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ABSTRACTS

Please note:

Abstracts contained in sessions are sorted by presentation time.
Further abstracts are sorted by family name.

Content

Session 5

Session 6

Evening session

Session 7

Session 8

Session 9

Additional abstract submissions

Session 1

Nanomedicine and biomaterials

Pseudo Natural Products – Chemical Evolution of Natural Product Structure

Herbert Waldmann (Max-Planck Insitute of Molecular Physiology)

Natural products have provided inspiration for chemical biology and medicinal chemistry research. However, their often complex structure, and, therefore, demanding synthesis as well as their frequent unavailability, hamper their application.

This raises the fundamental question whether the particular structural and biological properties of natural products can be translated to structurally less demanding compounds, readily accessible by chemical synthesis and yet still endowed with pronounced bioactivity.

The lecture will describe a logic for the simplification of natural product structure by means of "Biology Oriented Synthesis" (BIOS) and its evolution into the "Pseudo Natural Product" (PNP) concept. Application of natural product inspired compound collections designed and synthesized following these principles in cell-based phenotypic assays and subsequent identification of the cellular target proteins demonstrate that the BIOS and PNPs may enable innovation in both chemical biology and medicinal chemistry research.

Single-molecule visualisation of DNA G-quadruplex formation in live cells

Marco Di Antonio (Imperial College London)

G-rich sequences can form alternative DNA secondary structures called G-quadruplexes (G4s).1 Substantial evidence now exists to support that formation of G4 structures is related to gene-expression and the case for targeting G4s for therapeutic intervention is getting stronger.1 Nevertheless, there is a need to devise additional approaches to study G4s in living cells to build further understanding on their actual biological relevance. The in-situ observation of G4-formation in living cells would provide evidence that goes beyond observations by immunostaining and ChIP-Seq. In my talk, I will describe a new G4-specific fluorescent probe (SiR-PyPDS) that has properties that enable single-molecule detection of G4s. We use SiR-PyPDS to achieve real-time detection of individual G4 structures in living cells. Live-cell single-molecule fluorescence imaging of G4s is carried out under conditions that use low concentrations of the G4-binding fluorescent probe (20 nM) that enabled us to providing informative measurements representative of the population of G4s in living cells without globally perturbing G4 formation and dynamics.

Single-molecule fluorescence imaging and time-dependent chemical trapping of unfolded G4s in living cells by means of DMS treatment, revealed that G4s fluctuate between folded and unfolded states. We also demonstrated that G4-formation in live cells is cell-cycle dependent and inhibited by chemical inhibition of transcription and replication. The observation of single fluorescent probes binding to individual G4s provides a new experimental perspective on G4-formation and dynamics in living cells, which I will discuss during my talk.

New-generation Self-Immolative Spacers for Fast and Controlled Release of Anticancer Drugs

Alberto Dal Corso (University of Milan)

Self-immolative (SI) spacers are covalent constructs capable of undergoing a spontaneous disassembly starting from a stable and inactive state, in response to specific stimuli [1]. The growing interest in the generation of stimuli-responsive devices has led to the widespread application of SI spacers in different [areas], including synthetic and analytical chemistry, material sciences, and medicinal chemistry, especially in the context of prodrugs, antibody-drug conjugates, and several other drug-release strategies. We have recently described a proline-derived SI spacer that is able to release different types of anticancer drugs (possessing either a phenolic or secondary and tertiary hydroxyl groups) through a fast cyclization mechanism involving carbamate cleavage [2].

The high efficiency of drug release obtained with this spacer was found to be beneficial for the in vitro cytotoxic activity of protease-sensitive prodrugs, compared with a commonly used spacer of the same class. Starting from these findings, novel derivatives of this proline-derived SI spacer have been designed and synthesized, either to further accelerate the drug release rates or to develop a first-in-class spacer for dual-controlled drug release. These findings expand the repertoire of degradation machineries and are instrumental for the future development of highly efficient delivery platforms.

Infrared-emitting multimodal nanostructures for controlled in vivo magnetic hyperthermia

Riccardo Marin (Autonomous University of Madrid)

Deliberate and local increase of the temperature within solid tumours represents an effective therapeutic approach. Thermal therapies embrace this concept leveraging the capability of some species to convert the absorbed energy into heat. To that end, magnetic hyperthermia (MHT) makes use of magnetic nanoparticles that can effectively dissipate the energy absorbed under alternating magnetic fields. Indeed, MHT is one of the very few nanoparticle-based therapeutic modalities that is currently clinical trial and that has therefore the potential to be used in the clinics. However, magnetic nanoparticles cannot provide real-time thermal feedback during MHT. As a result, unwanted overheating might occur and on-the-fly adjustment of the therapeutic parameters (such as the frequency of the alternating magnetic field) is unfeasible.

