, B. L.; Baker, D. *Science* **2008**, *319*,1387-1391.

[3] Blomberg, R.; Kries, H.; Pinkas, D. M.; Mittl, P. R.; Grütter, M. G.; Privett, H. K.; Mayo, S. L.; Hilvert, D. *Nature* **2013**, *503*,418-421.

Paving the way towards the brain delivery of biotherapeutics: Modification of proteins with blood-brain barrier peptide shuttles

Macarena SÁNCHEZ NAVARRO,[1] Cristina DÍAZ-PERLAS, Meritxell TEIXIDÓ[1], Ernest GIRALT[1][2]

[1] Institute for Research in Biomedicine (IRB Barcelona), 08028 Barcelona, Spain.

[2] Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain.

Contact e-mail: macarena.sanchez@irbbarcelona.org

The number of biotherapeutics has increased dramatically since FDA approval of insulin in 1982. Among them, monoclonal antibodies or enzymes are candidates to treat several diseases, including those affecting the central nervous system (CNS). However, their use is mainly restricted by the presence of the blood-brain barrier (BBB). This highly metabolic, transport, and physiologic barrier protects the brain parenchyma from the passage of undesired molecules but limits the targeted delivery of pharmacological agents.

BBB peptide shuttles are compounds able to cross efficiently the BBB and to increase the transport of compounds linked to them. In this work, we propose the modification of given proteins with various protease resistant BBB-shuttles in order to increase its BBB penetration. We have used various chemical methods that allow the attachment of a controlled average number of peptides to different parts of the selected proteins. Each of the conjugates have been fully characterised, including number of shuttles and protein function.

The *in vitro* transport properties of the protein constructs has been evaluated in a human BBB cell-based model comprised of human endothelial cells derived from induced pluripotent stem cells and pericytes. Interestingly, improved transport has been obtained when GFP was modified with a venom derived BBB-shuttle, MiniAp-4, and with a branched version of THRre. In addition, this strategy has been applied to three different monoclonal antibodies, broadening its application.

The obtained results suggest that protein modification with BBB-shuttle may provide with an efficient method to promote protein delivery to the CNS.

References:

[1] Oller-Salvia, B.; Sánchez-Navarro, M; Giralt, E.; Teixidó, M. *Chem. Soc. Rev.*, **2016**, *45*, 4690.

[2] Oller-Salvia, B.; Sánchez-Navarro, M; Ciudad, S.; Guiu, M.; Arranz-Gibert, P.; Garcia, C.; Gomis, R.; Cecchelli, R.; García, J.; Giralt, E.; Teixidó, M. *Angew. Chem., Int. Ed.*, **2016**, *55*, 572-575.

[3] Díaz-Perlas, C.; Oller-Salvia, B.; Sánchez-Navarro, M; Giralt, E.; Teixidó, M., *Chem. Sci*., **2018**, *in press.*

Synthetic small-molecule RNA ligands: scope and applications

Duc Duy VO[(1)], Cathy STAEDEL[(2)], Thi Phuong Anh TRAN[(1)], Fabien DARFEUILLE[(2)], Audrey DI GIORGIO[(1)], Maria DUCA[(1)]

[1] Institute of Chemistry of Nice, Université Côte d'Azur, CNRS, Parc Valrose 06100 Nice, France.

[2] ARNA Laboratory, University of Bordeaux, 36 rue Léo Saignat, 33305 Bordeaux, France.

Contact e-mail: maria.duca@univ-cotedazur.fr

MicroRNAs (miRNAs) are a recently discovered category of small RNA molecules that regulate gene expression at the post-transcriptional level. Accumulating evidence indicates that miRNAs are aberrantly expressed in a variety of human cancers, thus being oncogenic and that the inhibition of oncogenic miRNAs (defined as the blocking of miRNAs' production or function) would find application in the therapy of different types of cancer in which these miRNAs are implicated (1).

Our work aims at the development of small-molecule drugs targeting specific oncogenic miRNAs production as illustrated in (2). Toward this aim, we perform both the synthesis of new RNA ligands and the screening of compounds libraries. Both approaches are based on a high throughput *in vitro* assays and demonstrated to be successful in identifying compounds able to interfere with the biogenesis of oncogenic miRNAs in a selective manner at the intracellular level. Thanks to these works, we demonstrated that it is possible to inhibit miRNAs production using synthetic small molecules and that this kind of approach could be applied in future anticancer therapies. Noteworthy, these RNA ligands could find a new original application in the induction of the differentiation of cancer stem cells (CSCs) upon modifying the miRNA network of these cells. This was validated in the case of glioblastoma, an incurable brain cancer, where glioblastoma stem cells are responsible for therapeutic failure. The chemical tools developed in these different projects could thus find extremely important applications as chemical biology tools for the improvement of our understanding of miRNAs biological pathways.

References:

[1] Ling H., Fabbri M., Calin, G.A. *Nature Rev. Drug. Disc.* **2013** 12, 847; Velagapudi S.P., Vummidi B.R., Disney M.D. Curr. Op. Chem. Biol. 2015 24,97.

[2] Staedel, C., Tran, T.P.A., Giraud, J., Darfeuille, F., Di Giorgio, A., Tourasse, N.J., Salin, F., Uriac, P., Duca, M.*Sci. Rep.***2018** *8*,1667;Vo, D.D., Becquart, C., Tran, T.P.A., Staedel, C., Darfeuille, F., Di Giorgio, A., Duca, M. *Org. Biomol. Chem.* **2018** 16, 6262; Vo, D.D., Tran, T.P.A. Staedel, C., Benhida, R., Darfeuille, F., Di Giorgio, A., Duca, M. *Chem. Eur. J.* **2016** *22*, 5350; Tran T.P.A., Vo D.D., Di Giorgio A., Duca M. *Bioorg. Med. Chem.* **2015** *23*, 5344; Vo D.D., Staedel C., Zehnacker L., Benhida R., Darfeuille F., Duca M. *ACS Chem. Biol.* **2014** *9*,711.

Optochemical Tools for Biological Processes

Benedikt HEINRICH, Lea ALBERT, Greta LINDEN, Lei ZHANG, Olalla VÁZQUEZ*

Department of Chemical Biology, Faculty in Chemistry, Philipps-Universität Marburg, Hans-Meerwein-Straße 4, 35043 Marburg, Germany.

Contact e-mail: olalla.vazquez@staff.uni-marburg.de

For a chemical biologist, light is a unique tool since it is orthogonal towards most elements of living systems, non-invasive allow us to address the questions where, when and to what extend a process is started or stopped devising spatiotemporal approaches to understand the underlying concepts of biological processes. Therefore, it is not surprising that photosensitive molecules have such a big impact and are continuously used in chemical biology[1].

Herein, we will discuss the recent developments of our young group in the use of light-sensitive molecules as chemical biology tools. In particular, we will focused on three different types of photosensitive molecules and our most recent results:

Fluorophores; we have currently reported a novel far-red fluorescent DNA binder: 6-TramTO-3, which constitutes an off-the-shelf reagent for real-time analysis of bacterial infection, including multidrug-resistant pathogens, for which the use of genetically encoded reporters is not feasible[2].

Photoswitches; we have recently described a cell-permeable photoswitchable peptidomimetic capable of modulating epigenetic cellular states by disruption of an essential protein–protein interaction within the MLL1 methyltransferase core complex[3].

Photosensitizers; we have just achieved both site-specific and conditional singlet oxygen generation simultaneously.

References:

[1] Ankenbruck, N.; Courtney, T.; Naro, Y.; Deiters, A. *Angew. Chem. Int. Ed.* **2018**, *57*, 2768.

[2] Schulte, L.N.; Heinrich, B.; Janga, H; Schmeck, B.T.; Vázquez, O. *Angew. Chem. Int. Ed.* **2018**, *36*, 11564.

[3] Albert, L.; Xu, J.; Wan, R.; Srinivasan V.; Dou, Y.; Vázquez, O. *Chem. Sci.* **2017**, *8*, 4612.

Therapeutic potential of blood glutamate grabbing in stroke: a proof of concept

José BREA[(2a)], Francisco CAMPOS[(1a)], Amparo PÉREZ-DÍAZ[(2)], Emiliano CUADRADO[(2)], Ramón IGLESIAS-REY[(1)], Andrés DA SILVA-CANDAL[(1)], Tomás SOBRINO[(1)], José CASTILLO[(*1)], María Isabel LOZA[(*2)]

[1] Department of Neurology, Clinical Neurosciences Research Laboratory, Hospital Clínico Universitario, Health Research Institute of Santiago de Compostela (IDIS), University of Santiago de Compostela, Santiago de Compostela, Spain.

[2] Innopharma pharmacogenomics platform. BioFarma Research Group, Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela (USC). Avenida de Barcelona, s/n, 15782 Santiago de Compostela, Spain.

Contact e-mail: pepo.brea@usc.es

a: These authors equally contributed to this work
*: Corresponding authors

A rapid elevation of glutamate into the extracellular space after ischemic strike induces brain edema and inflammation leading to neuronal death, increase in infarct volume and bad prognosis. In previous studies in human patients we observed that plasma glutamate levels correlate with brain edema after ischemic stroke. Therefore, blood glutamate scavenging has becoming a novel translational strategy validated by the Clinical Neurosciences Research Laboratory with successful protective effects during acute phase of ischemic stroke.

With the aim of identifying low molecular weight drugs acting as glutamate scavengers, we developed a miniaturized in vitro assay for measuring Glutamate-Oxaloacetate-Transaminase (GOT) activity, as well as, an off-target screening measuring succinate-dehydrogenase activity. Prestwick® chemical library was screened finding 6 hits that reduced glutamate levels over a 30%, being riboflavin the safer glutamate grabbing agent in vitro. Riboflavin showed a synergistic effect with low concentrations of oxaloacetate with a higher efficiency in decreasing glutamate concentration. In vivo analysis showed that 1mg/kg of riboflavin in ischemic animals induced a significant lowering of blood glutamate levels and a reduction of infarct volume at 7 days after ischemia.

A clinical proof of concept was carried out to clinically evaluate the novel mechanism in stroke. Acute (i.v.) administration of riboflavin to 25 ischemic patients in the first 4 hours after ischemic stroke led to a significant reduction of glutamate plasmatic levels after 6 hours of drug administration. These results validate the translation of both in vitro and in vivo results to decreasing human blood levels of glutamate.

Deciphering the mechanism of action of toxins delivered by the Type-VI Secretion System (T6SS) in *Pseudomonas aeruginosa*

María Ángela SAINZ-POLO[1], Laura M. NOLAN[3], Amy K. CAIN[4], Eleni MANOLI[3], Gordon DOUGAN[4], Julian PARKHILL[4], Alain FILLOUX[3], David ALBESA-JOVÉ[1,2]

[1] Structural Biology Unit, CIC bioGUNE, Bizkaia Technology Park, 48160 Derio, Spain.

[2] IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

[3] MRC Centre for Molecular Bacteriology and Infection (CMBI), Department of Life Sciences, Imperial College London, London SW7 2AZ, United Kingdom.

[4] Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

Contact e-mail: dalbesa@cicbiogune.es

Almost a million people worldwide die each year as a result of bacterial infections resistant to antibiotic treatment, a number which is predicted to rise tenfold by 2050. To fight the rapid rise of antimicrobial resistance, the scientific community is making a constant effort to understand the molecular mechanisms underlying microbial resistance.

Along these lines of research, we investigate *Pseudomonas aeruginosa*, a multidrug resistant pathogen, which has acquired sophisticated antibiotic resistance mechanisms. Importantly, this microorganism is the third most commonly isolated nosocomial pathogen and is lethal to patients with Cystic Fibrosis. In a setting of polymicrobial infections, this pathogen outcompetes other bacteria by using a Type-VI Secretion System (T6SS) to deliver toxic effectors into neighbouring bacteria or host cells. Thus, the T6SS provides a fitness advantage that allows the bacteria to thrive and facilitates host colonization[1,2]

Very recently, we have identified novel toxins delivered by the H1-T6SS in *P. aeruginosa* by a Transposon Directed Insertion Sequencing (TraDIS) approach (*unpublished results*). Based on these results, we have selected for further studies one of these toxins, which we have named Tse8 (type six exported 8). We have identified a mechanism of chemical inhibition and deciphered the 3D molecular structure of Tse8 at 1.9 Å resolution, showing it belongs to the Amidase Signature (AS) superfamily; enzymes in the family catalyse the hydrolysis of amide bonds (CO–NH2), although the family has diverged widely with regard to substrate specificity and function. Tse8 shares the canonical AS sequence motif found in the family members that consist of *ca.* 130 residues containing a region rich in serine and glycine residues and a unique, highly conserved, Lys84 – Ser162 – Ser186 catalytic triad used for amide hydrolysis.

References:

[1] Hachani, A.; Lossi, N. S.; Hamilton, A.; Jones, C.; Bleves, S.; Albesa-Jové, D.; Filloux, A. *J. Biol. Chem.* **2011**, *286* (14), 12317-12327.

[2] Planamente, S.; Salih, O.; Manoli, E.; Albesa-Jové, D.; Freemont, P. S.; Filloux, A. *EMBO J.* **2016**, *35* (15), 1613-1627.

Plasmonic ZIF8 metal-organic frameworks (MOFs) with self-assembled proteins as optical probes for imaging cell surface receptors

Gustavo BODELÓN, Sarah DE MARCHI-LOURENÇO, Lorena VÁZQUEZ-IGLESIAS, Jorge PÉREZ-JUSTE, Isabel PASTORIZA-SANTOS

Department of Physical Chemistry and Biomedical Research Centre (CINBIO), Universidade de Vigo, 36310 Vigo, Spain.

Contact e-mail: gbodelon@uvigo.es

Hybrid nanostructures composed of plasmonic metal nanoparticles and metal-organic frameworks (MOFs) are emerging tools for bioimaging applications due to the combination of optical properties of nanometals with the large internal surface area, tunable crystal porosity and unique chemical properties of MOFs. In this study we present MOF-based optical nanoprobes formed by a plasmonic core and a zeolitic imidazolate framework (ZIF-8) shell encoded with Raman active molecules. The surface of ZIF-8 nanocrystals allows the conjugation of recombinant poly-histidine-tagged proteins by means of the interaction of unsaturated Zn^{2+} and imidazole moiety of histidines. This strategy enabled the coordinative self-assembly of poly-histidine-tagged protein-G, as well as Spy-Catcher, used as adaptors to functionalize the optical nanoprobes with antibodies and Spy-tagged nanobodies, respectively. The targeting and imaging capabilities of the hybrid plasmonic nanocomposites towards cell receptors was assessed *in vitro* by means of surface-enhanced Raman scattering (SERS) spectroscopy.

New tool compounds for studying autophagy

Luca LARAIA[1],[2], Herbert WALDMANN[2]

[1] Department of Chemistry, Technical University of Denmark, Kemitorvet 207, 2800 Kgs. Lyngby, Denmark.

[2] Max Planck Institute of Molecular Physiology, Otto-Hahn-Str. 11, 44227 Dortmund, Germany.

Contact e-mail: luclar@kemi.dtu.dk

Autophagy is a conserved cellular nutrient recycling process that is responsible for the degradation of unnecessary proteins, organelles and pathogens. It is often upregulated in cancer to promote tumour survival and growth. As such, autophagy inhibitors are in high demand. Using high-content, image-based screening of a large compound collection, we identified several new autophagy inhibitor chemotypes (Figure 1). Target identification using chemical proteomics, morphological profiling and image-based proteome analysis revealed new inhibitors of the lipid kinase VPS34, mitochondrial complex 1, and the previously uncharacterized cholesterol transfer protein GRAMD1A. Extensive validation was carried out for all compounds and newly identified targets resulting in excellent tools to study autophagy.

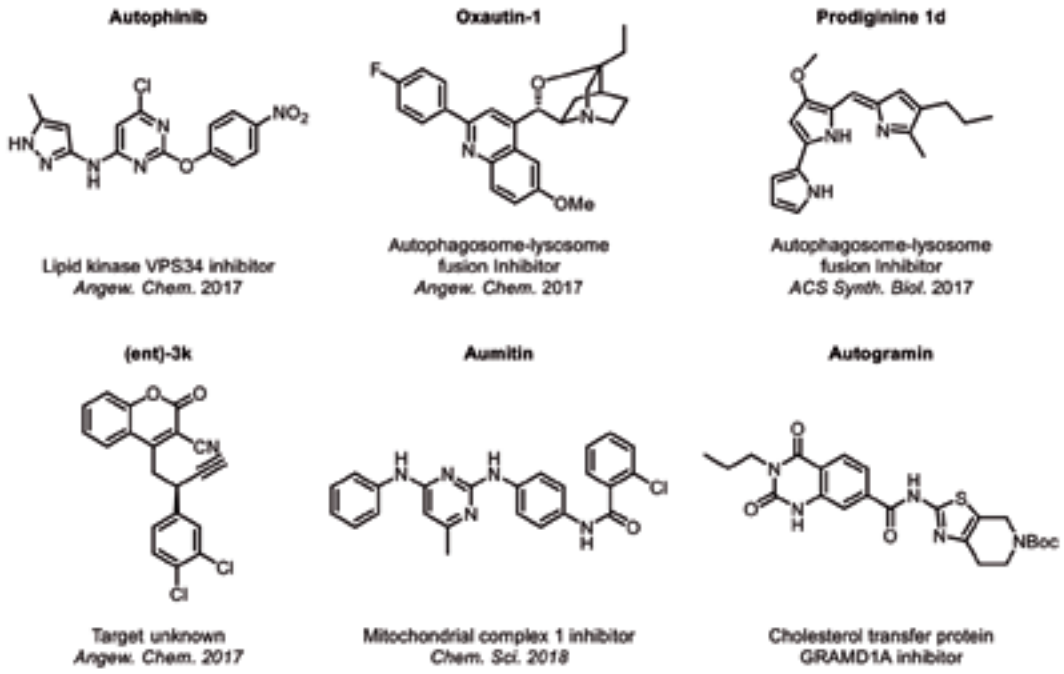


Figure 1. Structures and targets of newly identified autophagy inhibitor chemotypes

References:

[1] Robke L., Futamura Y., Konstantinidis G., Wilke J., Aono H., Mahmoud Z., Watanabe N., WuY-W., Osada H., Laraia L., Waldmann H., *Chem. Sci.* **2018**, 9, 11, 3014-3022.

[2] XuH., Laraia L., Schneider L., Louven K., Strohmann C., Antonchick A.P., Waldmann H., *Angew. Chem. Int. Ed.* **2017**, 56, 11232-11236.

[3] Laraia L., Ohsawa K., Konstantinidis G., Robke L., Wu Y., Kumar K., Waldmann H., *Angew.Chem. Int. Ed.* **2017**, 56, 2145-2150.

[4] Robke L., Laraia L., Carnero-Corrales M. A., Konstantinidis G., Muroi M., Richters A., Winzker M., Engbring T., Tomassi S., Watanabe N., Osada H., Rauh D., Waldmann H., Wu Y., Engel J., *Angew. Chem. Int. Ed*. **2017**, 56, 8153-8157.

Precise control of GPCR receptors with light and allosteric photoswitchable ligands in cells and living animals

Amadeu LLEBARIA

MCS, Laboratory of Medicinal Chemistry & Synthesis, Institute for Advanced Chemistry of Catalonia. (IQAC-CSIC), 08034 Barcelona.

Contact e-mail: amadeu.llebaria@iqac.csic.es

The administration of a photoactivated ligand in combination with illumination that is patterned in space and time can provide a novel degree of control and regulation of receptor activity. This method would allow precisely focusing the action of the ligand controlling the location and the temporal extension of its effects. When applied in vivo, the use of photopharmacology can reduce side effects by targeting receptors located in focused tissues, with potential for establishing personalized drug schedules to patient needs.

We have recently developed light-regulated negative allosteric modulators for metabotropic glutamate receptors (mGluRs). These include Alloswitch-1 and related phenylazopyridines [1] with NAM activity in mGlu5 and OptoGluNAM4.1, the first mGlu4 NAM active in vivo [2]. These photopharmacological tools are based in photoswitchable azobenzene scaffolds that serve for the real-time control of the receptors in cell assays. The molecules show a robust activity dependent of the illumination conditions allowing the real-time regulation of the intracellular effects of these GPCRs.

Moreover, when the molecules are applied in vivo and combined with external or internal light sources, we can register interesting light dependent behavioral effects in zebra fish embryos, tadpoles and rodents, including some pain models. We have shown that localized (in)activation with light of a specific area in the amygdala of live mice results in a control of chronic pain. [3] The key experiment for this uses a mGlu4 photoswitchable azobenzene ligand to control activity of endogenous receptors in vivo with light. With this molecule, we rapidly and reversibly inhibited chronic pain behavioral symptoms after illumination in the amygdala of rodent brain while measuring the painful response in the periphery. We have demonstrated a photopharmacological dynamic regulation of sensory and emotional information, bypassing central sensitization processes established for long periods of time and the validation of local mGlu4 targeting for persistent pain. This approach is effective to study the pharmacology of mGluRs and shows potential for spatiotemporal regulation of drugs targeting mGluRs.

Acknowledgements: This work was supported by Spanish Ministry of Science, Innovation and Universities and the Spanish Ministry of Economy, Industry and Competitiveness (PCIN-2013-017-C03-01, CTQ2014-57020-R, PCI2018-093047 and CTQ2017-89222-R) cofinanced by the European Regional Development Fund (FEDER);, Catalan government (2017-SGR-1604) and the ERANET Neuron projects "LIGHTPAIN" and "MAGNOLIA".

References:

[1] Gómez-Santacana, X., Pittolo S, Rovira X, Lopez M, Zussy C, Dalton JA, Faucherre A, Jopling C, Pin JP, Ciruela F, Goudet C, Giraldo J, Gorostiza P, Llebaria A.*et al. ACS Cent. Sci*. **2017**, *3*, 81.

[2] Rovira X, Trapero A, Pittolo S, Zussy C, Faucherre A, Jopling C, Giraldo J, Pin JP, Gorostiza P, Goudet C, Llebaria A. Cell Chem Biol. **2016**, *23*, 929.

[3] Zussy C, Gómez-Santacana X, Rovira X, De Bundel D, Ferrazzo S, Bosch D, Asede D, Malhaire F, Acher F, Giraldo J, Valjent E, Ehrlich I, Ferraguti F, Pin JP, Llebaria A, Goudet C. *Mol Psychiatry* **2018**, *23*, 509.

Preparation and Evaluation of Self-Illuminating Photosensitizers for Photodynamic Therapy of Cancer

L. PINTO DA SILVA[1], C. M. MAGALHÃES[1], P. FERREIRA[1], A. MONTENEGRO[1], E. BORGES[2], J. C. G. ESTEVES DA SILVA[1]

[1] CIQUP, Faculty of Sciences, University of Porto, R. Campo Alegre 687, 4169-007 Porto, Portugal.

[2] LAQV/REQUIMTE, Faculty of Sciences, University of Porto, R. Campo Alegre 687, 4169-007 Porto, Portugal.

Contact e-mail: luis.silva@fc.up.pt

Photodynamic therapy (PDT) is a minimally-invasive and clinically-approved cancer therapy, with limited side-effects when compared with other more common approaches (as chemo/radiotherapy and surgery). PDT consist on the administration of a photosensitizer to the patient, followed by its accumulation at the tumor site.[1] Irradiation of the tumor site by light activates the photosensitizer, which produces reactive oxygen species (mainly singlet oxygen) in photochemical reactions with O_2. However, due to problems regarding light-penetration into biologic tissue, PDT can only be applied to tumors on or just under the skin and on the lining of internal organs and cavities.[1]

Herein, we have developed new photosensitizers for PDT that can be self-activated inside tumor cells without the need for an external light source.[2] More specifically, these new molecules are capable of self-activation in the absence of light by undergoing a chemiluminescent reaction, which consist in the conversion of thermal energy into excitation energy.[3,4] Furthermore, these molecules are able to interact directly with O_2 to generate reactive oxygen species, without requiring previous energy transfer steps.[2]

Proof-of-concept was provided by: (i) determination of singlet oxygen production by monitoring the fluorescence of specific fluorescent probes; (ii) determination of the toxicity of these photosensitizers toward *in vitro* tumor cell lines. The development of this type of photosensitizer opens the door for using PDT to treat tumors irrespective of their size and localization in the body.

Acknowledgements: projects PTDC/QEQ-QFI/0289/2014, NORTE-01-0145-FEDER-000028 and POCI-01-0145-FEDER-016603

References:

[1] Magalhães, C.M.; Esteves da Silva, J.C.G.; Pinto da Silva, L. *ChemPhysChem* **2016**, *17*, 2286.

[2] Patent PPP59213 (Pending).

[3] Pinto da Silva, L.; Pereira, R.F.J.; Magalhães, C.M.; Esteves da Silva, J.C.G. *J. Phys. Chem. B* **2017**, *121*, 7862.

[4] Magalhães, C.M.; Esteves da Silva, J.C.G.; Pinto da Silva, L. *J. Lumin.* **2018**, *199*, 339.

Protein-directed Dynamic Systems: Insights into Real Time Chemical Processes

Andrea CANAL-MARTÍN[1,2], Javier SASTRE[1], Dolores MOLERO[3], Mª Encarnación FERNÁNDEZ-VALLE[3], Elena SÁEZ[3], María José SÁNCHEZ-BARRENA[4], Angeles CANALES[2], F. Javier CAÑADA[1], Jesús JIMÉNEZ-BARBERO[5], Alicia MANSILLA[6], Ana MARTÍNEZ[1], Ruth PÉREZ-FERNÁNDEZ[1]

1 Structural and chemical biology department, Centro de Investigaciones Biológicas, CIB-CSIC, 28040 Madrid, Spain.

2 Organic chemistry department, Universidad Complutense de Madrid, 28040 Madrid, Spain.

3 CAI de RMN, Universidad Complutense de Madrid, 28040 Madrid, Spain.

4 Crystallography and structural biology department, Instituto de Química Física Rocasolano, IQFR-CSIC, 28006 Madrid, Spain.

5 Molecular recognition and host-pathogen interactions, CIC bioGUNE, 48160 Derio, Bizkaia, Spain.

6 Instituto Ramón y Cajal de Investigación Sanitaria. Ctra. Colmenar Viejo, km. 9100, 28034 Madrid, Spain.

Contact e-mail: ruth.perez@csic.es

Evolution has long been associated to the emergence of the best-adapted organism. Complex disordered systems show an inherent property to self-organize and evolve in a way that was described as a "combinatorial optimization process". Dynamic combinatorial chemistry (DCC) studies mixtures of compounds formed through reversible reactions and the response of those mixtures (Dynamic combinatorial libraries, DCLs) to external stimuli. If a protein is added to the system and one or more molecules show affinity to it, these building blocks will, according to the Le Chatelier's principle, be amplified on the expense of the other non-bonding constituents[1].

NCS-1 (Neuronal Calcium Sensor 1) is a high-affinity Ca^{2+}-binding protein involved in pathologies that result from an abnormal synapse number [2]. The interaction between NCS-1 and the guanine exchange factor Ric8a regulates synapse number and probability of neurotransmitter release, thus constituting a pharmacological target for synaptopathies.

We present the discovery of novel modulators of *d*NCS-1 (*Drosophila* Neuronal Calcium Sensor-1) Ric8a complex, using acylhydrazone exchange as reversible chemistry. Furthermore, Ultrafast NMR (UF-NMR) techniques [3] have been applied to acylhydrazone exchange monitoring in real-time the chemical processes and obtaining information about the species involved in this dynamic system at physiological pH.

Acknowledgements: MINECO CTQ2015-69643-R and European Cooperation in Science and Technology (COST) action CMI304 «Emergence and evolution of complex chemical systems».

References:

[1] Mondal, M.; Hirsch, A. K. H. *Chem. Soc. Rev.* **2015**, *44*, 2455 and references herein.

[2] Baños-Mateos, S.; Chaves-Sanjuan, A.; Mansilla, A.; Ferrús, A.; Sánchez-Barrena, M. J. *Acta Cryst.* **2014** *F70*, 530.

[3] Herrera, A., Fernández-Valle, E., Martínez-Álvarez, R., Molero-Vílchez, D., Pardo-Botero, Z. D., Sáez-Barajas, E. *Magn. Reson. Chem.* **2015***, 53*, 952.

Computational characterization of enzymatic reactive intermediates for the discovery and design of new biocatalytic activities

Marc GARCÍA-BORRÀS(1), Kendall N. HOUK(1)

[1] Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095

Contact e-mail: marcgabo@ucla.edu

Biocatalysis is based on the application of natural catalysts, mainly enzymes, for new purposes which may include nonnative substrates and/or reactions. Although the first examples of biocatalysis were reported more than a century ago, it was revolutionized after the discovery of an *in vitro* version of Darwinian evolution called Directed Evolution (DE). Indeed, the invention of Directed Evolution of enzymes has been recognized by the 2018 Nobel Prize in Chemistry. Despite the recent advances in the field, major challenges remain to be addressed. Still, tens of thousands of variants need to be tested experimentally, and little information is available on how these mutations lead to enhanced enzyme proficiency.

Herein, we report recent successful cases where the combination of different multiscale computational techniques, including small truncated models (*theozymes*), molecular dynamics (MD) simulations and QM/MM calculations, have allowed us to unveil the molecular basis for enzymatic catalysis of different laboratory evolved enzymes generated by DE techniques.[1] The reactions studied include both natural and unnatural transformations catalyzed by enzymes, for instance Baeyer-Villiger monooxygenases[2] or carbene transferases.[3] These studied reactions all have in common the formation of reactive intermediate species along their reaction pathways, whose stabilization by the protein environment has been found to be crucial for enhancing the enzymatic activities and dictating the selectivities of such enzyme-catalyzed reactions. Our computational studies provide basic knowledge and atomistic descriptions about how mutations introduced by DE and site-directed mutagenesis improved and expanded the enzyme proficiency towards new reactivities and selectivities not observed in Nature, that may be useful for future rational protein engineering.

References:

[1] Romero-Rivera, A.; Garcia-Borràs, M.; Osuna, S. *Chem. Commun.*, **2017**, *53*, 284.

[2] Li, G.; Garcia-Borràs, M.; Fürst, M. J. L. J.; Ilie, A.; Fraaije, M. W.; Houk, K. N.; Reetz, M. T. *J. Am. Chem. Soc.*, **2018**, *140*, 10464.

[3] Lewis, R. D.[1]; Garcia-Borràs, M.[1]; Chalkley, M. J.; Buller, A. R.; Houk, K. N.; Kan, S. B. J.; Arnold, F. H. *Proc. Natl. Acad. Sci. U.S.A.*, **2018**, *115*, 7308. ([1]: R.D.L. and M.G.-B. contributed equally to this work.)

Introduction of post-translational modifications in vitro modulates Tau protein fibrillation

Mariapina D'ONOFRIO[(1)], Francesca MUNARI[(1)], Carlo Giorgio BARRACCHIA[(1)], Stefano CAPALDI[(1)], Giorgio ARRIGONI[(2)], Michael ASSFALG[(1)]

[1] Department of Biotechnology, University of Verona, Strada le Grazie 15 37134 Verona, Italy.

[2] Department of Biomedical Sciences, University of Padova, Via Ugo Bassi 58/B35131 Padova, Italy.

Contact e-mail: mariapina.donofrio@univr.it

Alzheimer's Disease (AD) is characterized by two lesions: intracellular neurofibrillary tangles of filaments made of hyperphosphorylated Tau protein and extracellular senile plaques containing predominantly the β-amyloid peptide. In this framework we focused our attention on Tau protein. A number of post-translational modifications, including phosphorylation, acetylation, or glycosylation, have been shown to be crucial in Tau function and dysfunction, however very little is known about the impact of another important post-translational modification, ubiquitination.

The present research aims at acquiring basic knowledge, that we believe is crucial, to understand whether ubiquitination of Tau is a key factor in its transition to toxic species, relevant in the pathogenesis of AD. To this aim, we obtained and characterized ubiquitin-Tau conjugates in vitro using different methodologies. Our results show that several lysine residues can be modified by monoubiquitin. The next step was then to determine whether ubiquitination could have a differential effect on Tau aggregation. We therefore, performed a series of in vitro aggregation assays, based on Thioflavin T fluorescence. Our preliminary results show that ubiquitination has a strong influence on Tau aggregation propensity, and modulates fibril formation. We believe that altogether these results will contribute to increase significantly our knowledge about the onset of Tau pathological species, starting from findings at the molecular level.

Acknowledgements: This work was supported by a grant from the Alzheimer's Association (AARG-17-529221).

Targeted release of epigenetic histone demethylase inhibitors via enzymatic bioreductive activation from prodrugs

Manfred JUNG[1,2], Eva-Maria HERRLINGER[1], Mirjam HAU[1,2], Dorothee REDHABER[3], Cornelius MIETHING[3]; Eric METZGER[4], Roland SCHÜLE[2,4]

1 Institute of Pharmaceutical Sciences, University of Freiburg, Germany, www.jungm.de/

2 CIBSS – Centre for Integrative Biological Signalling Studies, University of Freiburg.

3 Division of Hematology, Oncology and Stem Cell Transplantation, University Freiburg Medical Center.

4 Department of Urology and Center for Clinical Research, University of Freiburg Medical Center.

Contact e-mail: manfred.jung@pharmazie.uni-freiburg.de

Lysine Specific Demethylase 1 (LSD1) has evolved as a promising epigenetic therapeutic target for cancer treatment, especially leukemia, and first inhibitors of LSD1 have entered clinical trials.[1] Various derivatives of Tranylcypromine are known as potent and specific LSD1 inhibitors, e.g.[2,3] but the use of small molecules is generally impeded by the lack of cell-specificity. This feature is particularly important in the treatment of complex, multicellular organisms when lower side effects and higher drug concentrations are desired. To approach the challenge of site-specific drug release, we developed and synthesized selective prodrugs of the potent LSD1 inhibitor OG86[3] and analogues. The prodrugs get activated via reduction by the *E coli* nitroreductase NfsB (NTR) but are stable towards endogenous human enzymes. Thus, LSD1 gets inhibited only in heterologously NTR-expressing cells after the treatment with prodrugs. We show this cell-specific LSD1 inhibition with our prodrugs in the acute monocyte leukemic cell line THP1 versus lentiviral transfected THP1-NTR$^+$ cells that lead to LSD1

Acknowledgements: This study was supported by the Excellence Strategy of the German Federal and State Governments, (CIBSS - EXC 2189) and the Deutsche Forschungsgemeinschaft (DFG, CRC992).

References:

[1] Morera, L.; Lübbert, M.; Jung, M.. *Clin Epigenetics* **2016**, *8*, 57.

[2] Schulz-Fincke, J.; *et al*. *Eur J Med Chem* **2018**, *144*, 52.

[3] Maiques-Diaz, A.; *et al*., Cell Rep 2018, 22(13).

Dynamic stereoselection of kinetically-inert DNA-binding metallopeptide cylinders

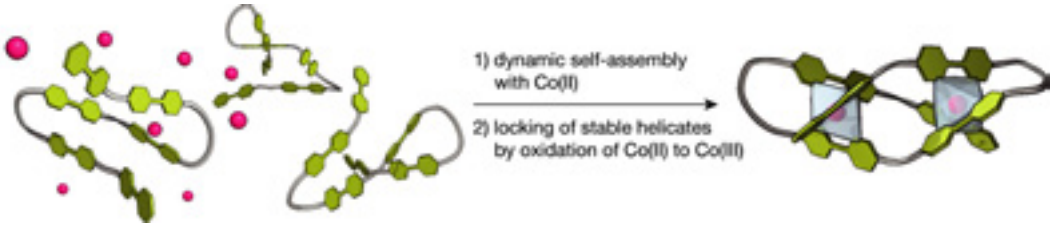
Jacobo GÓMEZ-GONZÁLEZ, David BOUZADA, Sonia BOGA, Diego GARCÍA PEÑA, Gustavo RAMA, Miguel VÁZQUEZ LÓPEZ, M. Eugenio VÁZQUEZ

Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CiQUS), Departamento de Química Inorgánica, Universidade de Santiago de Compostela. 15782 Santiago de Compostela, Spain.

Contact e-mail: eugenio.vazquez@usc.es

We propose that using peptide ligands avoids inefficient and labor-intensive multistep organic synthesis procedures, and allows the application of well-established peptide design tools (e.g., known structural preferences of peptides sequences, peptide scaffolds, etc.) for obtaining complex metallopeptide architectures. Indeed, we have previously described the synthesis of a 2,2'-bipyridine amino acid derivative, and its incorporation into peptide ligands that predictably fold into complexes with defined chirality in the presence of metal ions,[1] including self-assembled helicates.[2] Unfortunately, our original design featured two relatively hydrophobic (Pro-Gly-Gly) β-turn sequences to direct the folding of the peptide chain and encode the chirality of the final helicates, which made these peptide ligands poorly soluble and with a strong tendency to aggregate. Furthermore, the resulting peptide helicates, based on relatively labile Fe(II)-bipyridine coordination, were inherently dynamic, which limited their applications.

We report the stereoselective assembly of short peptides containing bipyridine ligands in the presence of metal ions. The folding of the peptidic ligands into three-stranded metallocylinders and the handedness of such supramolecular structures are encoded in the amino acid sequences by way of different combinations of amino acid residues of opposite chirality, and selected under thermodynamic control in the presence of Co(II) ions. The resulting metallocylinders can then be locked in the form of kinetically-inert Co(III) complexes by oxidation, thus providing a straightforward method for stereoselective selection of kinetically inert metallopeptides. Both the kinetically-labile Co(II) and the inert Co(III) helicates display good DNA binding properties with selectivity towards three-way DNA junctions.



***Acknowledgements**: Financial support from the Spanish grants CTQ2015-70698-R, the Xunta de Galicia (Centro singular de investigación de Galicia accreditation 2016–2019, ED431G/09) and the European Union (European Regional Development Fund - ERDF), is gratefully acknowledged. We also wish to thank the Fundación Asociación Española Contra el Cáncer for their support AECC (IDEAS197VAZQ).*

References:

[1] a) Rama, G.; Ardá, A.; Maréchal, J.-D.; Gamba, I.; Ishida, H.; Jiménez-Barbero, J.; Vázquez, M. E.; Vázquez López, M. *Chem. Eur. J.* **2012**, *18*, 7030; b) Gamba, I.; Rama, G.; Ortega-Carrasco, E.; Berardozzi, R.; Sánchez-Pedregal, V. M.; Di Bari, L.; Maréchal, J.-D.; Vázquez, M. E.; Vázquez López, M. *Dalton Trans*. **2016**, *45*, 881..

[2] a) Gamba, I.; Rama, G.; Ortega-Carrasco, E.; Maréchal, J.-D.; Martínez-Costas, J.; Vázquez, M. E.; Vázquez López, M. *Chem. Commun*. **2014**, *50*, 11097; b) Gómez-González, J.; Peña, D. G.; Barka, G.; Sciortino, G.; Maréchal, J.-D.; Vázquez López, M.; Vázquez, M. E. *Front. Chem*. **2018**, *6*, 520.

Selective Tankyrase Inhibitors Through a Structure-guided Deconstruction and Hybridization Approach

Upendra RAO ANUMALA[(1)], Jo WAALER[(3)], Yves NKIZINKIKO[(4)], Peter LINDEMANN[(1)], Katina LAZAROW[(1)], Petter Angell OLSEN[(3)], Sudarshan MURTHY[(4),] Ezeogo OBAJI[(4)], Jens Peter VON KRIES[(1),(2)], Lari LEHTIÖ[(4)], Stefan KRAUSS[(3)], Marc NAZARÉ[(1),(2)]

[1] Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Campus Berlin-Buch, 13125 Berlin, Germany.

[2] Berlin Institute of Health (BIH), Anna-Louisa-Karsch-Str. 2, 10178 Berlin, Germany.

[3] Hybrid Technology Hub - Centre of Excellence, University of Oslo and Oslo University Hospital, Norway.

[4] Faculty of Biochemistry and Molecular Medicine, Biocenter Oulu, University of Oulu, Finland.

Contact e-mail: nazare@fmp-berlin.de

WNT/β-catenin signaling regulates key cellular functions including proliferation, differentiation, migration, apoptosis, stem cell renewal and immune system modulation. Aberrant WNT/β-catenin signaling is found in multiple cancers. In particular, the recently described role of the WNT/β-catenin pathway in regulating immune cell infiltration in the tumor micro-environment suggests an impact of the pathway on immunotherapy.[1] The Poly-ADP-ribosyltransfrase tankyrase 1 and 2 are key targets in the WNT/β-catenin signalling pathway, regulating the turnover of the protein complex that controls β-catenin stability and translocation.

Albeit several small molecules have been identified that inhibit tankyrases 1 and 2 they often lack selectivity among the entire family of 17 PARP enzymes or efficacy in the WNT dependent adenoma and tumor models.[2]

Here we describe a successful structure-guided deconstruction and hybridization approach using two privileged substructures giving instant access to a new series of highly selective tankyrase inhibitors by preserving key protein-ligand contacts. The displayed high selectivity and favorable in-vitro ADME profile as well as good oral bioavailability in mice, rats and dogs makes this compound a high quality chemical tool for proof of concept studies.[3] Our tankyrase inhibitor series shows efficacy in WNT dependent adenoma and tumor models with the potential as preclinical candidates.

References:

[1] Pai, S. G.; Carneiro, B. A.; Mota, J. M.; Costa, R.; Leite, C. A.; Barroso-Sousa, R.; Kaplan, J. B.; Chae, Y. K.; Giles, F. J., Wnt/beta-catenin pathway: modulating anticancer immune response. *J. Hematol. Oncol.* **2017,** *10* (1), 101.

[2] Thorsell, A. G.; Ekblad, T.; Karlberg, T.; Low, M.; Pinto, A. F.; Tresaugues, L.; Moche, M.; Cohen, M. S.; Schuler, H., Structural Basis for Potency and Promiscuity in Poly(ADP-ribose) Polymerase (PARP) and Tankyrase Inhibitors. *J. Med. Chem.* **2017,** *60* (4), 1262-1271.

[3] Anumala, U. R.; Waaler, J.; Nkizinkiko, Y.; Ignatev, A.; Lazarow, K.; Lindemann, P.; Olsen, P. A.; Murthy, S.; Obaji, E.; Majouga, A. G.; Leonov, S.; von Kries, J. P.; Lehtio, L.; Krauss, S.; Nazare, M., Discovery of a Novel Series of Tankyrase Inhibitors by a Hybridization Approach. *J. Med. Chem.* **2017**, *60* (24), 10013-10025.

Flash Communications

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

Far-Red Fluorescent DNA Binder Allows Host-Pathogen Interaction Studies of Multidrug-Resistant Bacteria

Benedikt HEINRICH[(1)], Leon N. SCHULTE[(2)], Olalla VÁZQUEZ[(1)*]

[1] Chemical Biology Department, Chemistry Faculty, Philipps-University Marburg, Hans-Meerwein-Straße 4, 35043 Marburg, Germany.

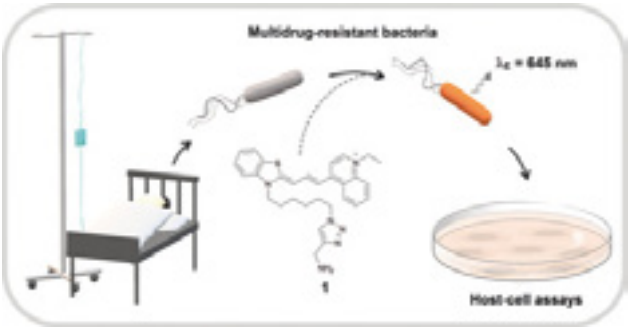
[2] Institute for Lung Research, Philipps-University Marburg, Hans-Meerwein-Straße 2, 35043 Marburg, Germany.