Accurate, rapid, and cost-effective localization of magnetic nanoparticles within a tissue represents another challenge, which could increase the efficacy and precision of MHT. In this talk, I present the combination of iron oxide magnetic nanoparticles with state-of-the-art infrared luminescent nanothermometers (Ag2S nanoparticles) in a nanocapsule that simultaneously overcomes these limitations. The novel optomagnetic nanocapsule acts as multimodal contrast agent for different imaging techniques (magnetic resonance, photoacoustic, infrared fluorescence, optical tomography, and X-ray computed tomography). Most crucially, this nanocapsule provides accurate (0.2 ºC resolution) and real-time subcutaneous thermal feedback during in vivo MHT, also enabling the attainment of thermal maps of the area of interest. These findings are a milestone on the road towards controlled magnetothermal therapies with minimal side effects.

Printing Biology: where printing meets synthetic biology

Giuseppe Arrabito (University of Palermo)

The assembly of life-like artificial systems is an emerging topic that contributes to the fundamental understanding of the molecular origins of life, and fuels the development of life-inspired platforms usable in different fields (e.g. molecular sensing, artificial biology, tissue engineering) [1]. The implementation of these platforms depends upon the ability to recapitulate the structural and functional features of biological systems, including multi-scale organization, adaptivity to environmental stimuli, collective behaviors [2]. The resulting research efforts have led to the recent definition of Printing Biology [3], a field resulting from the intersection between printing and the bottom up Synthetic Biology. Printing Biology aims at realizing reconfigurable multiscale systems (from nanometers to millimeters) with bespoke molecular composition, allowing for the determination of molecular interactions and features in conditions mimicking those of the living systems. As representative examples, the reproducible fabrication process of stable fL-scale compartments (the size selected by Nature for the formation of organelles and molecular condensates) by inkjet printing (IJP) and microcantilever spotting (µCS) will be shown.

The molecular composition of the compartments will be varied with the final aim to demonstrate the activity retention of different classes of the encapsulated biomolecules. Three different model applications will be shown, including DNA, proteins and phospholipids ink printable formulations. At first, the mechanism of DNA oligonucleotides ink imbibition by µCS into nylon porous supports is demonstrated (Figure 1). Subsequently, the immobilized DNA oligonucleotides (printed at different concentrations) are hybridized with a fluorolabeled complementary sequence permits to demonstrate the retaining of biological function [4], and the optical detection of oligonucleotides down to few tents of zeptomoles. As a second application, the retaining of CYP2E1 enzymatic activity from IJP compartments mimicking mitochondria is shown, highlighting the possibility to further induce spatial organization of the reaction products (Figure 2) [5]. Finally, preliminary experiments showing the realization of ordered phospholipids compartments containing fluorescein tagged phospholipids by µCS onto glass surfaces are reported. These systems allow for the realization of artificial platforms that could find applications in membrane-protein interaction studies.

Dual cross-link hydrogels with tunable viscoelasticity control stem cell differentiation

Chiara Pizzolitto (University of Trieste)

Mechanotransduction recapitulates the conversion of external mechanical information into intracellular biochemical response. To this, it was recently recognized that stem cell fate can be markedly influenced by surrounding extracellular matrix (ECM) mechanics. Most of our knowledge in cell mechanobiology has been built using purely elastic materials as model of ECM. However, native tissues do not exhibit purely elastic response but manifest viscoelasticity, that is a time- and frequency-dependence to loading. In fact, recent works have proved that stress-relaxation or plasticity, and more broadly, viscosity-driven processes can be considered as potent modulators of stem cell behavior.

To recapitulate native tissue mechanics and provide a more realistic ECM model, here we present unprecedented viscoelastic substrates showing adaptable viscoelasticity. In particular, I will present an innovative dual cross-link gel system based on a chitosan derivative, shortly named CTL, assembled via both temporary and permanent cross-linkers.[1] Concepts related to macromolecular chemistry and physics will be disclosed. Of note, temporary junctions are exploited to finely tune material viscoelasticity. I will show how resulting hydrogels result optimal substrates for cell anchoring upon coating with ECM proteins. Though endowed with similar elasticity, a viscosity-cell function relationship will be unveiled, identifying high viscosity hydrogels as superior materials in fostering stem cell differentiation toward a bone-like phenotype with respect to more elastic counterparts. Taken together, these results lay the groundwork for additional investigations on the role played by substrate viscoelasticity in directing cell-fate decisions.

Squaraine NIR dyes: a structure to function study for novel bilayer membrane probes

Francesca Cardano (University of Milan)

In the last decade, Near infra-red (NIR) fluorophores have been largely tested for bioimaging applications.[1] They typically show red-shifted absorption and emission, outstanding brightness and low photodegradation along with deep tissue penetration, small biological photodamage and negligible autofluorescence.[2] Several families of NIR dyes have been designed, synthesized and proposed to the market for selective staining of a plethora of biological structures, but a proper modernisation in the study of cutting edge probes specific for the complex and dynamic assemblies of the bilayer membrane, is still necessary.[3] Squaraines have been already introduced to visualize and deeply study biological membranes highlighting their relevance due to singular lightness and specificity.[4]

In this work, we have proposed symmetric and asymmetric squaraine dyes, decorated with carboxylic groups on the chromophoric core and different lengths aliphatic chains on the quaternary nitrogen positions of the scaffold itself. The formers facilitate the solubilization in biological media and lock the probes on the outer side of the amphiphilic bilayer, while the latter have been varied to investigate the respective interactions with the hydrophobic portion of the membranes. The photophysical properties, the kinetic of the insertion into large unilamellar vesicles (LUVs) bilayer membranes beside with the emission signal fluctuations related to the membrane phases properties have been analysed in relation to the probe molecular structures to provide key data to optimize the design of new NIR probes for bioimaging purposes.