Contact e-mail: benedikt.heinrich@chemie.uni-marburg.de

The implementation of the green fluorescent protein (GFP) has dramatically transformed modern molecular biology till the point of becoming an indispensable fluorescent reporter to study biological processes. Indeed, as an objective proof of GFP impact, it was awarded with the Nobel Prize in 2008[1]. The use of genetically encoded GFP is also essential to study host-pathogen interactions down to single cell resolution. However, GFP-based investigations rely on genetic manipulation, which is a limitation for the study of multi-drug resistant bacteria and their interplay in host-pathogen interactions. The fact that such bacteria are worldwide major health threat forces the development of innovative compatible tools.

Herein we report the design, synthesis and study of a new far-red fluorescent probe for labeling both multidrug-sensitive and –resistant bacteria and demonstrated their application in macrophage phagocytosis studies to decipher divergent infection strategies. Thus, using SyTO-9 as a starting point[2], new cyanine dye derivatives were designed and synthesized. Fluorescent labeling studies and comparison with known fluorophores such as ethidium bromide, carboxyfluorescein succinimidyl ester and SyTO9 revealed **6-TramTO-3** as our best candidate. We characterized the DNA binding mode, using fluorescence, UV-Vis, CD spectroscopy and microscopy. Importantly and in contrast to the described common fluorophores, **6-TramTO-3** combines excellent compatibility with bacterial growth, labeling efficiency and a pronounced fluorescence shift in fluorescence-activated cell sorting (FACS). We successfully labeled *E.coli* as well as multidrug-sensitive and -resistant *Klebsiella pneumoniae*, a major threat to hospitalized patients. For the first time, the use of our **6-TramTO-3** enabled the observation of different virulence strategies between both strains[3].

We anticipate that our new off-the-shelf reagent **6-TramTO-3** is the ideal solution for real-time analysis of bacterial infection, particularly in those cases that GFP technology cannot be applicable. We believe that **6-TramTO-3** will help to reveal strategies to combat a major worldwide health threat: multidrug-resistant bacteria.



References:

[1] Zimmer, M. *Chem. Soc. Rev.* **2009**, *38*, 2823.

[2] Tárnok, A. *Cytometry A.* **2008**, *73*, 477.

[3] Schulte, L.N.; Heinrich, B.; Janga, H; Schmeck, B.T.; Vázquez, O. *Angew. Chem. Int. Ed.* **2018**, *36*, 11564.

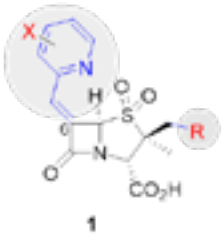
Novel Covalent Inhibitors of Carbapenem-hydrolyzing Class D β-lactamases Enzymes for Disabling Antibiotic Resistance in Superbugs

Diana RODRÍGUEZ[(1)], María MANEIRO[(1)], Concepción GONZÁLEZ-BELLO[(1)]*

[1] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela, calle Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain.

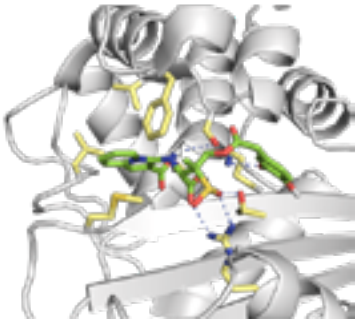
Contact e-mail: concepcion.gonzalez.bello@usc.es

The ability of antibiotics to cure infectious diseases is nowadays in serious danger due to the emergence and spread worldwide of strains that are multidrug-resistant to antibiotics, which means that at least three different classes of antibiotics no longer cure the infections. A recent study reported by WHO revealed that the situation is highly critical for healthcare-associated infections caused by the Gram-negative ESKAPE pathogens (Superbugs) *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*. These bacteria are resistant to carbapenems, which are the only remaining therapy that is often considered as antibiotics of last resort. Our research group is working in the development of inhibitors against the most prevalent cause of antibiotic resistance in Gram-negative bacteria, carbapenem-hydrolyzing class D β-lactamases, which hydrolyze the most widely used antibiotics in an acylation-deacylation-based process and are the most rapidly growing and diverse group. We have developed compounds **1**, which cause the covalent modification of the enzyme by formation of an adduct stable against hydrolysis (indolizine)[1]. These compounds are efficient inhibitors against those enzymes with an extraordinary *in vitro* activity for the most challenging carbapenem-hydrolyzing class D β-lactamases (MIC 2−4 μg/mL). In order to improve the inhibitor efficiency and half-life of the enzyme adducts, we have explored the effect of substituents in the pyridine ring on the inhibitor. Our recent results will be presented.



1

MIC 2 μg/mL (OXA-24)
MIC 4 μg/mL (OXA-23)
vs
Tazobactam 64 μg/mL



Acknowledgements: Financial support from the Spanish Ministry of Economy and Competiveness (SAF2016-75638-R), the Xunta de Galicia (Centro singular de investigación de Galicia accreditation 2016-2019, ED431G/09) and the European Regional Development Fund is gratefully acknowledged.

References:

[1] (a) Vallejo, J.A.; Martínez-Guitián, M.; Vázquez-Ucha, J.C.; González-Bello, C.; Poza, M.; Buynak, J.D.; Bethel, C.R.; Bonomo, R.A.; Bou, G.; Beceiro, A. *J. Antimicrob. Chemother.,* **2016***, 7*, 2171. (b) Vázquez-Ucha, J.C.; Maneiro, M.; Martínez-Guitián, M.; Buynak, J.; Bethel, C.R.; Bonomo, R.A.; Bou, G.; González-Bello, C.; Beceiro, A. *Antimicrob. Agents Chemother.,* **2017**, *61*, e01172.

[2] Rodríguez, D.; Maneiro, M.; González-Bello, C. manuscript in preparation.

Exploring bacterial surface display as a platform for developing engineered glycan-binding proteins

Alba VÁZQUEZ-ARIAS*, Jorge PÉREZ-JUSTE, Isabel PASTORIZA-SANTOS, Gustavo BODELÓN

Departamento de Química Física and CINBIO, Universidade de Vigo, 36310 Vigo, Spain.

* Contact e-mail: albavazquez@uvigo.es

Glycans are cell-surface carbohydrates that profoundly affect the function of glycoproteins, and play a critical role in a wide range of key biological processes including cell−cell adhesion, cell communication, development, inflammation, pathogen recognition and malignant transformation. Glycan-binding proteins have therefore enormous utility as tools to evaluate the expression of glycans for a variety of basic research and clinical applications. For instance, a huge number of carbohydrate-binding proteins are used to detect expression of tumor-associated glycans for diagnostic and prognostic purposes. Glycan-binding proteins can also be used for targeting specific cells for imaging, drug delivery, and to understand glycan-mediated processes. Unfortunately, most readily available glycan-binding proteins present either broad specificity or poor affinity for their target carbohydrates.

Here we explore the application of bacterial surface display as an efficient and cost-effective method for the production and engineering of human galectin-3, a multifunctional glycan-binding protein that has been shown to play a prominent role in diverse physiopathological settings such as autoimmunity, cancer, and heart failure[1].

In this study, we have demonstrated that the intimin display system from *E. coli* [2], allows the expression of functional galectin-3 on the surface of bacterial cells. Future work will aim to exploit this bacterial display system for the generation of galectin-3 mutants with increased binding affinities, as well as galectin inhibitors with potential diagnostic and therapeutic applications. We have shown that the display system described here should be a valuable platform to the limited analytical toolbox that is currently available for glycomics.

References:

[1] Krześlak A and Lipińska A. Galectin-3 as a multifunctional protein. Cell Mol Biol Lett. 2004.

[2] Salema V, Marín E, Martínez-Arteaga R, Ruano-Gallego D, Fraile S, Margolles Y, Teira X, Gutierrez C, Bodelón G, Fernández LÁ. Selection of single domain antibodies from immune libraries displayed on the surface of *E. coli* cells with two β-domains of opposite topologies. PLoS One. 2013.

MolPort – Screening Compounds & Building Blocks sourcing and procurement solution in a customized way

José GARCÍA-TENORIO[1]

[1] Sales Department, MolPort. Lacplesa iela 41, LV-1009 Riga. Latvia.

Contact e-mail: jose.garcia-tenorio@molport.com

The process of searching for compounds that are necessary for our works can really be so complicated and time consuming that many scientists get overwhelmed by the choices. Not just the number of suppliers offering or not the molecules we may need. But also, the different prices to assess, quantities, compound state, purity, logistics involved to bear in mind with customs clearance, different documentation, delays, cancellations, etc. And most important, the quality of what we eventually receive.

A Single Point of Contact that addresses all these factors, but not only that. Who cares to find the most optimized way for your project, in a customized manner. Helping you see what could really be the best to proceed with your works, based on >10 years' experience in the market. And whenever any issue or inquiry that may arise, we do take of it, addressing personally with each supplier involved, which whom we meet regularly. With several chemists in the team to understand the needs.

MolPort is a chemical compound marketplace that lists 7+ million stock compounds from 65+ suppliers. More than 80% of our available products database is synchronized with supplier data on a daily basis. Through frequent reliability checks, we know each supplier's specifics, costs, and delivery days. And their actual reliability and quality to provide the best service.

If you are looking for a single hard-to-find compound, sourcing thousands of compounds for a project or researching analogue synthesis for your hits, through MolPort you can do it quicker, simpler and with higher reliability. You can receive your package documented while we take care of the customs. We make sure that your final compound selection includes the very same available supplier direct prices with their volume discounts applied for you.

The difference with other competitors? Only the direct prices without a markup don't make it all. But the flexibility. The customization is not only a nice-to-have sometimes, but a must in some cases. We can deliver your molecules reweighed/reformatted (E.g. in DMSO) in standard tube-racks as well as in your specific plates or vials. Besides administration, logistics and accountability support, downloading our database for free, API webservices such as KNIME or Pipeline Pilot, our collaboration with Schrödinger and StarDrop / Optibrium…

Review our services now! Choose MolPort as your single point of contact for your research! Read more at www.molport.com

Trypanosomatids identification by Chem-NAT technology

María Angélica LUQUE-GONZÁLEZ[1,2], Mavys TABRAUE-CHÁVEZ[3], Bárbara LÓPEZ-LONGARELA[3], Rosario María SÁNCHEZ-MARTÍN[1,2], Matilde ORTIZ-GONZÁLEZ[1,4], Miguel SORIANO-RODRÍGUEZ[1,4], José Antonio GARCÍA-SALCEDO[1,4], Salvatore PERNAGALLO[3,5], Juan José DÍAZ-MOCHÓN[1,2,5]

1 Pfizer-Universidad de Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Parque Tecnológico de Ciencias de la Salud (PTS), Avenida de la Ilustración 114, 18016 Granada, Spain.

2 Dep. Medicinal and Organic Chemistry, School of Pharmacy, University of Granada, Campus Cartuja s/n, 18071 Granada, Spain.

3 DestiNA Genomica S.L. Parque Tecnológico Ciencias de la Salud (PTS), Avenida de la Innovación 1, Edificio BIC, 18016 Armilla, Granada, Spain.

4 Unidad de Enfermedades Infecciosas y Microbiología, Instituto de Investigación Biosanitaria ibs. GRANADA, Hospitales Universitarios de Granada / Universidad de Granada, Granada, Spain.

5 DestiNA Genomics Ltd. 7-11 Melville St, Edinburgh EH3 7PE, United Kingdom.

Contact e-mail: angelicaluque@ugr.es

Rationale: Parasites of the family Trypanosomatidae cause devastating diseases in humans and animals. An accurate diagnosis is needed for an effective treatment and control of these diseases; however, the shared symptomatology with other infections makes diagnosis challenging[1].

Objective: We aimed to develop a molecular assay for the unequivocal identification of *Trypanosoma cruzi, Trypanosoma brucei and Leishmania spp.*.

Methodology: A chemical-based approach for nucleic acid analysis (Chem-NAT technology) was combined with Matrix-Assisted Laser Desorption Ionization – Time-of-Flight Mass Spectrometry (MALDI-ToF) as readout tool in an innovative diagnosis test. The assay consists of three steps: 1) amplification with a single pair of primers of a region of 28S ribosomal RNA gene homologous for the three trypanosomatids under study, 2) analysis of the amplification product by the Chem-NAT technology and 3) MALDI-ToF readout. This chemical approach relies on Watson-Crick base pairing rules to template the specific incorporation of an aldehyde-modified SMART-Nucleobase into the abasic position of abasic PNA probes. Single base differences (Single Nucleotide Fingerprint 'SNF') in this homologous region results in a unique pattern of SMART-Nucleobase incorporation for each parasite and so their unequivocal identification[2].

Results: Chem-NAT and MALDI-TOF technologies has been were successfully combined in a a chemical-based molecular assay for the unequivocal differentiation of three trypanosomatids parasitest[3].

Acknowledgements: This research work has received funding from Junta de Andalucía, Consejería de Economía e Innovación (project number 2012-BIO1778), the Spanish Ministerio de Economía y Competitividad (Grants CTQ2012-34778, BIO2016-80519-R, FPI Grant BES-2013- 063020)). This research was partially supported by the 7th European Community Framework Program (FP7-PEOPLE-2012-CIG-Project Number 322276).

The authors thank DestiNA Genomica S.L. for assistance during the process of isolation of the genomic DNA, PCR amplification and dynamic chemistry reaction. We also thank Carlos Rodrigues-Poveda for technical advice and Mr. Hugh Ilyine for proof-reading.

References:

[1] Duncan, R., Advancing Molecular Diagnostics for Trypanosomatid Parasites COMMENT. *J Mol Diagn* **2014,** *16* (4), 379-381.

[2] Bowler, F. R.; Diaz-Mochon, J. J.; Swift, M. D.; Bradley, M., DNA analysis by dynamic chemistry. *Angewandte Chemie* **2010,** *49* (10), 1809-12.

[3] Luque-Gonzalez, M. A.; Tabraue-Chavez, M.; Lopez-Longarela, B.; Sanchez-Martin, R. M.; Ortiz-Gonzalez, M.; Soriano-Rodriguez, M.; Garcia-Salcedo, J. A.; Pernagallo, S.; Diaz-Mochon, J. J., Identification of Trypanosomatids by detecting Single Nucleotide Fingerprints using DNA analysis by dynamic chemistry with MALDI-ToF. *Talanta* **2018,** *176*, 299-307.

aAMDlib: Testing transferability of electron density models on chemical libraries with biological interest

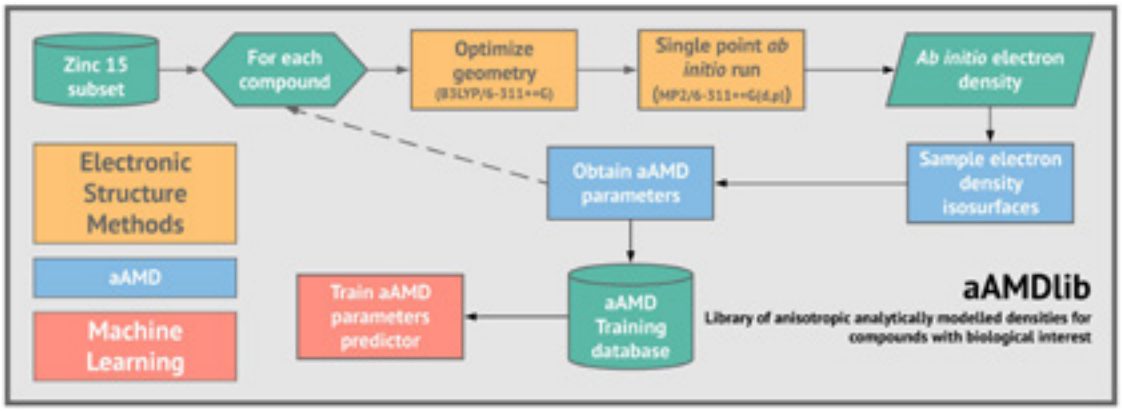
Bruno CUEVAS-ZUVIRÍA[1], Luis FERNÁNDEZ PACIOS[1,2]

[1] Centro de Biotecnología y Genómica de Plantas (CBGP, UPM-INIA), Campus de Montegancedo-UPM, 28223 Madrid, Spain.

[2] Departamento de Biotecnología-Biología Vegetal, ETSIAAB, Universidad Politécnica de Madrid (UPM), 28040 Madrid, Spain.

Contact e-mail: bruno.czuviria@upm.es

Classical force-field and quantum methodologies represent molecular systems with rather different accuracy and computational cost. In our *aAMDlib* approach (*a*nisotropic *A*nalytically *M*odelled *D*ensity *lib*rary), we aim to represent the electron density – an essential quantum descriptor– in an approximate, analytical form through an expansion on exponential functions together with a Fourier series to account for anisotropic effects. Based on previous work that demonstrated the high accuracy of our former *AMD* approach on atoms (1,2), we present here an extension of this procedure to molecular systems of biological interest represented by a selected set of compounds downloaded from chemical databases. The *aAMD* representation provides an efficient basis to compute a number of molecular properties formally dependent on the electron density at a computational cost equivalent to classical molecular mechanics treatments.



The performance of the proposed model is here explored by training it on a library of chemical compounds from ZINC15 database (3) for which electron densities are firstly obtained in quantum calculations. Once the *aAMD* parameters are determined, their transferability is studied by training a machine-learning regression algorithm on the prediction of those parameters. The reliability of the model is finally assessed by comparing properties predicted by quantum calculations with those obtained with the *aAMD* approach. Future work will address the transferability of the model to macromolecules such as proteins.

References:

[1]Pacios, L. F. Analytical Density-Dependent Representation of Hartree-Fock Atomic Potentials. *J. Comput. Chem.* **1993**, *14* (4), 410-421.

[2] Pacios, L. F. Kinetic Energies and Exchange Model Potentials Calculated with a Simple Analytical Representation of Atomic Densities. *J. Phys. Chem.* **1994**, *98* (14), 3688-3693.

[3] Sterling, T.; Irwin, J. J. ZINC 15--Ligand Discovery for Everyone. *J. Chem. Inf. Model.* **2015**, *55* (11), 2324-2337.

Hydroxylamine Derivatives for Specific Lysine Covalent Modification of Bacterial Type I Dehydroquinase

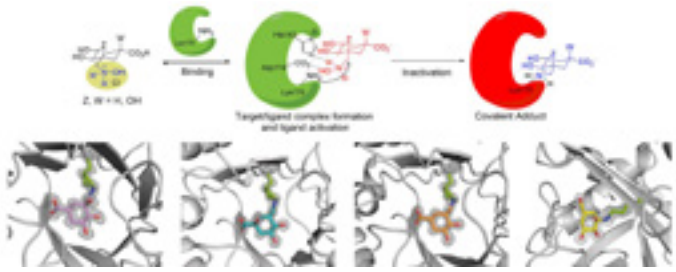
Ángela RODRÍGUEZ-COSTA[(1)], María MANEIRO[(1)], Emilio LENCE[(1)], Marta SANZ-GAITERO[(2)], José M. OTERO[(1)], Mark J. VAN RAAIJ[(2)], Concepción GONZÁLEZ-BELLO[(1)]*

[1] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela, calle Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain.

[2] Departamento de Estructura de Macromoléculas, Centro Nacional de Biotecnología (CSIC), Campus Cantoblanco, 28049 Madrid, Spain.

Contact e-mail: concepcion.gonzalez.bello@usc.es

Targeting cancer and infectious diseases with small molecules that covalently and irreversibly modify the target is an emerging area in drug discovery. In particular, efforts are currently being devoted to the design of irreversible compounds capable of modulating their reactivity when complementarity with the specific target takes place. These are rationally designed inhibitors bearing "latent electrophiles", which are functional groups that become activated toward covalent bond formation upon binding to a specific protein or enzyme but they are silent to non-specific targets. Most of the reported inhibitors have been designed to modify cysteine residues, however, targeting lysine residues are more challenging due to the low reactivity of the amino group under physiological conditions. We report here the unprecedented use of a hydroxylamine group as a latent electrophile for the specific covalent modification of the catalytic lysine residue of the bacterial type I dehydroquinase (DHQ1) enzyme[1]. DHQ1, which is present in several pathogenic bacteria, is a dehydratase enzyme that does not have any counterpart in human cells and has been pinpointed as a promising target in the search for new anti-virulence agents to combat widespread antibiotic resistance. Evidence in atomic detail for the covalent modifications caused by the ligands to the essential Lys170 by the formation of a stable amine is provided by the resolution of high quality crystal structures of DHQ1 from *Salmonella typhi* (*St*-DHQ1) chemically modified by the reported ligands. The results of docking and Molecular Dynamics simulation studies on the non-covalent target/ligand complexes, allowed us to explain in atomic detail how the reported compounds are activated by the enzyme for efficient chemical modification by Lys170. These studies might open up new opportunities for the development of novel lysine-targeted irreversible inhibitors bearing a hydroxylamine moiety as latent electrophile.



Acknowledgements: Financial support from the Spanish Ministry of Economy and Competiveness (SAF2016-75638-R), the Xunta de Galicia (Centro singular de investigación de Galicia accreditation 2016-2019, ED431G/09) and the European Regional Development Fund is gratefully acknowledged.

References:

[1] Maneiro, M.; Lence, E.; Sanz-Gaitero, M.; Otero, J. M.; van Raaij, M. J.; Thompson, P.; Hawkins, A. R.; González-Bello, C. submitted.

Thiol-disulfide reversible chemistry in non-conventional conditions

Andrea CANAL-MARTÍN[1,2], Ruth PÉREZ-FERNÁNDEZ[1]

[1] Structural and chemical biology department, Centro de Investigaciones Biológicas, CIB-CSIC, 28040 Madrid, Spain.

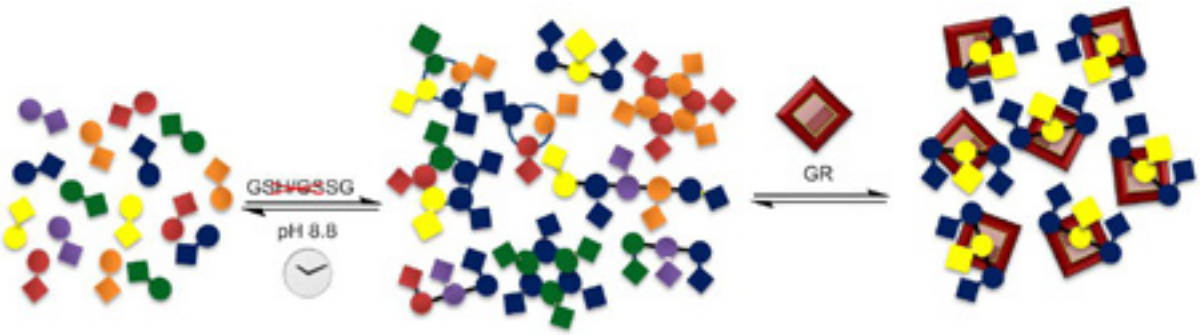
[2] Organic chemistry department, Universidad Complutense de Madrid, 28040 Madrid, Spain.

Contact e-mail: andrecan@ucm.es

Dynamic Combinatorial Chemistry (DCC) has demonstrated its potential in drug discovery speeding the identification of modulators of biological targets. However, the exchange chemistries typically take place under specific reaction conditions, with only a limited variety of tools capable of operating under physiological settings [1].

Disulfide exchange is one of the reversible chemistries compatible with biomolecules such as proteins. In a biological environment, this reversible chemistry requires a redox buffer, with oxidant and reductant agents able to develop the equilibrium in a shorter equilibration time and to control oxidation subprocess. GSH/GSSG buffer media allows to control these facts, though it biases adducts formation [2].

We report the use of disulfide exchange in a non-redox media with Glutathione Reductase (GR) as a template. This protein has been recently reported as an important antimalarial target [3].



Acknowledgements: CTQ2015-69643-R and European Cooperation in Science and Technology (COST) action CMI304.

References:

[1] Matysiak, B. M.; Nowak, P.; Cvrtila, I.; Pappas, C. G.; Liu, B.; Komáromy, D.; Otto, S. *J. Am. Chem. Soc.* **2017**, *139*, 6744.

[2] Corbett, P. T.; Leclaire, J.; Vial L.; West, K. R.; Wietor, J. L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652.

[3] Gallo, V.; Schwarzer, E.; Rahlfs, S.; Schirmer, R. H.; van-Zwieten, R.; Roos, D.; Arese1, P.; Becker, K. *PLoS ONE* **2009**, *4*, e7303.

Functionalization of Magnetic Nanoparticles with Gemcitabine via disulfide bonds: a new approach for the pancreatic cancer treatment

Nuria LAFUENTE-GÓMEZ(1), Paula MILÁN ROIS(1), Marco CORDANI(1), Yurena LUENGO(1), Gorka SALAS(1), Álvaro SOMOZA(1)

[1] Institute for Advanced Studies in Nanoscience (IMDEA Nanociencia), Centro Nacional de Biotecnología (CNB-CSIC) Associated Unit of Nanobiotechnology, Faraday 9, 28049 Madrid, Spain.

Contact e-mail: nuria.lafuente@imdea.org

Nanotechnology has received tremendous attention, in particular from the field of medicine. Breakthroughs in nanomedicine have had a high impact on the development of more efficient drug delivery and imaging systems. In this regard, the differences in the physicochemical properties between the environments of cancerous and healthy cells have motivated the design of smart nanoparticles[1].

In this work, we proposed a smart nanocarrier composed by dextran magnetic nanoparticles functionalized with gemcitabine, a chemotherapy drug, via disulfide bonds. The bonds are prone to rapid cleavage by glutathione (GSH) which is in higher concentration in tumor tissues (0,5-10 mM) compared with healthy ones (0,5-10 μM). This nanodevice is based on one previously described, which allows the release of drugs without any chemical modification[2].

The efficacy of the system has been evaluated in a variety of pancreatic cell lines: PANC-1, BxPC-3 and MIA PaCa-2. The sensitivity of these human cancer cells to gemcitabine is different: PANC-1 cells are the most resistant and MIA-PaCa-2 are the most sensitive. In addition, the breast cancer cell line MCF-7 was used to evaluate the therapeutic potential of this nanodevice for the treatment of this type cancer[3].

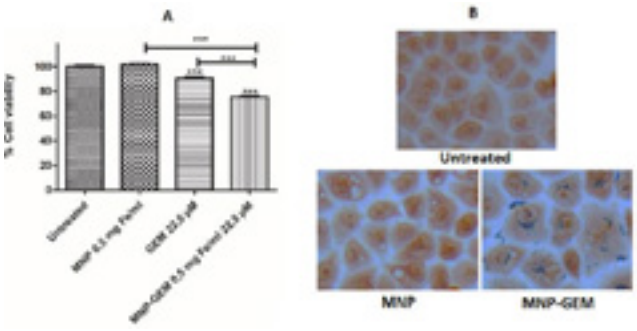


Figure 1.- A.-Cell viability assay in PANC-1 cells.
B.- Prussian blue staining of PANC-1 cells

Acknowledgments: This work was supported by the Spanish Ministry of Economy and Competitiveness (SAF2017-87305-R), Comunidad de Madrid (IND2017/IND-7809; S2017/BMD-3867), Asociación Española Contra el Cáncer, and IMDEA Nanociencia. IMDEA Nanociencia acknowledges support from the 'Severo Ochoa' Programme for Centres of Excellence in R&D (MINECO, Grant SEV-2016-0686). This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 685795 (NoCanTher). We also acknowledge the European Structural and Investment Fund.

References:

[1] Yoo, D.; Lee, T.; Shin, TH.; Cheon, J. Gu, Ni. *Accounts of Chemical Research.* **2011**,44, 863.

[2] Latorre, A.; Couleaud, P.; Aires, A.; Cortajarena AL.; Somoza, A.; *European Journal of Medicinal Chemistry.* **2014**, 82, 355.

[3] Kossatz, S.; Grandke, J.; Couleaud, P. *et al*. *Breast Cancer Research*. **2015**, 17, 1.

Accelerating (Bio)molecular Recognition and Assembly

Ferran FEIXAS[(1)], Christian CURADO[(1)], Sílvia OSUNA[(1,2)]

[1] Department of Chemistry, Institut de Química Computacional i Catàlisi, University of Girona, 17003 Girona, Spain.

[2] ICREA, 08010 Barcelona, Spain.

Contact e-mail: ferran.feixas@udg.edu

(Bio)molecular recognition and assembly are key concepts in chemistry, biology and drug design. Chemical and life processes are critically dependent on the association and dissociation of (bio)molecules. Understanding the mechanisms of these relevant processes is of utmost importance. Experimental methods provide information on stable structures, binding affinities, or kinetics but cannot offer a complete description of binding and unbinding pathways at the required atomic detail. Molecular dynamics can provide an atomistic dynamic view of such processes. However, a tremendous amount of conformational sampling is required to obtain accurate pathways and reliable stationary and kinetic properties. Current computational methods to study assembly, binding, and unbinding of (bio)molecules either require access to high-performance computing resources or the definition of a complex reaction coordinates to enhance conformational sampling.

Our goal is to develop a computational protocol to study, at atomic level, the mechanisms of self-assembly, association and dissociation of (bio)molecules at a reasonable computational cost. This protocol relies on the basic ideas of accelerated molecular dynamics (aMD),[1,2] an unconstrained enhanced sampling technique that does not require the a priori definition of any reaction coordinate. The novel strategy consists in redefining aMD by selectively boosting non-bonded interactions (aMD-nB) between interacting molecules. aMD-nB allows to enhance conformational sampling of associative and dissociative processes and to recover stationary and kinetic properties.

First, the new aMD-nB protocol is assessed for well-studied cases of protein folding, biomolecular recognition and protein-protein interactions. Second, we focus on showing the potential of this novel technique as a tool to efficiently explore the free energy landscape of drug binding and unbinding. In particular, we have reconstructed the pathways of inhibitor binding and unbinding to rationalze the origin of inhibitor selectivity and to improve drug design for a set of p38s and CDKs protein kinases involved in liver cancer.[3]

Acknowledgements: F.F. thanks the European Community for MSCA-IF-2014-EF-661160- MetAccembly grant. S. O. thanks the funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (ERC-2015-StG-679001).

References:

[1] Markwick, P. R. L.; McCammon, J. A. *Phys. Chem. Chem. Phys.* **2011**, 13, 20053.

[2] Miao, Y.; Feixas, F.; Eun, C.; McCammon, J. A. *J. Comp. Chem.* **2015**, 36, 1536.

[3] Tomás-Loba, A.; Manieri, E.; González-Terán, B.; Mora, A.; Leiva-Vega, L.; Santamans, A. M.; Rodríguez, E.; Feixas, F.; López, J. A.; Caballero, B.; Trakala, M.; Blanco, O.; Torres, J. L.; Roche-Molina, M.; Bernal, J. A.; Saavedra, D. M.; Ruiz-Cabello, J.; Brava-Sicilia, J.; Vázquez, J.; Marcos, M.; Malumbres, M.; Osuna, S.; Sabio, G. (submitted for publication) **2018**.

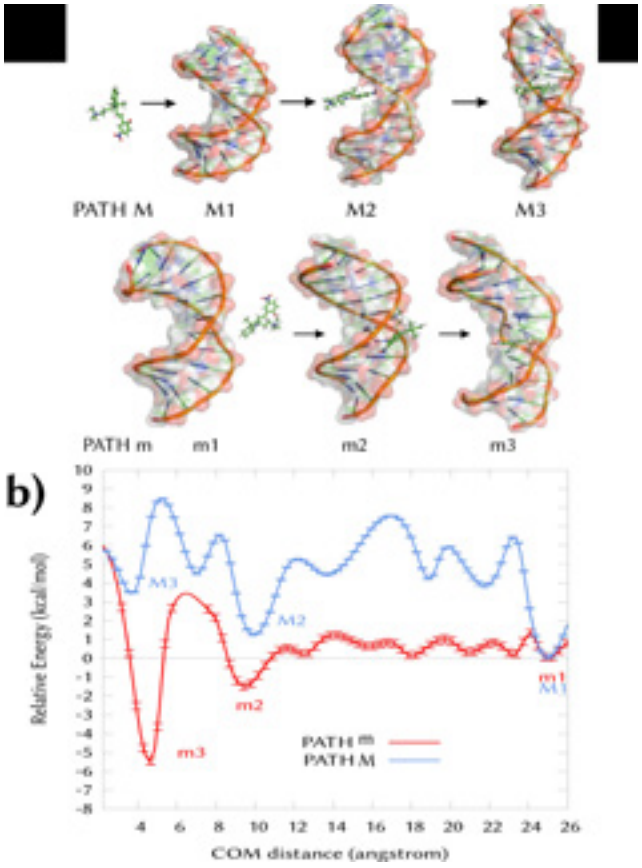
Drastic Effect of the Electrostatic Energy on the Binding mode of Spiropyran Photoprobe to dsDNA

Davide AVAGLIANO[(1)], Pedro A. SÁNCHEZ-MURCIA[(1)], Leticia GONZÁLEZ[(1)]

[1] Institute of Theoretical Chemistry, Faculty of Chemistry. University of Vienna.

Contact e-mail: davide.avagliano@univie.ac.at

Spiropyran belongs to a family of photoswitches known to bind dsDNA after photoisomerization triggered by UV irradiation.[1] Experimental studies evidenced intercalation as the binding mode for these molecules into the DNA,[2] but several aspects of such interaction are still unknown. By means of computational methods, such as umbrella sampling and unrestrained molecular dynamics simulations, we investigated the intercalation process of different relevant spiropyran derivatives. Our results clearly point out that these species favor binding through the minor groove (path m in Figure below) to dsDNA versus the major groove (path M). We characterized the final and intermediate states of the intercalation pathways and unraveled the critical role of the electrostatic component for targeting a specific binding mode.



Acknowledgements: D.A and LG thanks the Marie Curie Action, within the ETN project LightDyNAmics project, for the financial support.

References:

[1] J. Andersson, S. Li, P. Lincoln, J. Andréasson, *J. Am. Chem. Soc.* **2008**, *130*, 11836-11837.

[2] M. Hammarson, J. R. Nilsson, S. Li, P. Lincoln, J. Andréasson, *Chem. – A Eur. J.* **2014**, *20*, 15855-15862.

Neurodegenerative diseases: From small molecules to targeted therapies

Maria J. MATOS[1], Dolores VIÑA[2], Lourdes SANTANA[1], Eugenio URIARTE[1]

[1] Departamento de Química Orgánica, Facultad de Farmacia, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

[2] Centro de Investigación en Medicina Molecular y Enfermedades Crónicas (CIMUS) Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

Contact e-mail: mariajoao.correiapinto@usc.es

Neurodegenerative diseases are becoming increasingly prevalent with the aging of the general population. The twentieth century witnessed a significant demographic change in the human population of the industrialized world that is currently followed by a similar shift of life expectancy to upper age ranges in developing countries.

The effectiveness of a drug is dependent on accumulation at the site of action at therapeutic levels. However, challenges such as rapid renal clearance, degradation or non-specific accumulation requires drug delivery enabling technologies. Targeted drug delivery is a very promising concept, which still needs improvement for better clinical outcomes. Understanding some of the molecular changes associated to these ubiquitous and widespread diseases, has stimulated efforts to develop drugs that specially target key proteins involved in the development of a given type of it. Protein behaviour is scrupulously regulated by a plethora of post-translational modifications (PTMs). It is therefore desirable to develop methods to design rational PTMs to modulate specific protein functions.[1-3]

Here, we report different approaches and we illustrate their successful implementation, associated with Parkinson's disease. Computer-assisted design of potent small molecules, together with the development of new carriers, allows a wide range of possibilities for targeted therapies. We show that these new approaches augment the potential of small molecules, while preserving other important functionalities. Knowledge on biochemical processes brings the opportunity to provide treatments that are potentially less toxic and more effective than traditional therapeutic approaches.

Acknowledgements: This work was partially supported by the University of Santiago de Compostela (2018-PU067). Authors would like to thank the use of RIAIDT-USC analytical facilities, and ESF, FCT, POPH, QREN (SFRH/BPD/95345/2013) and Xunta da Galicia Plan of Research, Innovation and Growth 2011–2015 (Plan I2C, ED481B 2014/086–0 and ED481B 2018/007) for the financial support.

References:

[1] Matos, M. J.; Oliveira, B. L.; Martínez-Sáez, N.; Guerreiro, A.; Cal, P. M. S. D.; Bertoldo, J.; Maneiro, M.; Perkins, E.; Howard, J.; Deery, M. J.; Chalker, J. M.; Corzana, F.; Jiménez-Osés, G.; Bernardes, G. J. L. *J. Am. Chem. Soc.* **2018**, *140*, 4004.

[2] Matos, M. J. *Fut. Med. Chem.* **2018**, *10(9)*, 983.

[3] Matos, M. J.; Labão-Almeida, C.; Sayers, C.; Dada, O.; Tacke, M.; Bernardes, G. J. L. *Chem. Eur. J.* **2018**, *24*, 12250.

Specific Biosensors in Drug Discovery For Amyotrophic Lateral Sclerosis

Carlota TOSAT, Ana MARTÍNEZ, Valle PALOMO

Structural and Chemical Biology, Centro de Investigaciones Biológicas-CSIC, C/ Ramiro de Maeztu 9, 28040 Madrid, Spain.

Contact e-mail: carlota.tosat@cib.csic.es / vpalomo@cib.csic.es

Neurodegenerative diseases (NDs) constitute a major health, economic and social issue worldwide. Despite the efforts for understanding NDs there is a lack of knowledge of their molecular pathology which is crucial for developing new efficient treatments. Amyotrophic lateral sclerosis (ALS) is a ND characterized by motor neuron (MN) death that yields in progressive paralysis. The mechanism underlying selective MN death remains an essential question, becoming a critical target for drug development.[1]

Molecular profiling is an innovative powerful technology for unraveling complex molecular pathways that underlie physiological and pathological processes. Quantum dots (QDs) are very promising tools to detect molecular mechanisms at the subcellular level as their properties are ideal for multiplexing applications. QDs are nanoparticles formed by CdSe core and a ZnS coating that offer unique photo luminescent properties. They present an improved photostability, large molar extinction coefficients, broad absorption and narrow emission spectra that enables improved detection sensitivity and multiplexed analysis.[2] QDs have efficiently been conjugated to monoclonal antibodies (mAB) forming QD-AB probes that can be used in immunofluorescence, implementing a multiplexing QD based immunoassay.[3] Using this technology, different key proteins in ALS like TDP-43, p-TDP-43, CK1 will be analyzed at the single-cell level in human cell models from ALS patients such as lymphoblasts transformed from lymphocytes extracted from patients.

The scientific aim of this project is to further contribute to the molecular unraveling of ALS, finding patterns in patients, screening for biomolecular targets of these diseases; and to explore molecular changes in key protein targets upon pharmacological treatment to help select therapeutic candidates.

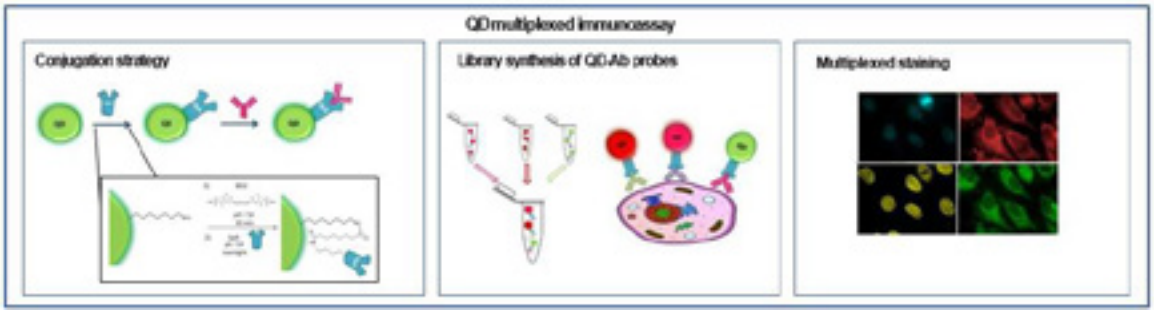


Figure 1. Methodology for the multiplexed molecular profile with QDs. a) Bioconjugation strategy of QD-SpA. b) Shematic illustration of the technology. c) Parallel multiplexed staining with five pre-assembled QD-SpA-Ab probes.[3]

References:

[1] Taylor, J.P.; Brown, R.H. Jr.; Cleveland, D.W. Decoding ALS: form genes to mechanism. *Nat*. **2016**, *539*, 197-206.

[2] Wegner, K.D.; Hildebrandt, N. Quantum dots: bright and versatile in vitro and in vivo fluorescence imaging biosensors. *Chem Soc Rev*. **2015**, *44*, 4792-834.

[3] Zrazhevskiy, P.; Gao, X. Quantum dot imaging platform for single-cell molecular profiling. *Nat Commun*. **2013**, *4*, 1619.

Amphiphilic peptide/Cas9 nanocomplexes for the delivery of gene editing nucleases

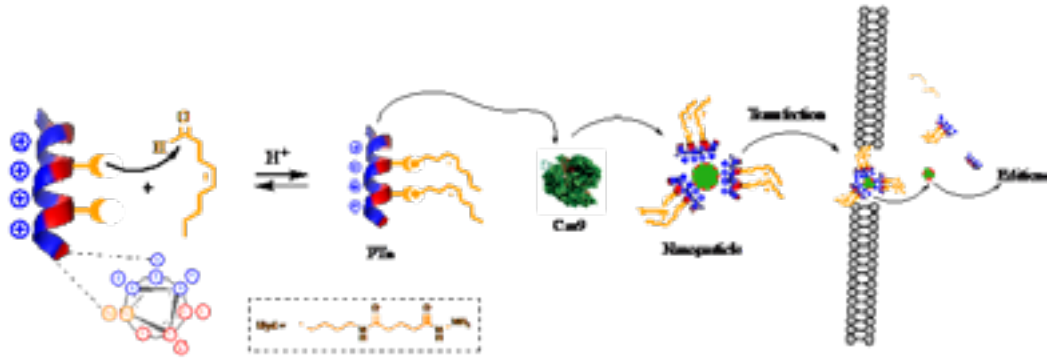
Marisa JUANES(1), Irene LOSTALÉ-SEIJO(1), Iria LOUZAO(1), Javier MONTENEGRO(1).

[1] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares, Campus Vida, Rúa de Jenaro de la Fuente, s/n, 15782 Santiago de Compostela, La Coruña.

Contact e-mail: marialuisa.juanes@usc.es

Since the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) were discovered and assigned to a bacterial adaptive immune system,[1] the excitement of the scientific community towards this technology has grown beyond any expectation.[2] In the fields of gene edition and biotechnology, the discovery of RNA guided endonucleases has emerged as one of the most important tools.[3] The selectivity and simplicity of the CRISPR/Cas9 strategy allows the straightforward targeting and editing of particular loci in the cell genome without the requirement of protein engineering. However, the transfection of plasmids encoding the Cas9 and the guide RNA could lead to undesired permanent recombination and immunogenic responses. Therefore, the direct delivery of transient Cas9 ribonucleoprotein constitutes an advantageous strategy for gene edition and other potential therapeutic applications of the CRISPR/Cas9 system.

In this regard, we here report a supramolecular strategy for the direct delivery of Cas9 by an amphiphilic penetrating peptide that was prepared by a hydrazone bond formation with a hydrophobic aldehyde tail. Upon the peptide/protein interaction, non-covalent ~50nm nanoparticles were formed. These aggregates performed with similar efficiency and less toxicity than one of the best methods described to date (i.e. lipofectamine).



Acknowledgements: We acknowledge the support from MINECO (RYC-2013-13784, CTQ2014-59646-R and SAF2017-89890-R), the Xunta de Galicia (ED431G/09 and 2016AD031), the ERDF, the ERC Starting Grant (DYNAP-677786) and the Human Frontier Science Program (RGY0066/2017).

References:

[1] K. S. Makarova, *et al., Nat. Rev. Micro.,* **2015**, *13*, 722.

[2] R. Barrangou and J. A. Doudna, *Nat. Biotechnol.,* **2016**, *34*, 933.

[3] V. Pattanayak, J. P. Guilinger and D. R. Liu, *Methods Enzymol.,* **2014**, *546*, 47.

Computational modeling and cysteine crosslinking studies of vimentin

Andreia MÓNICO[1], Joan GUZMÁN-CALDENTEY[1], María A. PAJARES[1], Sonsoles MARTÍN-SANTAMARÍA[1], Dolores PÉREZ-SALA[1]

[1] Department of Structural and Chemical Biology, Centro de Investigaciones Biológicas (CSIC), C/ Ramiro de Maeztu 9, 28040 Madrid, Spain.

Contact e-mail: amonico@cib.csic.es

The intermediate filament protein vimentin is a cytoskeletal component of metazoan cells. It is involved in several cellular processes, ranging from providing mechanical stability and cell elasticity to regulating the response to injury or inflammation (1). Vimentin possesses a single cysteine residue, C328, which plays a key role in the modulation of filament assembly (2). C328 serves as a sensor for oxidative and electrophilic stress in a manner dependent on zinc availability. In order to study the atomic details of this process, we have computationally modeled the 3D structure of a putative vimentin dimer (comprising amino acids from 264 to 406), and studied the possible binding sites for zinc. In addition, biochemical crosslinking and oxidative conditions indicate the existence of aggregates in which cysteine residues from different dimers or tetramers may fall within short distance in vimentin filaments. In fact, our protein-protein docking studies indicate the possible formation of a dimer of dimers where two cysteine residues are close enough to allow the crossed-linkage (Figure 1). Mutation studies are under progress to confirm the importance of this residue for vimentin assembly and response to lipooxidative stress.

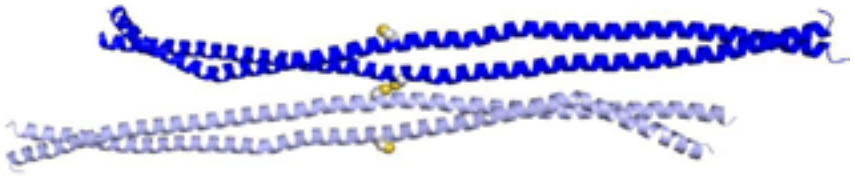


Figure 1. 3D Model of a putative vimentin dimer of dimers after 100ns MD simulation.

Acknowledgements: SAF2015-68590-R and CTQ2017-88353-R from MINECO/FEDER ; MSCA ITN Masstrplan from H2020, Grant No.: 675132

References:

[1] Ivaska J, Pallari HM, Nevo J, Eriksson JE. *Exp Cell Res* (**2007**) *313*, 2050.

[2] Pérez-Sala D, Oeste CL, Martínez AE, Carrasco MJ, Garzón B, Cañada. *Nat Commun* (**2015**) *6*, 7287.

Reprograming Uveal Melanoma Cells: A Combination Therapy Based On Gold Nanoparticles

Paula MILÁN ROIS[(1)], Alfonso LATORRE[(1)], Ciro RODRÍGUEZ DIAZ[(1)], Álvaro DEL MORAL[(1)], Álvaro SOMOZA[(1)]

[1] Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), Centro Nacional de Biotecnología (CNB-CSIC) Associated Unit, Faraday 9, 28049 Madrid, Spain.

Contact e-mail: paula.milan@imdea.org

Uveal melanoma (UM) is the most common primary intraocular malignant tumor in adults and around half of the patients develop metastasis and die shortly after because of the lack of effective therapies for metastatic UM. Consequently, new therapeutic approaches to this disease are welcome [1].

In this regard, microRNAs have been shown to have a key role in neoplasia progression and have the potential to be used as therapeutic tools. In addition, in different cancers including UM, a particular microRNA signature appears that is different from healthy cells. Thus, restoring the regular levels of microRNAs could restore the normal behavior of cells [2]. In this study, four microRNAs downregulated in UM have been chosen to reprogram cancer cells, to promote cell death or increase their sensitivity to the chemotherapeutic SN38. Furthermore, to improve the internalization, stability and/or solubility of the therapeutic molecules employed in this approach, gold nanoparticles (AuNPs) were used as carriers. Remarkably, this study found a synergistic effect when four oligonucleotides and the chemotherapeutic drug SN38 were combined.

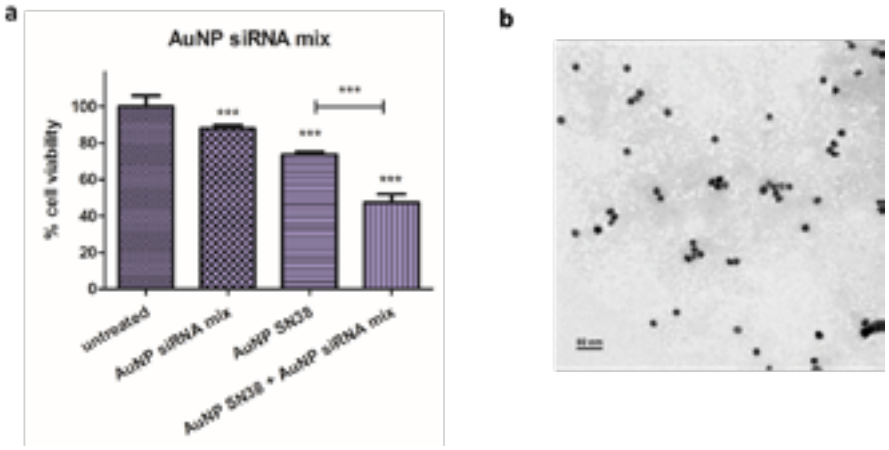


*Figure 1: (**a**) AuNP siRNA mix, AuNP SN38 and their combination (AuNP siRNA mix + AuNP SN38) (*** $p < 0.001$). (**b**) TEM images of AuNPs. Homogeneous size was observed.*

Acknowledgments: This work was supported by the Spanish Ministry of Economy and Competitiveness (SAF2017-87305-R), Fundación Científica Asociación Española Contra el Cáncer, and IMDEA Nanociencia. IMDEA Nanociencia acknowledges support from the 'Severo Ochoa' Programme for Centres of Excellence in R&D (MINECO, Grant SEV-2016-0686)

References:

[1] D. Reichstein.D. Curr. Opin. Ophthalmol **2017**, *28*, 219.

[2] Sato. T; *et al*.. Curr. Oncol. Rep **2008**, *10*, 431.

Green synthesis of designed protein-stabilized CdS quantum dots for bio-imaging applications

Antonio AIRES[1], Aitziber L. CORTAJARENA[(1,2*)]

[1] CIC biomaGUNE, Parque Tecnológico de San Sebastián, Paseo Miramón 182, 20014 Donostia-San Sebastián, Spain.

[2] Ikerbasque, Basque Foundation for Science, Mª Díaz de Haro 3, 48013 Bilbao, Spain.

Contact e-mail: aaires@cicbiomagune.es

Quantum dots (QDs) have received great attention due to unique optical, electronic and chemical properties, compared to traditional organic fluorescent dyes. In recent years, enormous efforts have been made to use QDs as fluorescent reporters for biomedical applications such as bioimaging.[1] However, conventional QD synthesis requires toxic reagents, extreme reaction conditions, intensive energy input and extensive post-synthetic processing to make the QDs water soluble for biological applications. To overcome these limitations, continued efforts have been made to produce QDs under aqueous conditions that do not require high temperature or post-synthetic processing. However, the aqueous routes are critically-dependent on surface capping agents. Many different organic molecules, peptides and proteins have been used efficiently as capping agents.[2]

The current study explores the use of an artificial repeat protein (CTPR) with a designed metal coordination site as a novel capping/stabilizing agent for green synthesis of QDs. The metal coordination site based on four histidines (H_4) was modeled on the crystal structure of the CTPR3 protein (1Na0), and four different CTPRs proteins with one, two, three and four metal coordination sides were assembled. These QD-stabilization capacity of the designed proteins was compared with a CTPR wild type protein without metal coordination site and a polyhistidine fusion CTPR protein that was previously shown to stabilize QDs.[3] The protein-stabilized QDs formation was evaluated by the appearance of characteristic fluorescence features. The CTPR wild type protein showed no fluorescence, whereas polyhistidine fusion CTPR protein and the designed CTPR proteins showed an emission peak at 520 nm when excited at 365 nm. The resulting protein stabilized CdS QDs present excellent fluorescent properties, photostability, and biocompatibility. In addition, the protein-QDs complexes are able to enter into living cells without any permeabilization treatment, making them useful for live cell imaging and labeling.

References:

[1] Jamieson, T.; Bakhshi, R.; Petrova, D.; Pocock, R.; Imani, M.; Seifalian, A. M., Biological applications of quantum dots. *Biomaterials* **2007,** *28* (31), 4717-4732.

[2] Zhou, W.; Baneyx, F., Aqueous, Protein-Driven Synthesis of Transition Metal-Doped ZnS Immuno-Quantum Dots. *ACS Nano* **2011,** *5* (10), 8013-8018.

[3] Aryal, B. P.; Benson, D. E., Polyhistidine fusion proteins can nucleate the growth of CdSe nanoparticles. *Bioconjugate chemistry* **2007,** *18* (2), 585-9.

Functionalization of elongated viral nanoparticles through chemical conjugation to molecules of different kinds

Carmen YUSTE-CALVO, Ivonne GONZÁLEZ-GAMBOA*, Luis F. PACIOS, Flora SÁNCHEZ, Fernando PONZ

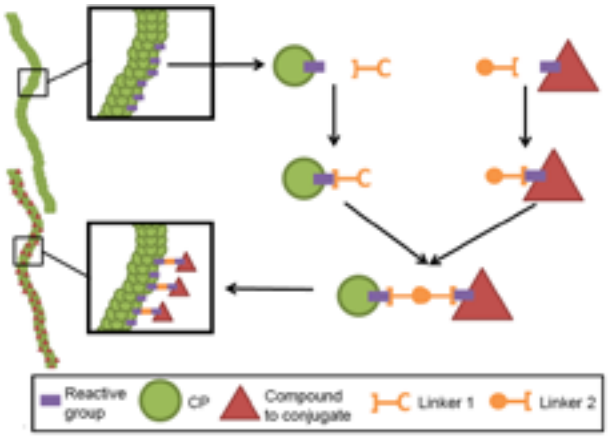
Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid - Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (CBGP, UPM-INIA), Campus Montegancedo, Autopista M-40, km 38. Pozuelo de Alarcón, 28223 Madrid, Spain.

*Present Adress: Centro de Biotecnología - FEMSA, Tecnológico de Monterrey, Ave. Eugenio Garza Sada 2501, Col. Tecnológico, CP 64849 Monterrey, Nuevo León, México.

Contact e-mail: fponz@inia.es

Viral nanoparticles (VNPs) are taking a leading role in the field of nanobiotechnology. In this context, plant viruses offer many advantages related with biosafety, as well as interesting structural characteristics, and cost-economy. This is the case of *Turnip mosaic virus* (TuMV), a potyvirus with a flexuous elongated structure which allows the development of functionalized nanoparticles with up to 2000 copies of different functionalizing compounds. The first approaches with this platform consisted of functionalization through genetic fusion of peptides to the virus coat protein (CP), rendering peptide-coated nanoparticles, deployable as tools for immunization [1], or for high sensitive diagnosis [1,2].

In order to develop nanoparticles functionalized with a wide range of compounds, we have implemented several chemical functionalizations based on the recently uncovered TuMV virion structure. Attempted chemical conjugations leaned on the spatial location of certain amino acids amenable to be attacked by different chemical compounds, creating very stable bonds. Compounds ranged from fluorophores to drugs, or other proteins or macromolecules. Different linkers were used in process, as shown in the figure.



VNP derivatizations will allow multiple functionalization of protein-based nanoparticles, thus open the path towards complex goals in nanoscience requiring different, possibly unrelated yet complementary, added functions.

Acknowledgements: This research was partially supported by several INIA grants. We thank Lucía Zurita for her technical assistance.

References:

[1] Sánchez, F.; Sáez, M.; Lunello, P.; Ponz, F. *J. Biotechnol.* **2013**, *168*, 409.

[2] González-Gamboa, I.; Manrique, P.; Sánchez, F.; Ponz, F. *J. Biotechnol*. **2017**, *254*, 17.

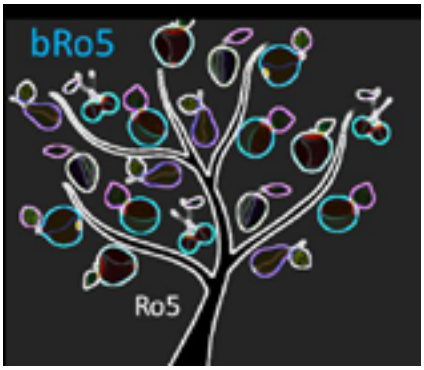
Ro5 vs bRo5: the winner is …

Giulia CARON[(1)], Maura VALLARO[(1)], Giuseppe ERMONDI[(1)]

[1] Molecular Biotechnology and Health Sciences Dept., University of Torino, via Quarello 15, 10135 Torino, Italy.

Contact e-mail: giulia.caron@unito.it

According to the most recent literature, the future of drug discovery also includes the transition from simple to complex structures where complex structures mostly refer to beyond Rule of 5 (bRo5) compounds (e.g. macrocycles and cyclic peptides). Potency and ADMETox-related properties (e.g. solubility, permeability) of bRo5 candidates require optimization but although pharma industry developed standardized protocols for optimizing small molecules, property-based optimization strategies for complex structures are still missing.



During the lead optimization process, medicinal chemists need information about the effect of structural changes on the physicochemical profile of candidates. Computational and experimental approaches are adopted to reach this aim. Traditionally, the lipophilicity in octanol/water is considered good enough to guide candidates' permeability optimization but this does not hold for bRo5 molecules since a single biphasic system cannot assess the chameleonic, environment-dependent, properties of flexible structures. To this respect media which better mimic the apolar nature of some internal membrane compartments are expected to be relevant.

In this talk I firstly introduce bRo5 compounds and discuss their relevance in modern drug discovery. Then I discuss the impact of flexibility on the molecular properties of these structures[1]. Finally, I illustrate which molecular properties are suited for the optimization of two series of bRo5 compounds a) drugs with macrocyclic structure[2], b) model charged cyclic hexapeptides specifically designed to explore the role of different molecular descriptors on permeability[3].

References:

[1] Caron, G.; Ermondi, G. *Drug Discov. Today* **2017,** *22***,** 835.

[2] Rossi Sebastiano, M.; Doak, B. C.; Backlund, M.; Poongavanam, V.; Over, B.; Ermondi, G.; Caron, G.; Matsson, P.; Kihlberg, J. *J. Med. Chem.* **2018**, *61,* 4189.

[3] Ermondi, G.; Vallaro, M.; Camacho-Leal, M. P.; Potter, T.; Visentin, S.; Caron, G. *Eur. J. Pharm. Sci.* **2018**, *122,* 85.

Structural basis of RNA polymerase I stalling at UV light-induced DNA damage

Marta SANZ-MURILLO[1], Jun XU[2], Georgiy A. BELOGUROV[3], Olga CALVO[4], David GIL-CARTON[5], María MORENO-MORCILLO[1][6], Dong WANG[2], Carlos FERNÁNDEZ-TORNERO[1]

[1] Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Cientificas (CSIC), 28040 Madrid, Spain.

[2] Skaggs School of Pharmacy & Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093-0625.

[3] Department of Biochemistry, University of Turku, FIN-20014, Turku, Finland.

[4] Instituto de Biología Funcional y Genómica, CSIC-Universidad de Salamanca, 37007 Salamanca, Spain.

[5] Structural Biology Unit, Cooperative Center for Research in Biosciences (CIC bioGUNE), 48160 Derio, Spain.

[6] Centro de Biología Molecular Severo Ochoa, CSIC, 28049 Madrid, Spain.

Contact e-mail: cftornero@cib.csic.es

For the regular function of the cell, DNA integrity is fundamental. Exogenous agents can damage the genetic material such as ultraviolet light, which among other lesions produces *cys-sin* cyclobutane pyrimidine dimers (CPDs). Transcription-coupled repair (TCR) is a NER subpathway that repairs bulky lesions such as CPD, which introduce distortions in the DNA double helix and stall transcription. In eukaryotic cells, there are three classes of RNA Polymerases (RNAPs) and each of them transcribes different type of genes. Pol I is a 14-subunit enzyme that synthesizes the ribosomal RNA, a major component of the ribosome. While the structural basis of CPD stalling and bypass by Pol II has been described, how Pol I identifies such lesions remains unclear. In the past, we used X-ray crystallography and cryo-EM to unveil the Pol I activation process [1][2]. We now aimed to elucidate the structural basis of CPD recognition by Pol I. For that, we determined the cryo-EM structure of Pol I in elongation complex containing a CPD lesion at a resolution of 3.6 Å. The structure shows an intermediate translocation state, where the CPD lesion is located over the bridge helix, a central element for RNA synthesis. This DNA configuration occludes the NTP entry site, which is incompatible with nucleotide addition opposite the lesion. We also observe that residue R1015 in the bridge helix, which is only conserved in Pol I, has an important role in lesion recognition by establishing a cation-π interaction with the 3'T in CPD. Besides, mutational analysis confirmed that this amino acid has an important effect in reducing the rate of CPD lesion bypass. In comparison with Pol II, the structure of Pol I stalled at a CPD lesion together with biochemical data suggests that lesion recognition differs between the two transcription systems [3].

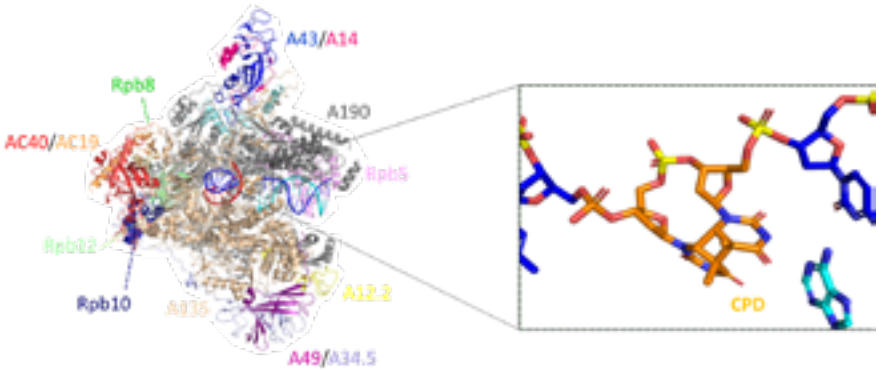


Figure 1. A. Structure of Pol I – CPD, indicating different subunits in the enzyme. B. Close-up view of CPD lesion.

References:

[1] Fernández-Tornero, C.; Moreno-Morcillo, M.; Rashid, UJ.; Taylor, NM.; Ruiz, FM.; Gruene, T.; Legrand, P.; Steuerwald, U.; Müller, CW. Crystal structure of the 14-subunit RNA Polymerase I. *Nature* **2013** 502:644-649.

[2] Torreira, E.; Louro, JA.; Pazos, I.; González-Polo, N.; Gil-Carton, D.; Duran, AG.; Tosi, S.; Gallego, O.; Calvo, O.; Fernández-Tornero, C. The dynamic assembly of distinct RNA Polymerase I complexes modulates rDNA transcription. *eLife* **2017** 6:e20832.

[3] Sanz-Murillo, M.; Xu, J.; Belogurov, GA.; Calvo, O.; Gil-Carton, D.; Moreno-Morcillo, M.; Wang, D.; Fernandez-Tornero, C. Structural basis of RNA polymerase I stalling at UV light-induced DNA damage. *Proc Natl Acad Sci USA* **2018** 115(36):8972-8977.

FP7, a synthetic TLR4 antagonist, reduces inflammation in the Gastrointestinal Tract

Fabio Alessandro FACCHINI[(1)], Davide DI FUSCO[(2)], Simona BARRESI[(1)], Alberto MINOTTI[(1)], Francesca GRANUCCI[(1)], Francesco PERI[(1)], Ivan MONTELEONE[(3)]

[1] Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza, 2, 20126 Milano, Italy.

[2] Department of Systems Medicine, University "Tor Vergata", Rome, Italy.

[3] Department of Biomedicine and Prevention, University of Rome "Tor Vergata", Rome, Italy.

Contact e-mail: f.facchini16@gmail.com

Background. The partial ineffectiveness and side effects of IBD current therapies drive basic research to find new therapeutic target and to look for new compounds with anti-inflammatory properties. Dysregulated TLRs signalling in gut mucosal immune cells is responsible of excessive innate immune responses, which compromise host tolerance towards microbial antigens, leading to chronic inflammation and to organ damage.[1] In particular, abnormal TLR4 activation in the gut of IBD patients enhances host sensitivity toward bacterial lipopolysaccharide (LPS), promoting gut inflammation.[2,3] The possibility to therapeutically modulate the signaling pathway mediated by TLR4 may be a promising strategy to reduce LPS responsiveness, restoring tolerance and ultimately ameliorating IBD inflammation.

Objectives. Considering the pivotal role played by TLRs in gut inflammation, in this study we aimed at evaluating the therapeutic effect of the synthetic TLR4 antagonist FP7.

Research methodology. We investigated the anti-inflammatory effect of FP7 both *in vitro* and *in vivo* and the mechanism of action of the compound was elucidated. Peripheral blood mononuclear cells (PBMCs) and *lamina propria* mononuclear cells (LPMCs) were isolated from IBD patients and exposed to LPS challenge in the presence or absence of FP7. The anti-inflammatory effect of FP7 was evaluated by real-time PCR and ELISA assay and the mechanism of action of the compound was investigated by flow cytometry and western blots analysis. The well-standardized experimental mouse model of dextran sulfate sodium (DSS)-induced colitis was used to evaluate FP7 anti-inflammatory effect *in vivo*.

Results. FP7 antagonizes the inflammatory responses induced by LPS *in vitro*, by competing with LPS for TLR4/MD-2 binding and, therefore, inhibiting the intracellular signaling. Moreover, FP7 abolishes LPS-induced CD14 and TLR4 internalization, suggesting that the compound could also interfere with the TRIF-dependent pathway. Therapeutic administration of FP7 reduces global inflammation of mice with DSS-induced colitis.

References:

[1] Cario E. *Inflammatory bowel diseases* **2010**, 16(9), 1583.

[2] Oostenbrug, LE; Drenth, JP.; *Inflammatory bowel diseases* **2005**, 11(6), 567.

[3] Smith, PD.; Smythies, LE. Mucosal immunology **2011**, 4(1), 31.

Insights into the molecular recognition and conformational preferences of the STn antigen derived glycopeptides as tools in cancer vaccination and diagnosis

Iris A. BERMEJO[(1)], Alicia ASÍN[(1)], Jesús Héctor BUSTO[(1)], Alberto AVENOZA[(1)], Jesús Manuel PEREGRINA[(1)], Ulrika WESTERLIND[(2)], Francisco CORZANA[(1)]

[1] Department of Chemistry, Universidad de La Rioja, C/ Madre de Dios 53, 26006 Logroño, Spain.

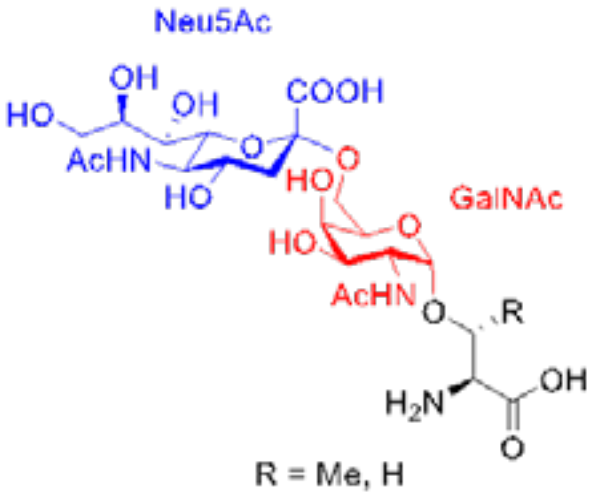
[2] Leibniz Institute for Analytical Sciences - ISAS, Otto-Hahn-Str. 6b, 44227 Dortmund, Germany.

Contact e-mail: irbermej@unirioja.es

MUC1 mucin is an *O*-glycoprotein presented on the surface of epithelial cells, which is closely related to cancer disease. In tumor cells, this protein is overexpressed and its carbohydrates are simple due to incomplete glycosylation. As a consequence, different tumor-associated carbohydrate antigens are exposed to the immune system. *O*-glycosylation begins with the Tn antigen, GalNAcα1-*O*-Ser/Thr, whose structural features and recognition by anti-MUC1 antibodies have been widely studied by our group.[1-2]

To expand the scope of our research, we decided to focus our attention on the more complex STn antigen (Neu5Aca2-6GalNAca1-*O*-Ser/Thr), containing a sialic acid moiety α-2,6 linked to the Tn antigen. Although the STn antigen is often co-expressed with the Tn antigen in cancer tissues, the former presents a notable advantage over the later: its expression is necessarily pathologic,[3] since in healthy cells sialic acid appears only terminating the carbohydrate chain.

Herein, we present for the first time a conformational analysis of the STn antigen engaged in the MUC1 scaffold. A deeper understanding of the STn antigen structure and their interactions with antibodies will contribute to design more powerful tools to be used in cancer vaccination and diagnosis.



References:

[1] Martínez-Sáez N.; Peregrina J.M.; Corzana F. *Chem. Soc. Rev.,* ***2017****,46,* 7154.

[2] Bermejo I.A.; Usabiaga I.; Compañón I.; Castro-López J.; Insausti A.; Fernández J.A.; Avenoza A.; Busto J.H.; Jiménez-Barbero J.; Asensio J.L.; Peregrina J.M.; Jiménez-Osés G.; Hurtado-Guerrero R.; Cocinero E.J.; Corzana F. *J. Am. Chem. Soc.* **2018**, *140,* 9952.

[3] Kudelka, M.R.; Ju, T.; Heimburg-Molinaro, J.; Cummings, R.D. *Adv. Cancer Res.* **2015**, *126*, 53.

Resolving extended N-glycans by NMR: New insights into influenza hemagglutinin N-glycan interactions

Beatriz FERNÁNDEZ DE TORO RONDA[(1)], Wenjie PENG[(2,3)], Andrew THOMPSON[(3)], Gema DOMÍNGUEZ[(4)], F. Javier CAÑADA[(1)], Javier PÉREZ CASTELLS[(4)], James C. PAULSON[(3)], Jesús JIMÉNEZ BARBERO[(5,6)], Ángeles CANALES[(7)]

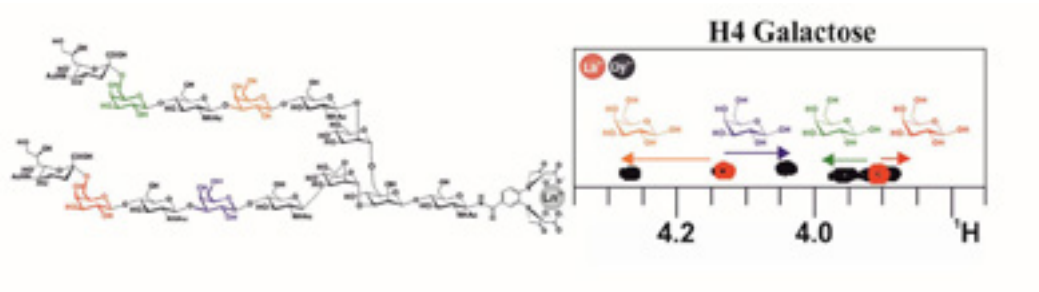
1 Dpto Biología Estructural y Química, Centro de Investigaciones Biológicas CSIC, Madrid, Spain.
2 Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, China.
3 Depts. of Mol Med and Immunol and Microbiol, The Scripps Research Institute, California, USA.
4 Dpto Química, Fac. Farmacia, Universidad San Pablo CEU, Madrid, Spain.
5 CIC bioGUNE, Bizkaia Science and Technology Park, 48160 Bilbao, Spain.
6 IKERBASQUE, Basque Foundation for Science, 48009 Bilbao, Spain.
7 Dpto Química Orgánica I, Fac. Ciencias Químicas, Univ Complutense de Madrid, Spain.

Contact e-mail: beatriz.fernandez.toro@cib.csic.es

Seasonal flu (influenza virus infection) is one of the major threats to human health, causing of up to 500 thousand deaths worldwide annually.[1,2] Hemagglutinin, a glycoprotein presented in the surface of the influenza virus, binds sialylated N-glycans located on the surface of host epithelial cells.

The structural elucidation and the conformational studies of N-glycans remain a challenge due to the complexity, heterogeneity and flexibility of carbohydrates. Moreover, the isochronous chemical shifts presented for the same units located at different branches make impossible the distinction of their signals in the NMR spectra.

Taking advantage of the lanthanide approach, an unambiguous assignment of each carbohydrate unit has been reached for bianntenary N-glycans containing one and two LacNAc units, permitting their conformational elucidation and the recognition study of these N-glycans by the HK/68 hemagglutinin strain.[3]



References:

[1] https://www.cdc.gov/flu/

[2] Petrova, V. N.; Russell, C. A. *Nature Reviews Microbiology* **2017**, *16*.

[3] Fernández de Toro, B.; Peng, W.; Thompson, A. J.; Domínguez, G.; Cañada, F. J.; Pérez-Castells, J.; Paulson, J. C.; Jiménez-Barbero, J.; Canales, Á. *Angewandte Chemie International Edition* **2018**, *57*.

Selection of aptamers recognizing the glycosylation site of PSA: aptasensors for detection of cancer-related glycosylation changes

Ana DÍAZ-FERNÁNDEZ(1), Rebeca MIRANDA-CASTRO(1),
Noemí DE-LOS-SANTOS-ÁLVAREZ(1), María Jesús LOBO-CASTAÑÓN(1)

[1] Departamento de Química Física y Analítica, Universidad de Oviedo, Calle Julián Clavería 8, 33006 Oviedo (Spain).

Contact e-mail: mjlc@uniovi.es

Cancer is associated with aberrant glycosylation and each type of malignant issue is characterized by a distinct change in the glycan structure. Therefore, analysis of tumor-linked glycan alterations is proposed as a valuable tool for diagnostic and prognostic purposes. The glycosylation profile of tumors is currently characterized by long and complicated protocols, based on final MS detection or with the use of lectins. However, these procedures cannot be applied for routine analysis in early detection of cancer. In consequence, to translate these biomarkers from discovery to clinical diagnostics there is a clear need for extremely selective methods, able to differentiate the glycan profile characteristic of disease conditions. Aptamers can be tailored to specifically detect aberrant glycosylation at each protein or even at each glycosylation site, and the use of aptamers as synthetic receptors appears to be a promising approach to replace the current methods [1]. In the case of the prostate cancer, it has been recently described that there is a variation in the fucose and the sialic acid in the glycan structure of the Prostate Specific Antigen (PSA), the only biomarker approved for clinical use in this type of cancer, is a better marker of the aggressiveness of prostate cancer [2].

In this work we present a strategy to direct the selection of aptamers toward the glycosylation site of the protein PSA, based on a SELEX procedure with counter-selection steps against the recombinant PSA (unglycosylated). After six rounds of selection and two counter-selection against recombinant PSA, the aptamers in the last cycle are identified by next generation sequencing and classified into families using bioinformatics tools. The selected aptamers for each family are characterized by electrochemical binding assays and Surface Plasmon Resonance to evaluate their affinity for the PSA and the capability of recognizing the glycans. With this strategy we describe a simple method for directing the selection of aptamers toward the glycan structure of a native glycoprotein.

The best aptamer able to recognize the glycan of the PSA is employed for the design of the first electrochemical aptamer-based sensor, using a sandwich assay and gold electrodes as a support. The sensor responds to different levels of PSA in serum and discriminates human PSA from the unglycosylated protein, reaching levels well-below the 4 ng/mL cut-off which allows the evaluation of PSA levels with clinical significance in the diagnosis of prostate cancer.

Acknowledgements: Ana Díaz-Fernández thanks Junta Provincial de Asturias de la Asociación Española Contra el Cáncer (AECC) for the award of a predoctoral grant. The work has been financially supported by Spanish Government (project CTQ2015-63567-R).

References:

[1] Díaz-Fernández A.; Miranda-Castro, R.; de-los-Santos-Álvarez, N.; & Lobo-Castañón M. J. Post-translational modifications in tumor biomarkers: the next challenge for aptamers? *Anal Bioanal Chem*. **2018**, *410*, 2059.

[2] Gilgunn, S.; Conroy, P.J.; Saldova, R.; Rudd, P.M.; O'Kennedy, R.J. Aberrant PSA glycosylation- a sweet predictor of prostate cancer. *Nat. Rev. Urol*., **2013**.*10*, 99.

Poster Communications I

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

Translocation Transfer, a JPI-AMR Network on antibiotic permeability

P. GRIBBON[1], W. FECKE[2], M. WINTERHALTER[3]

[1] Fraunhofer IME, Hamburg.

[2] EU-OPENSCREEN ERIC.

[3] Jacobs University Bremen.

Contact e-mail: Philip.Gribbon@ime.fraunhofer.de; wolfgang.fecke@eu-openscreen.eu; M.Winterhalter@jacobs-university.de

There is an urgent need for discovery and development of new drugs to combat multi-resistant organisms. The search for new drugs is cumbersome, particularly because the current business model for antibiotics in the pharmaceutical industry has stalled because of the poor return on investment. In response to the pharmaceutical industry stepping back from antibiotic discovery, multiple public efforts, including the JPIAMR and IMI ND4BB, as well as the efforts of Biomedical Science (BMS) European Research Infrastructures community have stepped in to fill the gap. In this project, we will set up a knowledge sharing network, **Translocation-transfer** (**TT**) bringing together experts from with two major publically funded programs, with the goal to improve the process of academically driven antibiotic drug discovery by capitalising on recently gained insights into a key bottleneck in anti-bacterial research, namely how compound penetration properties determine efficacy and resistance properties. Three existing communities who will form the **TT** network are: i) the partners associated with the multinational program *Translocation* (www.translocation.eu), part of IMI ND4BB; ii) partner sites from EU-OPENSCREEN, the European Research Infrastructure for chemical biology and screening (www.eu-openscreen.eu) and iii) partners from the wider global community working on AMR issues and research. *Translocation* (1/2013-6/2018) was one of the largest antibiotic research programs in the world specifically devoted to understanding and to devising ways of increasing antibiotic penetration into bacteria. EU-OPENSCREEN began operation in April 2018 and from 2019 onwards will run some 50 chemical biology and academic drug discovery projects per year, across a network of 25 screening sites, based in 8 European countries on behalf of users from across Europe. It is anticipated that at least 20% of EU-OPENSCREEN projects will involve antibiotic drug discovery element. The initial goal of the TT network will be to transfer knowledge between *Translocation* and EU-OPENSCREEN to fully incorporate compound permeation and efflux considerations into academic antibiotic drug discovery. We have the active participation of the Pew Charitable Trust, which will contribute to the long term systematic dissemination of findings from the co-funded funded *Translocation* project to help academic antibiotic drug discovery efforts on a global scale.

Identification of small molecules targeting the IL-17 inflammatory pathway

Elia ÁLVAREZ-COIRADAS[(1)], Cristian R. MUNTEANU[(2)], Iria GÓMEZ-TOURIÑO[(1)], Laura DÍAZ-SÁEZ[(3)], Kilian HUBER[(4)], Richard ROBERTS[(4)], María Isabel LOZA GARCÍA[(1)], Eduardo DOMÍNGUEZ[(1)]

[1] BioFarma, Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), University of Santiago de Compostela, Avenida de Barcelona s/n, 15782 Santiago de Compostela, A Coruña, Spain.

[2] RNASA, Computer Science Faculty, University of A Coruña, Campus de Elviña s/n, 15071 A Coruña, Spain.

[3] Target Discovery Institute, Structural Genomics Consortium, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7FZ, United Kingdom.

[4] Kaertor Foundation, EMPRENDIA Building, 15706 Santiago de Compostela, Spain.

Contact e-mail: elia.alvarez@usc.es

Interleukin 17 (IL-17) is a proinflammatory cytokine that not only plays a pivotal role in host defence against extracellular pathogens, but also acts as an immune checkpoint in several autoimmune diseases. As a result, the IL-17 family has become an attractive pharmacological target for inflammatory autoimmune diseases such as psoriasis[1], for which therapeutic antibodies have been approved for clinical use [2].

Targeting IL-17 with small molecules might be feasible [3], although novel methods to identify novel chemical matter are needed. Virtual screening using docking and molecular dynamics of our chemical library of 60 000 compounds studied against IL-17 available structures yielded 67 potential ligands. Then, we developed a biophysical label-free assay based on Dynamic Mass Redistribution technology that allows the detection of the interaction between proteins and small molecules. The optimum experimental conditions for the immobilization of the extracellular domain of IL-17RA were determined, and a screening of the ligands in the label-free assay was carried out. 2 molecules sharing a common chemical scaffold were found active as potential binders with micromolar binding affinity confirmed by a SPR assay. The functional activity of these ligands as inhibitors of IL-17A-IL17RA interaction was tested by a cytokine CXCL1 release assay based on blocking IL-17A proinflammatory stimulation of HT-29 colorectal cancer cells. Combining biochemical and cell-based assays with structure-based design from this novel chemotype can facilitate the identification of compounds functionally targeting IL-17 inflammatory pathway.

References:

[1] N. Amatya, AV. Garg, SL. Gaffen, *Trends Immunol*., **2017**, *38*, 310.

[2] E. Sbidian, A. Chaimani, G. Do, C. Hua, C. Mazaud, C. Droitcourt, *et al.*, *Cochrane Database Syst Rev*., **2017**, *12*, CD011535.

[3] S. Liu, LA. Dakin, L. Xing, JM. Withka, PV. Sahasrabudhe, W. Li, *et al.*, *Sci Rep*., **2016**, *6*, 30859.

Enabling efficient 3D culture assays in high throughput format with bluecatbio bluewasher

Haim BARR[1], Alexander PLOTNIKOV[1], Noga KOZER[1], Leonardo SOLMESKY[1]

[1] HTS Unit, The Nancy and Stephen Grand Israel National Center for Personalized Medicine, The Weizmann Institute of Science, 234 Herzl St, Rechovot, Israel.

Contact e-mail: leonardo.solmesky@weizmann.ac.il

High Throughput Screening (HTS) has traditionally been the workhorse technology for drug discovery. HTS processes hundreds of thousands of compounds in a non-biased fashion with goal of discovering new chemical matter for novel biological targets. The complexity of biology necessitates a reductionist approach in HTS, where an assay system is usually reduced to an isolated protein and a "mix and read" detection step. This enables parallel processing of thousands of compounds. However, the biological system is devoid of the context of *in situ* tissue structure which is believed to play a significant role in determining efficacy of therapeutic compounds. Advanced cell culture systems which use 3 dimensional growth of one or more cell lineages can potentially bridge this gap. Tumor spheroids or organoids are much more challenging than monolayers to propagate and maintain uniformity. The culture must be grown for several weeks and survive media exchanges. Further, heterogeneity of the sample will impede the ability to quantify response to compound, particular if imaging is used as the assay endpoint.

The bluecatbio blue washer is a new technology that simplifies media exchange and plate washing. While traditional washers use vacuum and aspiration manifolds to clear media from assay plates, the blue washer uses a centrifugal spin to evacuate media. This process has been shown to be gentle, fast and efficient in traditional cultures.

In our study we evaluated the ability of the blue washer to streamline sample preparation of spheroid cultures, as well as contributing to throughput by reducing reagent costs and working in compressed 1536 well mode.

Using dithiothreitol as a pharmacological tool to study the effect of extracellular disulphide bridges in serotonin 2_A receptor functionality

Marta CIMADEVILA[(1)], Alba IGLESIAS[(1)], María Isabel CADAVID[(1)], José Manuel BREA[(1)], María Isabel LOZA[(1)]

[1] BioFarma Research Group, Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CiMUS), Universidade de Santiago de Compostela, Avenida de Barcelona 22, 15782 Santiago de Compostela, Spain.

Contact e-mail: marta.cimadevila@usc.es

G Protein Coupled Receptors (GPCRs) extracellular domains are emerging as a determining factor in receptor functionality, not only for orthosteric ligands, but also as an allosteric modulation site[1]. Dithiothreitol (DTT) is a reducing agent that has been used to demonstrate the dimerization of numerous G protein coupled receptors (GPCRs), as well as to study the effect of reducing the rigidity of the binding site in intracellular signalling[2]. Serotonin 2_A ($5\text{-}HT_{2A}$) receptor is known to behave as a dimer in the presence of 20 mM DTT, but with an impaired capacity of binding and signalling[3].

Our hypothesis is that disulfide bridges formed among extracellular cysteines in $5\text{-}HT_{2A}$ receptor are capable of modulating its pharmacological response to drugs. Thus, the aim of this work was to employ DTT as a pharmacological tool to study the implication of extracellular disulphide bridges in $5\text{-}HT_{2A}$ receptor functionality.

To that end, a pretreatment with 20 mM DTT for 10 minutes at 37 ºC was carried out in both membranes expressing serotonin $5\text{-}HT_{2A}$ receptor or CHO $5\text{-}HT_{2A}$, living cells. Then, [^{3}H]ketanserin displacement assays, calcium mobilization and IP accumulation measurements were carried out evaluating 5-HT, (±)DOI and LSD as a representative example of agonists, and clozapine and haloperidol as the same for antagonist activity.

A significant decrease in 25% of specific binding was observed in the presence of DTT, without affecting affinity. Moreover, a 25% descent in all tested compounds maximal response is observed, independently of its agonistic or antagonistic nature, but again without affecting the compounds potency. Considering these results, we can conclude that DTT is altering $5\text{-}HT_{2A}$ binding pocket, which is reflected into its ability to proper accommodate ligands of different types. This incapacity is fully transferred into an impaired signalization.

References:

[1] Wooley MJ, Conner AC. *Understanding the common themes and diverse roles of the second extracellular loop (ECL2) of the GPCR super-family*. **2017**, 449, 3.

[2] De Filippo E, Namasivayan V, Zappe L, El-Tayeb A, Schiedel AC, Müller CE. Role of extracelular cysteine residues in the adenosine A2A receptor. **2016**, *12*, 313.