Session 2

Targeted protein degradation

How PROTAC degraders work and why the ternary complex matters

Alessio Ciulli (University of Dundee)

Degrader molecules (also known as PROTACs) recruit proteins to E3 ubiquitin ligases for targeted protein degradation. Formation of a ternary complex between the PROTAC, the ligase and the target leads to the tagging of the target protein by ubiquitination, and subsequent proteasomal degradation.

In 2015, we disclosed MZ1, a potent BRD4 degrader made of our fragment-based designed VHL ligand, and a pan-BET inhibitor. Since then, my Lab has illuminated fundamental structural and biophysical understanding of PROTAC molecular recognition and mechanism of action, including solving the first crystal structure of a PROTAC ternary complex showing how MZ1 brings together VHL and its target protein BRD4. These fundamental insights into the mode of action provide guiding principles to rationally design degraders and other proximity-inducer modalities for translation chemical biology and drug discovery.

Identification and Characterization of novel molecular glue degraders

Georg Winter (Research Center for Molecular Medicine of the Austrian Academy of Sciences)

Targeted protein degradation (TPD) is a new therapeutic modality based on drugs that destabilize proteins by inducing their proximity to E3 ubiquitin ligases. In this presentation, I will discuss how we develop phenotypic drug screens to find novel small molecule degraders that function as "molecular glues". Molecular glues are of particular interest as they can degrade otherwise unligandable proteins by orchestrating direct interactions between target and ligase. I will describe a scalable strategy toward glue degrader discovery that is based on chemical screening in hyponeddylated cells, coupled to a multi-omics target deconvolution campaign. This approach led us to identify compounds that induce ubiquitination and degradation of cyclin K by prompting an interaction of CDK12–cyclin K with a CRL4B ligase complex. Notably, this interaction is independent of a dedicated substrate receptor, thus functionally segregating this mechanism from all described degraders. Collectively, our data outline a versatile and broadly applicable strategy to identify degraders with nonobvious mechanisms and thus empower future drug discovery efforts.

A novel class of small molecule degraders targeting prion protein folding intermediate

Andrea Astolfi (University of Perugia)

Decades of research efforts have conclusively provided overwhelming evidence that the cellular prion protein (PrPC) represents an optimal pharmaceutical target to tackle prion diseases, a set of fatal and incurable neurodegenerative disorders characterized by the conformational conversion of the physiological PrPC into a misfolded and infectious isoform referred to as PrP scrapie (PrPSc). Indeed, PrPC plays a key role in the disease etiology and knock-out experiments demonstrated that its therapeutic suppression can be considered safe.[1] Over the years different strategies have been proposed to tackle this target based on traditional drug discovery approaches, such as the identification of small molecules able to promote the PrPC relocalization from cellular membrane to intracellular endosomes, as well as PrPC binders that prevent its conversion to PrPSc.

However no therapy is yet available, and prion disease still represents a currently unmet medical need.[2] Very recently, we have applied a novel drug discovery approach devoted to lowering PrPC levels by hampering a complete folding process. We refer to this strategy as Pharmacological Protein Inactivation by Folding Intermediate Targeting (PPI-FIT).[3] The reconstruction of the PrP folding pathway through all-atoms MD simulation allowed the identification of a metastable intermediate of the PrP folding pathway characterized by a druggable pocket. Virtual screening of a commercial small molecule library resulted in the identification of thirteen potential binders, four of which capable of selectively lowering the load of PrP into the cellular membrane and promote its degradation. Additionally, one of these compounds inhibits prion replication in a dose-dependent fashion.

De novo identification of a fully synthetic FKBP12-FRB Molecular Glue

Felix Hausch (Technical University Darmstadt)

The gain-of-function pharmacology of Molecular Glues, prominently exemplified by the immunosuppressants FK506 and Rapamycin, holds great potential to address otherwise intractable targets. So far, the known Molecular Glues have been largely discovered by serendipity or in a target-agnostic manner.

To explore the probability for the existence of Molecular Glues, we performed a targeted screen for Molecular Glues for FKBP12-FRB(FKBP-Rapamycin binding domain of mTOR), using Rapamcin as an established control. From our in-house FKBP-targeted ligand library, we identified a weak hit, that was validated in an FP-based secondary assay.

Surprisingly, the structure of the ternary complex revealed a new binding mode of this hit compared to Rapamycin, which allow chemical optimization resulting in a fully synthetic FBKP12-FRB Molecular Glue with sub-micromolar efficacy. Our results show that with a focused library and a tailored assay cascade a targeted de-novo screening for Molecular Glues is feasible.