[3] Iglesias A, Cimadevila M, la Fuente RA, Martí-Solano M, Cadavid MI, Castro M, Selent J, Loza MI, Brea J. *Serotonin 2A receptor disulfide bridge integrity is crucial for ligand binding to different signalling states but not for its homodimerization*. **2017**, *815*, 138.

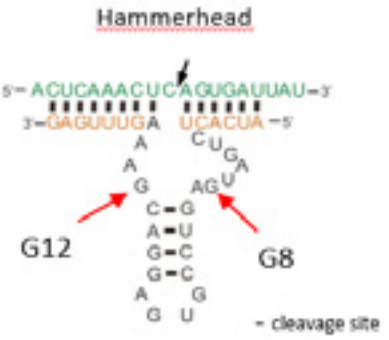
Modified hammerhead ribozyme with guanosine derivatives

Tomasz CZAPIK(1), Ryszard KIERZEK(1)

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego str. 12/14, Poznań, Poland.

Contact e-mail: rkierzek@ibch.poznan.pl

Hammerhead ribozymes are RNA capable of catalyzing cleavage at specific site within an RNA molecule. Discovered first in 1986 hammerhead ribozymes are still investigated. They are found in many forms of life despite they were first discovered in virus-like RNAs[1]. Ribozymes in general are widely studied for they interesting structural aspects, such as overall 3D structure or local active sites structure[2]. Using various modified nucleosides introduced to hammerhead strand a catalytic core with fixed nucleoside composition was determined[3].



Hammerhead ribozyme activity is dependent to Mg^{2+} ion concentration, and it was thought that Mg^{2+} was directly involved in mechanism of substrate strand hydrolysis[4]. Recently it has been showed that nucleoside residue from catalytic core are responsible for phosphodiester bond hydrolysis. Especially two guanosines G8 and G12 are involved in mechanism of catalytic hydrolysis[5].

In presented studies we use minimal hammerhead model and introduce into it guanosine derivatives. We substituted G8 and G12 with 8-bromoguanosine, 2'-deoxy-, arabino- and 2'-fluoroarabinoguanosine. In other positions of ribozyme UNA-uridine, and UNA-isoguanosine was used. For each ribozymes catalytic activities were analyzed using standard cleavage assay and acrylamide gel electrophoresis. Here we present observed reaction rates for chemically modified hammerhead ribozymes.

Acknowledgements: Authors will like to acknowledge Polish National Center (grants: UMO-2013/08/A/ST5/00295 and UMO-2017/25/B/NZ1/02269 (RK)) and Polish Ministry of Science and Higher Education for financial support.

References:

[1] Hammann C.; Luptak A.; Perreault J.; de la Peña M. *The ubiquitous hammerhead ribozyme RNA.* **2012**, *18 (5)*, 871-885.

[2] Nelson, J.A.; Uhlenbeck, O.C. *Minimal and extended hammerheads utilize a similar dynamic reaction mechanism for catalysis. RNA* **2008**, *14*, 43-54.

[3] Blount K. F.; Uhlenbeck O. C. *The Structure-Function Dilemma of the Hammerhead Ribozyme. Annu. Rev. Biophys. Biomol. Struct.* **2005**, *34*, 415-440.

[4] Fedoruk-Wyszomirska, A.; Szymański, M.; Wyszko, E.; Barciszewsk, M. Z.; Barciszewski, J. *Highly Actice Low Magnesium Hammerhead Ribozyme. J. Biochem.* **2009**, *145(4)*, 451-459.

[5] Venyaminova, A. G.; Savydova, A. S.; Venyaminova, A. G. *Artificial hammerhead ribozymes: engineering and applications. Russian Chemical Rev.* **2011**, *80*, 127-143.

Screening for modulators of catecholamine neurotransmitter synthesis

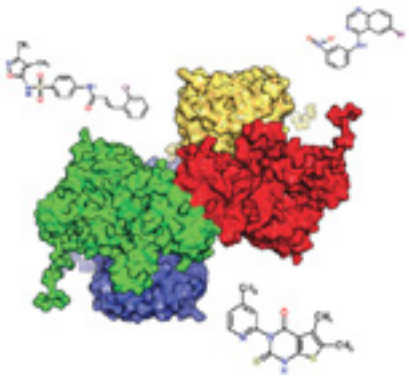
Marte I. FLYDAL, Maria TRAN, Emil HAUSVIK, Knut TEIGEN, Aurora MARTÍNEZ

Department of Biomedicine, University of Bergen, 5009 Bergen, Norway.

Contact e-mail: aurora.martinez@uib.no

Dopamine, noradrenaline and adrenaline are catecholamines functioning as hormones and neurotransmitters, essential for regulation of motor coordination, behaviour, learning and memory. Tyrosine hydroxylase (TH) catalyzes the first and rate-limiting step in the synthesis of catecholamines, generating L-DOPA from L-tyrosine. Dysfunctional TH leads to aberrant catecholamine synthesis and *TH*-mutations cause the rare neurometabolic disorder TH deficiency (THD), manifesting as L-DOPA responsive dystonia, and complex encephalopathy with neonatal onset in the most severe cases. THD is a rare, devastating disease, treated by L-DOPA, but not all patients respond to this medication, which also has several side-effects. Most mutations leading to THD result in instability and misfolding of TH, still maintaining some residual activity. THD and other dopamine deficiencies thus appear suitable for pharmacological chaperone therapy.

We have searched for compounds that increase TH stability using a differential scanning fluorimetry (DSF)-based high-throughput screening (HTS)[1]. We screened three chemical libraries: a) diversity collection with 10 000 cpds (Sigma-TimTec), b) library of FDA-approved drugs (1280 cpds) (Prestwick Ltd), and c) innovative compounds amenable to modification and favorable drug-like and ADME-Tox properties (344 cpds) (Prestwick Ltd). Whereas the initial screening resulted in the identification of several non-selective, inhibitory compounds that preserve TH activity by weak binding to the catalytic iron[2-3], the screening of the Prestwick libraries has also lead to identification of 8 novel primary hits, including novel non-inhibitory compounds with high pharmacological chaperone potential. Five of these primary hits have been prioritized based on concentration dependent binding assays and are being tested in additional binding and functional assays, as well as for stabilization in cells expressing wild-type and mutant TH. Furthermore, they will be tested in a mouse model with the *Th*-p.R203H mutation, equivalent to a recurrent human mutation associated with severe THD, which is non-responsive to L-DOPA. Novel therapies are thus necessary for these patients.



References:

[1] Brennecke, P.; Racina, D.; Aubi, O., EU-OPENSCREEN: A Novel Collaborative Approach to Facilitate Chemical Biology. *SLAS Discovery* **2019,** *In Press*.

[2] Hole, M.; Underhaug, J.; Diez, H.; Ying, M.; Rohr, A. K.; Jorge-Finnigan, A.; Fernandez-Castillo, N.; Garcia-Cazorla, A.; Andersson, K. K.; Teigen, K.; Martinez, A., Discovery of compounds that protect tyrosine hydroxylase activity through different mechanisms. *Biochim Biophys Acta* **2015,** *1854* (9), 1078-89.

[3] Hole, M.; Jorge-Finnigan, A.; Underhaug, J.; Teigen, K.; Martinez, A., Pharmacological Chaperones that Protect Tetrahydrobiopterin Dependent Aromatic Amino Acid Hydroxylases Through Different Mechanisms. *Curr Drug Targets* **2016,** *17* (13), 1515-1526.

RNA secondary structure motifs of the influenza A virus as a target for RNA interference mediated by chemically modified siRNAs

Julita KESY, Marta SOSZYNSKA-JOZWIAK, Paula MICHALAK, Elzbieta LENARTOWICZ, Barbara SZUTKOWSKA, Ryszard KIERZEK, Elzbieta KIERZEK

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland.

Contact e-mail: julitak@ibch.poznan.pl, Elzbieta.Kierzek@ibch.poznan.pl

The influenza A virus genome consists of 8 negative-sense RNA molecules. The pathogen is highly prone to genetic changes. Rapid antigen changes and transmission cause outbreaks of seasonal epidemics and pandemic threat. At all stages of viral replication cycle crucial processes are guided by genomic RNA and its intermediates. RNA folds into stable structural motifs which were reported to carry important role in viral proliferation. Detailed analyses revealed that despite the genetic diversity, certain motifs are conserved among influenza strains. The conserved RNA motifs seem to be a target carrying great potential when the current therapeutic strategies become ineffective. We propose targeting segment 5 mRNA of the influenza virus by siRNA-mediated RNA-interference. Careful selection of target motifs was based on the secondary structure of viral RNA, predicted in bioinformatics' analyses and experimentally defined by our team. Selected regions are highly structure-conserved among type A influenza strains, potentially functional and accessible. To further improve siRNA properties we incorporated phosphorothioates and 2'fluororibonucleotides in a certain positions of siRNA strands. Modified duplexes were shown to gain increased nuclease resistance and serum stability. We performed cell culture tests in which siRNAs were transfected into the MDCK cell line infected by influenza virus A/California/04/2009 (H1N1). Viral inhibition was evaluated in a 24hour postinfection by realtime PCR quantitative analysis of viral genomic RNA. A substantial inhibitory effect was obtained by modified variants of siRNA at 8 nM concentration. The effect was strongly dependent on the level of modification and its pattern. The most potent duplex contained 2'fluororibonucleotides at specified positions and reduced the viral RNA copy number by c.a. 90%. Target selection based on analysis of RNA structural motifs may pose promising alternative for conventional siRNA design. The obtained results support the understanding of the structural motifs impact on the influenza replication cycle.

High-throughput screening in the early phase of GPCR drug discovery

Jana KOTULOVA[1], Sona GURSKA[1], Marian HAJDUCH[1], Petr DZUBAK[1]

[1] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic.

Contact e-mail: jana.kotulova@upol.cz

G protein-coupled receptors (GPCRs) are important medicinal chemistry targets (~34 % of all Food and Drug Administration approved drugs) and the finding of novel active molecules could offer new perspectives for drug development. Our efforts aim to contribute to the characterization of intricate relationship between a receptor and an active molecule.

In order to increase throughput and cost-effectiveness in a reasonable time frame, we have established a robust and fully automated high-throughput screening (HTS) method for the identification of active derivatives. Moreover, we also developed a complex pipeline for the characterization of GPCR ligands.

We especially focus on adenosine, opioid, and cannabinoid receptors. Here, we present our system for analysis of adenosine receptor (AR) ligands that is comprised of (1) primary screen to identify preliminary active molecules, (2) secondary screen for active confirmation, (3) orthogonal assay, and (4) counter-screen for further elimination of false positives or false negatives. In addition, we can specify the mechanism of action of confirmed hits in profiling studies using molecular biology and advanced proteomic methods. Most importantly, our HTS system is applicable for other GPCR screens utilizing the same technology and could represent guidance on hit identification of other GPCRs.

Acknowledgements: This work was supported by Grant Agency of the Czech Republic (19-08124S), internal grant of Palacky University (IGA_LF_2018_031) and by the Czech Ministry of Education, Youth and Sports (LO1304, CZ-OPENSCREEN-LM2015063, EATRIS-LM2015064).

Employment of novel phenotypic *in vitro* models for high throughput screening of new drugs for neuropathic pain

Antón L. MARTÍNEZ[(1)], Amparo PÉREZ[(1)], José Manuel BREA[(1)], Xavier MONROY[(2)], Manuel MERLOS[(2)], Javier BURGUEÑO[(2)], María Isabel LOZA[(1)]

[1] Center of Research on Molecular Medicine and Chronic Diseases (CIMUS). Universidade de Santiago de Compostela, Santiago de Compostela (A Coruña).

[2] Laboratorios Esteve, Parc Cientific de Barcelona, Barcelona.

Contact e-mail: antonleandro.martinez@usc.es

Despite its high prevalence, neuropathic pain remains an unsolved therapeutic need due to the limited efficacy and the high rate of adverse effects of the drugs currently employed for its treatment[1]. Thus, there is a clinical need to develop translational models for screening drugs for neuropathic pain.

Our aim was to perform a screening cascade employing two novel assays for screening new drugs for neuropathic pain employing Prestwick® chemical library, the primary assay was designed for the identification of drugs useful for relieving the hyperexcitability of DRG neurons exposed to inflammatory mediators, and the secondary assay for searching drugs for the treatment of iatrogenic neuronal degeneration.

We assessed the efficacy of 1120 compounds for relieving the hyperexcitability of immortalized DRG (dorsal root ganglion) neurons after an exposition to a combination of inflammatory mediators as the primary assay and we found six hits that could relieve this hyperexcitability with an IC_{50} between 1 and 7 μM. Afterwards, we checked the efficacy of the hits for counteracting the nerve degeneration induced by two antitumor drugs and one antiviral drug as the secondary assay, and we find two drugs belonging to the dihydropyridine family of antihypertensive drugs that protect neurons against iatrogenic neuronal degeneration ($p<0.01$).

References:

[1] Gilron, I.; Dickenson, A. *Expert opinion on emerging drugs* **2014**, *19*, 249.

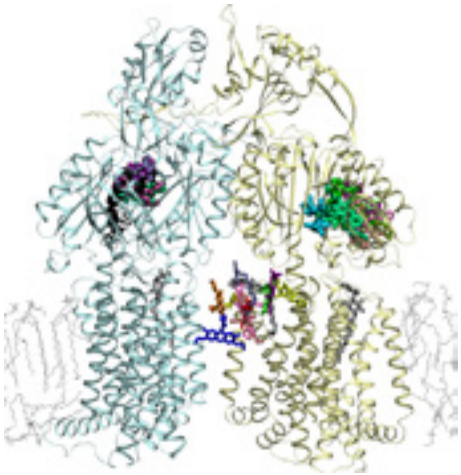
Binding of antibiotics to multidrug efflux transporters AcrB of *E.coli* and MexB of *P. aeruginosa* investigated by molecular simulations

Giuliano MALLOCI[(1)], Alessio ATZORI[(1)], Francesca CARDAMONE[(1)], Chiara FAIS[(1)], Andrea BOSIN[(1)], Giovanni SERRA[(1)], Attilio Vittorio VARGIU[(1)], Paolo RUGGERONE[(1)]

[1] Department of Physics, University of Cagliari, I-09042 Monserrato (CA), Italy.

Contact e-mail: paolo.ruggerone@dsf.unica.it

Understanding the molecular determinants for recognition, binding and transport of antibiotics by multidrug efflux systems is important for basic research and useful for the design of more effective antimicrobial compounds. The drug/proton antiporters AcrB and MexB, respectively part of the major drug efflux pumps AcrAB-TolC in *Escherichia coli* and MexAB-OprM in *Pseudomonas aeruginosa*, are characterized by an impressive ability to transport antibiotics belonging to many different classes, and as such they confer multi-drug resistance phenotype. To date, however, only a few compounds have been co-crystallized with AcrB/MexB and, as a consequence, the molecular features allowing to discriminate between good and poor substrates of these pumps have not yet been identified[1,2]. To investigate how subtle chemical differences affect recognition of substrates by these transport proteins, we combined molecular docking and molecular dynamics simulations to study the interaction of AcrB and MexB with representative antibiotics from four classes: carbapenems, cephalosporins, penicillins, and fluoroquinolones. Our analysis reveals different binding preferences of the selected compounds within the large deep binding pockets of AcrB and MexB. The relationship between these preferences and the physico-chemical properties of the different compounds provides precious information for the design of new antibiotics able to escape the efflux[3].



Acknowledgements: This research has received support from the National Institutes of Allergy and Infectious Diseases project n. AI136799 and the Innovative Medicines Initiatives Joint Undertaking under Grant Agreement n. 115525 resources which are composed of financial contribution from the European Union 7th framework programme (FP7/2007-2013) and EFPIA companies in kind contribution.

References:

[1] Ruggerone, P.; Murakami, S.; Pos, K. M.; Vargiu, A. V.; *Curr. Topics in Med. Chem.,* **2013**, *13*, 3079.

[2] Ramaswamy, V. K.; Vargiu, A. V.; Malloci, G.; Dreier, J.; Ruggerone, P. *Sci. Reports,* **2017**, *7*, 8075.

[3] Atzori, A.; Malviya, V. N.; Malloci, G.; Dreier, J.; Pos, K. M.; Vargiu, A. V.; Ruggerone, P. *Biochimica et Biophysica Acta – Biomembranes,* **2019**, *1861*, 62.

New capsaicin derivatives that rapidly desensitize TRPV1 receptors as an emerging treatment option in irritable bowel syndrome

Agata SZYMASZKIEWICZ[1], Jakub WLODARCZYK[1], Mikołaj SWIERCZYNSKI[1], Arkadiusz SZTERK[2], Vincenzo DI MARZO[3,4], Martin STORR[5,6], Marta ZIELINSKA[1], Jakub FICHNA[1]

1 Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Lodz, Poland.

2 National Medicines Institute, Warsaw, Poland.

3 Endocannabinoid Research Group, Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy.

4 Canada Excellence Research Chair on the Microbiome-Endocannabinoidome Axis in Metabolic Health, Université Laval, Quebec City, Canada.

5 Walter Brendel Center of Experimental Medicine, Ludwig Maximilians University Munich, Germany;

6 Center of Endoscopy, Starnberg, Germany.

Contact e-mail: szymaszkiewicz.a@gmail.com

Introduction: Transient receptor potential vanilloid-1 receptors (TRPV1) are involved in the pain sensation and control of gastrointestinal (GI) functions. Activation of TRPV1 induces visceral pain; desensitization or blockage of TRPV1 evokes analgesic activity. Fast desensitization of TRPV1 was suggested as an emerging approach in the therapy of irritable bowel syndrome (IBS).

The aim of our study was to assess the action of N-palmitoyl-vanillamide (PV) and stearoyl vanillylamide (SV) on GI motility in mice.

Methods: In vivo, the action of TRPV1 agonists (0.1–1 mg/kg) in the GI tract was assessed in mice using fecal pellet output and colonic bead expulsion tests. Both compounds were administered intraperitoneally, i.p. Comparisons between variables were performed with one-way ANOVA test; $p<0.05$ were considered as statistically significant.

Results: In vivo, after i.p. administration of PV or SV a significant inhibition (by 70%) of fecal pellet expulsion was observed. PV at a lower dose (0.1 mg/kg *i.p.*) accelerated colonic motility during first 60 minutes following injection. In contrary, at higher doses (0.25 and 1 mg/kg, *i.p.*) PV inhibited colonic motility.

SV at the dose of 0.1 mg/kg, *i.p.*, decreased the time between insertion and expulsion of a glass bead from the distal colon during first 60 minutes after administration. At higher doses, SV inhibited colonic motility 90 minutes following the injection; there was no action during first 60 minutes.

Conclusion: Systemic administration of PV and SV induced significant alterations in GI motility. TRPV1 fast-desensitizing compounds, due to their antinociceptive action and ability to modulate GI motility could become promising agents in the therapy of IBS.

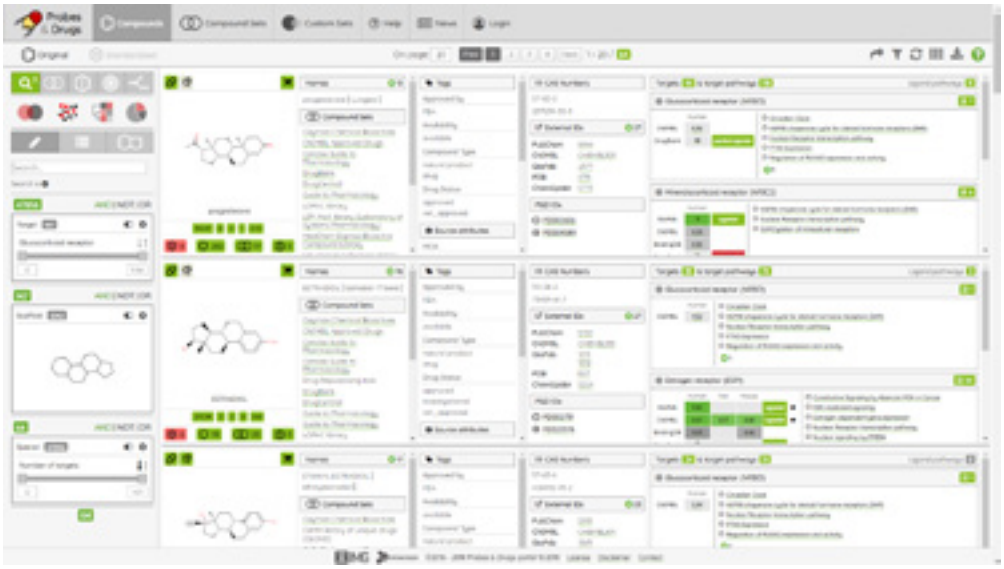
Probes & Drugs portal: interactive approach to Open Data exploration in chemical biology

Ctibor SKUTA(1), Petr BARTUNEK(1)

1 CZ-OPENSCREEN: National Infrastructure for Chemical Biology, Institute of Molecular Genetics AS CR, v.v.i., Prague, Czech Republic.

Contact e-mail: ctibor.skuta@img.cas.cz

Probes & Drugs portal[1] (P&D) is a public resource which is trying to reflect the current state of high-quality chemical tools. Since its release (July 2017), it became one of the primary software tools in chemical biology and, probably, the most comprehensive source of compounds labeled as chemical probes. Its compound database currently consists of more than 50 well-described sets extracted from large well-established bioactivity databases, such as ChEMBL[2] or Guide to Pharmacology[3], as well as small focused compound libraries and databases. P&D also contains pre-picked commercial compound libraries from selected vendors.



P&D was developed to simplify and enable anyone to perform the well-informed selection of up-to-date, high quality chemical tools for biological screening. As such, it offers tools to visualize and explore chemical space, build complex queries enhanced by Boolean logic, and filter compounds based not only on their target potency, but also promiscuity and selectivity. In combination with chemical intelligence and the possibility to create user-defined compound sets, P&D is a unique discovery platform in the field of bioactive compound tools.

References:

[1] Skuta, C., *et al*., Probes & Drugs portal: an interactive, open data resource for chemical biology. *Nature methods* **2017,** *14* (8), 759-760.

[2] Gaulton, A., *et al*., ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Res* **2012,** *40* (Database issue), D1100-7.

[3] Pawson, A. J., *et al*., The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. *Nucleic Acids Res* **2014,** *42* (Database issue), D1098-106.

Sweet G-quadruplex ligands as effective antiparasitic drugs

Juan Carlos MORALES[(1)], Efres BELMONTE-RECHE[(1)], Filippo DORIA[(2)], Marta MARTÍNEZ-GARCÍA[(1)], M. ZUFFO[(2)], Aurore GUÉDIN[(3)], A. STUCCHI[(2)], Jenny CAMPOS-SALINAS[(1)], M. CABELLO-DONAYRE[(1)], José María PÉREZ-VICTORIA[(1)], Jean-Louis MERGNY[(3,4)], Mauro FRECCERO[(2)]

[1] Department of Biochemistry and Molecular Pharmacology, Instituto de Parasitología y Biomedicina López Neyra, CSIC, PTS Granada, Avda. del Conocimiento, 17, 18016 Armilla, Granada, Spain.

[2] Department of Chemistry, University of Pavia, V. le Taramelli 10, 27100 Pavia, Italy.

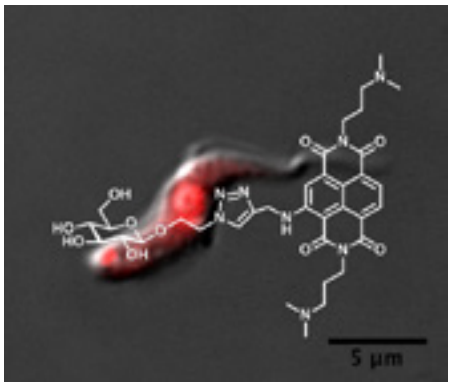
[3] ARNA Laboratory, Université de Bordeaux, Inserm U1212, CNRS UMR5320, Institut Européen de Chimie Biologie (IECB), 2 Rue Robert Escarpit, 33607 Pessac, France.

[4] Institute of Biophysics, AS CR, v.v.i. Kralovopolska 135, 612 65 Brno, Czech Republic.

Contact e-mail: jcmorales@ipb.csic.es

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. We have found putative G-quadruplex forming sequences on the genome of parasites *T. brucei*, *L. major* and *P. falciparum* suggesting they could also be explored as therapeutic targets.

G-quadruplex ligands based on sugar conjugated naphthalene diimide scaffold (carb-NDI), which were previously reported as antiproliferative agents,[1] showed very relevant antiparasitic activity.[2] 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 one of the carb-NDIs and the aglycone NDI localize in the nucleus and kinetoplast of T. brucei suggesting they can reach their potential quadruplex targets. Finally, cytotoxicity and zebrafish toxicity studies reveal the possibility of diminishing G4-ligands intrinsic toxicity through glucose and mannose conjugation.



References:

[1] Arevalo-Ruiz, M.; Doria, F.; Belmonte-Reche, E.; De Rache, A.; Campos-Salinas, J.; Lucas, R.; Falomir, E.; Carda, M.; Perez-Victoria, J. M.; Mergny, J. L.; Freccero, M.; Morales, J. C., Synthesis, Binding Properties, and Differences in Cell Uptake of G-Quadruplex Ligands Based on Carbohydrate Naphthalene Diimide Conjugates. *Chem. Eur. J.* **2017,** *23* (9), 2157-2164.

[2] Belmonte-Reche, E.; Martinez-Garcia, M.; Guedin, A.; Zuffo, M.; Arevalo-Ruiz, M.; Doria, F.; Campos-Salinas, J.; Maynadier, M.; Lopez-Rubio, J. J.; Freccero, M.; Mergny, J. L.; Perez-Victoria, J. M.; Morales, J. C., G-Quadruplex Identification in the Genome of Protozoan Parasites Points to Naphthalene Diimide Ligands as New Antiparasitic Agents. *J. Med. Chem.* **2018,** *61* (3), 1231-1240.

Molecular recognition of Complement proteins by sialic acid. Computational approaches

Elena GÓMEZ-RUBIO, Enrique CRISMAN, Santiago RODRÍGUEZ DE CÓRDOBA, Sonsoles MARTÍN-SANTAMARÍA

Centro de Investigaciones Biológicas, CIB-CSIC, C/ Ramiro de Maeztu, 9, 28040 Madrid, Spain.

Contact e-mail: elena.gomez@cib.csic.es, smsantamaria@cib.csic.es

The complement system plays a critical role in the innate defense against common pathogens. Complement activation by three different pathways results in the generation of C3b and its unspecific binding to cell surfaces. Furthermore, the presence of C3b also amplifies the activation of the complement alternative pathway (AP)[1]. Due to this situation, a regulator is necessary to avoid complement action on host cells. Factor H (FH) is the main regulator of AP upon binding to surface-attached C3b in host cells. To prevent self-cell damage the discerning between host and non-host cells by FH is critical and specific sialic acids or glycosaminoglycan present on the cell surfaces have to be recognized as host markers, increasing FH binding affinity for C3b to avoid complement action [2]. Besides FH, there is another group of proteins, Factor H-related proteins (FHRs), involved in AP regulation (Figure 1). FHR-1 competes with FH for the C3b binding, and the genetic variations in FHRs can cause chronic diseases, including atypical hemolytic uremic syndrome, C3 glomerulopathy, and age-related macular degeneration [3].

We have used multiple computational methods such as docking, conformational analysis, virtual screening and MD simulation to: i) study the recognition of sialic acid derivatives by FH and FHR-1, ii) explore the conformation of C3d-bound to FHR-1, and iii) search for novel chemical scaffolds able to selectively bind FHR-1 vs FH. Our studies can be of great help to understand the different behavior of FH and FHR-1, and search for selective modulators.

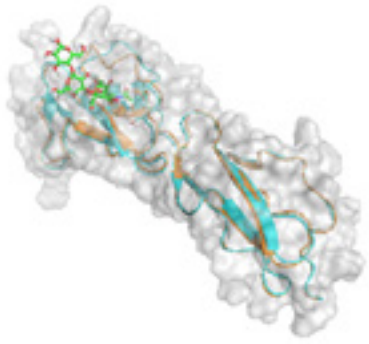


Figure 1. *Superimposed X-ray crystallographic structures of FH (blue) in complex with 3'-sialyllactose (green) (PDB code 4ONT), and FHR1 (orange) (PDB code 4MUC).*

Acknowledgements: Red COMPLEMENTO II-CM" (Ref. S2017/BMD-3673L, 2018-2021)

References:

[1] Kolev, M.; Le Friec, G.; Kemper, C. Nat Rev Immunol. 2014, 14(12), 811-20.

[2] Bläum B.S.; Hannan, J.P.; Herbert, A.P.; Kavanagh, D.; Uhrín, D.; Stehle, T. Nat Chem Biol. 2014, 11(1), 77-82.

[3] Józsi, M.; Tortajada, A.; Uzonyi, B; Goicoechea de Jorge, E.; Rodríguez de Córdoba, S. Trends Immunol. 2015, 36, 374-84.

Structure-based design of new MUC1 derivatives for detection of antibodies in patients with cancer

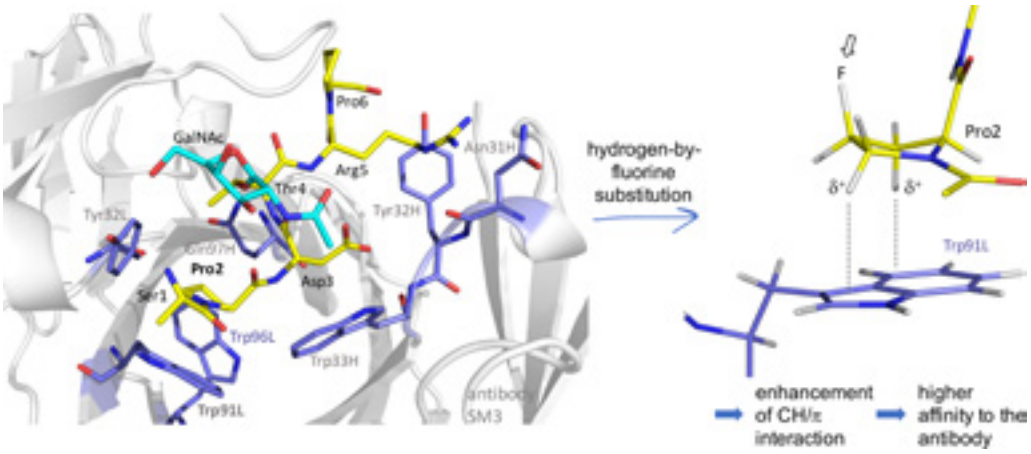
Alicia ASÍN, Ester JIMÉNEZ-MORENO, Jesús M. PEREGRINA, Francisco CORZANA

Departamento de Química, Universidad de La Rioja, Centro de Investigación en Síntesis Química, 26006 Logroño, Spain.

Contact e-mail: alicia.asin@unirioja.es

MUC1 is one of the most studied mucins because it is a glycoprotein overexpressed in around 80% of human cancers.[1] Recent studies[2] have demonstrated the presence of anti-MUC1 antibodies in patients who suffer cancer. Therefore, the design of MUC1-based antigens could be used as diagnostic tools for the detection of anti-MUC1 antibodies in human serum.

Within this context, our group has carried out the synthesis of two non-natural MUC1 derivatives featuring fluorinated proline residues.[3] These compounds present a notable enhancement in the binding affinity against two anti-MUC1 antibodies in comparison to the natural antigens and have been successfully used to improve the detection of low concentrations of cancer-associated anti-MUC1 antibodies from serum of patients with prostate cancer. According to X-ray studies and MD simulations, the fluorinated residues stabilize the antigen-antibody complex by enhancing fundamental CH/π interactions (Figure).



Considering the existence of other amino acids in the MUC1 that could be modified to enhance these CH/π interactions, we have synthesized several fluorinated amino acids and incorporated them into the MUC1 sequence to determine their binding affinity against anti-MUC1 antibodies.

Acknowledgements: *We thank the Ministerio de Economía y Competitividad (projects: CTQ2015-67727-R and UNLR13-4E-1931). A. A. thanks the Asociación Española contra el Cancer (AECC-La Rioja) for a fellowship.*

References:

[1] Taylor-Papadimitriou, J.; Burchell, J. M. Mucins and Cancer; Future Medicine Ltd: Unitec House, London, UK, 2013.

[2] Chen, H.;Werner, S.;Tao, S.;Zörnig, I.;Brenner, H.*Cancer Lett.* **2014**, *346*, 178−187.

[3] Somovilla, V. J.; Bermejo, I. A.; Albuquerque, I. S.; Martínez-Sáez, N.; Castro-López, J.; García-Martín, F.; Compañón, I.; Hinou, H.; Nishimura, S.; Jiménez-Barbero, J.; Asensio, J. L.; Avenoza, A.; Busto, J. H.; Hurtado-Guerrero, R.; Peregrina, J. M.; Bernardes, G. J. L.; Corzana, F. *J. Am. Chem. Soc.*, **2017**, *139*, 18255-18261.

Substrate specificity of catalytic subunit of human protein kinase CK2 and its variants

Ewa BUGAJSKA(1), Sławomir KASPEROWICZ(1), Kinga MIECZKOWSKA(1), Jarosław POZNAŃSKI(1)

[1] Department of Biophysics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Pawińskiego 5a, 02-106 Warsaw, Poland.

Contact e-mail: ewa.b@ibb.waw.pl

CK2 is a ubiquitous serine/threonine protein kinase, being one of the most pleiotropic of all protein kinases. It plays a key role in cell growth, differentiation and cell death. It becomes the therapeutic target in cancer treatment, since its level is significantly increased in cancer cells[1].

We use the catalytic subunit of human protein kinase CK2 (hCK2α) and its three variants ($H_{160}Q$, $H_{160}S$, $H_{160}F$) to investigate their effect on substrate specificity. We have tested the set of compounds originated from halogenated benzotriazoles. Halogenated ligands have been widely developed as potent inhibitors of protein kinases. Among them 4,5,6,7-tetrabromobenzoteriazole (TBBt) is one of the first potent and selective inhibitor of CK2α [2,3].

Former studies conducted by Dr Winiewska demonstrated that the protonation state of H160, which is proximal to the ATP binding site, modulates the ligands binding and affects pH-dependent affinity. Substrate specificity was analyzed using combination of molecular biology, optical spectroscopy and thermodynamic methods. Using site-directed mutagenesis I have replaced His160 by Gln, Ser and Phe. Thermal stability of the wild type protein and its three variants for their apo form and complexes with a series of ligands was analyzed by means of thermal shift assay. The binding affinity for the strongest ligands was also measured by microscale thermophoresis (MST).

$H_{160}Q$ and $H_{160}S$ replacement significantly increases protein thermal stability. All repalcements affect preferences towards particular ligands.

Acknowledgements: This work was supported by NCN grant 2017/25/B/ST4/01613. The equipment used was sponsored in part by the Centre for Preclinical Research and Technology (CePT).

References:

[1] Cozza, G.; Pinna, L. A.; Moro, S., *Curr Med Chem* **2013,** *20* (5), 671-93.

[2] Wąsik, R.; Wińska, P.; Poznański, J.; Shugar, D., *PloS one* **2012,** *7* (11), e48898-e48898.

[3] Poznański, J.; Shugar, D., *Biochim Biophys Acta* **2013,** *1834* (7), 1381-6.

New naphtho- and anthraquinone derivatives as Cholinesterase inhibitors for the treatment of Alzheimer's disease

Marta CAMPORA[(1)], Marco CATTO[(2)], Michele TONELLI[(1)], Bruno TASSO[(1)]

[1] Dipartimento di Farmacia, Università degli studi di Genova, Viale Benedetto XV 3, 16132 Genova, Italy.

[2] Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari "Aldo Moro", Via Orabona 4, I-70125 Bari, Italy.

Contact e-mail: campora@difar.unige.it

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a gradual decline of cognitive processes. The multifactorial pathogenesis of AD includes accumulation of aggregates of β-amyloid (Aβ) and loss of cholinergic function, with consequent deficit of acetylcholine (ACh). The inhibition of acetylcholinesterase (AChE), that is responsible for the breakdown of ACh, has proven useful to relieve some cognitive and behavioral symptoms of AD. In advancing AD, AChE levels in the brain are declining, whilst a progressive increase of butyrylcholinesterase (BChE) is observed, which is also able to hydrolize ACh, even if at lower rate. We already described a number of quinolizidine-containing bi- and tricyclic systems, which provided dual ChEs inhibitory activity, sometimes accompanied to Aβ aggregation inhibition [1-2]. In order to achieve novel ChEs inhibitors, either dual or, even better, selective for BChE, we have developed a novel series of naphto- and anthraquinones linked through a polymethylene chain to an aryl piperazine or piperidine ring (Figure 1).

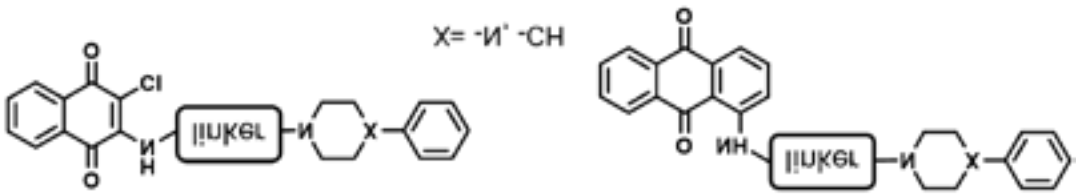


Figure 1: Chemical structures of the investigated naphto- and anthraquinone derivatives.

The basic framework has been selected in order to reduce the high hydrophobicity related to the quinolizidine moiety and to maintain an efficient inhibition of both ChEs and of the AChE anionic site (PAS) that it is implicated in the production of toxic aggregates of Aβ. Moreover, the aromatic ring in the side chain might contribute to establish efficient interactions with a sequence of aromatic aminoacids of Aβ, involved in the initial phases of Aβ aggregation [3]. The newly synthesized derivatives have been evaluated *in vitro* for their inhibitory activities against both ChEs and Aβ aggregation with the aim of adressing a single molecule with a multitarget mechanism of action of potential interest for the treatment of AD.

References:

[1] Tonelli, M.; Catto, M. *Publication* **2015**, *10*, 1040.

[2] De Ferrari, G. V.; Canales, M. A. *Biochemistry* **2001**, *40*, 10447.

[3] Kirkitadze, M.D.; Kowalska, A. *Acta Biochim*. **2005**, *52*, 417.

Metformin reveals a mitochondrial copper addiction of mesenchymal cancer cells

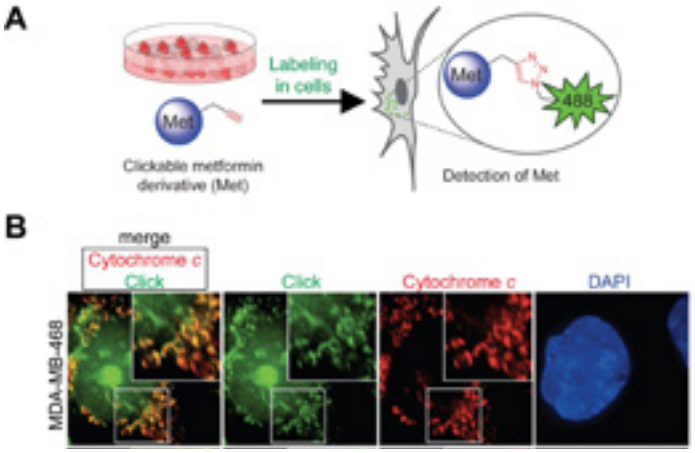
Sebastian MÜLLER(1), Tatiana CAÑEQUE(1), Raphaël RODRÍGUEZ(1)

[1] Chemical Biology of Cancer Team, Labellisée Ligue Contre le Cancer. PSL Research University, CNRS UMR3666 – INSERM U1143, Institut Curie, 26 rue d'Ulm, 75005 Paris, France.

Contact e-mail: tatiana.caneque-cobo@curie.fr

The clinically approved drug metformin, used in the management of type 2 diabetes, has been shown to selectively kill persister cancer cells through mechanisms that are not fully understood[1]. Although it has been proposed that metformin acts on mitochondria and alters metabolism in cancer cells [2], there is of yet no direct visual evidence that biguanides physically accumulate in mitochondria.

To provide further mechanistic insights, we developed a drug surrogate that phenocopies metformin and can be labeled in situ by means of click chemistry. We found this molecule to be more potent than metformin in several cancer cell models. This technology enabled us to provide visual evidence of mitochondrial targeting with this class of drugs. A combination of fluorescence microscopy and cyclic voltammetry indicated that metformin targets mitochondrial copper, inducing the production of reactive oxygen species in this organelle, mitochondrial dysfunction and apoptosis. Importantly, this study revealed that mitochondrial copper is required for the maintenance of a mesenchymal state of human cancer cells, and that metformin can block the epithelial-to-mesenchymal transition, a biological process that normally accounts for the genesis of persister cancer cells, through direct copper targeting [3].



References:

[1] Janzer, A.; German, N. J.; Gonzalez-Herrera, K. N.; Asara, J. M.; Haigis, M. C.; Struhl, K. *Proc Natl Acad Sci USA.* **2014**, *111*, 10574.

[2] Andrzejewski, S.; Gravel, S. P.; Pollak, M.; St-Pierre, J. *Cancer Metab.* **2014**, *2*, 12.

[3] Müller, S.; Versini, A.; Sindikubwabo, F.; Belthier, G.; Niyomchon, S.; Pannequin, J.; Grimaud, L.; Cañeque, T.; Rodriguez, R. *PLoS ONE* **2018**, *13*. e0206764.

Inhibition of bacterial conjugation by disrupting TrwB oligomerization

Roberto VICARIO-MARTÍN[1], Lide ARANA[2], Lucía PÉREZ-REGIDOR[1], Elena GÓMEZ-RUBIO[1], Sonsoles MARTÍN-SANTAMARÍA[1], Itziar ALKORTA[2,3]

[1] Centro de Investigaciones Biológicas, CIB-CSIC, C/ Ramiro de Maeztu, 9, 28040 Madrid, Spain.

[2] Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), Barrio Sarriena s/n, 48940 Leioa, Biscay, Spain.

[3] Instituto Biofisika (CSIC, UPV/EHU), Barrio Sarriena s/n, 48940 Leioa, Biscay, Spain.

Contact e-mail: r.vicario.martin@gmail.com; itzi.alkorta@ehu.es

Infections by multidrug-resistant bacteria are one of the greatest threats to modern medicine. Bacterial conjugation (Fig. 1) is the main process by which bacteria acquire antibiotic resistance, where a conjugative plasmid is transferred from a donor to a recipient bacterium. These plasmids encode not only antibiotic resistance genes, but also the genes necessary for bacterial conjugation. Thus, the recipient acquires both antibiotic resistance traits and the capacity to transfer the plasmid to new recipient bacteria, contributing to the dissemination of antibiotic resistance among bacteria. One of the key proteins essential in bacterial conjugation is the coupling protein (T4CP) and each conjugative plasmid codifies its own T4CP. They form a protein family whose representative is TrwB, the T4CP of R388 plasmid. TrwB is a homohexameric membrane protein with a central pore[1,2]. The structure of a soluble mutant protein of TrwB (TrwBΔN70) unveils that contains a nucleotide binding domain in each monomer[2], suggesting that TrwB could use the energy from ATP hydrolysis for plasmid transfer. Since oligomerization is necessary for ATPase[3], specific inhibition of oligomerization of TrwB could lead to control its activity.

In this work we have followed a computational strategy to identify putative TrwB modulators by means of virtual screening (VS), and molecular dynamics simulations. We have undertaken a computational analysis of the TrwBΔN70 monomeric and hexameric structures of the apo and ADP-bound protein previously published[2], to identify different possible binding pockets. In the hexamer structure we have focused in the channel, and in the monomer structure we have focused on the protein-protein interfaces required for the oligomerization (blue, yellow, green and magenta in Fig 2). Specifically, from a drug repurposing strategy, we have performed the VS of nearly 3000 compounds from ZINC database of generic drugs to find potential inhibitors of TrwB oligomerization.

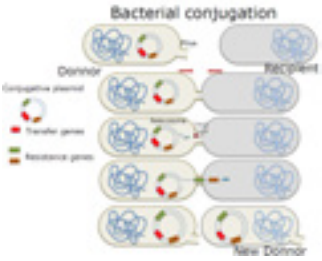


Figure 1

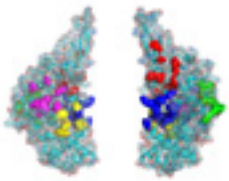


Figure 2

References:

[1] Hormaeche, I., Alkorta, I., Moro, F., Valpuesta, J. M., Goñi, F. M., de la Cruz, F., Hormaeche, I. *J Biol Chem* **2002,** *277*,46456.

[2] Gomis-Rüth, F. X., Moncalián, G., de la Cruz, F., Coll, M. *J Biol Chem* **2002**, 277:7556

[3] Matilla, I., Alfonso, C., Rivas, G., Bolt, E. L., de la Cruz, F., Cabezon, E. *J Biol Chem* **2010**, 285:17537.

Weak agonistic LPS restores intestinal immune homeostasis

Flaviana DI LORENZO[(1)], Alex STEIMLE[(2)], Julia Stefanie FRICK[(2)], Antonio MOLINARO[(1)], Alba SILIPO[(1)]

[1] Department of Chemical Sciences, University of Naples Federico II, Naples, Italy.

[2] Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany.

Contact e-mail: silipo@unina.it

Administration of *Bacteroides vulgatus* mpk is known to protect from colonic inflammation in mouse models for experimental colitis. Here we show that the bacterium even restores gut homeostasis in an already inflamed intestine and that this effect is completely mimicked by its lipopolysaccharide (LPS). Indeed, its LPS[1] is able to convert intestinal dendritic cells into a tolerant and tolerogenic phenotype thus mediating the recovery of the intestinal homeostasis. We establish the structure of the LPS as consisting of hypo-acylated and *mono*-phosphorylated lipid A and a unique galactofuranose containing core oligosaccharide resulting in a weak immunostimulating molecule. Computational models account for a possible MD-2/TLR4-mediated mechanism that might be responsible for its inflammation silencing characteristics. This work reverses the concept of LPS as a harmful molecule, indeed insights gained from the structural and molecular analysis will pave the way to design novel inflammation-silencing drugs as a potential alternative approach for treatment of intestinal inflammatory disorders.

References:

[1] Di Lorenzo F, *et al*. *FEMS Microbiol Rev*, **2019**, doi.org/10.1093/femsre/fuz002

Identification of the core chemical structure in SureChEMBLpatents

María-José FALAGUERA[(1)], Jordi MESTRES[(1)]

[1] Systems Pharmacology, Research Programme on Biomedical Informatics (GRIB), Universitat Pompeu Fabra (UPF) Hospital del Mar, Medical Research Institute (IMIM), Parc de Recerca Biomèdica de Barcelona (PRBB), Dr. Aiguader, 88, 08003 Barcelona, Spain.

Contact e-mail: mjfalagueramata@imim.es

Public repositories containing chemical structures present in patents that claim pharmacological actions, such as SureChEMBL[1], had made possible the access to previously hidden data that would help the advance in drug discovery field[2]. This notwithstanding, these repositories had been obtained by applying automated text mining techniques for the identification of chemical names and structures and thus, lack of proper manual expert curation that ensures the high quality standards necessary to collect all pharmacologically relevant molecules contained in the patent claim[3]. To improve the quality of the molecules contained in SureChEMBL, we have assembled a protocol for the automatic identification of the core chemical structure claimed by a given patent. The core found will be used to filter out those molecules annotated to the patent that are likely to be outside the claim.

Using these protocol we have been able to identify in 80% of US-pharma patents are core structure with a recall of 0.8. The recall was defined as the percentage of molecules annotated to the patent in ChEMBL, assuming that ChEMBL molecules were already curated. From the 6.7 million molecules initially annotated to those patents in SureChEMBL, we ended up with 3.7 million that are most likely to be defined by the respective patent claims. This means that we removed almost 3 million molecules that were originally part of those patents in SureChEMBL but in fact were reagents or other intermediate products not relevant to the patent claim.

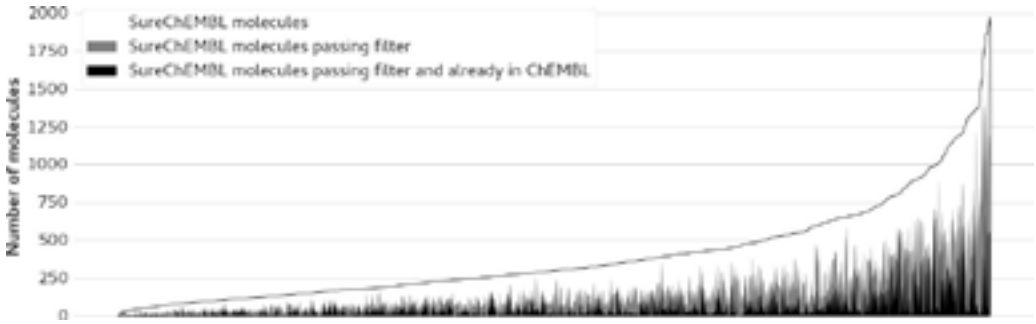


Figure. Variation in the amount of molecule-patent associations annotated to a subset of the patents analysed. X-axis represents each of the patents in the subset. Y-axis represents the size of the following datasets: molecules initially annotated to the patent in SureChEMBL (white), SureChEMBL molecules passing the filtering by core (gray), molecules already annotated to the patent in ChEMBL that were also found by our protocol(black).

References:

[1] Papadatos, *et al*. SureChEMBL: a large-scale, chemically annotated patent document database. Nucleic acids research, **2015**, 44(D1), D1220-D1228.

[2] Vidal, D., *et al*. Ligand-based approaches to in silico pharmacology. In Chemoinformatics and Computational Chemical Biology, **2011**, (pp. 489-502). Humana Press, Totowa, NJ.

[3] Senger, S. Assessment of the significance of patent-derived information for the early identification of compound–target interaction hypotheses. Journal of Cheminformatics, **2017,** 9(1), 26.

Synthesis and biological evaluation of novel (thio) semicarbazone-based benzimidazoles as antiviral agents

Valeria FRANCESCONI[(1)], Lieve NAESENS[(2)], Michele TONELLI[(1)]

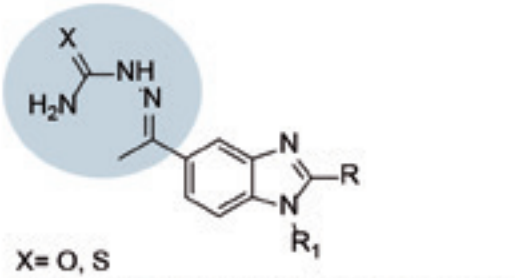
[1] Dipartimento di Farmacia, Università di Genova, Viale Benedetto XV 3, 16132 Genova, Italy.

[2] Rega Institute for Medical Research, KU Leuven, Herestraat 49, B-3000 Leuven, Belgium.

Contact e-mail: francesconi.phd@difar.unige.it

In the drug discovery efforts to counteract microbial infections, (thio)semicarbazones have attracted considerable attentions and many derivatives of this class have been described for their promising bioactivity. In virtue of their interference with vital biochemical processes in the living cells such as deoxyribonucleotide synthesis, cell wall biosynthesis and maintaining thiol contents, (thio)semicarbazones have been reported to exert antibacterial, anticancer, antifungal and antimalarial activities. In particular the transformation of some 5-acetyl-2-phenylbenzimidazoles into the corresponding (thio)semicarbazone analogues were proven to be able to inhibit the Bovine Viral Diarrhoea Virus (BVDV) and the Hepatitis C Virus (HCV) replication [1].

During the last years, our research group developed a large variety of benzimidazole-based derivatives with different pharmacological aims, exploring the analgesic, antiviral and anti-tumor activities. In particular we described 1-dialylaminoalkyl substituted 2-benzylbenzimidazoles and 2-[(benzotriazol-1/2-yl) methyl)benzimidazoles for exhibiting a promising antiviral activity against different single-stranded RNA (ssRNA) virus strains [2,3]. Within these classes, the 5-acetyl benzimidazoles resulted to be less effective antiviral agents, thus here we have explored the derivatization of this group on the benzimidazole ring, synthesizing new (thio)semicarbazone derivatives (Figure 1) in order to evaluate the influence of this modification on the antiviral activity. The new compounds have been evaluated in cell-based assays for the cytotoxicity and the antiviral activity against a panel of ssRNA viruses.



X= O, S
R= 2-benzyl, 2-(benzotriazol-1/2-yl)methyl
R_1= dialkylaminoalkyl, alkyl

References:

[1] Vitale, G.; Corona, P. *J. Med. Chem.* **2012**, *53*, 83.

[2] Tonelli, M; Novelli, F. *Bioorg. Med. Chem.* **2014**, *22*, 4893.

[3] Tonelli, M; Paglietti, P. *Chem. Biodiversity.* **2008**, *5*, 2386.

Enzymatic Engineering of Acyltransferase LovD

Guillermo GARCÍA-MARQUINA[1], Fernando LÓPEZ-GALLEGO[2], Gonzalo JIMÉNEZ-OSÉS[3]

[1, 3] Departmento de Química, Centro de Investigación en Síntesis Química, Universidad de La Rioja, C/ Madre de Dios, 53, 26006 Logroño (La Rioja), Spain.

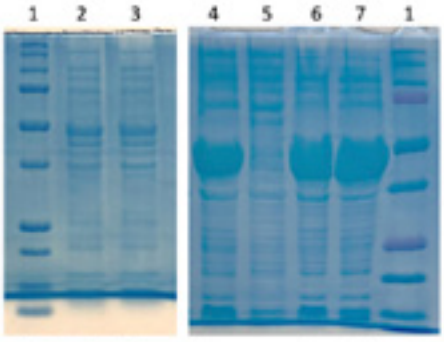
[2] Instituto de Síntesis Química y Catálisis Homogénea (ISQCH), Departamento de Química Orgánica, Universidad de Zaragoza, C/ Pedro Cerbuna, 12, 50009 Zaragoza (Aragón), Spain.

Contact e-mail: guillermo.garciam@unirioja.es

The natural acyltransferase LovD is involved in lovastatin synthesis[1]. The 29-mutant variant recently obtained by directed evolution by Tang and Codexis, Inc (LovD9) accepts a small free acyl thioester eliminating the need for its natural acyl carrier protein partner LovF [2]. This enzyme is 1000-fold more efficient in the synthesis of simvastatin than the wild-type LovD. Computational studies revealed that evolution fixes the active site in the proper arrangement for catalysis, overriding the dependency on protein-protein interactions occurring in the native variant.

A new pool of computationally designed variants was generated in order to probe the mechanisms of laboratory evolution and test the ability to achieve divergent but catalytically efficient engineering pathways. These designs were screened through different computational filters and the most promising candidates were selected for experimental validation.

Ten selected mutants were expressed in *E. coli* (together with LovD and LovD9 as controls) and their activity was screened using HPLC. One of them was not expressed, another one was expressed in an insoluble form, two were expressed in a partially soluble form and the other six were expressed in a completely soluble form. Unfortunately, none of them showed enzymatic activity yet, whereas LovD and LovD9 showed a reduced and high activity in simvastatin production, respectively.



PAGE 12% of protein extracts. 1: Molecular weight marker. 2, 4, 6: Insoluble fractions of LovD9, LovD9-3 and LovD9-4, respectively. 3, 5, 7: Soluble fractions of LovD9, LovD9-3 and LovD9-4, respectively.

References:

[1] Xie, X.; Meehan, M. J.; Xu, W.; Dorrestein, P. C.; Tang, Y. *J. Am. Chem. Soc.* **2009**, *131*, 8388-8389.

[2] Jiménez-Osés, G.; Osuna, S.; Gao, X.; Sawaya, M. R.; Gilson, L.; Collier, S. J.; Huisman, G. W.; Yeates, T. O.; Tang, Y.; Houk, K. N. *Nat. Chem. Biol.* **2014**, *10*, 431-436.

Hallucinogenic ligand (±)DOI modulate agonist and antagonist D2 signaling by activating 5-HT2A protomers of a 5-HT2A/D2 heterooligomer

Laura GÓMEZ-GARCÍA[(1)], Sonia GÓMEZ[(1)], Marcos VILLAR, María Isabel CADAVID[(1)], José Manuel BREA[(1)], María Isabel LOZA[(1)].

[1] Biofarma Research Group, University of Santiago de Compostela, Santiago de Compostela, Spain.

Contact e-mail: laura.gomezgarcia@usc.es

G-protein coupled receptors (GPCRs) oligomerization phenomena may to lead to alterations in the intracellular signaling, modulating the effect of the physiological ligands of these receptors. The 5-HT_{2A} receptors have been observed to be expressed as heterodimers with the D_2 receptor, causing alterations in intracellular signaling [1]. Both receptors are targets for antipsychotic drugs used to treat schizophrenia and others neurological disorders [2]. Our hypothesis was that 5-HT_{2A}/D_2 heterooligomer may condition the activation of these receptors by hallucinogenic ligands. The aim of the present work was to study the effect exerted by the hallucinogenic, 5-HT_{2A} agonist, (±)DOI [1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine] over agonists and antagonists effect at D_2 receptor.

For this purpose, we have used a cell line of human embryogenic kidney (HEK293) constitutively over-expressing D_2 receptors and expressing 5-HT_{2A} receptor in a doxycycline-dependent inducible way. Dopamine D_2 signaling was determined by measuring cAMP levels by employing cAMP–G_s Dynamic Kit (Cisbio). We have demonstrated that the activation of 5-HT_{2A} by (±)DOI decreased the efficacy of dopamine to inhibit the production of cAMP with Emax values of 58.9±8.5% and 29.9±7.0%* in absence and presence of (±)DOI, respectively (*$p<0.05$ compared with dopamine control curve in absence of (±) DOI). This effect was reverted in the presence of the 5-HT_{2A} antagonist Ketanserin. In addition, typical antipsychotic haloperidol and atypical antipsychotic clozapine showed different inhibition profiles of the dopamine-induced D_2 activation.

In view of the results, we conclude that the activation of $5HT_{2A}$ receptor by the hallucinogenic agonist (±) DOI modulated the signal pathway dependent on $G_{i/o}$ from the D_2 receptor in HEK293 cells co-expressed both receptors. This modulation may condition the response of both receptors to antipsychotic drugs.

Acknowledgements: Ministry of Economy and Competitiveness (MICINN (SAF2014-57138-C2-1-R) Xunta de Galicia (GRC2014/011) Grants to support predoctoral stage in the SUG universities, in public research organizations in Galicia and other entities of the Galician system of R & D, co-funded partially by the ESF program Galicia 2014-2020 (ESF- investing in your future).

References:

[1] Albizu, L.; Holloway, T.; Gonzalez-Maeso, J.; C.Sealfon, S. *Neuropharm*., **2011**, 770-777.

[2] Lukasiewicz, S.; Polit, A.; Kedracka-Krok, S.; Wedzony, K.; Mackowiak, M.; Dziedzicka-Wasylewska, M. *Biochim. Biophys*. Acta, **2010**, 1347-1358.

DIANA CA assay for profiling of inhibitors against carbonic anhydrases (CAs): fast and simultaneous determination of compounds inhibition potency against a panel of catalytically active CAs

Jan TYKVART[(1)], Jiří SCHIMER[(1)], Jitka ZEMANOVÁ[(1)], Václav NAVRÁTIL[(1)]

[1] DIANA Biotechnologies, s.r.o., Nad Safinou II 366, Vestec 252 50, Czech Republic.

Contact e-mail: jan.tykvart@dianabiotech.com

Carbonic anhydrases (CAs) catalyze reversible hydration of carbon dioxide to bicarbonate. In human, 16 isoforms of CAs have been discovered with varying catalytic efficiencies, tissue distribution and physiological roles. CA inhibitors (CAIs) are established diuretics and antiglaucoma drugs. Moreover, it is assumed that CAIs may have potential as novel anti-obesity, anti-cancer and anti-infective drugs [1]. For example, targeting isoforms CAIX via toxic CAI conjugates may lead to better treatment of chemoresistant tumors [2]. Unfortunately, all potent CAIs inhibit most of CA isoforms. Thus, the discovery of novel and selective CAIs represents one of the main challenges of exploiting CAs for novel treatments.

Here, we present a novel assay in multiwell plate format that enables to determine the inhibition potency of CAIs against all major human CA isoforms. The DIANA CA assay is based on our proprietary DIANA technology (DNA-linked Inhibitor ANtibody Assay), which is applicable for either ultrasensitive enzyme quantification or screening of enzyme inhibitors [3]. In the presented set-up, the analyzed compounds are incubated with the DIANA active site probe with selected CAs isoforms, which are captured in the wells. The inhibition potency is measured based on the compound ability to outcompete the probe from the enzyme active site.

The DIANA CA assay has several unique advantages over the current methods: (i) wide dynamic range enabling the determination of the inhibition potency (Ki value) from a single well measurement, (ii) extremely high signal-to-noise ratio and (iii) simultaneous measurement of several CA isoforms. We have validated the method using a panel of well characterized CAIs and showed that the obtained inhibition constants are in a good agreement with reported values.

To our knowledge, DIANA CA assay is the first assay enabling quantitative determination of inhibitory potencies of CAIs against a panel of human CAs, which is suitable for high-throughput screening.

References:

[1] Supuran, C.T. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. **2008** Nat. Rev. Drug Discov. 7, 168-181. https://doi.org/10.1038/nrd2467.

[2] Wichert, M., & Krall, N. (2015). Targeting carbonic anhydrase IX with small organic ligands.**2015** *Current Opinion in Chemical Biology*, 26, 48-54. https://doi.org/10.1016/j.cbpa.2015.02.005

[3] Navrátil, V., Schimer, J., Tykvart, J., Knedlík, T., Vik, V., Majer, P., Konvalinka, J., Šácha, P. DNA-linked Inhibitor Antibody Assay (DIANA) for sensitive and selective enzyme detection and inhibitor screening. **2017** Nucleic Acids Res. 45, e10–e10. https://doi.org/10.1093/nar/gkw853.

Targeting G-Quadruplexes using small NIR fluorescent molecules

Jorge GONZÁLEZ-GARCÍA[1]

[1] European Institute of Chemistry and Biology (IECB), University of Bordeaux, 2 Rue de Escarpit, Pessac 33600, France.

Contact e-mail: j.gonzalez-garcia@iecb.u-bordeaux.fr or jorge.gonzalez@uv.es

In the past few years, non-canonical nucleic acid structures have emerged as molecular controlling gates of biological processes acting as epigenetic markers. Unusual nucleic acid structures include triplexes, *i*-motifs, three-way junctions, holiday junctions or G-quadruplexes (G4s). The later one is formed from stacks of two or more planar guanine tetrads that arise from hydrogen bonding network of four guanines whereas these structures are assembled and stabilized by alkali metal cations. A large number of putative G-quadruplex forming sequences have been identified in the human genome and evidences suggest their pivotal role in key biological processes.[1] Therefore, these G4 structures have been proposed as potential targets by small molecules for therapeutic intervention.[2]

Even G-quadruplexes have been fully proved to exist *in vitro*; its existence *in vivo* still remains an active debate. Some of the most direct evidence has been obtained by using antibodies to visualize G4 structures in fixed cells.[3] Because of the limitation of the antibody technology, a large number of optical probes has been reported to date to visualize these structures in live cells (rather than fixed cells). Mostly, small-molecule optical probes are based on changes in the emission intensity in the visible range. However, this approach in microscopy has important drawbacks such as photon scattering, high absorption and autofluorescence of cells. To overcome this issue, we recently developed a series of small near infrared fluorescent probes, which emission intensity is tightly regulated by the interaction with G-quadruplexes.

In this talk, I will present the most recent studies in this area including: (i) development of novel NIR optical probes for targeting G4s; (ii) interaction of these probes with a panel of G-quadruplexes of different topology in addition to duplexes and other non-canonical DNA structures and (iii) demonstration that these molecules can be used to target and visualize G4s in live cells.

Acknowledgements: This work was supported by an IdEx fellowship (University of Bordeaux).

References:

[1] Rhodes, D.; Lipps, H. J. *Nucleic Acids Res.*, **2015**, *43*, 8627.

[2] Neidle, S.; Balasubramanian, S. *Quadruplex Nucleic Acids*, RSC: Cambridge, 2006.

[3] Biffi, G.; Tannahill, D.; McCafferty, J.; Balasubramanian, S. *Nat. Chem.*, **2013**, *5*, 182.

Synthesis of novel metalated-oligonucleotide conjugates as potential anticancer agents

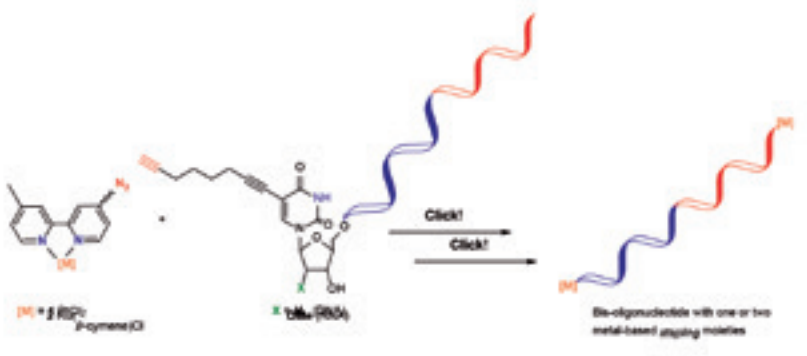
Patricia GRATAL[(1)], Marta VALENCIA[(1)], Lourdes GUDE*[(1)]

[1] Department of Organic Chemistry and Inorganic Chemistry, Andrés M. del Río Chemistry Research Institute (IQAR), University of Alcalá, 28805 Alcalá de Henares, Madrid, Spain.

Contact e-mail: patricia.gratal@uah.es

The search of novel anticancer agents with an increased activity and selectivity, and ideally less toxicity than cisplatin is an area of intensive investigation. Among the variety of alternative drug design strategies, the incorporation of polyaromatic DNA intercalating agents, the modification of the metal coordination sphere to include biologically active molecules or the preparation of platinated and modified oligonucleotides are significant examples. In fact, in the last years, increasing efforts have been made to develop therapeutics based on modified nucleic acids targeting genomic sequences[1,2], thus paving the way for future applications in medicine.

One of the goals of our present work is to assemble metalated-oligonucleotide conjugate systems capable of carrying out a dual inhibition of the telomerase-telomere system through simultaneous recognition (by both Watson-Crick base pairing and covalent binding) of the two components in an *in vivo* setting. As metal centers, Pt(II) and Ru(II) have been chosen, whereas the assembly process has been approached by using the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, better known as "*click chemistry*".



In this communication, we report our preliminary results on the synthesis and characterization of these complex multifunctional conjugates, including recent data on cell permeability and biological activity.

Acknowledgements: Funding from Ministerio de Ciencia, Innovación y Universidades (Grant CTQ2015-72625-EXP-AEI) and from Comunidad de Madrid-European Social Fund (Youth Employment Initiative, YEI, PEJ-2017-AI/SAL-6160) is gratefully acknowledged.

References:

[1] Angelbello, A. J.; Chen, J. L.; Childs-Disney, J. L.; Zhang, P.; Wang, Z.-P.; Disney, M. D. *Chem. Rev*. **2018**, *118*, 1599.

[2] Avitabile, C.; Cimmino, A.; Romanelli, A. *J. Med. Chem*. **2014**, *57*, 10220.

Implementation of new screening concepts for the modulation of MAP kinase based signaling

Adam Levente PÓTI[(1)], Attila REMÉNYI[(2)]

[1] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, 1117 Budapest, Magyar tudósok körútja 2.

Contact e-mail: poti.adam.levente@ttk.mta.hu

Mitogen activated protein kinases (MAPKs) are part of multi-tiered kinase cascades regulating cellular processes such cell division, apoptosis, and differentiation. MAPKs interact with their substrates via linear binding motifs. These short protein regions bind to the so-called MAPK docking groove and determine the specificity of the interaction between MAPKs and their partner molecules.[1-2] Pathological MAPK signaling has been implicated in the development of many human diseases. For example, deregulated and persistent activation of the JNK or p38 signaling pathways is a hallmark of neuronal apoptosis, while the ERK signaling pathway plays a key role in several steps of tumorigenesis including cancer cell proliferation, migration, and invasion. Therefore these proteins are important targets for pharmacological research. Our work focuses on the design of new screening concepts in order to find small molecule inhibitors capable of binding to the docking grooves of the ERK2, JNK1 and p38 MAPKs.

References:

[1] Ágnes Garai, András Zeke, Gergő Gógl, Imre Törő, Ferenc Fördős, Hagen Blankenburg, Tünde Bárkai, János Varga, Anita Alexa, Dorothea Emig, Mario Albrecht, Attila Reményi, *Sci. Signal.*, **2012**, *5*, 245,

[2] András Zeke, Tomas Bastys, Anita Alexa, Ágnes Garai, Bálint Mészáros, Klára Kirsch, Zsuzsanna Dosztányi, Olga V Kalinina, Attila Reményi1, *Mol Syst Biol*., **2015**, *11*, 837.

Fine-tuning DREAM signaling using chemical tools for neurodegenerative disease treatment

Marta GUTIÉRREZ-RODRÍGUEZ[(1)], Pilar CERCOS[(1)], Carolina IZQUIERDO[(1)], Diego A. PERAZA[(2,3)], Alejandro LÓPEZ-HURTADO[(4,5)], Paz GONZÁLEZ[(4,5)], Xose M. DOPAZO[(4,5)], Rosario HERRANZ[(1)], Teresa GONZÁLEZ[(6,7)], Britt MELLSTRÖM[(4,5)], Mercedes MARTÍN-MARTÍNEZ[(1)], Jose R. NARANJO[(4,5)], Carmen VALENZUELA[(6,7)]

[1] Instituto de Quimica Medica, IQM-CSIC, Madrid, Spain.

[2] Instituto de Investigaciones Biomedicas Alberto Sols, CSIC-UAM. Madrid, Spain.

[3] Spanish Network for Biomedical Research in Cardiovascular Research (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain.

[4] Spanish Network for Biomedical Research in Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain.

[5] Centro Nacional de Biotecnología, CNB-CSIC, Madrid, Spain.

Contact e-mail: mgutierrez@iqm.csic.es

DREAM (Downstream Regulatory Element Antagonist Modulator), also known as KChIP-3 or calsenilin, is a multifunctional calcium binding protein that controls the expression level and/or the activity of several proteins related to calcium homeostasis, neuronal excitability and neuronal survival. As an auxiliary protein in the plasma membrane, DREAM interacts with, and regulates, the gating of K_V4 potassium channels, L- and T-type voltage-dependent calcium channels, NMDA receptors and the transcriptor factor ATF6, which is involved in the unfolding protein response machinary. Considering that altered neuronal calcium homeostasis and the accumulation of poorly folded proteins are common features of many neurodegenerative pathologies, the DREAM modulation could open new avenues for the treatment of different neurodegenerative diseases. We recently showed that reduced DREAM expression or blockade of DREAM activity by repaglinide is neuroprotective in Huntington's disease (HD) [1]. However, up to now, only three DREAM binding molecules have been identified. Hence, there is a clear need for the development of chemical tools to modulate and characterize DREAM activity and its interactions.

In this communication we report the identification of novel DREAM modulators following a multidisciplinary strategy that involves structure-based design, organic chemistry, site-directed mutagenesis studies, *in vitro* and *in vivo* experiments. Our findings open a new avenue in the search of effective treatments of neurodegenerative diseases.

Acknowledgements: Spanish Ministery of Economy, Industry and Competitivity (AEI-FEDER, EU grants): BFU2015-67284-R, SAF2017-89554-R, SAF2016-75021-R, SAF2015-66275-C2-2-R; Instituto de Salud Carlos III CIBERNED and CIBERCV programs; the Madrid regional government/Neurodegmodels; and CSIC PIE 201580E073.

References:

[1] Naranjo, J.R. *et al*. Activating transcription factor 6 derepression mediates neuroprotection in Huntington disease. *J. Clin. Invest.* **2016**, 126, 627.

The Orphan GPCR GPR6: Model Development and Docking Studies

Israa ISAWI[1], Paula MORALES[2], Dow HURST[1], Patricia REGGIO[1]

1 Chemistry & Biochemistry Dept., UNCG, Greensboro, NC, United States.

2 Instituto de Química Médica, Consejo Superior de Investigaciones Científicas, Madrid, Spain.

Contact e-mail: ihisawi@uncg.edu

G protein coupled receptor 6 (GPR6) is a cannabinoid-related Class A GPCR orphan receptor, with high abundance in the central nervous system and high constitutive activation of adenylyl cyclase. GPR6 has been shown to recognize several phytocannabinoids, including cannabidiol and cannabinoid inverse agonists. As demonstrated by different research groups, GPR6 represents a possible target for the treatment of different conditions such as Parkinson's disease, Alzheimer´s disease, or Huntington's Disease. Several recent patents have claimed the use of GPR6 modulators for the treatment of several neurological conditions [1].

The goal of this study is to develop a human GPR6 homology model that helps us to elucidate the structural determinants governing ligand-receptor interactions. The X-ray crystal structure of the Sphingosine-1-phosphate receptor 1 (S1P1) was used as a template [2]. This GPCR shares 33% similarity with the GPR6 sequence. Important sequence differences between S1P1 and GPR6 in transmembrane helices (TMHs) 1,2,4,6 and 7 were identified, and the Conformational Memories technique was used to explore the flexibility introduced by helix bending residues [3]. The extracellular and intracellular loop geometries were calculated using Modeller v9.1. The resultant model was then energy minimized using a standard protocol and then embedded in a fully hydrated POPC bilayer environment for equilibration of the model via Molecular Dynamics (MD) simulations. An equilibrated bundle with low RMSD was chosen to be used for ligand docking studies. Ongoing studies using the GPR6 inactive state model are helping us unravel key molecular interactions of quinoxaline derivatives and other reported inverse-agonists. These interactions will be presented.

[Support: NIDA KO5 DA021358]

References:

[1] Isawi, I.; Morales, P.; Reggio, P. H. *Drug Metabolism Reviews* **2018**, *50* (1), 74-93.

[2] Hanson, M. A.; Roth, C. B.; Jo, E.; Griffith, M. T.; Scott, F. L.; Reinhart, G.; Desale, H.; Clemons, B.; Cahalan, S. M.; Schuerer, S. C.; Sanna, M. G.; Han, G. W.; Kuhn, P.; Rosen, H.; Stevens, R. C. *Science* **2012**, *335* (6070), 851-855.

[3] Guarnieri, F. *et al. J. Amer. Chem. Soc.* **118,** 5580 (1996).

Rational Design of glycomimetic inhibitors of GalNAc-T2

Sandra PEREIRA[(1)], Pedro MERINO[(1)], Ignacio DELSO[(2)], Sonsoles MARTÍN-SANTAMARÍA[(3)]

[1] Instituto de Biocomputación y Física de Sistemas Complejos (BIFI). Universidad de Zaragoza. Facultad de Ciencias, 50009 Zaragoza, Spain.

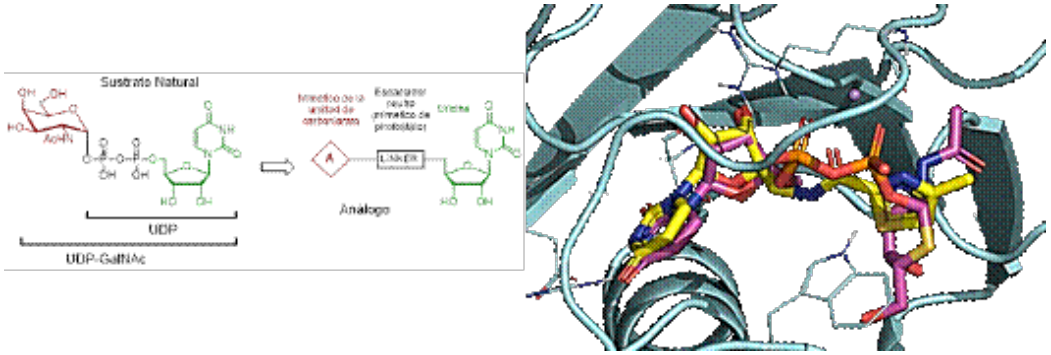
[2] Instituto de Síntesis Química y Catálisis Homogénea (ISQCH). Universidad de Zaragoza-CSIC. Facultad de Ciencias, 50009 Zaragoza, Spain.

[3] Department of Structural and Chemical Biology, Centro de Investigaciones Biológicas, CIB-CSIC. C/ Ramiro de Maetzu, 9, 28040 Madrid, Spain.

Contact e-mail: spereira@unizar.es

Glycosyltransferases (GTs) catalyze the biosynthesis of a new glycosidic linkage by transferring a monosaccharide from an activated sugar donor to an acceptor substrate which can be another sugar molecule, a lipid, peptide, DNA, or an organic molecule [1]. Glycosylated compounds directly exert a wide range of functions, including energy storage, maintenance of cell structural integrity, information storage and transfer, molecular recognition, cell–cell interaction, cellular regulation, immune response, virulence and chemical defense[2].

Only nine sugar donors are known to be involved in protein glycosylation in mammal organisms which is the most abundant post-translational modification in nature. Six of these sugar donors contain the uridine moiety that is in line with the existence of GTs employing UDP sugars as the most predominant in nature. Therefore, the design of inhibitors mimic nucleotide phosphate sugar by modification of diphosphate group or sugar residue could lead to the development of compounds with therapeutic applications [3].



References:

[1] Breton, C.; Fournel-Gigleux, S.; Palcic, MM. *Curr Opin Struct Biol.* **2012**; 22(5): 540-9.

[2] Liang, DM.; Liu, JH.; Wu, H.; Wang, BB.; Zhu, HJ.; Qiao, JJ. *Chem Soc Rev*. **2015**; 44(22):8350-74.

[3] Hang, HC.; Yu, C.; Ten Hagen, KG.; Tian, E.; Winans, KA.; Tabak, LA.; Bertozzi, CR. *Chem Biol*. **2004**, 11(3), 337-45.

Double Fluorogenic π-Extended Rhodamines

Attila KORMOS[(1)], Alexandra EGYED[(1)], Dóra KERN[(1)], Krisztina NÉMETH[(1)], Péter KELE[(1)]

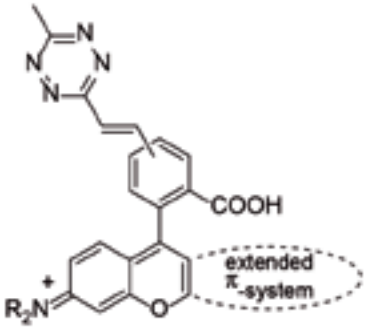
[1] Research Centre for Natural Sciences, Hungarian Academy of Sciences, 1117 Budapest, Magyar tudósok körútja 2, Hungary.

Contact e-mail: kormos.attila@ttk.mta.hu

The emergence of super-resolution microscopy techniques revolutionized the study of biological functions in living cells. There is still need for further development of the microscopy methods, but the main challenge is the unavailability of dyes with the proper optical properties suitable for site-specific labeling of biomolecules inside living cells.

Fluorogenic (or turn-on) probes have the advantage that they exist in a non-fluorescent form. Their fluorescence is only turned on while reacting with the target structure. This means that there is minimal to no background fluorescence caused by the unreacted or unspecifically bound species eliminating the need of purification procedures. The preceding years have brought substantial developments in the design and synthesis of bioorthogonally applicable fluorogenic labels mainly based on the quenching effects of azide and tetrazine moieties. The quenching effect of these bioorthogonal motifs typically becomes less efficient on more conjugated systems, i.e. on probes with red-shifted emission wavelength. In order to reach efficient quenching, thus fluorogenicity even in the red range of the spectrum we introduced the concept of multiple fluorogenicity, lately[1–3].

Herein we present the synthesis, fluorogenic characterization and preliminary biological application of bioorthogonal double fluorogenic π-extended rhodamines with emission maxima between 600–750 nm. The double fluorogenicity is achieved by the quenching effect of the tetrazine and the polarity dependent lactonization and ring opening of the rhodamine core.



Funding by the "Lendület" Program of the HAS (LP2013-55/2013) is gratefully acknowledged.

References:

[1] Demeter, O.; Kormos, A.; Koehler, C.; Mező, G.; Németh, K.; Kozma, E.; Takács, L.; Lemke, E. A.; Kele, P. *Bioconjugate Chem.* **2017**, *28*, 1552.

[2] Kozma, E.; Estrada Girona, G.; Paci, G.; Lemke, E. A.; Kele, P. *Chem. Commun.* **2017**, *53*, 6696.

[3] Kormos, A.; Koehler, C.; Fodor, A. E.; Rutkai, Zs. R.; Martin, M. E.; Mező, G.; Lemke, E. A.; Kele, P. *Chem. Eur. J.* **2018**, *24*, 8841.

Isothermal amplification of aptamers for the sensitive detection of cancer biomarkers

Ramón LORENZO-GÓMEZ(1), Rebeca MIRANDA-CASTRO(1),
Noemí DE-LOS-SANTOS-ÁLVAREZ(1), María Jesús LOBO-CASTAÑÓN(1)

[1] Departamento de Química Físca y Analítica, Universidad de Oviedo, Julián Clavería, 8, 33006 Oviedo, Spain.

Contact e-mail: mjlc@uniovi.es

Early diagnosis of cancer is of uttermost importance to increase the survival rate of patients. Current research focus on liquid biopsy, a method that relies on the existence of specific biomarkers in the serum of cancer patients. These biomarkers are mainly secreted proteins present in extremely low concentrations in a complex medium. For this reason, biomarker quantitation requires strategies to boost sensitivity. Nucleic acid-based receptors such as aptamers are especially appropriate because they can be easily manipulated with molecular biology tools[1] (e.g. polymerases, ligases), so they can be integrated in analytical schemes forbidden to receptors like antibodies.

In this work, we propose rolling circle amplification (RCA), an isothermal DNA amplification strategy, to enhance the sensitivity. As nucleic acids, aptamers can be designed with a short DNA sequence that hybridizes with a circularizable DNA padlock. Amplification of the DNA circle using polymerases yield an extremely long single-stranded DNA, which contain multiple copies of the complementary sequence to the circular template. The RCA extended aptamers can be electrochemically detected using short DNA probes labelled with hapten tags which hybridize with the multiple repetitions obtained after RCA and enable further enzymatic amplification.

Herein we demonstrate that previously described aptamers against certain potential cancer biomarkers can be shortened without losing their affinity and then adapted to RCA-based assays leading to a decrease of three order of magnitude in apparent affinity. Some parameters affecting the amplification were carefully optimized greatly reducing both the time and the consumption of relatively expensive biological reagents. The proposed amplification strategy has been adapted to different assay platforms and schemes proving that might be an alternative to conventional ones.

Acknowledgements: R.L.G thanks the Spanish Ministerio de Educación, Cultura y Deporte for a predoctoral FPU grant. This work has been financially supported by Spanish Government (project CTQ2015-63567-R) and Principado de Asturias Government (FC-15-GRUPIN14-025).

References:

[1] Li F, Zhang H, Wang Z, Newbigging AM, Reid MS, Li X-F, Le XC. Aptamers Facilitating Amplified Detection of Biomolecules. *Anal. Chem.* **2015**, *87*, 274.

New insight in the modulation of TLR4: agonistic and antagonistic effect of small molecules

Andrea LURAGHI(1), Fabio FACCHINI(1), Florent COCHET(1), Michela NEGRI(1), Jessica NEGRINI(1), Alessio ROMERIO(1), Francesco PERI(1)

[1] Università degli studi di Milano-Bicocca, Department of Biotecnology and Bioscience, Piazza della scienza 2, 20126 Milano, Italy.

Contact e-mail: a.luraghi2@campus.unimib.it

The innate immune response is the first line defense against microbial infections and is promptly activated after recognition of a wide range of microbial pathogens. Toll-Like receptors (TLRs) are host proteins responsible to detect conserved microbial and viral component, named pathogen-associated molecular patterns (PAMPs), in order to initiate innate immunity. Among TLRs, TLR4 is the mammal sensor of gram-negative bacterial LPS. TLR4 recognized the conserved portion of LPS, the Lipid A, which triggers receptor dimerization, initiating the inflammatory response. TLR4 activation may also be induced by endogenous mediators released in case of cell damage, named danger-associated molecular patterns (DAMPs). Despite the beneficial role played by TLR4 in counteracting infections[1], dysregulated TLR4-mediated signaling could lead to a series of acute and chronic disease such as sepsis or large array of inflammatory disease [2]. For such reasons, the modulation of TLR4 by the means of small and non-toxic molecules could help on one hand reducing chronic inflammation (antagonist of LPS) and on the other hand stimulating the immune response (agonist) in the cases in which is naturally inhibited (tumor immunotherapy) or in the field of vaccine adjuvant. Because of that, in our laboratory has been synthetized a small library of monosaccharide derived from glucosamine and inspired by LPS pharmacophore (lipid A) [3], able to stimulate or to inhibit TLR4 just varying the ration between fatty acid chains and phosphate groups (Figure 1).

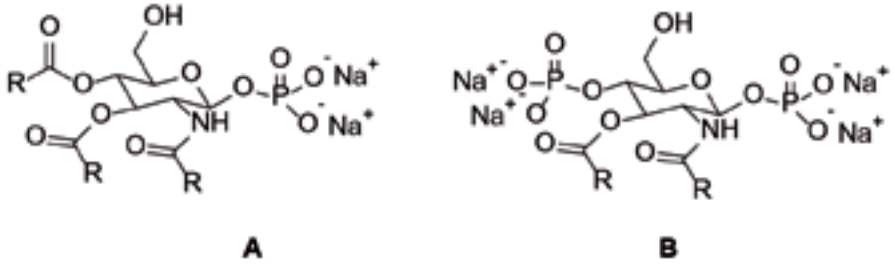


Figure 1: (A) general structure of agonist compound and (B) general structure of antagonist

References:

[1] *Science* **2017**, *356*, 1026.

[2] *Nature Reviews Immunology* **2006**, *6*, 823.

[3] *Journal of Medicinal Chemistry* **2018**, *61*, 2895.

Drug disc overy for mitophagy modulators

Inés MAESTRO[1], Patricia BOYA[2], Ana MARTÍNEZ[1]

[1] Department of Structural and Chemical Biology, Centro de Investigaciones Biológicas-CSIC, C/ Ramiro de Maeztu, 9, 28004 Madrid, Spain.

[2] Departament of Cellular and Molecular Biology, Centro de Investigaciones Biológicas, CSIC, C/ Ramiro de Maeztu, 9, 28004 Madrid, Spain.