Session 3

Artificial intelligence & computational drug design

Using Artificial Intelligence and Chemical and Biological Data for Drug Discovery: Opportunities and Pitfalls

Andreas Bender (University of Cambridge)

While Artificial Intelligence (AI) had a profound impact on areas such as image and speech recognition, comparable advances in drug discovery are rare. In this contribution, we will firstly discuss in which ways chemical and biological data differs fundamentally from data available in other domains, both in its quantity and its underlying characteristics. Subsequently, case studies will be presented where the use of chemical and biological data, in combination with computational algorithms, has been successfully applied to questions related to compound mode of action, efficacy and safety. We will conclude by outlining what is needed in the future in order to advance the application of algorithms in the drug discovery field further, in particular with respect to the *in vivo* relevance of any predictions that are being made.

Combining molecular simulation and machine learning approaches for structure-based drug design

Rebecca Wade (Heidelberg Institute for Theoretical Studies)

Structure-based drug design approaches increasingly require the handling of very large amounts of data, such as large compound libraries for screening or many protein conformations generated by molecular dynamics simulations. There is also the need to integrate diverse types of experimental and computational data into the design process. I will describe examples of how we are addressing these issues by combining molecular simulation and machine learning approaches [1-5]. We focus in particular on the challenges and opportunities for drug design provided by protein binding pocket dynamics. I will present the development and recent applications of a machine learning approach to identify pocket conformations with high druggability in TRAPP, a toolbox of computational approaches to identify TRAnsient Pockets in Proteins for ligand design (https://trapp.h-its.org/). Protein binding site flexibility is one of the factors that can affect drug-target binding kinetics. Growing evidence that the efficacy of a drug can be correlated to target binding kinetics has led to the development of many new methods aimed at computing rate constants for receptor-ligand binding processes, see: kbbox.h-its.org. I will introduce the t-random acceleration molecular dynamics simulation (tRAMD) method to compute relative residence times and discuss how interaction fingerprint (MD-IFP) and machine learning analysis of tRAMD trajectories can be used to decipher the determinants of drug-target residence times.

From probe to drug: Polypharmacology across drug discovery

Albert Antolin (The Institute of Cancer Research, London)

Most small molecules interact with several target proteins but this polypharmacology is seldom comprehensively investigated or explicitly exploited during drug discovery. Here, we present the use of computational and experimental methods to identify and systematically characterize the kinase cross-pharmacology of representative HSP90 and PARP inhibitors. We demonstrate that the HSP90 inhibitors ganetespib and luminespib and the PARP inhibitors rucaparib and niraparib display unique off-target kinase pharmacology as compared to other clinical inhibitors of the same class, with important implications for personalized prescription.

We also demonstrate that the early PARP chemical tool PJ34 displays a different polypharmacology than several FDA-approved PARP inhibitors, with important implications for target validation and the practise of chemical biology. We finally demonstrate that polypharmacology evolved during the optimisation to discover luminespib and that the hit, leads and clinical candidate all have different polypharmacological profiles. We therefore recommend the computational and experimental characterization of polypharmacology earlier in drug discovery projects to unlock new multi-target drug design opportunities as well as identifying undesired toxicity and unexplained cellular effects.

The changes in prolyl oligopeptidase structure upon inhibition modify its ability to decrease alpha-synuclein aggregation

Katarzyna Walczewska-Szewc (Nicolaus Copernicus University)

The formation of extended misfolded protein aggregates is one of the main reasons for neuronal malfunction and, eventually, brain damage in many neurodegenerative diseases [1]. In Parkinson's disease alpha-synucleins are implicated in the accumulation of the aggregates. The origin of such aggregation is not yet known, however, there is a compelling evidence that it can be reduced by inhibition of prolyl oligopeptidase (PREP) [2].

This effect cannot be simply related to the inhibition of the catalytic function of the enzyme, as not all PREP inhibitors stop the alpha-synuclein aggregation [3]. Finding differences in the dynamics of the enzyme inhibited with diverse compounds would allow us to pinpoint the regions of the protein involved in the interaction between PREP and alpha-synuclein. Here, we study the action of three PREP inhibitors, each of which affects alpha-synuclein aggregation to different extent. Using molecular dynamics modelling, we determine molecular mechanisms underlying the PREP inhibition and identify structural differences in each inhibitor-PREP system. We suggest that even subtle differences in the dynamics of the enzyme affect its interactions with alpha-synucleins. Thus, identification of these regions may be crucial in preventing formation of alpha-synuclein aggregates. Acknowledgement: The computational results were obtained using the facilities of the Interdisciplinary Centre for Modern Technologies, NCU, Poland.

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

Alejandra Matamoros-Recio (Center for Biological Research (CIB) Margarita Salas / CSIC)

Toll-like receptors (TLRs) are pattern recognition receptors involved in innate immunity. In particular, TLR4 binds to lipopolysaccharides (LPS), a membrane constituent of Gram-negative bacteria and, together with MD-2 protein, forms a heterodimeric complex which leads to the activation of the innate immune system response. TLR4 activation has been associated with certain autoimmune diseases, noninfectious inflammatory disorders, and neuropathic pain, suggesting a wide range of possible clinical settings for the application of TLR4 antagonists, while TLR4 agonists would be useful as adjuvants in vaccine development and in cancer immunotherapy.[1,2] Specific molecular features of extracellular, transmembrane, and cytoplasmic domains of TLR4 are crucial for coordinating the complex innate immune signaling pathway. Although structural and biochemical data is currently available for the independent TLR4 domains, this only provides a partial fragmented view, because full-length proteins are flexible entities and dynamics play a key role in their functionality. Therefore, many structural and dynamical features of the TLR4 mode of action remain largely unknown.[3]

Computational studies of the different independent domains composing the TLR4 were undertaken, using ab-initio calculations, homology modeling, protein-protein docking, all-atom molecular dynamics simulations, and thermodynamics calculations, to understand the differential domain organization of TLR4. From the information gathered from our independent TLR4 domains studies, we have modeled, by all-atom MD simulations, the structural assembly of plausible full-length TLR4 models embedded into realistic plasma membranes, with different chemical compositions, accounting for the active (agonist) state of the TLR4. We have also applied computational techniques to characterize, at the atomic level, the molecular recognition processes by reported TLR4 modulators, thus proposing a mechanism for their biological activity. These observations unveil relevant molecular aspects involved in the mechanism of receptor activation, and adaptor recruitment in the innate immune pathways, and will promote the discovery of new TLR4 modulators and probes.

Session 4

Platforms in drug discovery

Natural Small Molecules in Chemical Immunology

Angelo Fontana (University of Naples "Federico II")

The immune system protects us against external pathogens and internal threats such as impaired body cells. In the last years, it has become increasingly apparent that innate immunity can play surprising roles in the etiology of many chronic diseases. The effect of dysregulation of this part of the immune response is particularly evident in the progression of neurodegenerative disease, as well as is a critical element in many chronic conditions, including infective syndromes, cancer, and autoimmune disorders. The pandemic of SARS-COV2 has also brought to our attention the role of the innate immune system in the exacerbated inflammatory reaction observed in severe COVID-19 cases, as well as how immune dysregulation can significantly modify the clinical outcomes of affected patients.

A deeper understanding of the mechanisms and factors controlling innate immune response should provide new therapeutic targets in cancer and chronic pathologies. For example, the role of the key interaction between Dendritic cells (DCs) and T-cells have been strongly reconsidered to fight cancer with the introduction of the immune checkpoint inhibitors (Granier et al., 2017; Robert, 2020). However, the study of these processes is complicated by the presence of several cell types, the absence of a single organ, and the fact that many immune cells can change phenotype upon activation. Furthermore, the immune system continually works to maintain the balance between tolerance and reactivity. In this context, besides the classical ability to detect, target, and kill pathogens, innate immunity has a fundamental role in shaping the cytokine and chemokine milieu and setting a complex chain of events leading to activation and differentiation of T and B cell effectors.

Molecules able of activating antigen presentation by innate immune cells are considered suitable tools to boost the physiological immune and switch to a tolerogenic/immunosuppressive behavior. Dendritic Cells (DCs) are the most efficient professional antigen-presenting cells (APC) and constitute a bridge between the innate and adaptive immune systems (Banchereau and Steinman, 1998; Mellman, 2013; Domogalla et al., 2017). Recently, we have reported a novel class of sulfoglycolipids, collectively named Sulfavants, that trigger unconventional DC maturation and in vivo antigen-specific immunization(Manzo et al., 2017b, 2017a, 2019). The initiation of a systemic immune response by the stimulation of innate immune cells correlates to adjuvanticity and PRR-mediated signaling. Sulfavants are under preclinical trials as vaccine adjuvants, and their efficacy has been already proven in a murine model of a vaccine against melanoma (Manzo et al., 2017a). Interestingly, these products are not cytotoxic but treated mice do not show the progress of the tumor for more than 10 days after subcutaneous injection of B16F10 melanoma cells.

This result prompted us to search for other small molecules that can activate DCs and, potentially, enhance the immune response against neoplastic cells (Gallo et al., 2020). The rationale for the pursuit of small molecule-based immunotherapies is the broad range of cellular processes that can be targeted. In this field, the potential anticancer and immunological activity of natural products represents an excellent and unexplored source for the development of new drugs (Newman and Cragg, 2020). In a more general view, we are pursuing two lines of researches concerning the development of small molecules that either boost the innate (adjuvanticity) and adaptive immune effectors (DCs, T cells, macrophages, and microglia) by unconventional mechanisms, or control the type of the immune response initiated by specific antigens and stimuli mostly by reducing the inflammatory burst. In consideration of the role of the DC-T cell axis, our studies range from vaccine adjuvants to novel candidates for the treatment of chronic diseases, such as neurodegenerative diseases, as well as from implementation of screening strategies of natural extracts to the chemical synthesis of bioactive molecules and probes.