Contact e-mail: ines.maestro@cib.csic.es

Mitophagy is the selective degradation of mitochondria by autophagy [1]. It often occurs to defective mitochondria following damage or stress. It promotes turnover of mitochondria and prevents accumulation of dysfunctional mitochondria which can lead to cellular degeneration.

In fact, mitophagy is known to be altered in several pathological conditions [2], such as in cancer, heart diseases and especially in neurodegenerative diseases like Parkinson's Disease, Alzheimer's Disease or Amyotrophic Lateral Sclerosis.

So, due to its relevant role in those conditions, there is a need for new ways to restore those situations through mitophagy regulation. For that, big efforts are being done to find new modulators of mitophagy.

The objective of this work is to find mitophagy regulators from the Medicinal and Biological Chemistry (MBC) library [3] using a cell line expressing the tandem mCherry-GFP-FIS (Figure 1). With image analysis tools, several hits were found discovered.

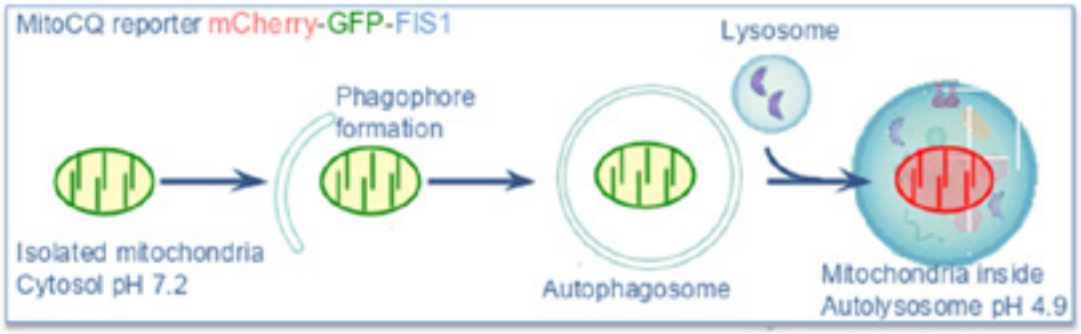


Figure 1. Description of MitoQC reporter

References:

[1] Ashrafi, G.; Schwarz, T.L.; The pathways of mitophagy for quality control and clearance of mitochondria. *Cell Death Differ* **2013** *20*, 31-42.

[2] Um, J.H.; Yun, J. Emerging role of mitophagy in human diseases and physiology. *BMB Rep* **2017,** *50 (6)*, 299-307.

[3] Sebastian-Perez, V.; Roca, C.; Awale, M.; Reymond, J. L.; Martinez, A.; Gil, C.; Campillo, N. E., Medicinal and Biological Chemistry (MBC) Library: An Efficient Source of New Hits. *J Chem Inf Model* **2017,** *57* (9), 2143-2151.

Computational generation of novel ligands of glucocorticoid receptor

M. Isabel AGEA[1,2], Ya CHEN[2], Johannes KIRCHMAIR[2,3,4], Martin ŠÍCHO[1,5], Daniel SVOZIL[1,5]

1 Department of Informatics and Chemistry & CZ-OPENSCREEN: National Infrastructure for Chemical Biology, Faculty of Chemical Technology, University of Chemistry and Technology, Prague, Czech Republic.

2 Center for Bioinformatics, Universität Hamburg, Hamburg, Germany.

3 Department of Chemistry, University of Bergen, Bergen, Norway.

4 Computational Biology Unit (CBU), University of Bergen, Bergen, Norway.

5 CZ-OPEN-SCREEN: National Infrastructure for Chemical Biology, Institute of Molecular Genetics, AS CR v.v.i., Prague, Czech Republic.

Contact e-mail: agealorm@vscht.cz

Molpher[1] is a computational approach for the exploration of the chemical subspace defined by a given set of existing molecular structures. The algorithm can be used, for example, for the generation of virtual libraries enriched with compounds active on a target of interest. The aim of this study was to demonstrate the capacity of Molpher to produce a virtual library enriched with activators of the glucocorticoid receptor (GR). For this purpose, Molpher was fed with known GR activators that we collected from ChEMBL17 and an in-house database to create a GR virtual library. A second virtual library was generated by random selection of compounds of the ZINC database. Both datasets were then profiled with a random forest model that classifies compounds into GR activators and non-GR activators, thereby taking into account the model's applicability domain via conformal prediction. This experiment indicated the presence of a substantially higher number of GR activators in the GR virtual library as compared to the random one. Prospective validation of Molpher against a new version of ChEMBL (ChEMBL24) revealed that the algorithm is able to generate new actives that are based on previously unknown scaffolds. Finally, we employed a docking approach to identify novel compounds that are likely GR activators. Experimental efforts to confirm these predictions are under way.

References:

[1] Hoksza, D.; Skoda, P.; Voršilák, M.; Svozil, D. *Molpher: a software framework for systematic chemical space exploration.* J Cheminform. **2014** *Mar 21*;6(1):7.

Molpher-lib: Programming Interface for Chemical Space Exploration

M. ŠÍCHO[1], D. SVOZIL[1,2]

[1] CZ-OPENSCREEN: National Infrastructure for Chemical Biology, Department of Informatics and Chemistry, Faculty of Chemical Technology, University of Chemistry and Technology Prague, Prague, Czech Republic.

[2] CZ-OPEN-SCREEN: National Infrastructure for Chemical Biology, Institute of Molecular Genetics, AS CR v.v.i., Prague, Czech Republic.

Contact e-mail: martin.sicho@vscht.cz

Molpher-lib is a chemical space exploration software library. It offers a feature-rich interface that can be used for implementation of various chemical space exploration strategies. This way focused virtual libraries of compounds can be designed and existing ones can be enriched by novel chemistry. Molpher-lib is based on a unique atom-based approach called molecular morphing [1]. This approach acts on the premise that if structural features of known bioactive compounds are combined, the resulting hypothetical molecules will likely have similar activity themselves. Various strategies that go even beyond molecular morphing can be implemented with Mopher-lib such as automatic sampling of chemical space around a particular scaffold or simple fragment-based *de novo* structure design. In this work, we present some basic Molpher-lib capabilities as well as more involved features such as substructure locking or implementation of customized morphing operators, which we consider particularly important when extending the capabilities of Molpher-lib beyond the original molecular morphing approach and towards more optimal chemical space exploration.

References:

[1] Hoksza, D.; Škoda, P.; Voršilák, M.; Svozil, D. Molpher: A Software Framework for Systematic Chemical Space Exploration. Journal of Cheminformatics 2014, 6 (1), 7.

Simultaneous Quantification of Phenytoin and Fosphenytoin in Mouse Blood and Brain using High-Performance Liquid Chromatography – a tool for *in vivo* studies

Patrícia C. PIRES[1,2,*], Márcio RODRIGUES[1,2], Gilberto ALVES[1,2], Adriana O. SANTOS[1,2,#]

[1] Health Sciences Research Centre (CICS-UBI), University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.

[2] Faculty of Health Sciences, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.

Contact e-mail: * patriciapires93@gmail.com; # asantos@fcsaude.ubi.pt

Simultaneous quantification of the antiepileptics phenytoin (PHT), hydrophobic, and its hydrophilic prodrug fosphenytoin (FOS) has been a challenge for analytical chemistry. The few already developed methods are mainly for human, dog or rabbit plasma or for media used in *ex vivo* drug permeability studies in Ussing Chambers [1]. However, there are none for brain tissue, and also none for the most common animal model in *in vivo* studies – mice.

Therefore, we developed a high-performance liquid chromatography (HPLC) method for the simultaneous quantification of FOS and PHT in mouse whole blood and brain.

Inhibition of FOS conversion to PHT was accomplished by adding orthophosphoric acid to the tissues immediately after collection. Samples were processed by liquid-liquid extraction using diethyl ether, and analyte detection was done at 215 nm, with separation being achieved within 20 minutes of each run. Elution was executed in isocratic mode at 1 mL/min through a C18-column and pre-column, at 30 ºC. The mobile phase was composed of 36% methanol and 64% aqueous phase (sodium acetate buffer, 10 mM, pH 5, with 0.25% triethylamine).

Concentrations ranged 0.3-15 μg/mL for FOS and 0.3-30 μg/mL for PHT, in both blood and brain. After applying a weighted least squares linear regression to the data [2], the method showed appropriate inter and intra-day linearity ($R^2 > 0.99$), accuracy and precision (bias and coefficient of variation < 15%), as required by the Food and Drug Administration guideline[3]. Absolute recovery percentages were between 74-79% and 50-53% for FOS, 85-89% and 78-81% for PHT, in brain and blood, respectively, and 73-74% for the internal standard in both matrices.

In conclusion, we successfully developed and validated a new HPLC method for the simultaneous quantification of FOS and PHT in mouse whole blood and brain that might be useful for future *in vivo* pharmacokinetic studies.

References:

[1] Viegas, Daniel A.; Rodrigues, Márcio; Francisco, Joana; Falcão, Amílcar; Alves, Gilberto; Santos, Adriana O. *Development and application of an ex-vivo fosphenytoin nasal bioconversion/permeability evaluation method*, European Journal of Pharmaceutical Sciences **2016**, *89*, 61.

[2] Almeida, A.M.; Castel-Branco, M.M.; Falcão, A.C.. *Linear regression for calibration lines revisited: weighting schemes for bioanalytical methods*, Journal of Chromatography B **2002**, *774*, 215.

[3] Food and Drug Administration. *Bioanalytical Method Validation - Guidance for Industry*, **2018**.

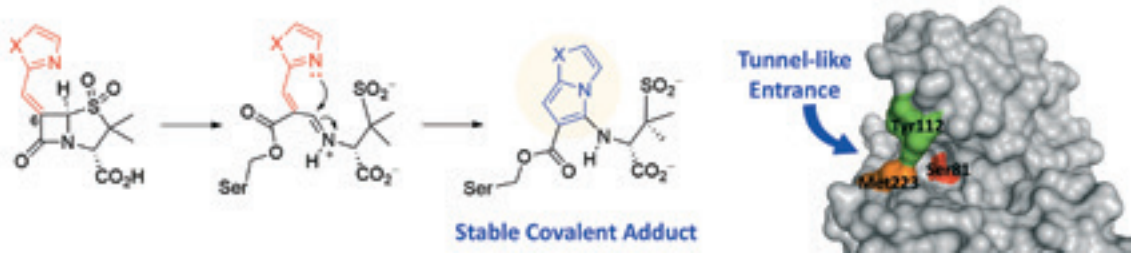
Novel Penicillin-based Sulfones to Unlock Bacterial Resistance to Antibiotics

Esther COLCHÓN[(1)], Concepción GONZÁLEZ-BELLO[(1)]*

[1] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela, C/ Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain.

Contact e-mail: concepcion.gonzalez.bello@usc.es

After many years of success in the battle against infectious diseases, we are now losing ground in this fight with the worldwide increasing appearance of "superbugs", which are resistant to most antibiotics in clinical use. With improvements in the management of chronic diseases and increasingly ageing population, antibiotic resistance is rising to such dangerous levels that the World Health Organization have estimated that it could kill 10 million people per year by 2050. Among the strategies currently employed for the discovery of novel anti-infective drugs, a great success has been achieved with those approaches that minimize the emergence and impact of resistance to antibiotics by the use of an '*antibiotic adjuvant*' in combination with an antibiotic[1]. Among them, *β-Lactamase* inhibitors are the most successful and clinically used antibiotic adjuvants. In particular, our research group is focused in the inhibition of carbapenem-hydrolyzing class D β-lactamases (CDLDs), which are the most rapidly growing and diverse group of β-lactamases that are found among the most clinically challenging species including *A. baumannii* and *P. aeruginosa*. These enzymes are able to hydrolyze carbapenems, which are the only remaining therapy that is often considered as antibiotics of last resort. It has been proposed that the ability of CDLDs to hydrolyze carbapenems is provided by a tunnel-like entrance to the active site that forms a hydrophobic barrier and controls the access to the active site to only certain substrates. In our group we are exploring the inhibition of CDLDs by penicillin-based sulfones able to form, after reaction with the catalytic serine of the enzyme, of an aromatic adduct in the active site[2]. This aromatic moiety seems to be able to block the tunnel-like entrance that would protect the enzyme adduct of the subsequent hydrolysis required for catalytic turnover. We present here our recent results in this project.



Acknowledgements: Financial support from the Spanish Ministry of Economy and Competiveness (SAF2016-75638-R), the Xunta de Galicia (Centro singular de investigación de Galicia accreditation 2016-2019, ED431G/09) and the European Regional Development Fund is gratefully acknowledged.

References:

[1] González-Bello, C. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 4221.

[2] Coleman, K.; Griffin, D. R. J.; Page, J. W. J.; Upshon, P. A. *Antimicrob. Agents Chemother.* **1989**, *33,* 1580.

Poster Communications II

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

Prodrugs for the treatment of inflammatory disease

Viola PREVITALI[(1)], Mads H. CLAUSEN[(1,*)]

[1] Center for Nanomedicine and Theranostics, Department of Chemistry, Technical University of Denmark, Kemitorvet 207, DK-2800 Kgs. Lyngby, Denmark.

[*]Contact e-mail: mhc@kemi.dtu.dk

Insufficient drug selectivity causes severe side effects and host toxicity even in many of the first-line therapy treatments of diseases. Prodrugs are masked forms of pharmacologically active agents designed to undergo in vivo activation by specific stimuli. The use of prodrugs is generally proposed for the improvement of the ADME and "drug-like" properties of compounds. However, an additional feature is their application in targeted drug delivery, i.e. the released of the bioactive molecule by disease-specific stimuli [1]. Several pathologies, like cancer and chronic inflammatory diseases, are associated with increased levels of reactive oxygen species (ROS), due to the generation of inflammation [2]. This unique environment at the inflammatory tissue can therefore be used as a trigger stimulus. In this work we propose the use of prodrugs for the reduction of the undesired effects of drugs prescribed for inflammation-related diseases. Making them inactive until they get activated predominantly or exclusively in inflammatory tissue was the strategy suggested (see Figure 1).

In order to achieve this goal, a series of promoieties sensitive to ROS were developed and synthesized [3]. They were then coupled to different existing drugs and investigated. Promising results on the stability of the compounds in different physiological conditions, good ADME properties, activation at different ROS concentrations and comparable activity to the parent drug in cell-based and in vivo rodent models were obtained. This indicates that the prodrug strategy is a promising tool for the improvement of current therapies for inflammatory diseases associated with serious side effects. This project presents a unique site-selective prodrug strategy based on ROS activation suitable for a wide range of diseases and different marketed drugs.

Figure 1: Expected mode of action of prodrugs sentitive to ROS.

References:

[1] Rautio, J.; Meanwell, N. A.; Di, L.; Hageman, M. J. *Nat. Rev. Drug Discov.* **2018***, 17, 559*.

[2] Mittal, M.; Siddiqui, M. R.; Tran, K.; Reddy, S. P.; Malik, A. B. *Antiox. Redox Signal.* **2014**, *20*, 1126.

[3] Peiró Cadahía, J.; Bondebjerg, J.; Hansen, C. A.; Previtali, V.; Hansen, A. E.; Andresen, T. L.; Clausen, M. H. *J. Med. Chem.* **2018**, *61*, 3503.

Ligand Versatility and Nanoreactors: Two Different Approaches to *In Situ* Pd Catalysis

José R. COUCEIRO[1], Miguel MARTÍNEZ-CALVO[1], Ana SOUSA-CASTILLO[2], Paolo DESTITO[1], Miguel A. CORREA-DUARTE[2], José L. MASCAREÑAS[1]

[1] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Universidade de Santiago de Compostela, Spain.

[2] Department of Physical Chemistry, Singular Center for Biomedical Research (CINBIO), Universidade de Vigo, Vigo, Spain.

Contact e-mail: jose.rodriguez.couceiro@usc.es

Palladium is a transition metal commonly used as a biorthogonal catalyst due to its high catalytic activity and relative stability in biological environments including living cells. Arguably, Pd nanoparticles have been the most common platform for biorthogonal catalysis, while the use of discrete Pd catalysts has increased in recent years[1,2].

Discrete Pd ligands are believed to be unstable in biological media and that has limited their use. In our lab, however, we are interested in understanding what are the requirements for an efficient intracellular Pd catalysis and how the functionalization of the ligands affects the activity and stability of the Pd inside the cell. With this purpose, we prepared a set of ligands derived from pyridine, phosphine and thioether and tested their effect on Pd catalysis either *in vitro* or living cells. Surprisingly our results of the analysis of Pd-promoted propargyl and alloc deprotection of amines showed an inverse correlation between the catalytic activity of a Pd complex in water and its activity in biological relevant media and living cells. Phosphine containing Pd complexes were the most stable in biological media and therefore able to promote deprotections in cell lysates and living cells while pyridine and thioether are more efficient catalysts in water and phosphate buffered saline solution[3].

These results demonstrate that chemical modification of Pd ligands is a versatile methodology to modulate Pd biorthogonality. In parallel, we are also interested in a second strategy to promote biorthogonal reactions that consist in the encapsulation of the catalytic Pd species. Our perspective goes beyond the mere protection of the active species but in the construction of functional nanoreactors in the cellular environment. With this aim, we have synthesized hollow silica nanospheres (500 nm in diameter) that accommodate in their interior Pd nanoparticles (5 nm in diameter). The porosity of the silica cover allows the entrance and exit of reagents and reaction products while maintaining the Pd away from the macromolecules of the biological media. With this strategy, we have been able of conducting Pd-promoted deprotection and Suzuki reactions with success both in complex biological buffers and in the presence of living cells.

References:

[1] Li, J; Yu, J; Zhao, J: Wang, J; Zheng, S; Lin, S; Chen, L; Yang, M; Jia, S; Zhang, X; Chen, PR. *Nature Chemistry* **2014**, *6*, 352.

[2] Miller, MA; Askevold, B; Mikula, H; Kohler, RH; Pirovich, D; Weissleder, R. *Nature Communications* **2017**, *8*, 15906.

[3] Martínez-Calvo, M; Couceiro, JR; Destito, P; Rodríguez, J; Mosquera, J; Mascareñas, JL. *ACS Catalysis* **2018**, *8*, 6055.

Development and validation of a luminescence-based high throughput screening assay for the identification of LsrK inhibitors

Viviana GATTA(1), Päivi TAMMELA(1)

1 Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland.

Contact e-mail: viviana.gatta@helsinki.fi

Quorum sensing is a bacterial communication strategy mediated by release and detection of small molecules called autoinducers. Due to its role in the activation of phenomena like virulence factor expression, biofilm formation, adhesion and host colonization, the process has been widely investigated for the development of antivirulence agents able to prevent infection establishment without compromising bacterial survival and, consequently, less prone to stimulate resistance[1]. In gut enteric bacteria, (4S)-4,5-dihydroxy-2,3-pentanedione (DPD), a precursor of autoinducer 2 (AI2), is internalized by *lsr* transporter and phosphorylated by AI2 kinase LsrK. Since only the phosphorylated form of DPD is able to initiate the quorum sensing cascade[2], LsrK inhibition should result in quorum sensing inactivation and inhibition of virulence. However, no systematic screening has been previously carried out against this target. To facilitate the discovery of LsrK inhibitors, we set up and validated a target-based, automation-compatible and scalable assay for HTS of chemical libraries. Different assay set-ups based on ATP detection were evaluated and compared in terms of reagent stability, incubation time, DMSO tolerance, read-out stability and quality parameters in order to select an assay scalable up to 384-well plate format. The validated assay was used to perform screening of a 2000-compound library with the identification of 107 positive hits which were retested against LsrK at a lower concentration. The same set of compounds was also tested against glycerol kinase for preliminary assessment of selectivity. The data from the two experiments were combined with information from literature to produce a final list of 22 compounds for dose-response experiment. The screening campaign led to the identification of 12 compounds with an IC_{50} ≤10 μM. Two compounds with an IC_{50} of 1 μM were identified proving the effectiveness and sensitivity of our assay and qualifying it as new method for the identification of LsrK inhibitors.

References:

[1] Rutherford ST, Bassler BL; Cold Spring Harb Perspect Med; 2012; 2 (11); a012427.

[2] Xavier KB, Bassler BL; J Bacteriol; 2005;187(1); 238.

Green Microwave Assisted Synthesis of Several Quinazolin-4(3H)-ones and their DNA Photo-cleavage Activity under UV-Visible Irradiation

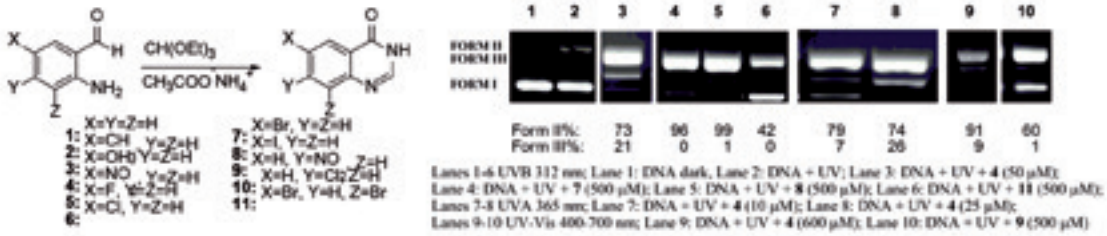
Anastasios PANAGOPOULOS[(1)], Thomas BALALAS[(2)], Achilleas MITRAKAS[(1)], Aikaterini-Rosalia KATSANI[(1)], Michael I. KOUKOURAKIS[(1)], Alexandros E. KOUMBIS[(2)], Konstantinos E. LITINAS[(2)], Konstantina C. FYLAKTAKIDOU[(*,1)]

[1] Molecular Biology and Genetics Department and Department of Medicine, School of Health Sciences, Democritus University of Thrace, University Campus, Dragana, 68100 Alexandroupolis, Greece.

[2] Chemistry Department, Laboratory of Organic Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

Contact e-mail: kfylakta@mbg.duth.gr, akoumbis@chem.auth.gr

Photo-chemo and photo-dynamic therapies are minimally invasive approaches for the treatment of cancer[1] and powerful weapons for fighting bacterial resistance to antibiotics[2]. Quinazolinones are considered privileged anticancer and antibacterial synthetic or natural compounds and some of them are commercially available drugs[3]. We have synthesized in one step, in large scale, and in excellent yields and purity 11 quinazolinon-4(3H)-ones (QZN), from the reaction of readily available anthranilic acids, ethyl orthoformate and ammonium acetate, using green chemistry and microwave irradiation. We have then, irradiated them in the presence of pBR322 plasmid DNA at UVB, UVA and Visible light. The activity varied on the wavelength of irradiation, depending on the UV absorption of the compounds. 6-Br (**7**), 6-I (**8**) and 6,8-di-Br-QZN (**11**) were active at 312 nm, at 500 μM concentration, whereas 6-NO_2-QZN (**4**) below 50 μM. 6-NO_2 (**4**) and 7-NO_2-QZN (**9**) were considerably active at 365 nm even at 10 and 50 μM, respectively. Finally, under Visible light the latter compounds were active at 500 μM. The activity of all compounds proved to be time, oxygen and concentration dependent and pH independent. 6-NO_2-QZN (**4**), being overall the best DNA photocleaver of the series, was irradiated in the presence of Glioblastoma cell lines U87MG and T98G and Skin malignant melanoma cell line A-375, at 365 nm, killing them at 50 μM concentration. Thus, quinazolinones and specifically 6-NO_2-QZN (**4**) may serve as lead photo-sensitizer for the development of promising novel photo-chemo-therapeutics.



Acknowledgements: Part of this work was realized using the facilities of OPENSCREEN-GR (Hellenic Small Molecules Center, Aristotle University of Thessaloniki).

References:

[1] Sengupta, D.; Mazumdar, Z.H.; Mukherjee, A.; Sharma, D.; Halder, A.K.; Basu, S.; Jha, T. J. Photochem. Photobiol. B: Biology, **2018**, *178*, 228.

[2] Hamblin, M. R. Current Opinion in Microbiology, **2016**, *33*, 67.

[3] Khan, I.; Ibrar, A.; Ahmed, W.; Saeed, A. Eur. J. Med. Chem. **2015**, *90*, 124.

Metal-dependent cell internalization of designed basic peptides: applications in dynamic cellular delivery

Soraya LEARTE-AYMAMÍ, Alejandro GUTIÉRREZ GONZÁLEZ,
M. Eugenio VÁZQUEZ, José L. MASCAREÑAS

[1] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), and Departamento de Química Orgánica. Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

Contact e-mail: soraya.learte@usc.es

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 its consensus target DNA site (5-GTCAT-3). However, addition of cis-$Pd(en)Cl_2$ promotes a high-affinity interaction with exquisite selectivity for this sequence. 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 of this peptide.[1]

To obtain exact information on the molecular mechanism of the cell internalization process we have carried out different studies; a modelling of our system, a study with different inhibitors of endocytosis and a screening with modified peptides to know the structural requirements of the peptide for an effective metal-switched uptake.

This is the first demonstration of a triggering mechanism for the cell penetration of a short, basic peptide using a metal complex; this tactic promises to find further applications in dynamic cellular delivery that could be used for the targeted delivery of cargos without capacity to cross membranes or to control the cellular uptake of molecules of interest.

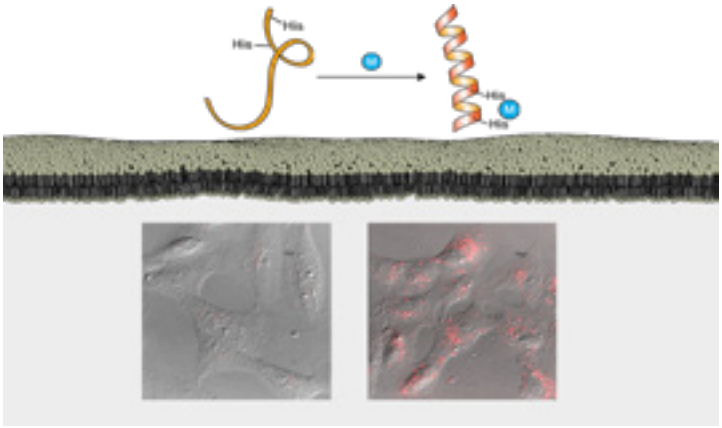


Figure 1. Metal-promoted cell internalization of peptide ($brHis_2$).

Acknowledgements: Soraya Learte Aymamí thanks the Spanish MINECO for her FPI fellowship.

References:

[1] Soraya Learte-Aymamí, Natalia Curado, Jéssica Rodríguez, M. Eugenio Vázquez, and José L. Mascareñas, J. Am. Chem. Soc., 2017, 139.,16188-16193.

Photogeneration of Quinone Methides as Latent Electrophiles for Lysine Targeting

Emilio LENCE[1], Raúl PÉREZ-RUIZ[2,3], Oscar MOLINS-MOLINA[2], Concepción GONZÁLEZ-BELLO[1], Miguel A. MIRANDA[2], M. Consuelo JIMÉNEZ[2]

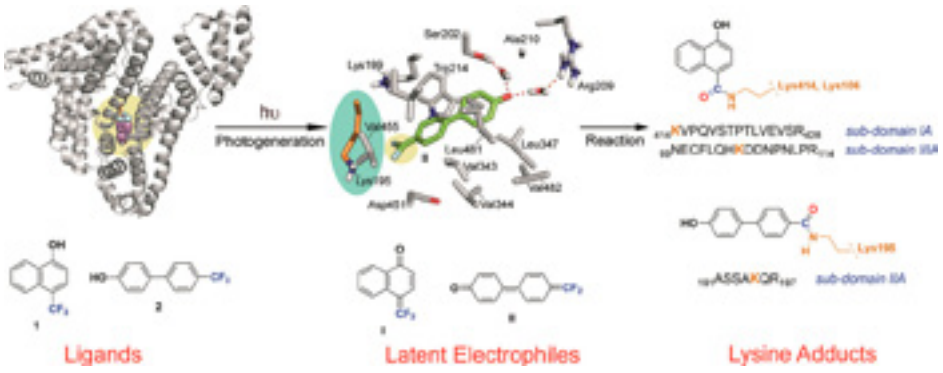
[1] Departamento de Química Orgánica, Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Universidade de Santiago de Compostela, Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain.

[2] Departamento de Química, Instituto de Tecnología Química UPV-CSIC, Universitat Politécnica de València, Camino de Vera s/n, 46071 Valencia, Spain.

[3] Photoactivated Processes Unit, IMDEA Energy Institute, Av. Ramón de la Sagra 3, 28935 Móstoles, Madrid, Spain.

Contact e-mail: emilio.lence.quintana@gmail.com, concepcion.gonzalez.bello@usc.es.

Latent electrophiles are nowadays very attractive chemical entities for drug discovery, as they are unreactive unless activated upon binding with the specific target. In this work, the utility of 4-trifluoromethyl phenols as precursors of latent electrophiles, quinone methides (QM), for lysine-targeting is demonstrated. These Michael acceptors were photogenerated for specific covalent modification of lysine residues using human serum albumin (HSA) as a model target. The reactive QM-type intermediates **I** or **II**, generated upon irradiation of 4-trifluoromethyl-1-naphthol (**1**)@HSA or 4-(4-trifluorometylphenyl)phenol (**2**)@HSA complexes, exhibited chemoselective reactivity toward lysine residues leading to amide adducts, which was confirmed by proteomic analysis.[1] For ligand **1**, the covalent modification of residues Lys106 and Lys414 (located in subdomains IA and IIIA, respectively) was observed, whereas for ligand **2**, the modification of Lys195 (in subdomain IIA) took place. Docking and molecular dynamics simulation studies provided an insight into the molecular basis of the selectivity of **1** and **2** for these HSA subdomains and the covalent modification mechanism. These studies open the opportunity of performing protein silencing by generating reactive ligands under very mild conditions (irradiation) for specific covalent modification of hidden lysine residues.



Acknowledgements: Financial support from the Spanish Ministry of Economy and Competiveness [CTQ2016-78875-P, SAF2016-75638-R and BES-2014-069404 (predoctoral fellowship to O.M.-M.)], the Generalitat Valenciana (PROMETEO/2017/075), the Community of Madrid (2016-T1/AMB-1275), the Xunta de Galicia (Centro Singular de Investigación de Galicia accreditation 2016–2019, ED431G/09 and postdoctoral fellowship to E.L.), and the European Regional Development Fund (ERDF) is gratefully acknowledged.

References:

[1] Pérez-Ruiz, R.; Molins-Molina, O.; Lence, E.; González-Bello, C.; Miranda, M. A.; Jiménez, M. C. *J. Org. Chem.* **2018**, *83*, 13019.

Glycosyl Aldehydes as New Scaffolds for the Bioorthogonal Oxime Bond Formation of Neoglycoconjugates

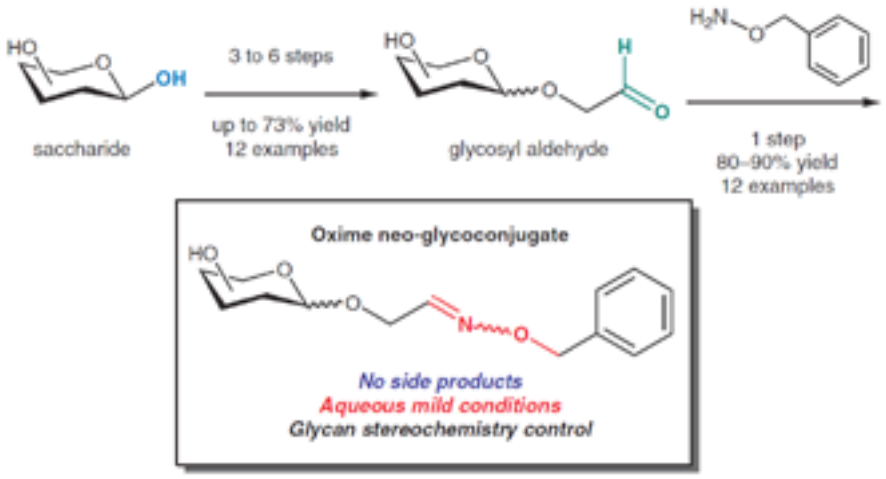
Alicia RIOBOO[(1)], José J. REINA[(1)], Javier MONTENEGRO[(1)]

[1] Centro Singular de Investigación en Química Biolóxica (CiQUS), Universidade de Santiago de Compostela (USC). Rúa Jenaro de la Fuente, s/n, 15782 Santiago de Compostela, España.

Contact e-mail: alicia.rioboo@rai.usc.es

The employ of oxime or hydrazone bond formation for the preparation of neoglycoconjugates represents an useful and straightforward biorthogonal tool in chemical biology.[1] However, when the reducing end of the glycan moiety is involved in this strategy, the opening of the glycan cyclic hemiacetal and the formation of the opened tautomer occurs, which cause the loss of the configuration and the stereochemical information.[2]

Herein, we present the synthesis of a new library of glycosyl aldehydes to be used as scaffold for the oxime bond formation of neoglycoconjugates. They allow the oxime bond formation conjugating the aldehyde group present in the reducing end of carbohydrates. This way, these glycosyl aldehydes represent an accessible alternative to avoid the loss of chiral information.[3]



Acknowledgements: We acknowledge the support from MINECO (RYC-2013-13784, CTQ2014-59646-R and SAF2017-89890-R), the Xunta de Galicia (ED431G/09 and 2016AD031), the ERDF, the ERC Starting Grant (DYNAP-677786), the Human Frontier Science Program (RGY0066/2017) and the Foundation Segundo Gil Dávila.

References:

[1] Fernández-Tejada, A.; Cañada, F. J.; Jiménez-Barbero, J. *ChemMedChem* **2015**, *10*, 1291.

[2] Kwase, Y. A.; Cochran, M.; Nitz, M. *Protecting-Group-Free Glycoconjugate Synthesis: Hydrazide and Oxyamine Derivatives in N-Glycoside Formation*; Werz, D. B.; Vidal, S. Eds.; Modern Synthetic Methods in Carbohydrate Chemistry: From Monosaccharides to Complex Glycoconjugates; Wiley-VCH Verlag GmbH & Co KGaA: Weinheim, Germany, 2013.

[3] Reina, J. J.; Rioboo, A.; Montenegro, J. *Synthesis* **2018**, *50*, 831.

Ex Vivo Drug Sensitivity Testing of Primary Cells for Precision Cancer Medicine

Jani SAARELA(1), Laura TURUNEN(1), Philipp IANEVSKI(1), Evgeny KULESSKIY(1), Sergey KUZNETSOV(1), Karoliina LAAMANEN(1), Elina LEHTINEN(1), Maria NURMI(1), Swapnil POTDAR(1), Tanja RUOKORANTA(1), Katja SUOMI(1), Krister WENNERBERG(2), Päivi TAMMELA(1)

[1] Institute for Molecular Medicine Finland (FIMM), HiLIFE unit, University of Helsinki, Tukholmankatu 8, 00290 Helsinki, Finland.

[2] Biotech Research & Innovation Centre (BRIC), University of Copenhagen, Ole Maaløes Vej 5 2200 København N, Denmark.

Contact e-mail: jani.saarela@helsinki.fi

The High Throughput Biomedicine unit at FIMM Technology Centre provides a wide range of biomedical high throughput assays [1]. In collaboration with local research groups and clinicians, we have set up a drug sensitivity and resistance testing (DSRT) platform using a set of 528 approved and investigational oncology drugs. Using the DSRT platform, we perform *ex vivo* drug testing of primary cancer cells from patients with leukemia or multiple myeloma. Solid tumor samples as well as cancer cell lines can also be analysed using the same platform.

We have to date produced approximately 3800 drug sets as dose response assay ready plates using acoustic dispensing. The acoustic dispensing allows for making pre-drugged single drug plate sets and/or drug combination plates within hours after sampling of the cells. The plates are also readily sent to researchers anywhere in the world for running comparable assays at other sites. The drugging reproducibility is excellent generating results with correlations of 0.98 or higher in replicate assays. We have developed in-house software solutions to aid these processes: a script for quick creation of transfer list for combination plates and automated analysis pipelines with web-based software interfaces to enable the screening biologists to analyze the screening results effectively.

The drug sensitivity responses, measured by cell viability and death readouts, are integrated with molecular profiling such as exome or whole genome sequencing, transcriptomics and phosphoproteomics [2]. Currently, we are setting up a standardized flow cytometry pipeline for sample analysis, which provides detailed information about the drug responses of cell subpopulations. The DSRT platform enables drug repositioning, provides new combinatorial possibilities and allows for linking drug sensitivities to predictive biomarkers. DSRT data are being used to develop methods and models to identify selective and synergistic drug combinations. The platform has already benefitted many patients and led to new treatment options for those patients who do not have any alternative therapies available.

Acknowledgements: Tero Aittokallio, Caroline Heckman, Olli Kallioniemi, Satu Mustjoki, Kimmo Porkka.

References:

[1] Kulesskiy E.; Saarela J. *J. Lab. Autom.* **2016**, *21*, 27.

[2] Pemovska T.; Kontro M. *Cancer Discov*. **2013**, *3*, 1416.

[3] Pemovska T.; Johnson E. *Nature*. **2015**, *519*, 102.

Polymer Therapeutics for treating neurodegenerative disorders: exploring the intranasal route to bypass the BBB

Inmaculada CONEJOS-SÁNCHEZ, Fernanda RODRÍGUEZ-OTORMIN, Esther MASIÁ, Irene DOLZ-PÉREZ, Tetiana MELNYK, Martina PALOMINO-SCHÄTZLEIN, María J. VICENT

[1] Polymer Therapeutics Lab, Centro de investigación Príncipe Felipe, C/Eduardo Primo Yúfera, 3, 46012 Valencia, Spain.

Contact e-mail: mjvicent@cipf.es

The growing incidence and increasing societal costs caused by neurodegenerative disorders in an ageing population, together with the lack of effective treatments, point out the need for novel approaches in order to address their enormous burden. Only in Europe it has been estimated that 35 % of all disease burden is attributable to brain-related disorders. In particular, Alzheimer's Disease (AD) is one of the largest global public health challenges to be faced. Moreover, Central Nervous System (CNS) drug discovery and development is a challenging task due to the presence of the most impenetrable biological barrier in the human body- the Blood Brain Barrier (BBB). Indeed, only 2 % of small-molecule drugs and almost 0 % of biologic drugs do reach the brain, thus limiting the development of efficient treatments for brain diseases.[1]

Focused on designing non-invasive strategies for brain targeting and active delivery through nanotechnological approaches, we are developing a polymer-based platform for intranasal delivery using polyglutamates, underpinned on previously established findings of our lab.[2,3] These versatile, biodegradable carriers with controlled architecture and self-assembled behavior hold several key features. First, they are crosslinked with reversible disulfide chemistries, prone to disassemble under reductive media to facilitate enhance chemical adsorption to on the mucosa via disulfide interchange with cysteine-rich glycoproteins and also to allow greater diffusion/penetration rates in brain. In parallel, surface modification with targeting motifs has been exploited to improve the ability to cross the mucosal barrier in synergy with the disulfide cross-linking motifs. Screening studies have been performed through mucodiffusion evaluation by NMR techniques and *ex vivo* permeation assays in a sheep mucosal model. Second, they can bear a rationally designed combination therapy. In this regard, we have identified adequate neuroprotectant-neurorescuer drug combination therapy for the treatment of AD and linking chemistry of the resultant combinations is now under development.

If successful, this novel delivery method for intranasal delivery will be of interest for other CNS-related diseases.

Acknowledgements: This work has been supported by the predoctoral grants (FRO and TM) of Junta Provincial de Valencia of the Spanish Association Against Cancer (AECC), European Research Council (grant ERC-CoG-2014-648831 "MyNano") and the Spanish Ministry of Science and Innovation (SAF2016-80427-R). Part of the equipment employed in this work has been funded by Generalitat Valenciana and co-financed with FEDER funds (PO FEDER of Comunitat Valenciana 2014–2020).

References:

[1] Rodriguez-Otormín, F *et al*. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. **2018**.

[2] Duro-Castano, A. *et al*., *Adv Mater* **2017**, 29:39.

[3] Patent: Vicent, M. J.; Duro Castaño, A; Nebot, V.J. WO2017025298 (A1)

Translocation, a private public partnership to investigate the permeability barrier for antibiotics into Gram-negative bacteria

P. GRIBBON[1], M. WINTERHALTER[2]

[1] Fraunhofer IME, Hamburg.

[2] Jacobs University Bremen.

Contact e-mail: Philip.Gribbon@ime.fraunhofer.de; M.Winterhalter@jacobs-university.de

The TRANSLOCATION project was part of the IMI's "New Drugs for Bad Bugs" (www.ND4BB.eu) initiative (www.ND4BB.eu) which was designed to address scientific and financial challenges associated with antibacterial drug discovery and development.

From an antibacterial drug discovery point of view, TRANSLOCATION (www.translocation.eu) focused on understanding and overcoming the low permeability of the Gram-negative bacterial cell envelope which can severely limit a drug's ability to reach its target. The objective was to increase the understanding of how small molecules penetrate into and are effluxed out of Gram-negative bacteria and to create and validate tools and assays that can be used to improve the design of new drugs to treat resistant Gram-negative infections. To facilitate data sharing, TRANSLOCATION created and populate a repository of antibacterial data and the framework to allow the analysis of that data to establish best practices for future antibacterial drug discovery efforts. TRANSLOCATION achieved these objectives by combining theoretical and experimental techniques, determining the structure, function, presence, and underlying molecular mechanisms of recognition and transport by bacterial outer membrane proteins associated with small molecule uptake or efflux (e.g. porins, siderophore receptors, other uptake systems, efflux systems).

Here we will present some results (see details on www.translocation.eu for publications):

- A protocol to detect fluorescent drug uptake at the single bacterium and population levels in *E. coli* was developed
- Electrophysiological recording as a new technique to quantify penetration
- MS-based protocol for cpd uptake was designed
- The outer membrane proteome of *P. aeruginosa* and *A. baumannii* in different in vitro and in vivo conditions was characterized.
- Approximately 40 new high-resolution X-ray structures of porins, siderophore receptors and other related proteins were solved
- A variety of novel vectors to exploit the iron uptake systems were synthesized and studied
- A scoring function to both predict and explain small molecule penetration through porins of Enterobacteriaceae was developed
- Orthologues of the PiuA and PirA, Fe-receptors important for siderophore-conjugate antibiotic transport into Pseudomonas have been identified in *A. baumannii*
- New protocol to increase the throughput of X-ray studies of efflux systems
- Structural and dynamic features affecting uptake, binding, and transport of compounds by RND transporters
- New insights in the ferri-enterobactin uptake pathway in *P. aeruginosa*
- Proteome of *P. aeruginosa* grown in the presence or not of siderophore-linezolid conjugates in planktonic growth conditions of eukaryotic cell infection assays
- Improved understanding of the potential of both maltodextrin uptake and fatty acid uptake as routes for drug discovery
- Synthesis of tris-catechol vectors able to transport the linezolid into *P. aeruginosa* periplasm and induce the expression of the enterobactin pathway

A virtual screening method to predict permeation of molecules in Gram negative bacteria

Silvia ACOSTA GUTIÉRREZ[(1)], Igor BODRENKO[(1)], Matteo CECCARELLI[(1)]

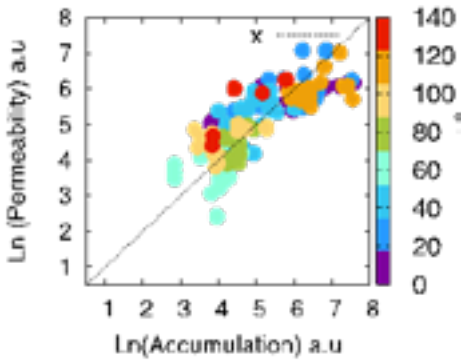
[1] Department of Physics, University of Cagliari, Cittadella universitaria, 09042 Monserrato, Italy.

Contact e-mail: matteo.ceccarelli@dsf.unica.it

The demand of new drugs for combating multidrug-resistant bacteria appears more urgent for Gram-negative bacteria. The presence of the additional outer membrane, which hinders the access of molecules to internal targets, renders the development of anti-infectives challenging. Today we have few exploratory cases where physical/chemical rules have been proposed to modulate permeation through the outer membrane.

In order to explore quantitatively the transport route represented by porins in enterobacterial species, we have recently developed a scoring function that is able to rank molecules according to their permeation [1]. This scoring function is based on general molecular properties (size, charge, electric dipole) and trained with permeation data of 9 beta-lactam antibiotics through 8 porins from enterobacteriaceae. Then, it was verified using in vitro time-kill assays and electrophysiology.

We selected a large number of not beta-lactam molecules for which accumulation data in *E.coli* are available from literature (153 molecules, [2]). After calculating the molecular descriptors for each molecule, we used the scoring function to predict their permeation through the main porin from *Escherichia coli*, OmpF. The computed correlation between permeation and accumulation is not high, R=0.47, considering the entire set of molecules. However, not considering those few molecules likely not using porins to enter the cell, we obtained a higher correlation, R=0.80.



Though internal accumulation does not imply that permeation occurs through porins, the high correlation obtained indicates that in fact porins represents the main pathway for the entry of molecules in *E.coli*. The capability of the scoring function to predict the permeation of a dataset chemically diverse from the one used to train the scoring function, it is a proof that (i) the scoring function can predict well permeation, and (ii) it has general application, as by construction does not depend on specific chemical groups. Thus, the efficiency of our scoring function (thousand of molecules per day) would allow its use in screening large virtual databases to identify new scaffolds with good permeation.

Acknowledgements: we acknowledge the contribution from IMI-ND4BB, project Translocation nr. 115525 from EU and EFPIA contributions.

References:

[1] Acosta-Gutierrez, S. *et al*. *ACS Infect. Dis.* **2018**, *4*, 1487.

[2] Richter, M. F. *et al*. *Nature* **2017**, *545*, 299.

New [18F]-PET Radiotracers for *in vivo* diagnosis of tauopathies

Ana María GONZÁLEZ-FUENTE[1][2], Francisco SÁNCHEZ-SANCHO[1], Aurelio GARCÍA-CSÄKY[2]

[1] Instituto Química Médica (IQM-CSIC), C/ Juan de la Cierva, 3, 28006 Madrid, Spain.

[2] Instituto Plurisciplinar, Universidad Complutense de Madrid, Paseo Juan XXIII, 1, 28040 Madrid, Spain.

Contact e-mail: francisco.sanchez@csic.es

Alzheimer 's disease (AD) belongs to the pathology group named Tauopathies. Currently more than 24 million people suffer from Alzheimer disease and other dementias. This represents a serious health problem, especially if we take into account that the diagnosis of AD can only be confirmed by a postmortem histological analysis. So we need a more effective and non-invasive techniques for early diagnosis [1].

AD presents two biomarkers: tau protein and amyloid β peptide. Tau protein is the main microtubule-associated protein, responsible for axonal stabilization which allows the proper neuronal transport. In its aberrant form, tau undergoes a process of hyperphosphorilation that leads to an abnormal aggregation, forming Paired Helicoidal Filaments (PHFs) and Neurofibrillary Tangles (NFTs) in the intraneuronal medium. This process drives to the loss of the biological activity of tau and as a consequence, the cognitive decline. In addition, the other biomarker in Alzheimer's disease, the β-amyloid peptide aggregates as an extracellular deposit, forming senile plaques (SPs), which can trigger a local inflammatory response [2].

Positron emission tomography (PET) is a powerful technique to be used as an image diagnostic tool to detect those biomarkers, due to its non-invasive high spatial resolution and sensitivity, to obtain the desired information of AD and other tauopathies. Therefore, the final purpose of the development of a selective PET radiotracer in the early detection of tauopathies acquires great importance.

Notably, tau becomes our target for a reason: the close relationship between the location and density of the NFTs and the progressive decline of memory, which allows an early diagnosis of the disease and identification of the damaged areas. In the past decade, β -Amyloid [18F]-PET radiotracers were developed and some of them have been already approved for clinical use by the FDA and EMA, allowing the evaluation of Aβ deposition over time. However, in recent years an increasing number of compounds have been studied as selective tau [18F]-PET radiotracers to get a more comprehensive insight into the causes, diagnoses, and potential treatment of tauopathies. Our studies are included in this last group.

We have designed and tested new compounds based on three main criteria: their affinity and selectivity for tau protein, their kinetic profile and their unique structural features. We have identified new compounds, not previously described to date, that can be considered good candidates for further development. Furthermore, related bibliographic and experimental data from our group and others, point to their adequate pharmacokinetic profile and their capacity to cross the blood-brain barrier.

To reach our goal, an *in vitro* evaluation of the affinity and nature of the interaction between the ligands and the target protein has been performed using Surface Plasmon Resonance (SPR) [3]. This highly sensitive technique, has allowed real-time studies of the interaction between the organic compounds and tau protein (or β-amyloid peptide), both in its monomeric and aggregated form, in order to find structural-activity relationships (SAR) and the structural requirements for tau selectivity.

New studies have been designed to optimize the hit-to-lead process, obtain more detailed information about the binding epitope and develop a rational design of new improved ligands.

References:

[1] Brier, M. R. *et al. Sci. Transl. Med.* **2016**, 8, 338, 1.

[2] Cho, H. *et al. Neurology* **2016,** 87,

[3] Liu, L.; Xia, N.; Wang, J.; *RSC Adv.* **2012**, 2, 2200.

Improved linkage design for the discovery of multitarget ligands as powerful drugs for neurodegenerative diseases

Valle PALOMO[(1)], Alfonso GARCÍA-RUBIA[(1)], Vanesa NOZAL[(1)], Carlos ROCA[(1)], Concepción PÉREZ[(2)], Ana MARTÍNEZ[(1)]

[1] Centro de Investigaciones Biológicas-CSIC, Ramiro de Maeztu 9, 28040 Madrid.

[2] Instituto de Química Médica-CSIC, Juan de la Cierva 3, 28006 Madrid.

Contact e-mail: vpalomo@cib.csic.es; ana.martinez@csic.es

Multitarget drugs are molecular entities that are designed to present more than one biological activity. They are arising as powerful tools to tackle complex diseases including bacterial resistances, cancer or neurodegenerative diseases.[1] Typically, the rational strategies to design multitarget drugs are linkage, fusion and incorporation or merge.[2] Here we present the creation of a multitarget drug combining active fragments in a way that could inhibit an additional third target, improving the linkage design. In this way, we envisaged the creation of a multitarget compound following the linkage strategy in which instead of incorporating a spacer with a mere function of connecting the original molecular motifs, such a linker could entail a third biological activity useful for the treatment of these pathologies.

These novel derivatives could become powerful modulating agents for neurodegenerative diseases. Multitarget compounds are ideally suited for the treatment of these pathologies due to their unknown etiology, multifactorial pathology and lack of efficient treatments. To achieve this aim we have combined fragments that inhibit kinases involved in the main pathomolecular pathways of Alzheimer's disease such as tau aggregation, neuroinflammation and decreased neurogenesis, looking for a third action in BACE1 responsible of β-amyloid production.

Results show that the compounds prepared are active in the three biological systems targeted. Computational chemistry led to proposed binding sites of these compounds to their targets and biological experiments prove their potential for the treatment of neurodegenerative diseases.

References:

[1] Ramsay, R.R.; Popovic-Nikolic, M. R.; Nikolic, K.; Uliassi, E.; Bolognesi, M. L. A perspective on multi-target drug discovery and design for complex diseases. *Clin. Transl. Med.* **2018**, *7,* 3.

[2] Savelieff, M. G.; Nam, G.; Kang, J.; Lee, H. J.; Lee, M.; Lim, M. H. Development of Multifunctional Molecules as Potential Therapeutic Candidates for Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis in the Last Decade. *Chem Rev*. **2018,** Aug 10. doi: 10.1021/acs.chemrev.8b00138.

A second-generation of tryptophan-containing small molecules extremely potent against HIV and EV-A71 clinical isolates

Olaia MARTÍ-MARÍ[(1)], Belén MARTÍNEZ-GUALDA[(1)], Sofia DE LA PUENTE-SECADES[(1)], Ernesto QUESADA[(1)], Liang SUN[(2)], Leen DELANG[(2)], Carmen MIRABELLI[(2)], Sam NOPPEN[(2)], Dominique SCHOLS[(2)], Johan NEYTS[(2)], Federico GAGO[(3)], María-José CAMARASA[(1)], Ana SAN-FÉLIX[(1)]

[1] Instituto de Química Médica (IQM-CSIC), Madrid, Spain.

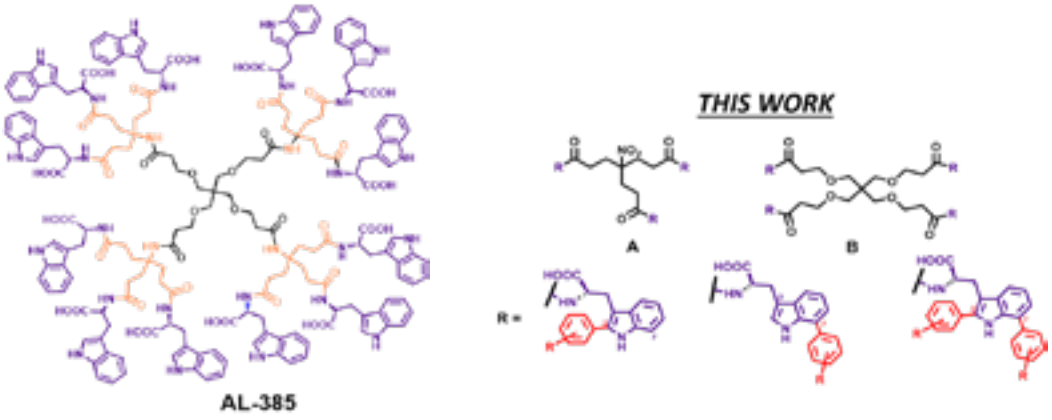
[2] Department of Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium.

[3] Department of Biomedical Sciences, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain.

Contact e-mail: anarosa@iqm.csic.es

We have recently reported a family of tryptophan (Trp) dendrimers that are dual inhibitors of HIV and EV-A71.[1,2] The prototype compound (**AL-385,** MW: 3575.84 Da) is a tetrapodal derivative with a pentaerythritol core, 4 trivalent spacer arms and 12 tryptophan (Trp) units on the periphery. These Trp moieties are linked to the central scaffold through their amino groups, consequently their carboxylic acids are free and exposed to the surface.

In the present study, a scaffold simplification strategy was applied to reduce the structure complexity and molecular weight of the prototype. With this aim, tri- (**A**) or tetrapodal (**B**) Trp derivatives bearing differently substituted phenyl rings at C2 or/and C7 positions of the indole ring were prepared. The synthesis of these compounds implies the formation of new C-C bonds through metal-catalyzed (Pd II) cross-coupling reactions.[3] Biological results demonstrated that the novel half-sized Trp derivatives (1500 Da versus 3575.84 Da) are considerably more active against HIV-1/HIV-2 (10 to 100-fold times) and EV71 (8-fold times) than the prototype while having little or no adverse effects on the host cells (at concentrations up to 100 μM). As demonstrated earlier for the dendrimer prototype, these compounds inhibit early steps of the replicative cycle of HIV-1 and EV-A71 by interacting with their respective viral surfaces (glycoprotein gp120 of HIV and 5-fold vertex of the EV71 capsid).



References:

[1] Rivero-Buceta, E.; Doyagüez, E. G.; Colomer, I.; Quesada, E.; Mathys, L.; Noppen, S.; Liekens, S.; Camarasa, M-J.; Pérez-Pérez, M-J.; Balzarini. J.; San-Félix, A. *Eur. J. Med.Chem*. **2015**, 34-43.

[2] Rivero-Buceta, E.; Sun, L.; Martínez-Gualda, B.; Doyagüez, E. G.; Donkers, K.; Quesada, E.; Camarasa, M-J.; Delang, L.; San-Félix, A; Neyts, J.; Leyssen. P. *Antimicrob. Agents and Chemother,* **2016**, *60*, 5064-5067.

[3] Preciado, S.; Mendive-Tapia, L.; Albericio, F.; Lavilla, R. *J. Org. Chem.* **2013**, *78*, 8129-8135.

From peptide to small molecule dimerization disruptors of homodimeric Trypanothione Reductase as innovative and potent antileishmanial agents

Alejandro REVUELTO[1], Héctor DE LUCIO[2], Marta RUIZ-SANTAQUITERIA[1], Kilian Jesús GUTIÉRREZ[1], Pedro A. SÁNCHEZ-MURCIA[3], Federico GAGO[3], Antonio JIMÉNEZ-RUIZ[2], María José CAMARASA[1], Sonsoles VELÁZQUEZ[1]

[1] Instituto de Química Médica (IQM-CSIC), Juan de la Cierva 3, Madrid, Spain.

[2] Laboratorio de modelado molecular, Universidad de Alcalá, Unidad asociada al CSIC, Madrid, Spain.

[3] Departamento de Biología de Sistemas, Universidad de Alcalá, Madrid, Spain.

Contact e-mail: sonsoles@iqm.csic.es

Leishmaniasis is one of the major neglected tropical diseases (NTDs) in terms of number of cases and death rate. Current available treatments possess high toxicity, side-effects and the emergence of resistance which justify the research for more effective and innovative antileishmanial drugs. Trypanothione Reductase (TryR) is an enzyme essential for the antioxidant defences of these parasites. Most, if not all, of the efforts made to inhibit TryR have relied on the design of molecules directed towards the active site. Since the biologically functional form of TryR is a homodimer, our groups have devised, an unexploited alternative inhibition strategy that attempts to disrupt protein-protein interactions (PPIs) at the homodimer interface.[1] As a "proof of concept" of this novel approach we designed and tested a small library of linear peptides and peptidomimetics[2,3] that represent rational variations of the α-helix spanning residues P435 to M447 from TryR (including the proposed «hot spots» at the dimer interface). The PKIIQSVGISN$_L$KN$_L$ 13-*mer* peptide (**TRL35**) emerged as a potent enzyme-dimerization disruptor that also behaves as a strong inhibitor of the oxidoreductase activity of TryR of *Leishmania infantum* (*Li*-TryR). However, conjugation with cell penetrating peptides was required to facilitate their cellular uptake and to kill the parasites in cell culture.[2,3]

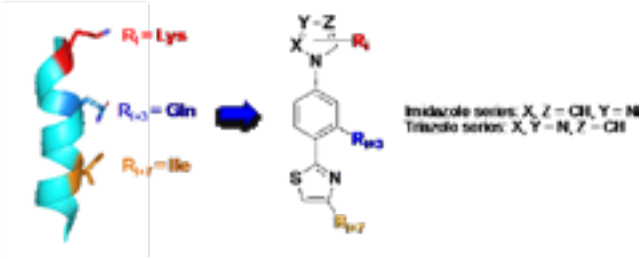


Figure. From **TRL35** *peptide to small-molecule inhibitors*

In order to improve the drug-like properties of the peptide-based prototypes, we herein report the discovery of the first potent non-peptide disruptors of homodimeric *Li*-TryR through a combination of a proteomimetic approach and scaffold hopping. Different heterocyclic scaffolds were used to project the key side-chains at the *i, i+3* and *i+7* positions of the peptide prototype (essential for the PPI) (Figure). These small molecules displayed potent *in vitro* leishmanicidal activities against extracellular and intracellular *L. infantum* parasites and interesting fluorescent properties that allows their direct visualization inside the parasites.

References:

[1] Toro, M.; Sánchez-Murcia, P. A. *et al*. *ChemBioChem* **2013**, *14*, 1212.

[2] Ruiz-Santaquiteria, M., Sanchez-Murcia, P. A. *et al*. *Eur. J. Med. Chem.* **2017**, *135*, 49.

[3] Ruiz-Santaquiteria, M., De Castro, S. *et al*. *Eur. J. Med. Chem.* **2018**, *149*, 238.

Toll-like receptor 4. Computational chemistry tools for drug repurposing and drug design

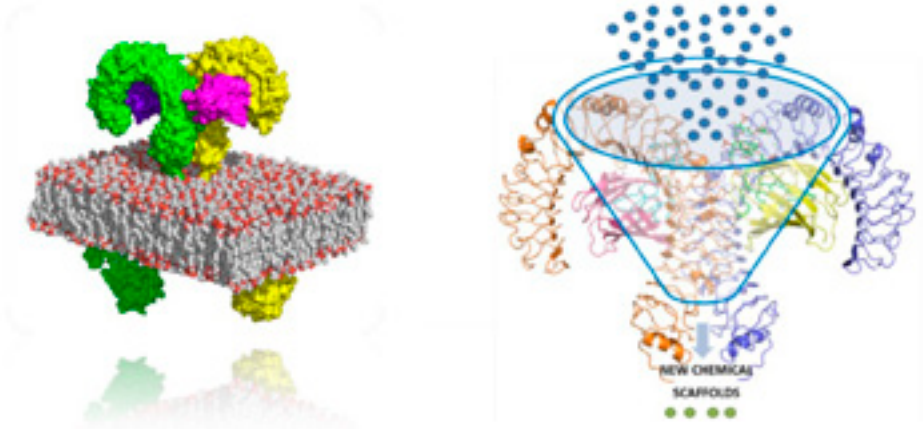
Joan GUZMÁN-CALDENTEY[(1)], Alejandra MATAMOROS, Lucía PÉREZ-REGIDOR, Jean-Marc BILLOD, Sonsoles MARTÍN-SANTAMARÍA[(1)]

[1] Centro de Investigaciones Biológicas, CIB-CSIC, C/ Ramiro de Maeztu, 9, 28040 Madrid, Spain;

Contact e-mail: juanguzman@cib.csic.es, smsantamaria@cib.csic.es

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.[1]. TLR4 activation has been associated with certain autoimmune diseases, non-infectious inflammatory disorders, and neuropathic pain, suggesting a wide range of possible clinical settings for application of TLR4 antagonists, while TLR4 agonists would be useful as adjuvants in vaccine development and in cancer immunotherapy.[2].

We have undertaken computational studies on the full structure of the TLR4/MD-2 system inserted in the membrane by means of coarse-grained and molecular dynamics simulations.[3]REFWe have also applied computational techniques (docking and MD simulations) to characterize at atomic level the molecular recognition processes by reported TLR4/MD-2 modulators, involving glycolipids and several agonist and antagonist peptides.[4REF] The possible binding to different pockets of the TLR4/MD2 system was studied, thus proposing a mechanism for their biological activity. Moreover, virtual screening and drug repurposing strategies,[5] REFfollowed by biological assays, have allowed us to identify new TLR4 antagonists with non LPS-like chemical structure useful for the development of novel TLR4 modulators.



References:

[1] Park, B. S.; Song, D. H.; Kim, H. M.; Choi, B-S.; Lee, H.; Lee, J-O. *Nature*. **2009**. 458, 1191-1196.

[2] Kuzmich, N. N.; Sivak, K. V.; Chubarev, V. N.; Porozov, Y. B.; Savateeva-Lyubimova, T. N.; Peri, F. *Vaccines*. **2017**, 5, 34, 1-25.

[3] Billod, J.-M.; Lacetera, A.; Guzmán-Caldentey, J.; Martín-Santamaría, S. *Molecules*. **2016**. 21(8).

[4] Shanmugam, A.; Rajoria, S.; George, A. L.; Mittelman, A.; Suriano, R.; Tiwari, R. K. *PLoS One*. **2012**. 7(2).

[5] Pérez-Regidor, L.; Zarioh, M.; Ortega, L.; Martín-Santamaría, S. *Int. J. Mol. Sci*. **2016**. 14(23) 2672-83.

DC-SIGN receptor expression on platelet surface in severe and nonsevere dengue patients

Sojit TOMO[1,2], M. SINDHUJADEVI[2], Vijay KUMAR[2], S. SEVATH[2], M. S. DAISY[2], B. P. AGIESHKUMAR[3], R. SOUNDRAVALLY[2]

[1] AIIMS, Jodhpur, INDIA.

[2] Jawaharlal Institute of Post-graduate Medical Education and Research (JIPMER), Puducherry, INDIA.

[3] Central Inter Disciplinary Research Facility, Sri BalajiVidyapeeth Deemed University, Puducherry, INDIA.

Contact e-mail: sojitomo@gmail.com

Platelets play a major role in dengue viral infection. Thrombocytopenia due to platelet destruction is seen in many dengue viral infected patients. Multiple mechanisms are thought to play varied roles in dengue virus-induced thrombocytopenia to cause the spectrum of Non severe dengue and Severe dengue. DC-SIGN (Dendritic Cell-Specific Intercellular Adhesion molecule -3- Grabbing Non-Integrin), a receptor present on monocytes and platelets, is found to mediate dengue virus infection of monocytes. Study of DC-SIGN receptor expression on platelet surface of severe and non severe dengue patients help us to better understand the pathophysiology of severity of the disease.

This was an analytical cross-sectional study carried out at JIPMER hospital, Puducherry. 50 patients with dengue infection were recruited. 3ml at day of admission, day 3 and day of discharge.Platelet-rich plasma extracted from the study group was assessed for DC-SIGN analysis using BD FACS CaliburTM.

In the present study, we observed a decrease in expression of DC-SIGN on platelets in severe dengue patients on the day of admission, day3 and day of discharge in comparison to nonsevere dengue. Further, expression of DC-SIGN on platelets in non severe dengue patients was decreased in patients positive for the NS1 antigen when compared with NS1 Negative. Also, expression of DC-SIGN on platelets in non severe dengue patients was decreased in patients negative for the IgM when compared with IgM positive patients.

Our results suggest that DC-SIGN, which is the receptor for viral capture, might be down-regulated on platelets in patients with dengue infection. Lower DC-SIGN on platelets in viremia states signifies the assertive role of these receptor in viral-mediated platelet destruction. This could be part of the protective response from the host to prevent platelets from taking part in the ongoing conflict between immune system and dengue virus.

Chemical approaches for improving *in vitro* diagnosis sensitivity in allergy to β-lactam antibiotics

Ángela MARTÍN-SERRANO ORTIZ[1,2], Adriana ARIZA[1], Patrik STENSTRÖM[3], Pablo MESA[3], Michael MALKOCH[3], María José TORRES[1,2], María Isabel MONTAÑEZ[1,2]

1 IBIMA - Regional University Hospital of Malaga, UMA, Malaga (Spain).

2 BIONAND - Andalusian Centre for Nanomedicine and Biotechnology, Malaga (Spain).

3 Department of Fibre and Polymer Technology, KTH Royal Institute of Technology, 10044 Stockholm (Sweden).

Contact e-mail: amartinserrano@uma.es

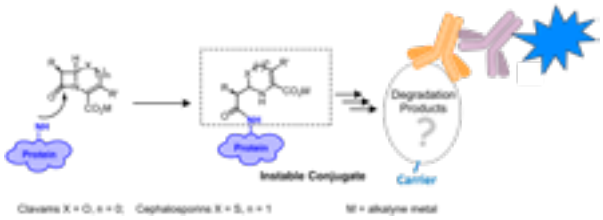
Currently, only a low percentage of cases suspected of allergy to antibiotics are finally confirmed [1]. *In vivo* tests are often the first and only option for diagnosis, however they could be risky for patients. Thus, *in vitro* tests are a more convenient and safer alternative for diagnosis: immunoassays quantify specific IgE (sIgE), the analyte of interest for immediate allergy diagnosis, by the use of labeled secondary antibodies.

The sensitivity of immunoassays for diagnosing allergy to antibiotics depends, among other factors, on: (i) the similarity between the structure used in the assay as emulator of the antigenic determinant (AD) formed *in vivo* and the structure actually formed, and (ii) the intensity of the detection signal at low concentrations of sIgE to drugs.

The general objective of this work is to improve sensitivity of current immunoassays for diagnosing immediate allergic reactions to betalactam antibiotics (BLs). More specifically, we aimed to research the chemical structures recognized by sIgE, as well as to accomplish the amplification of immunoassays detection signal.

BL derivatives were synthesized using a multicomponent assembly and their Structure-Activity Relationship (SAR) study performed using RadioAllergoSorbent Test (RAST) inhibition for immunological evaluation. Also, to pursue the amplification of immunoassays detection signal, fluorescent dendrons allowing site-specific conjugation to biomolecules and bearing 1, 2 or 8 fluorescent units were synthesized, characterized, and their suitability for labeling antibodies and fluorescence amplification capacity evaluated [2].

Results showed that synthetic BL derivatives are specifically recognised by sIgE from patients allergic to the corresponding BL or to BLs sharing a similar side chain in their structure. Also, synthesized fluorescent dendrons are suitable for site-specific labeling of a model antibody and the labeling with the dendron bearing 2 fluorescent units enhanced fluorescence by one order of magnitude compared with that bearing 1 fluorescent unit [2].



Acknowledgements: To FEBS and "I Premio UNICAJA a la innovación en biomedicina y salud".

References:

[1] Doña, I.; Blanca-Lopez, N.; Torres, M.J.; Garcia-Campos, J.; Garcia-Nunez, I.; Gomez, F.; Salas, M.; Rondon, C.; Canto, M.G. & Blanca, M. J Investig Allergol Clin Immunol. **2012**, *22*, 363.

[2] Martín-Serrano Ortiz, Á.; Stenström, P.; Mesa Antunez, P.; Andrén, O.C.J.; Torres, M.J.; Montañez, M.I.; Malkoch, M. *J Polym Sci A Polym Chem*. **2018**, *56*, 1609.

Synthesis of new fluorescent dehydroamino acids for protein labelling

Paula OROZ, Gonzalo JIMÉNEZ-OSÉS

Departamento de Química, Centro de Investigación en Síntesis Química, Universidad de La Rioja, 26006 Logroño, Spain.

Contact e-mail: paoroz@unirioja.es

Site-selective chemical modification has merged as a potential tool for protein functionalization in order to install new functionalities such as fluorescent probes, cytotoxic payloads, etc [1]. When modification is through a reaction with electrophiles, cysteine [2] and lysine [3] sidechains are often targeted, due to their high nucleophilicity. Chemo- and regioselective labelling of certain amino acids in a variety of proteins has been achieved 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.

In this work, the design, synthesis and evaluation for protein labelling of new fluorescent dehydroalanine derivatives, which are soluble in water, is presented. The superior reactivity and chemoselectivity of these reagents as Michael acceptors observed with both small-molecule nucleophiles and proteins, is described.

Acknowledgements: MINECO (projects CTQ2015-70524-R and RYC-2013-14706).

References:

[1] Boutureira, O.; Bernardes, G.J.L. *Chem. Rev.,* **2015**, *115*, 2174-2195.

[2] Vinogradova, E. V; Zhang, C.; Spokoyny, A. M.; Penlute, B. L.; Buchwald, S.L., *Nature,,* **2015**, *526*, 687-691.

[3] Matos, M. J.; Oliveira, B. L.; Martínez-Sáenz, N.; Guerreiro, A.; Cal, P. M. S. D.; Bertoldo, J.; Maneiro, M.; Elizabeth, P.; Howard, J.; Deery, M. J.; Chalker, J.M.; Corzana, F.; Jiménez-Osés, G.; Bernardes, G.J.L., *J. Am. Chem. Soc.,* **2018**, *140*, 4004-4017.

CX4945-based scaffolds for the synthesis of dual inhibitors of Protein Kinase 2 (CK2) and Histone Deacetylases(HDACs)

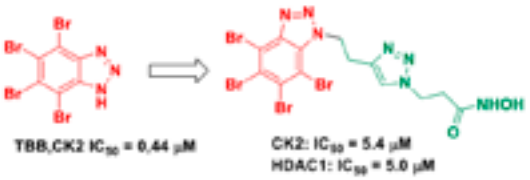
L. RANGASAMY(1), I. ORTÍN(1), B. DE PASCUAL-TERESA(1), A. RAMOS(1)

[1] Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad San Pablo CEU, Urbanización Monteprincipe, 28925 Alcorcón, Madrid, Spain.

Contact e-mail: irene.ortinremon@ceu.es, loganathan.rangasamy@ceu.es

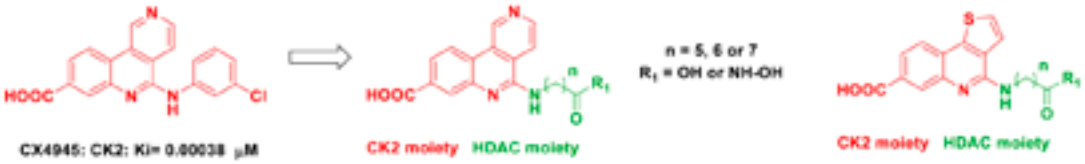
Combination of chemotherapeutic regimens aiming at multiple molecular targets are commonly used in the treatment of cancer to improve efficacy, decrease toxicity, and prevent the development of drug resistance. An alternative strategy which is gaining interest in drug discovery is the development of a single chemical entity that contains a combination of pharmacophores that are capable of modulating multiple targets simultaneously. Since Protein kinase 2 (CK2) and histones deacetylases (HDACs)[1] are closely related relevant proteins in cancer therapy, we envisioned that concurrent inhibition of these two enzymes, by the design of a novel potential multi-target single molecule, might enhance drug efficacy and overcome the current pharmacokinetic limitations, as well as likely provide beneficial additive or synergistic biological effects.

Our first described dual HDAC/CK2 inhibitors were designed using **TBB** (4,5,6,7-tetrabromo-2H-benzotriazole) as scaffold to promote CK2 inhibition and the hydroxamate as zinc binding group (ZBG) to coordinate the Zn atom present in the active site of HDAC (Scheme 1). [2]



Scheme 1

More recently **CX-4945** [3] a highly potent and selective CK2 inhibitor was reported. In this project we are using this compound together with related heterocycles, as scaffolds for the design and synthesis of several series of dual-targeting inhibitors with improved inhibitory profile (Scheme 2).



Scheme 2

Acknowledgements: Financial support from CTQ2014-52604-R (MINECO/FEDER, UE) is kindly acknowledged. L.R was supported by a Marie-Curie Individual Fellowship (746225).

References:

[1] Hesham H. M, Lasheen D. S, Abouzid K. A.M, *Med. Res. Rev*. **2018**,1.

[2] Purwin M., Hernandez-Toribio J., Coderch C., Panchuk R., Skorokhyd N., Filipiak K., de Pascual- Teresa B., Ramos A. *RSC Advances* **2016**, *6*,66595.

[3] Pierre F., Chua P. C., O'Brien S. E., Siddiqui-Jain A., Bourbon P., Haddach M., Michaux J., Nagasawa J., Schwaebe M. K., Stefan E., Vialettes A., Whitten J. P., Chen T. K., Darjania L., Stansfield R., Anderes K., Bliesath J., Drygin D., Ho C., Omori M., Proffitt C., Streiner N., Trent K., Rice W. G., Ryckman D. M. J.Med.Chem **2011**, 54, 635.

Hypoxia sensing based on bio-inspired peptide sensors

Pablo IGLESIAS[(1)], Cristina PENAS[(2)], José A. COSTOYA[(1)*], Elena PAZOS[(3)*]

[1] Departamento de Fisioloxia, Facultade de Medicina and Centro de Investigación en Medicina Molecular y Enfermedades Crónicas (CiMUS). Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS). Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain.

[2] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CiQUS), Departamento de Química Orgánica, Universidade de Santiago de Compostela. 15782 Santiago de Compostela, Spain.

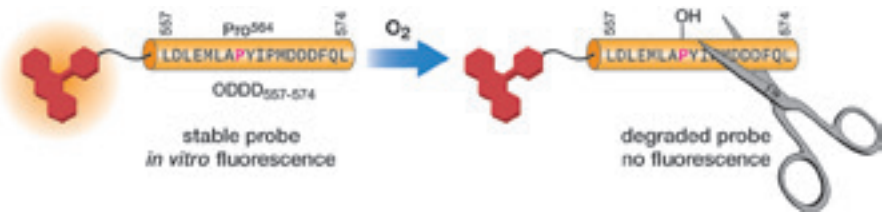
[3] Departamento de Química, Facultade de Ciencias and Centro de Investigacións Científicas Avanzadas (CICA), Universidade da Coruña, 15071 A Coruña, Spain.

Contact e-mail: elena.pazos@udc.es

Some tumour types are characterized by a fast growth that leads to deficient intratumoral vascularization and low oxygen availability. To overcome this lack of oxygen (hypoxia), tumoural cells activate the neoangiogenic program by upregulating the transcription factor HIF-1α.[1] As such, this transcription factor has been used as prognostic marker of tumoural aggressiveness and its activity a readout of tumoural proliferation and ability to metastasize.[2] Therefore, the development of novel and more efficient techniques of visualization of tumoural processes is a high priority in molecular imaging.

We envisioned a fluorescent peptide sensor of hypoxia that would mimic the oxygen-sensing capability of HIF-1α, so that the stability of the sensor, and therefore the fluorescence intensity, would be higher under hypoxic conditions. For its design we decided to use a short 16-mer peptide derived from the HIF-1α oxygen dependent degradation (ODD) domain (Leu^{557} to Leu^{574}), which is hydroxylated in the Pro^{564} residue and then degraded during normoxia,[3] but is stable towards degradation under hypoxic conditions. In addition to this oxygen-sensing domain, the sensor includes an octa-arginine peptide that mediates cell internalization and a fluorochrome (5,6-ROX) that acts as fluorescent reporter of the integrity of the peptide.

In vitro assays in human breast cancer cells showed that the proteolytic stability of the designed probe is controlled under hypoxia and that its fluorescence signal depends on the oxygen availability. We have further confirmed that the probe is degraded by the proteasome upon hydroxylation of the ODD domain, thus demonstrating our hypothesis for the design of the probe based on an inhibition of the sensor degradation in a HIF-dependent hypoxic response.



References:

[1] Wong, B.W.; Marsch, E.; Treps, L.; Baes, M.; Carmeliet, P. *EMBO J.* **2017**, *36*, 2187.

[2] LaGory, E. L.; Giaccia, A. J. *Nat Cell Biol.* **2016**, *18*, 356.

[3] Harada, H.; Hiraoka, M.; Kizaka-Kondoh, S. *Cancer Res.* **2002**, *62*, 2013.

Developing *de novo* designed models of the type III copper proteins

Fabio PIRRO[(1)], Marco CHINO[(1)], Ornella MAGLIO[(1,2)], Flavia NASTRI[(1)], Angela LOMBARDI[(1)]

[1] Department of Chemical Sciences, University of Napoli "Federico II,", Via Cintia, 46, Napoli, Italy.

[2] Institute of Biostructure and Bioimaging, National Research Council, via Mezzocannone, 16, Napoli, Italy.

Contact e-mail: fabio.pirro@unina.it

Catechol Oxidase and tyrosinase are prominent members of the type III copper (T3Cu) proteins and they play a key role in diverse biological processes [1]. The T3Cu site, two magnetically coupled copper ions, allows the binding and subsequent activation of molecular oxygen. Tyrosinase catalyzes the orthohydroxylation of monophenol (monophenolase activity) and the subsequent oxidation of the diphenolic product to the quinone (diphenolase activity), whereas catechol oxidase shows diphenolase, but lacks monooxygenase activity. X-ray crystallographic investigations of the native enzymes suggest that the monophenolase activity is possible only in presence of a structural water, whose role is the deprotonation of the phenolic substrate. However, the structural determinants to differentiate the two classes of enzymes are still unclear [2].

De novo protein design is one of the approaches of choice to study metalloenzymes. A polypeptide sequence that is not directly related to any natural protein allows the elucidation of unbiased structural determinants. Indeed, employing this approach, we have already developed the DF proteins, a series of artificial metalloenzymes, that recapitulate not only the structural, but also the functional features of the natural diiron-oxo-proteins [3].

Here, we describe the development of DR1, a *de novo* designed metalloprotein inspired by the T3Cu proteins. The computational design consisted primarily in the positioning of the first coordination sphere to recreate the T3Cu site. Subsequently, the second coordination sphere and the hydrophobic core were optimized to stabilize the metal-binding site and the intended native-like tertiary structure. Finally, the overall stability of the final model was evaluated using molecular dynamics simulations.

Our preliminary characterization demonstrates that DR1 binds two copper ions as designed and, more importantly, catalyzes the oxidation of 3,5-di-tert-butyl catechol to the o-quinone, showing diphenolase activity. Starting from DR1, new models will be designed to unveil the structural determinants to induce the monophenolase activity.

References:

[1] Kaintz, C.; Mauracher, S. G.; Rompel, A. *Chapter One - Type-3 Copper Proteins: Recent Advances on Polyphenol Oxidases.* Christov, C. Z.; Advances in Protein Chemistry and Structural Biology; Academic Press: 2014; Vol. 97.

[2] Decker, H.; Solem, E.; Tuczek, F. *Inorganica Chimica Acta* **2018**, 481, 32.

[3] Chino, M.; Maglio, O.; Nastri, F.; Pavone, V.; DeGrado, W. F.; Lombardi, A. *European Journal of Inorganic Chemistry* **2015**, 2015 (21), 3371.

Discovery of Novel Rimonabant-like peripheral selective CB_1 ligands

Rubén PRIETO[(1)], Nora PIAY[(2)], Diego TRIÑANES[(1)], Jhonny AZUAJE[(1)], Jacobo SOILÁN[(1)], José BREA[(2)], María I. LOZA[(2)], Alessandra CONTINO[(3)], Angela STEFANACHI[(3)], Eddy SOTELO[(1*)]

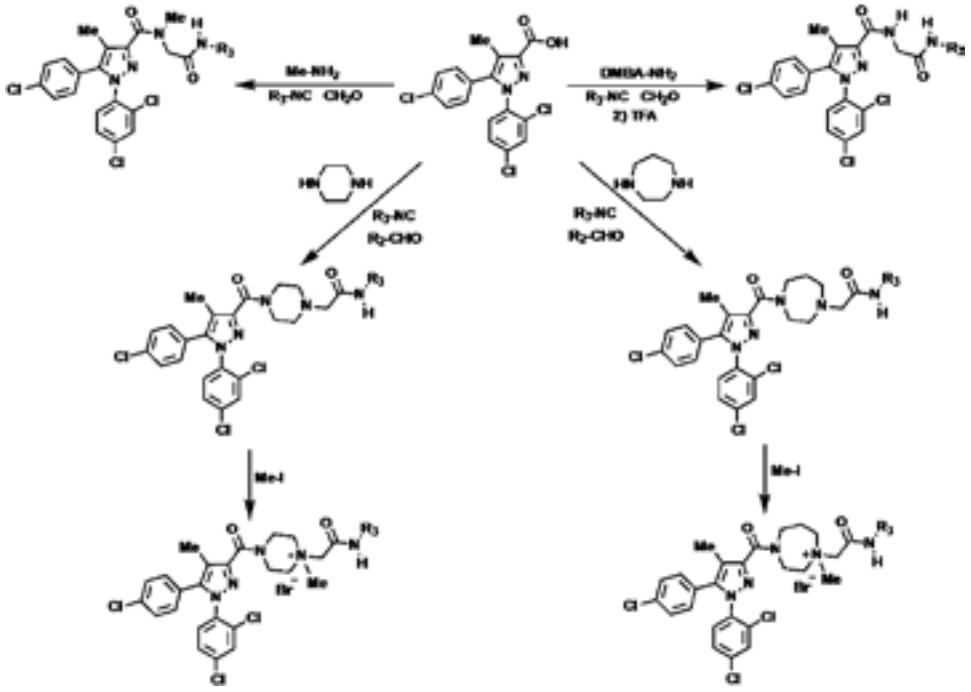
[1] Center for Research in Biological Chemistry and Molecular Materials (CiQUS), University of Santiago de Compostela, 15782 Santiago de Compostela, Spain.

[2] Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela, 15782 Santiago de Compostela, Spain.

[3] Department of Pharmacy and Drug Science, Bari University, Bari, Italy.

[*]Contact e-mail: e.sotelo@usc.es

Rimonabant is a CB_1 inverse agonist approved in 2006 for the treatment of obesity that was removed from the market in 2008 due to serious psychiatric side effects.[1] Growing evidences confirmed Rimonabant´s neuropsychiatric liabilities are consequence of its binding to central CB_1 receptors, but also that peripheral CB_1 receptor blockade produces similar appetite suppression and weight loss.[2] Accordingly, the search for novel peripheral selective CB_1 ligands have emerged as a promising approach for obesity control.[3] In the frame of a project aimed to identify new lead compounds by using MCR-based approaches, we herein document the discovery and optimization of novel series of brain non-penetrant Rimonabant analogues. The new ligands, that were assembled by Ugi or Ugi-split reactions, exhibit high polar surface area and low Log P while retain excellent CB_1 affinity and selectivity profile.



References:

[1] Lee, H.K., Choi, E.B., Pak, C.S., *Curr. Top. Med. Chem.,* **2009**, 9, 482.

[2] Tam, J., Vemuri, V.K., Liu, J., Batkai, S., Mukhopadhyay, B., Godlewski, G., Osei-Hyaman, D., Ohnuma, S., Pickel, J., Kunos, G., *J. Clin. Invest*. **2010**, *120*, 3735. *Chem. Lett*. **2013**, *4*, 1031.

[3] Flup, A., Bortoff, K., Seltzman, H., Zhang, Y., Mathews, J., Snyder, R., Fennell, T., Maitra, R., *J. Med. Chem*., **2012**, 55, 2820.

Evaluation of Gemcitabine based targeted analogues for the treatment of solid tumors

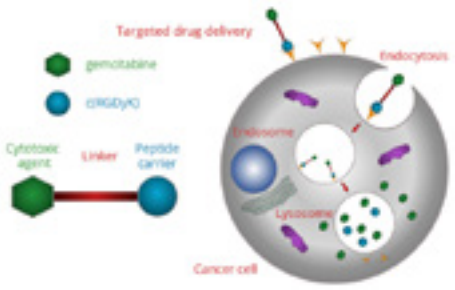
Angeliki VELENTZA-ALMPANI[(1)], Theodora CHATZISIDERI[(2)], George LEONIDIS[(2)], Eleni SKAVATSOU[(1)], Vasiliki SARLI[(2)], Constantin TAMVAKOPOULOS[(1)]

[1] Biomedical Research Foundation, Academy of Athens, Center of Clinical Research, Experimental Surgery and Translational Research, Division of Pharmacology-Pharmacotechnology, Soranou Ephessiou Street 4, GR-11527 Athens, Greece.

[2] Department of Chemistry, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece.