The power of chemoselectivity: Functional protein-conjugates for intra- and extracellular targeting

Christian Hackenberger (Leibniz Research Institute for Molecular Pharmacology)

In this presentation, I will focus on the chemical modification of functional proteins for for pharmaceutical and medicinal applications.1 In my laboratory, we use a combined approach of recently developed chemoselective reactions and enzymatic ligations, for instance the so-called P5-2 or Tub-tag3-labeling, for bioconjugation.

By generating stable antibody-drug conjugates (ADCs),2b structurally defined multivalent scaffolds4 or cell-permeable antibodies via conjugating cyclic cell-penetrating peptides,5 we provide new modalities for most challenging pharmaceutical targets, including next generation cancer therapeutics or novel inhibitors against viral infections.

Covalent Inhibitors re–invented

Stefan Laufer (Tübingen University)

Covalent Inhibitors belong to the oldest and most successful drugs. Prominent examples are e.g. Acetylsaliclic Acid, ß-Lactone Antibiotics or Gastric Proton Pump Inhibitors. A major breakthrew in cancer therapy of the last decades was targeted therapy with protein kinase inhibitors. Still unmet needs in this field are target residence time, selectivity and rapid development of target kinase mutations. "Targeting the Cysteinome": We applied this strategy to unsolved problems in the field of JAK3, JNKs and mutant EGFR kinases.

JAK3 signaling is a key driver in the development of lymphoid cells and modulation of immune response. Due to its isolated expression in lymphocytes a selective JAK3 inhibitions is considered to be a promising strategy for the development of new immunosuppressant drugs. Via a covalent-reversible inhibition approach we were able to develop new highly potent JAK3 inhibitors with high isoform specifity as well as an outstanding kinome wide selectivity. A novel binding mode was observed in the x-ray structure.

The emergence of mutations within the catalytic domain of EGFR has led to resistances against small molecular drugs. By the application of a scaffold hopping approach, we successfully developed picomolar covalent-irreversible inhibitors against gefitinib resistant EGFR mutants with high cellular activity (14 nM). Moreover we further improved the reversible binding patterns of this chemotype to yield compounds showing high activities in the low nanomalar range against the clinically challenging osimertinib resistant L858R/T790M/C797S triple mutant.

In continuing efforts to enhance both, JNK3 selectivity and activity of our pyridinyl-imidazole-based kinase inhibitors, we successfully applied the approach of covalent targeting of non-catalytic Cysteins to our dual JNK3/p38α MAP kinase inhibitors. The most promising covalent inhibitor (JNK3, IC50 = 0.3 nM) shows high metabolic stability in human liver microsomes and displays excellent selectivity in a screen against 410 kinases. Covalent binding to Cys-154 of the enzyme was confirmed by incubation of the inhibitors with wild-type JNK3 as well as with a JNK3-C154A mutant followed by mass spectrometry.

Design and implementation of small-molecule RNA binders for anticancer and antimicrobial therapies

Maria Duca (Côte d'Azur University – CNRS)

Non-coding RNAs recently raised as a major drug target and one of the greatest challenges of current medicinal chemistry (1). Various approaches have been described to target biologically relevant RNAs but the use of small molecules is one of the most promising for therapeutic applications and recent approval of Risdiplam as a mRNA splicing regulator for the treatment of SMA further underlined the potential of RNA targeting in clinic (2). During recent years, we focused our activities on the targeting of various non-coding RNAs such as viral, bacterial or oncogenic RNAs with a particular attention toward microRNAs (miRNAs). These 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 (3).

Our work aims at the development of original small-molecule drugs targeting specific oncogenic miRNAs production as illustrated in (4). 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. The chemical tools developed in these different projects could thus find extremely important applications as chemical biology tools for important clinical applications not only in anticancer therapies but also in target validation programs and for the discovery of new antimicrobials.

Academic Drug Discovery capabilities at SciLifeLab, Sweden

Annika Jenmalm-Jensen (SciLifeLab)

Science for Life Laboratories (SciLifeLab) is a fully integrated national center for technology and data driven molecular life sciences. SciLifeLab comprises a wide variety of capabilities relevant for academic drug discovery of which two platforms are fully devoted to this: the SciLifeLab drug discovery and development (DDD) platform and the Functional Biology and Target Discovery platform. At the meeting I will give an overview of the SciLifeLab national Infrastructure and the overall capabilities relevant for academic drug discovery.

EU-OPENSCREEN Academic Compound Library – Sourcing compounds from the chemistry community for new discoveries

Päivi Tammela (University of Helsinki)

EU-OPENSCREEN ERIC is a non-profit research infrastructure, which operates on a global scale and offers access to academic high-throughput screening facilities and medicinal chemistry groups in the ERIC member countries. Scientists from academia and industry can implement their screening projects at EU-OPENSCREEN´s partner laboratories using our European Chemical Biology Library (ECBL). Part of this collection, the EU-OPENSCREEN Academic Compound Library, will be collected from academic communities. Compounds submitted into this collection will be screened against a wide range of biological assays, thereby delivering extensive information about their biological activities and providing opportunities for novel collaborations with EU-OPENSCREEN users from all over Europe and beyond. EU-OPENSCREEN ERIC together with its Academic Library Working Group has established the framework and procedures for compound collections from academic communities, and these will be presented in this talk.