Contact e-mail: ctamvakop@bioacademy.gr

Cancer represents a global pandemic, as it is the second leading cause of death in 2018 [1]. Chemotherapy is one of the most preferred treatments for many cancer types, leading to the disruption of the abnormal proliferation of cancer cells. This approach causes patients to experience unpleasant side effects resulting in cancer resistance and tumor relapse. Gemcitabine is a nucleoside analogue used for the chemotherapeutic treatment of various solid tumors inhibiting the cell cycle, whilst preventing tumor growth and expansion. Although gemcitabine is an approved drug, its efficacy is limited due to its rapid metabolic inactivation, induction of cancer resistance and development of off-targeted toxicities. To overcome these limitations, we developed two novel gemcitabine-based conjugates with the cyclic RGD peptide (cRGDyk), TC-113 and TC-116.



cRGDyk peptides are small molecules that bind with high affinity to αvβ3 integrin that is proven to be overexpressed in various cancer types[2]. Through this conjugation we could achieve better efficacy and less off-targeted toxicities, as the drug will be specifically driven towards the tumor site, where integrins will be highly present.

The A549 NSCLC cell line was chosen in order to perform MTT viability assays and determine the antiproliferative capacity of these conjugates. Both TC-113 and TC-116 were shown to be potent with TC-116 presenting an IC50 value of 31 nM (close to gemcitabine's value), whereas TC-113 presented a value of 532 nM. These results suggest that TC-113 is more stable, something that was confirmed after the development of an analytical method for the simultaneous determination of gemcitabine, cRGDyk and TC-113/TC-116 by HPLC-UV. We tested the stability of both conjugates in medium, mouse and human plasma, showing that TC-113 is more stable in all the above conditions.

The results provide insight into future design of molecules with potential for *in vivo* efficacy.

References:

[1] Rebecca L. Siegel, Kimberly D. Miller, Ahmedin Jemal, "*Cancer Statistics*", Ca Cancer J Clin, 2017, 67, 7-30.

[2] Yi Ma, Guanhua Ai, Congying Zhang, Menglu Zhao, Xue Dong, Zhihao Han, Zhaohui Wang, Min Zhang, Yuxi Liu, Weidong Gao, Siwen Li, Yueqing Gu, "*Novel Linear Peptides with High Affinity to αvβ3 Integrin for Precise Tumor Identification*", Theranostics, 2017, 7, 1511- 1523.

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

Ciro RODRÍGUEZ DÍAZ[(1)], Ana LATORRE[(1)], Romina LORCA[(2)], Alfonso LATORRE[(1)], Álvaro SOMOZA[(1)]

[1] Instituto Madrileño de Estudios Avanzados en Nanociencia, Centro Nacional de Biotecnología- Unidad Asociada de Nanobiotecnología, Cantoblanco, 28049 Madrid, Spain.

[2] Universidad de Santiago de Chile, Facultad de Química y Biología, Departamento de Química de los Materiales, Alameda 3363, Estación Central, Santiago de Chile. Código Postal 9170022

Contact e-mail: ciro.rodriguez@imdea.org

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 multiple 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. A) Fluorogenic Cu (I) catalyzed azide-alkyne cycloaddition reaction scheme. B) The representation of the ligand-catalyst complex employed herein.

The synthesis of the ligand involves the functionalization of AuNP with different oligonucleotides. Remarkably, we have noticed a clear dependence of the sequence employed on the efficacy of the coupling and higher efficiency compared with traditional ligands.

Acknowledgments: This work was supported by the Spanish Ministry of Economy and Competitiveness (SAF2017-87305-R), Comunidad de Madrid (IND2017/IND-7809; S2017/BMD-3867), Asociación Española Contra el Cáncer, 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 also acknowledge the European Structural and Investment Fund.

References:

[1] Yang, M.; Li, J.; Chen, P. *Chem. Soc. Rev.*, **2014**, *43*, 6511.

[2] Droumaguet, C.; Wang, C.; Wang, Q. *Chem. Soc. Rev.*, **2010**, *39*, 1233.

Evaluation of novel nucleoside analogues for lung cancer treatment: an approach based on metronomic chemotherapy

E. SKAVATSOU, T. KARAMPELAS, C. TAMVAKOPOULOS

Biomedical Research Foundation, Academy of Athens, Center of Clinical Research, Experimental Surgery and Translational Research, Division of Pharmacology-Pharmacotechnology, SoranouEphessiou Street 4, GR-11527 Athens, Greece.

Contact e-mail: ctamvakop@bioacademy.gr

Non-Small Cell Lung Cancer (NSCLC) is responsible for an estimated 80% of total lung cancer cases worldwide [1]. Even though chemotherapy is the preferred treatment modality for NSCLC, causes patients to experience unpleasant side effects and cancer recurrence. Gemcitabine is a nucleoside analogue used against NSCLC, inhibiting the cell cycle, but with limited long-term efficacy due to its rapid metabolic inactivation. Metronomic chemotherapy (MTR), relying on the daily oral administration of a drug, at low doses, is a multi-targeted therapy, as it inhibits tumor angiogenesis, modulates immunity pathways and effects tumor initiating cells reducing the toxicity of traditional maximal tolerated dose chemotherapy (MTD) [2]. Our goal is to provide a new angle in the MTR approach, by examining the efficacy of the daily administration of an oral prodrug of gemcitabine, OralGem, to improve gemcitabine's therapeutic properties.

The A549 lung cancer line was used to establish an *in vitro* model that simulated the MTD versus the MTR conditions. Cells were cultured either in the presence of a high concentration of gemcitabine or in medium in which lower concentrations were added daily in order to study alterations in the expression of angiogenic factors. An *in vivo* xenografted animal model was set up to study the effects of MTR chemotherapy on tumor's expansion, angiogenesis and toxicity.

Daily addition of gemcitabine in A549 cells led to a decreased expression of VEGFA compared to the high dose incubation. In NOD/SCID xenografted mice, the MTR administration of OralGem led to a decreased expression of VEGFA and CD31, suggesting a suppressed angiogenic profile. Finally, MTR administration of Oral Gem led to an increase in the expression levels of Thrombospondin-1, compared to MTD chemotherapy.

MTR administration of OralGem combines restriction of angiogenesis and vessel normalization. Multiple low dosing of OralGem shows improved efficacy compared to MTD administration.

References:

[1] Rebecca L. Siegel, Kimberly D. Miller, Ahmedin Jemal, "*Cancer Statistics*", Ca Cancer J Clin, 2017, 67, 7-30

[2] Rajasekaran T, Ng QS, Tan DS, Lim WT, Ang MK, Toh CK, Chowbay B, Kanesvaran R, Tan EH, "*Metronomic chemotherapy: A relook at its basis and rationale*", Cancer Lett, 2017, 388,328-333

PEI Covalent-Modified Carbon Nanotubes for Gene Editing Mediated by CRISPR/Cas9

Ana LATORRE[(1)], Teresa NARANJO[(1)], Milagros CASTELLANOS[(1)], Emilio PÉREZ[(1)], Álvaro SOMOZA[(1)]

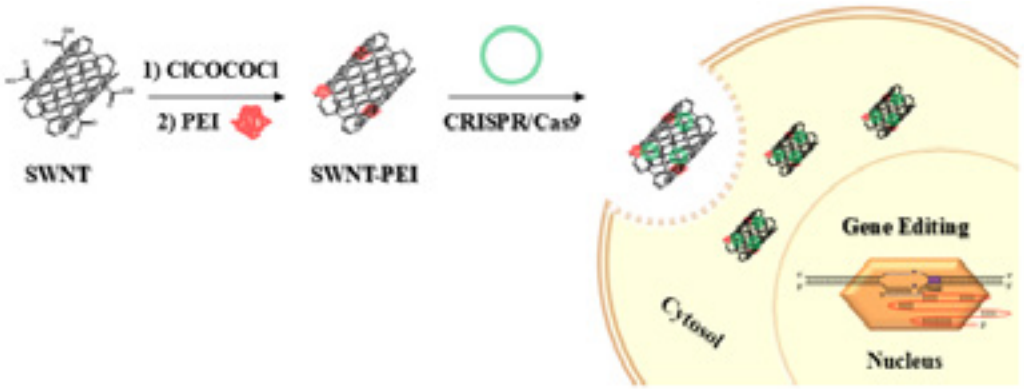
[1] Instituto Madrileño de Estudios Avanzados en Nanociencia, Centro Nacional de Biotecnología- Unidad Asociada de Nanobiotecnología, Cantoblanco, 28049 Madrid, Spain.

Contact e-mail: anabelen.latorre@imdea.org

CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeats) has an essential role in the protection of bacteria and archaea against different infections of plasmid and bacteriophage using a guide RNA to locate the specific sequence of the foreign DNA and inducing double-strand breaks, thus preventing its integration in the host genome[1].

In the past few years, it has been demonstrated that the CRISPR/Cas9 system can be used as a gene editing system of different kinds of cells and organisms, including mammals. However, this system presents multiple challenges when used in eukaryotic cells because it is not expressed as in prokaryotic organisms. For this reason, it is necessary to introduce the corresponding DNA, mRNA or the Cas9 protein by different means. Also, the stability of the Cas9 protein and the CRISPR complex, as well as, its biodistribution in organisms have a significant effect on the efficiency of gene editing[2]. For these reasons, a variety of delivery systems are being developed to ease the application of this gene editing tool in cell culture and animal models.

In this work, we have evaluated a functional CRISPR/Cas9 plasmid designed specifically to cleave different genes in human embryonic kidney (HEK293T) cells and its delivery using PEI covalent-modified single-wall carbon nanotubes (SWNT-PEI). In addition, to better assess the delivery capabilities of these carbon nanotubes, we have used an eGFP reporter plasmid that allowed us to quantify the process through fluorescence techniques.



Acknowledgments: This work was supported by the Spanish Ministry of Economy and Competitiveness (SAF 2017-87305-R), Asociación Española Contra el Cáncer, IMDEA Nanociencia, Severo Ochoa Excellence Award and Program for centers of Excellence in R&D (MINECO, Grant SEV-2016-0686)

References:

[1] Mojica, F.; Díez-Villaseñor, C.; García-Martínez, J. and Soria, E. *J. Mol. Evol.* **2005**, *Vol. 60*, 174.

[2] Lino, C.; Harper, J.; Carney, J. and Timlin, J. *Drug delivery* **2018**, *Vol. 25*, 1234.

[3] Glass, Z.; Lee, M.; Li, Y. and Xu, Q. *Trends in Biotechnology* **2018**, *Vol 36*, 137.

Naphthalimide-Based Fluorescence Macrophage Sensors. Localization Selectivity through Substituent Chemical Manipulation

Rosario HERRANZ(1), Francisco FUEYO-GONZÁLEZ(1), Juan A. GONZÁLEZ-VERA(2), Ángel ORTE(2), Mar FERNÁNDEZ-GUTIÉRREZ(3)

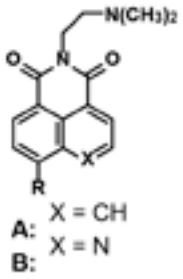
1 Instituto de Química Médica (CSIC). Juan de la Cierva 3, 28006 Madrid, Spain.

2 Department of Physical Chemistry, Faculty of Pharmacy, University of Granada, Campus Cartuja, 18071 Granada, Spain.

3 Instituto de Ciencia y Tecnología de PolímerosInstituto de Ciencia y Tecnología de PolímerosInstituto de Ciencia y Tecnología de Polímeros (CSIC), CIBER-BBN. Juan de la Cierva 3, 28006 Madrid, Spain.

Contact e-mail: rosario@iqm.csic.es

The 1,8-naphthalimide scaffold is found among the most fruitful structures in the design of fluorophores with application in the development of fluorescence biosensors, due to its favorable photophysical properties, such as strong absorption and emission, large Stokes shifts, and photostability[1]. Considering that introduction of a nitrogen atom into the 1,8-naphtalimide skeleton could red-shift the fluorescence emission and increase water solubility, we have recently studied the design, synthesis and photophysical properties of 8-methoxy-4,5-quinolimides[2]. The photophysical properties of both scaffolds depend on the attached substituents and can be tuned by chemical manipulation. We have synthesized and will communicate new naphthalimide (**A**) and quinolimide (**B**) derivatives with a dimethylaminoethyl chain at the imide nitrogen atom, which behave as macrophage fluorescence sensors. Depending on the nature of the R substituent, these fluorophores can be used to imagine either the macrophage cytoplasm or the nucleus. The nucleus sensors bind DNA as intercalants.



Acknowledgements: The work was supported by the Spanish Ministerio de Economía y Competividad grants SAF2012-32209, FU2015-67284-R, CTQ2017-85658-R, and the CSIC grant 201580E073.

References:

[1] Banerjee, S.; Veale, E. B.; Phelan, C. M.; Murphy, S. A.; Tocci, G. M.; Gillespie, L. J.; Frimannsson, D. O.; Kelly, J. M.; Gunnlaugsson, T. *Chem. Soc. Rev.* **2013**, *42*, 1601.

[2] Gonzalez-Vera, J. A.; Fueyo-Gonzalez, F.; Alkorta, I.; Peyressatre, M.; Morris, M. C.; Herranz, R. *Chem. Commun.* **2016**, *52*, 9652.

QAFFP – novel activity fingerprint derived by large scale QSAR modeling

Ctibor ŠKUTA(1), Isidro CORTES-CIRIANO(2), Gerard J. P. VAN WESTEN(3), Igor TETKO(4), Andreas BENDER(2), D. SVOZIL(1,5)

1 CZ-OPENSCREEN: National Infrastructure for Chemical Biology, Institute of Molecular Genetics, AS CR v.v.i., Prague, Czech Republic.

2 Centre for Molecular Informatics, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, U.K.

3 Leiden Academic Centre for Drug Research, Universiteit Leiden, Leiden, Netherlands.

4 Helmholtz Zentrum München, München, Germany.

5 CZ-OPENSCREEN:National Infrastructure for Chemical Biology, Department of Informatics and Chemistry, Faculty of Chemical Technology, University of Chemistry and Technology Prague, Prague, Czech Republic.

Contact e-mail: svozild@vscht.cz

An activity fingerprint is a vector representing a molecule and consisting of compound's affinity or potency against a reference panel of protein targets. An activity fingerprint describes compound's biological behavior, contrary to commonly used topology-based based fingerprints, such as the ECFP figerprint, that captures compound's chemistry, i.e. its chemical structure.

The major drawback of an activity fingerprint is a need to experimentally profile new ligands across a reference panel of protein targets. To alleviate this issue, we developed in silico QSAR-based activity fingerprint (QAFFP), individual elements of which are predicted by random forest regression models trained on bioactivity data from the ChEMBL database. Two variants of the QAFFP fingerprint were developed: 1) rv-QAFFP (real-valued QAFFP) that consists of affinity values predicted by a random forest the applicability domain of which was estimated by a conformal prediction [1], and 2) b-QAFFP (binary QAFFP) that is constructed from the rv-QAFFP fingerprint using an affinity cutoff. Altogether, 1,360 QSAR models were constructed, from which a subset of 440 highly reliable models (covering 376 molecular targets) was used in QAFFP. The performance of both binary and real valued QAFFP fingerprints was compared with that of the ECFP4 fingerprint in two important cheminformatics applications: in similarity searching and in the classification of compounds as being active or inactive. In similarity searching, the b-QAFFP fingerprint provides, compared to the ECFP4 fingerprint, comparable or better performance in terms of discriminating between actives and inactives, in terms of its early recognition potential and in terms of its ability to correctly classify individual data points. In classification, both b-QAFFP and rv-QAFFP provide comparable results with that obtained using the ECFP4 fingerprint. On the basis of these results, we propose the QAFFP fingerprint as a novel descriptor suitable for virtual screening.

References:

[1] Norinder, U.; Carlsson, L.; Boyer, S.; Eklund, M: Introducing conformal prediction in predictive modeling. A transparent and flexible alternative to applicability domain determination. *Journal of Chemical Information and Modeling*, **2014**, *54*(6), 1596.

Conformational study of cannabinoid ligands in model membrane systems using solution NMR

Paula MORALES[1,2], Nadine JAGEROVIC[2], M. Ángeles JIMÉNEZ[1]

[1] Departamento de Química-Física Biológica, Instituto de Química Física Rocasolano (IQFR-CSIC), C/ Serrano 119, 28006 Madrid, Spain.

[2] Instituto de Química Médica, CSIC, C/ Juan de la Cierva, 3, 28006 Madrid, Spain.

Contact e-mail: pmorales@iqfr.csic.es; paula.morales@iqm.csic.es

The cannabinoid receptors (CB_1 and CB_2) and their ligands have been widely studied in the last decades due to their pathophysiological relevance and therapeutic potential. These G protein-coupled receptors (GPCRs) bind lipid-derived ligands and are different from other receptors (*e.g.* aminergic receptors) in that their ligand diffuses from the lipid bilayer into the receptor binding pocket through a transmembrane portal. As demonstrated by recently reported CB_1 receptor crystal structures, the *N*-terminus can cover the extracellular region occluding access to ligands, which may enter to the binding crevice through the transmembrane helices 1 and 7 (TMH1/7 portal)[1]. Molecular dynamics simulations of the activation of the CB_2 receptor with the endogenous ligand 2-AG showed the diffusion of the ligand out of bulk lipid at the TMH6/7 interface[2].

Since cannabinoid ligands reach the receptor upon membrane bilayer diffusion, it is crucial to understand their possible interactions with membranes of different lipid composition. In this scenario, we addressed solution NMR conformational studies of different cannabinoids in membrane models.

In particular, we evaluated synthetic cannabinoid agonists previously reported by us, chromenopyrazoles derivatives[3], along with the non-psychoactive phytocannabinoid cannabidiol (CBD). The conformation of these molecules was examined in organic solvents such as trifluoroethanol and in zwitterionic dodecylphosphocholine detergent micelles as membrane mimics. Analysis of the NOESY spectra showed evidence of their interaction with these lipids. This approach allows us to optimize the characterization of lipophilic ligands such as cannabinoids in a medium that more closely resembles biological membranes.

Acknowledgements: Supported by Spanish MCIU project CTQ2017-84371-P (co-financed by FEDER). PM is a recipient of a "Juan-de-la-Cierva" post-doctoral fellowship FJCI-2016-29227. The NMR experiments were performed in the "Manuel Rico" NMR laboratory, LMR, CSIC, a node of the Spanish Large-Scale National Facility ICTS R-LRB.

References:

[1] Shao, Z.; Yin, J.; Chapman, K.; Grzemska, M.; Clark, L.; Wang, J.; Rosenbaum. *Nature* **2016**, *540*, 602.

[2] Hurst, D. P.; Grossfield, A.; Lynch, D. L.; Feller, S.; Romo, T. D.; Gawrisch, K.; Pitman, M. C.; Reggio, P. H. *J. Biol. Chem.* **2010**, *285*, 17954.

[3] Morales, P.; Gómez-Cañas, M.; Navarro, G.; Hurst, D.P.; Carrillo-Salinas, F.J.; Lagartera, L.; Pazos, R.; Goya, P.; Reggio, P.H.; Guaza, C.; Franco, R; Fernandez-Ruiz, J; Jagerovic, N. *J. Med. Chem.* **2016**, *59*, 6753.

How to measure hydrophobicity? A new extensive thermodynamic parameter for characterization of new drug candidates.

Anna SZYMANIEC-RUTKOWSKA(1), Ewa BUGAJSKA(1), Sławomir KASPEROWICZ(1), Kinga MIECZKOWSKA(1), Agnieszka M. MACIEJEWSKA(1), Jarosław POZNAŃSKI(1)

[1] Department of Biophysisc, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5A, 02-106 Warsaw, Poland.

Contact e-mail: szymaniec@ibb.waw.pl

Lipinski's revolutionary work[1] concluded that upon selection of potential drug candidates numerous physicochemical factors, which improve probability that molecule with promising activity will lead a drug, should be taken into account. The promising drug candidate should be soluble enough to sufficiently dissolve in blood or other body fluids, but also moderately hydrophobic to be transported by diffusion through biological membranes.

The first microscopic theory of hydrophobic interactions was formulated by Frank and Evans in 1945. In their "iceberg theory", water molecules in the first solvation shell of a hydrophobic compound, resemble the structure of ice. Later Hermann[2] showed that the solvation free energy is strongly correlated with the number of water molecules surrounding the solute. Further studies on hydrophobicity lead K.Lum, D. Chandler and J.D. Weeks[3] to their theory in which change in the solvation free energy is proportional to the molecular surface for larger compounds, contrary to particles of radius smaller than 10Å, for which solvation free energy is proportional rather to their volume.

On the basis of this theories we introduce a semi-empirical extensive thermodynamic parameter (β)[4] for characterisation of potential drug candidates. Volume of the single molecule in solution can be estimated from density-concentration dependency, but also theoreticly on the basis of known structure of the investigated molecule. **β** is calculated as the difference between these two volumes, and can be interpreted as the free energy of the solute solvation.

We have tested our model for the rationally selected small set of halogenated ligands, some of which are competitive inhibitors of human protein kinase CK2. We have shown that β parameter correlates with the standard ADME parameters routinely determined upon early steps of drug design procedure, but also with the melting temperature (T_m) of the protein-ligand complex, which is a comonly used desctiptor of the strength of protein-ligand interactions (Thermal Shift Assay).

Acknowledgements: This work was supported by NCN grant 2015/19/B/ST4/02156. The equipment was sponsored in part by Centre for Preclinical Research and Technology (CePT).

References:

[1] Lipinski, C. A., *Discov. Today Technol,* **2004,** *1*(14), 337.

[2] R.B. Hermann*, J. Phys. Chem.* **1971**, 75, 363.

[3] Lum, K.; Chandler, D.; Weeks, J. D., *J. Phys. Chem. B,* **1999**, *103* (22), 4570.

[4] Zielenkiewicz, W.; Poznanski, J., *J. Solution Chem.* **1998**, *27* (3), 245.

Multicomponent self-assembled peptide-based soft materials for biomedical application

Rosa MARTÍ-CENTELLES,[1] Jenifer RUBIO-MAGNIETO,[1] Beatriu ESCUDER[1]

[1] Departament de Química Inorgànica i Orgànica, Universitat Jaume I, 12071 Castelló, Spain.

Contact e-mail: centellr@uji.es

Self-assembling peptides are receiving great attention in the field of Nanomedicine due to their particular dynamics and great biocompatibility. In particular, supramolecular hydrogels based on short peptides have been used for drug delivery, cell culture and tissue repair among other applications.[1] Supramolecular hydrogels are materials assembled exclusively by weak non-covalent interactions with unique properties such as reversibility and responsiveness to a wide variety of stimuli including temperature, pH, enzymes, mechanical forces and light among others.[2] In addition, those properties can be tuned by combination of different materials into a multicomponent system.[3]

Here we present two multicomponent systems, one consisting of two short amphipathic peptides that form hydrogels at neutral pH (Figure 1A) and another one consisting of a short peptide hydrogelator network encapsulated into polypeptide-based polymersomes (Figure 1B). Both systems have been designed as carriers for transdermal drug delivery.

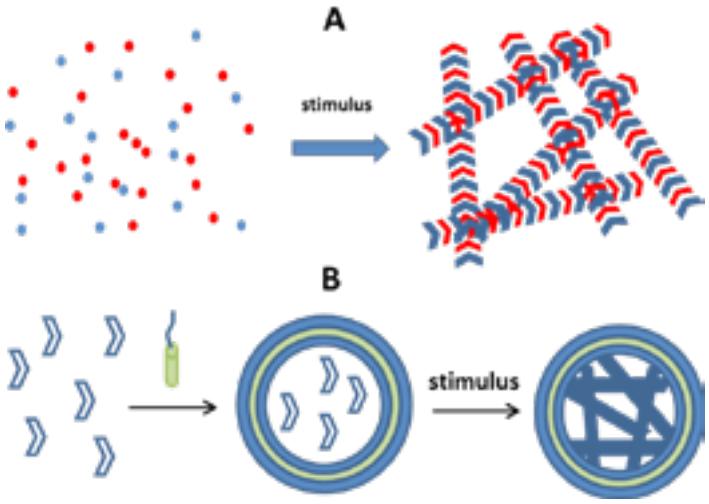


Figure 1

Acknowledgements: Ministerio de Economía, Industria y Competitividad (Grant CTQ2016-76287-R) and Universitat Jaume I (Grant UJI-2017-22) are acknowledged for funding.

References:

[1] Du, X; Zhou, J.; Shi, J.; Xu, B. *Chem. Rev.*, **2015**, *115*, 13165.

[2] Segarra-Maset, M. D.; Nebot, V. J.; Miravet, J. F.; Escuder, B. *Chem. Soc. Rev.*, **2013**, *42*, 7086.

[3] a) Raeburn, J.; Adams, D. J. *Chem. Commun*., **2015**, *51*, 5170. b) Cornwell, D. J.; Smith, D. K. *Mater. Horiz*., **2015**, *2*, 279.

[4] Tena-Solsona, M.; Alonso-de Castro, S.; Miravet, J. F.; Escuder, B. *J. Mater. Chem. B*, **2014**, *2*, 6192.

Visible-light decageable lysine derivatives for epigenetic studies

András TELEK(1), Gergely B. CSERÉP(1), Krisztina NÉMETH(1), Péter KELE(1)

[1] Institute of Organic Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, H-1117 Magyar tudósok krt. 2, Budapest, Hungary.

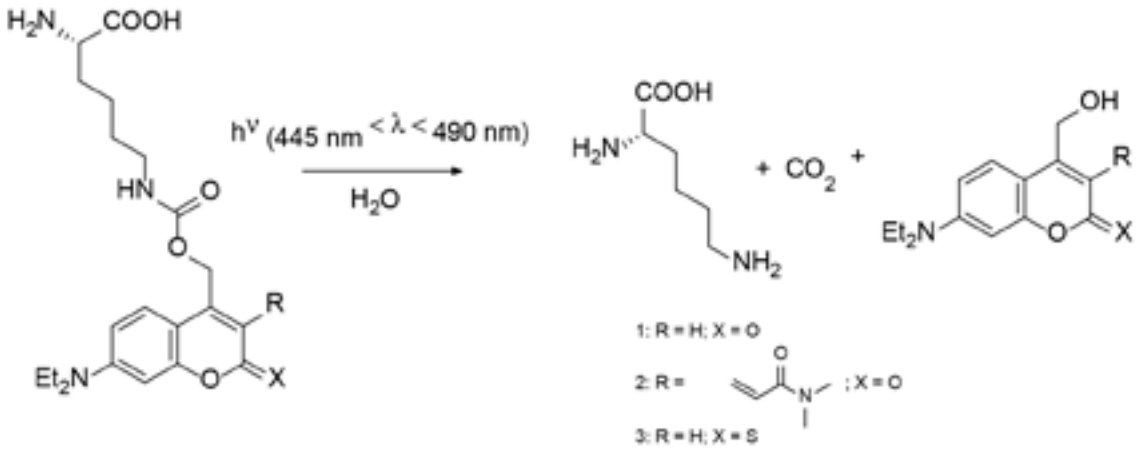
Contact e-mail: telek.andras@ttk.mta.hu

Activation and deactivation of gene expression in nature is regulated with high spatiotemporal resolution by a very complex system. Manipulation of this complex system can help us to get better understanding of gene regulation provided that it is carried out with similarly precise spatiotemporal resolution. Such precision of external control can be achieved by light to induce instantaneous changes at specific sites in order to carry out dynamic investigations. Light-controlled gene activation/deactivation can be accomplished by the use of photocaged non-canonical amino acids encoded into regulatory proteins e.g. histons.[1]

Amongst the many visible-light decageable protecting groups described in literature, 7-diethylamino-coumarinbased derivatives meet the best the criteria required for usage in proteins.[2] To this end, we designed and synthesized a set of coumarin-caged non-canonical aminoacids (ncAAs). These lysine derived ncAAs carry a visible-light sensitive cageing group at the ε-amino group. Lys is a key element in many processes regulating gene expression, and it also plays important roles in the active site of many enzymes.[3]

The prepared compounds proved to be stable under physiological conditions, and are efficiently decaged by blue-light irradiation, so their applicability in biological experiments seem to be promising.

In photolysis experiments we used a commercially available blue LED light source with emission maximum at around 462 nm. For the incorporation of the non-canonical amino acids we applied amber suppression technique involving aminoacyl-tRNA-synthase (PylRS) and tRNA ($tRNS^{Pyl}$) from *Methanosarcina mazei*.



References:

[1] Mayer, G.; Heckel, A. *Angew. Chemie Int. Ed.* **2006**, *45*, 4900.

[2] Fournier, L. *et al*. *Chem. - A Eur. J.* **2013**, *19*, 17494.

[3] Bannister, A. J.; Kouzarides, T. *Cell Res.* **2011**, *21*, 381

Synthesis and Structural Analysis of Tn AntigenMimics

Pablo TOVILLAS, Claudio D. NAVO, Francisco CORZANA, Jesús M. PEREGRINA

Universidad de La Rioja, Facultad de Ciencia y Tecnología. Dept. Química. C/ Madre de Dios, 53. 26006 Logroño, Spain.

Contact e-mail: patovill@unirioja.es

The Tn antigen is a specific human tumor-associated carbohydrate antigen (TACA) formed by N- acetyl-galactosamine (GalNAc) α-*O*-linked to either serine (Ser) or threonine (Thr) residues. In spite of its simple structure, the Tn antigen has been attracting a great interest because it has been correlated with many type of tumors. Thus, TACAs are considered promising targets for the development of cancer immunotherapy.[1]

In this field, we have reported the synthesis of several Tn antigen mimetics,[2] incorporating structural modifications that are able to imitate the conformational preferences of the natural antigen in solution and enhance the binding affinity to anti-MUC1 antibodies.[3] In this regard, we present here the synthesis of some α-*C*-linked glycomimetics with exotic conformational preferences such as inversion of the typical chair conformation in the galactosamine ring (**Figure 1**). All these compounds were subjected to a thorough conformational analysis in solution using NMR data, Quantum Mechanical (QM) calculations and Molecular Dynamics (MD) simulations.

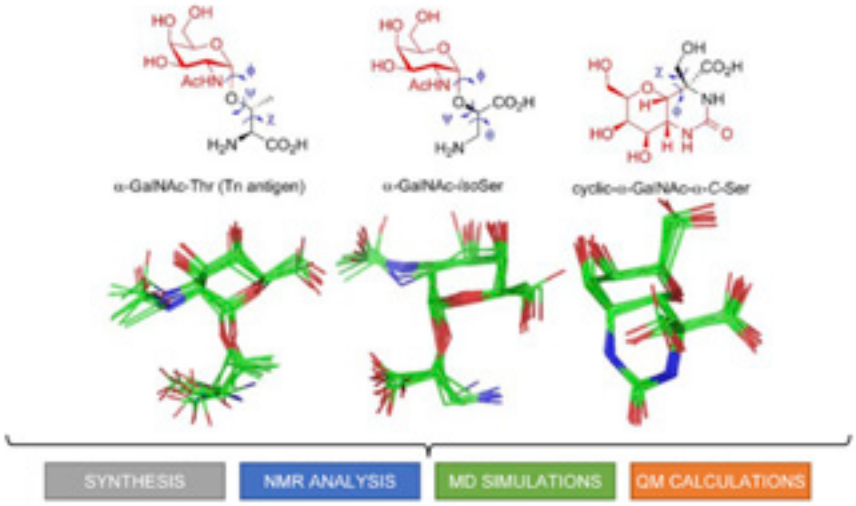


Figure 1: α-O- and α-C-linked Tn antigen mimics derived from acyclic/cyclic α- and β-amino acids.

Acknowledgements: MINECO (projects CTQ2015-67727-R, and FPI fellowships to P.T. and C.D.N.)

References:

[1] Sáenz-Martínez, N.; Peregrina, J. M., Corzana, F. *Chem. Soc. Rev.* **2017**, *46*, 7154-7175.

[2] Tovillas, P.; García-González, I.; Oroz, P.; Mazo, N.; Avenoza, A.; Corzana, F.; Jiménez-Osés, G.; Busto, J. H.; Peregrina, J. M. *J. Org. Chem.* **2018**, *83*, 4973-4980.

[3] Sánchez Fernández, E. M.; Navo, C. D.; Martínez-Sáez, N.; Gonçalves-Pereira, R.; Somovilla, V. J.; Avenoza, A.; Busto, J. H.; Bernardes, G. J. L.; Jiménez-Osés, G.; Corzana, F.; García Fernández, J. M.; Ortiz Mellet, C.; Peregrina, J. M. *Org. Lett.* **2016**, *18*, 3890-3893.

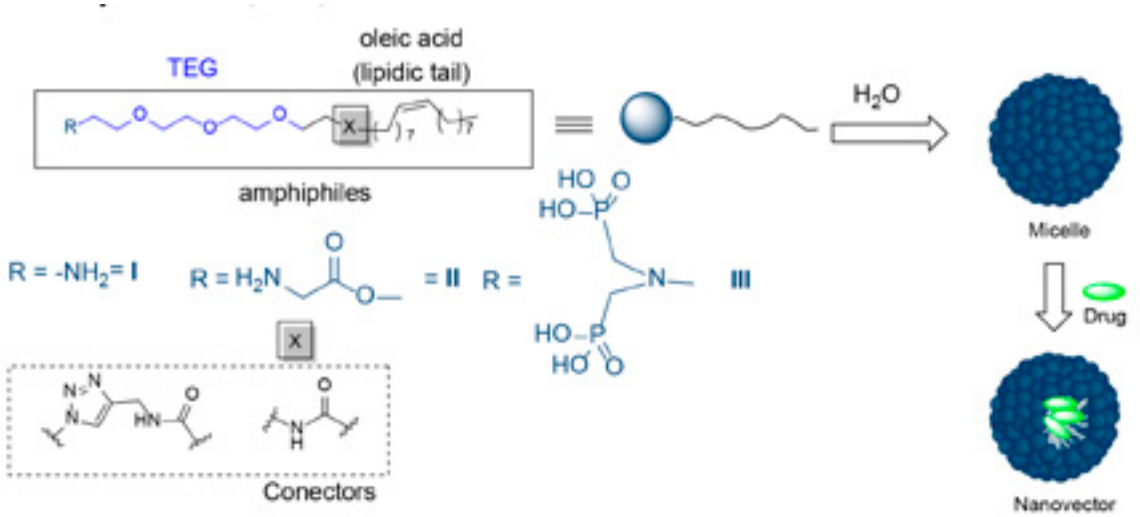
Synthesis of new nanovectors for drug delivery

Victoria VALDIVIA[(1)], Chiara PAGGIARO[(1)], Inmaculada FERNÁNDEZ[(1)]

[1] Departmento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, C/ Profesor García González nº 2, 41012 Sevilla, Spain.

Contact e-mail: vvaldivia@us.es

In the last years, amphiphilic molecules have been highly used in the development of nanostructures, representing a great promise for the targeted delivery, improved bioavailability, and drugs controlled release. Amphiphilic compounds have also proved to be useful in gene therapy as a method for the treatment of chronic and genetic diseases.[1] These molecules consist of a polar head and a lipophilic tail that are distributed in an aqueous medium to form different types of structures such as micelles, monolayer vesicles, bars, sheets and tubes.



We present the synthesis of different amphiphilic compounds based on a versatile polar head, a tetraethylene glycol spacer and a lipophilic tail derived from the oleic acid in order to obtain micelles in water. The structure of micelles with a hydrophilic shell and a hydrophobic core segment allows them to be charged with different lipophilic drugs, resulting on the increase of their bioavailability. Amino group as a polar head of compounds **I** permits the incorporation of specific ligands, as TAT peptide for the target delivery of drugs into the brain across the blood-brain-barrier. Compounds **II,** with glycine as a polar head, are useful in gene therapy, as well as for the target delivery of drugs into the brain. Compounds **III,** with phosphonate group as a polar head, have been prepared in order to increase the polarity and biocompatibility of the amphiphiles taking advantage of the similarity with important phosphorus-containing biomolecules.[2]

References:

[1] Jung Son S., Brimble M.A., Yang S., W. R. Harris P., Reddingius T., W. Muir B., Hutt O.E., Waddington L., Guan J., and Savage P.G *Aust. J. Chem.* **2013**, 66, 23-29.

[2] Barrière C, Latour V, Fau P, Caminade A.M. *Tetrahedron Letters*, **2012**, *53*, 1908-1911.

Biotechnological functionalization of viral-derived nanoparticles with health-promoting plant phenolics

Edith VELÁZQUEZ-LAM, Jaime TOMÉ-AMAT, Fernando PONZ

Centro de Biotecnología y Genómica de Plantas (CBGP, UPM-INIA). Autopista M40, km 38. Campus Montegancedo. Pozuelo de Alarcón, 28223 Madrid. Spain.

Contact e-mail: fponz@inia.es

Over the years, viral-derived nanoparticles (VNPs) have started to become a widely accepted technological platform for various applications[1]. There are a number of advantages of introducing VNPs in areas such as vaccines, drug-delivery system, gene therapy, and nanomaterials to name a few.

Turnip mosaic virus (TuMV) is a member of the *Potyviridae* family. TuMV virions contain over 2000 copies of a single protein (CP) and have an elongated flexuous structure[2].This structure shows potential for technological applications since it is possible to use the surface of the capsid to add either chemically or genetically a high number of biomolecules, thus exposed to the environment. In the present work we have used TuMV-derived VNPs chemically conjugated to biomolecules of plant origin with antimicrobial and anti-tumor activity.

Plant phenolics are natural molecules widely available amongst nature, which have been attributed a broad spectrum of properties such as antioxidant, anti-inflammatory, anti-allergic, antitumor, antimicrobial, amongst other effects[3] Using a modified version of Mannich conjugation, these phenolic molecules were chemically conjugated to CP in order to produce VNP-phenolic complexes. Different phenolic molecules were selected in order to produce these nanoparticles. Once complexes were produced they were separated from free non-reacted molecules, characterized by scanning electron microscopy (SEM), and stored until further use.

In order to assess if the VNP-phenolics nanoparticles retained antimicrobial and antitumor activities after conjugation, cell proliferation and antibiotic sensitivity tests were performed. Both sets of tests showed that the aforementioned properties were maintained, even enhanced, for the different complexes produced. VNPs represent a flexible technological platform with a vast range of applications including human, veterinary, and plant health-promoting properties.

References:

[1] Khudyakov, Y.; Pumpens, P. *Viral Nanotechnology,* 1st ed.; CRC Press: Boca Raton, 2015.

[2] Viral Zone. SIB Swiss Institute of Bioinformatics. https://viralzone.expasy.org/50 (accessed Nov 2,2018)

[3] Gorzynik-Debicka, M.; Przychodzen, P.; Cappello, F.; Kuban-Jankowska, A.; Marino Gammazza, A.; Knap, N.; Wozniak, M.; Gorska-Ponikowska, M. Potential Health Benefits of Olive Oil and Plant Polyphenols. *Int J Mol Sci* **2018**, 19 (3). doi: 10.3390/ijms19030686.

The enemy under siege: Blocking the catalytic action of GAPDH, an immunoevasion factor of the complement system, with the natural compounds curcumin and anacardic acid

Javier QUEROL GARCÍA[(1)], Sara GÓMEZ[(1)], Sergio NAVAS-YUSTE[(1)], Ana V. MARIN[(2)], Anaïs JIMÉNEZ[(2)], Marta SUBÍAS[(1,3)], Àlex GONZÁLEZ-ALSINA[(4)], Gara SÁNCHEZ[(1,5)], Karla DE LA PAZ[(1)], José R. REGUEIRO[(2)], Sebastián ALBERTI[(4)], Santiago RODRÍGUEZ DE CÓRDOBA[(1,3)], Francisco J. FERNÁNDEZ[(1,5)], Mª Cristina VEGA[(1)]

[1] Biología Estructural y Química, CIB-CSIC, 28040 Madrid, Spain.

[2] Institut Universitari d'Investigació en Ciències de la Salut, University of the Balearic Islands, Mallorca, Spain.

[3] Departamento de Medicina, Facultad de Medicina, Universidad Complutense de Madrid, 28040 Madrid, Spain.

[4] CIBER de Enfermedades Raras, Madrid, Spain.

[5] Present addres: Abvance Biotech srl, Madrid, Spain.

Contact e-mail: cvega@cib.csic.es

The ubiquitous and highly abundant glycolytic enzyme D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is central to the metabolism of biological organisms, including human pathogenic bacteria. For bacteria which depend on the glycolytic pathway for survival, GAPDH represents an enticing target for inhibitor discovery. High-resolution X-ray structures of GAPDH are invaluable for the development of antibacterial compounds since they reveal the most detailed description of catalytic and/or druggable sites. Here, we present the crystal structures of three GAPDH enzymes from three gram-positive pathogens relevant to human infection biology: *A. vaginae*, *S. pyogenes* and *C. perfringens*. We have analyzed the inhibitory potency and modality of inhibition of two natural products widely studied for their antimicrobial properties. These two molecules, anacardic acid and curcumin, have shown promise to combat bacterial infections in the clinical setting, even though the cellular targets upon which they act remain elusive to date. Our data show that both anacardic acid and curcumin can inhibit GAPDH for two of the three GAPDH enzymes in *in vitro* assays (*C. perfringens* GAPDH is however unaffected by them), exploiting both uncompetitive and noncompetitive inhibitory modalities. In addition, both inhibitors can impair the cellular growth of clinical isolates of *S. pyogenes* strains. Taken together, these data support the view that GAPDH from gram-positive bacterial pathogens could be a relevant pharmaceutical target for antibacterial development. Finally, we investigated whether anacardic acid and/or curcumin inhibition could interfere with the complement immunoevasive properties previously ascribed to GAPDH.

Acknowledgements: MCV acknowledges S2017/BMD-3673, SAF2016-81876-REDT and SAF2015-72961-EXP

References:

[1] Gómez S, Querol-García J, Sánchez-Barrón G, Subias M, González-Alsina À, Franco-Hidalgo V, Albertí S, Rodríguez de Córdoba S, Fernández FJ and Vega MC *Front. Microbiol.* **2019**, *10*, 326.

[2] Querol-García J, Fernández FJ, Marin AV, Gómez S, Fullà D, Melchor-Tafur C, Franco-Hidalgo V, Albertí S, Juanhuix J, Rodríguez de Córdoba S, Regueiro JR and Vega MC Anaphylatoxin. *Front. Microbiol.* **2017**, *8*, 541.

[3] Fernández FJ, Gómez S, Vega MC. *Seminars Cell Dev Biol.* **2019**, *85*, 98.

Authors Index

6th European Chemical Biology Symposium

ECBS/LS-EuChemS

Madrid (Spain) 3 - 5 April 2019

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

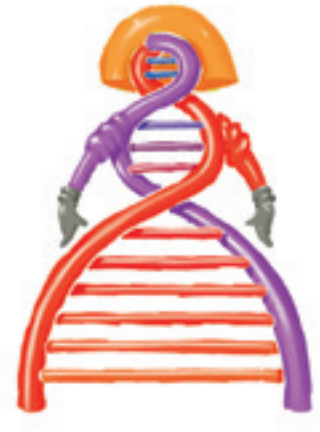
Y

Z

EUROPEAN CHEMICAL BIOLOGY SYMPOSIUM

6th ECBS/LS EuChemS

3-5 APRIL 2019 / MADRID-SPAIN



ORGANIZED BY:

EuChemS
European Chemical Society

RSEQ
Real Sociedad Española de Química

eu openscreen

PARTNERS:

CSIC
Consejo Superior de Investigaciones Científicas

Universidad de Alcalá

EFMC

International Chemical Biology Society

RSEQ

SPONSORED BY:

BECKMAN COULTER
Life Sciences

Enamine

ChemPubSoc Europe

LinuxVixion, S.L.