Immobilized Metal Affinity Chromatography as a Potential Drug Discovery Platform

Lukas Roth (University of Sydney)

In 1990, approximately 80% of medicines approved in the U.S. were either natural products or their derivatives(1). In the early 2000s there was a significant drop in the number of natural products in clinical studies, coinciding with the expansion of high throughput screening (HTS) techniques(1). However, the limited structural diversity inherent to HTS and the emerging threat of antimicrobial resistance has reinvigorated the focus on exploiting natural products for the discovery and development of new medicines(2). Immobilized metal affinity chromatography (IMAC), a technique originally designed for the isolation of histidine-tagged proteins, has shown promise in isolating and purifying bioactive compounds(3). IMAC relies on the fundamentals of coordination chemistry to reversibly retain compounds with known metal ion affinity.

Although originally developed for recombinant protein purification, this simple method has been shown to readily purify hydroxamic acid siderophores, such as desferrioxamine B (DFOB) and other clinical agents, from bacterial cultures(3, 4). Most IMAC work, in the context of siderophore isolation and purification, has utilized Ni(II) as the metal ion, but there is potential in substituting Ni(II) with other metal ions, such as Cu(II), Fe(III), Ga(III) and Zn(II)(4). The modified IMAC resin beds may consequently act as metalloenzyme surrogates and select for different metabolites as directed by distinct coordination chemistries. This could open up a new platform to discover metalloenzyme inhibitor drug candidates as the isolated metabolites, by virtue of their metal binding affinity, may demonstrate activity against various metalloenzymes. As an initial proof of concept, we have exposed a mixture of in use metalloenzyme inhibitors to IMAC columns charged with various biologically relevant metals with promising results(5). The IMAC ligand-metal complex is a reasonable surrogate of the active site of a metalloenzyme and the method is capable of reversibly binding a variety of antihypertensive, anti-inflammatory and anticancer drugs.

Combinatorial discovery of synthetic biohybrid ligands for RNA-hairpins and for the SARS-CoV-2-spike protein

Sebastian Pomplun (Massachusetts Institute of Technology)

The de novo discovery of ligands for challenging and novel drug targets often requires the cumbersome screening of individual compounds from large libraries. Here we present a fully chemistry based affinity selection - mass spectrometry (AS-MS) platform: within days synthetic polyamide compound libraries with > 100 million members can be produced, screened against targets of interest and originate hits with nanomolar affinity for their targets. We use AS-MS for the rapid discovery of synthetic high-affinity peptide binders for the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. The peptides display excellent selectivity for RBD over human serum proteins and can detect picomolar RBD concentrations in a biological matrix. We further expanded the AS-MS platform for the discovery of compounds targeting oncogenic pre-miRNA hairpins.

In nature nucleic acids are often controlled by large supramolecular protein/oligonucleotide complexes as in the case of ribosomal protein synthesis. Rather than forming large complexes to coordinate the role of different biopolymers, we dovetail protein amino acids and nucleobases into a single low molecular weight precision polyamide polymer. We established efficient chemical synthesis and de novo sequencing procedures and prepared combinatorial libraries with up to 100 million biohybrid molecules. This biohybrid material has a higher bulk affinity to oligonucleotides than peptides composed exclusively of canonical amino acids. Using affinity selection mass spectrometry, we discovered variants with a high-affinity for pre-microRNA hairpins. Our platform points toward the development of high throughput discovery of sequence defined polymers with designer properties, such as oligonucleotide binding.

Label-free functional proteomics links the anti-angiogenic properties of the pyrazolyl-urea GeGe-3 to Calreticulin binding

Elva Morretta (University of Salerno)

In the last twenty years, 5-pyrazolyl-ureas have been largely investigated for their poly-pharmacological potential. In this scenario, ethyl 1-(2-hydroxypentyl)-5-(3-(3-(trifluoromethyl) phenyl)ureido)-1H-pyrazole-4-carboxylate (GeGe-3) emerged as a promising anti-angiogenic compound, inhibiting Human Umbilical Vein Endothelial Cells (HUVEC cells) proliferation and endothelial tube formation, impairing inter-segmental angiogenesis during zebrafish embryos development and blocking tumour growth in transplanted subcutaneous Lewis Lung Carcinomas [1]. Regrettably, although different primary targets implicated in cell division and/or calcium homeostasis have been hypothesized for this compound, all the binding tests gave negative results. Thus, to link GeGe-3 anti-angiogenic potential to a suitable protein partner, the molecule interactome has been deeply investigated in HUVEC cells through label-free functional proteomics approaches, namely Drug Affinity Responsive Target Stability (DARTS) [2] and targeted Limited Proteolysis coupled to Multiple Reaction Monitoring Mass Spectrometry (t-LiP-MRM) [3].

These approaches share the principle that, interacting with a molecule, a protein undergoes conformational changes that result in its lower sensitivity to limited proteolysis, when performed in native conditions. Thus, in a first step, the coupling of DARTS with high resolution mass spectrometry allowed the identification of GeGe-3 most reliable interacting protein, Calreticulin, as later on validated by Western Blotting. Subsequently t-LiP-MRM, which allows to discover the target protein structural alterations due to complex formation with GeGe-3, served the purpose of pinpointing Calreticulin regions directly or distally involved in the interaction with the compound. T-LiP-MRM obtained results were corroborated by molecular docking analyses. Calreticulin is a major Ca2+ binding protein involved in intracellular Ca2+ homeostasis, cells adhesion, migration, proliferation, differentiation and apoptosis, as well as in cell-cell interactions [4, 5]. To shed light on the biological consequences of GeGe-3 interaction with such an interesting protein partner, in cell assays were performed. The obtained results disclosed GeGe-3 potential mechanism of action as anti-angiogenic factor: due to its binding to calreticulin, the molecule is able to alter Ca2+ intracellular shift in HUVEC cells, consequently modifying their cytoskeletal proteins organization.

Development of small molecule inhibitors of the endocytic cytoskeleton

Evgeny Kulesskiy (University of Helsinki)

Clathrin-mediated endocytosis (CME) is the primary cellular route of cell surface receptors uptake, which in turn regulate the strength and specificity of downstream signalling. Changes in CME have been linked to increasing of cancer cell survival, proliferation and migration. FCHSD2 protein is a key activator of actin polymerization during CME. Importantly, it has been linked to chemotheraphy resistance and other diseases such as diabetes. FCHSD2 is recruited to clathrin-coated pits (CCPs) by intersectin via an SH3-SH3 interaction. Such interaction might be an interesting therapeutic target for inhibition of cancer cell metastasis and chemoresistance. Here, we are presenting two biochemical assays for a compound screening for a molecule that can break the interaction between FCHSD2 and Intersectin.

The first assay is based on a split Nanoluc-luciferase where the second SH3 domain of FCHSD2 (F2S2) and the fourth SH3 domain of Intersectin (ITSd) were fused to Large and Small Nanoluc fragments respectively. After initial screens of Specs Consortium collection (30 000 compounds) and MicroSource Spectrum library (2 000 compounds), we found 148 hits which showed significant inhibition of luminescent signal. To exclude compounds that directly affect on Nanoluc luminescence a second screen was performed using the split-FAST tag fluorescent labelling system. This confirmatory screen yield 9 compounds that will be followed up for their specificity, mechanism of inhibition and cellular effects.

The synthesis and activity of analogues of the HDAC inhibitor panobinostat with added hydrogen bonding capacity

Callum Rosser (University of Sydney)

Histone deacetylase (HDAC) enzymes are crucial structural modulators of chromatin, which affect differentiation, cell proliferation and homeostasis of eukaryotic cells [1]. Overexpression of HDACs plays a role in cancer, neurological diseases, infection, and inflammation [1]. Currently, HDAC inhibitors have been approved for use in non-solid cancers, with benefit coupled with serious adverse effects. The low specificity of inhibitors to the HDAC isoforms and other Zn(II) containing metalloproteinases is proposed to cause these adverse effects [2]. This has prompted a search for optimised inhibitors to reduce these side effects. Molecular modelling studies have identified acidic amino acid residues at the surface of HDAC2 capable of forming hydrogen bonds to the cap group region of an HDAC inhibitor [3].

This project aimed to develop a library of cinnamyl-hydroxamate compounds that incorporate a hydrogen bonding group (carboxamide) to probe these acidic amino acid residues. The naturally occurring amino acid tryptophan, allowed easy addition of the carboxamide group into two regions of the inhibitor using two different synthetic routes. The carboxamide group was incorporated in alternative stereochemical configurations, via the use of L- or D-tryptophan, to explore enantioselective effects. Four cinnamyl-hydroxamate HDAC inhibitors were synthesised and screened for HDAC inhibitory activity in HeLa nuclear extract. Compounds with the carboxamide group in the linker region (5 (225 nM, S), 6 (240 nM, R)) were less potent than approved HDAC inhibitor panobinostat. Compounds with the carboxamide on the cap group (7 (264 nM, S), 8 (1564 nM, R)) possessed similar inhibitory activity to compounds 5 and 6 in the S configuration but reduced inhibitory activity in the R configuration. This project has provided a rationale for the design of new HDAC inhibitors that probe interactions with the acidic amino acid residues at the surface of the HDAC binding pocket.

Understanding the mechanisms governing the interaction of drugs with mucus using a novel biosimilar mucus model

Cosmin Butnarasu (University of Turin)

A constitutive mucus layer covers all the wet epithelial tissues ensuring lubrication and protection against external threats. Mucus can represent a strong barrier to tackle even for oral or pulmonary administered drugs (Figure 1). Despite the critical role played on drug absorption, very little is known about the molecular properties that mediate the interaction of drugs with mucus (Butnarasu, 2019). Moreover, due to its high biological complexity and heterogeneity, it is difficult to recreate a robust and reproducible in vitro model suitable for high throughput screening purposes. We have developed a biosimilar mucus model that mimics a pathological mucus (Pacheco, 2019). A natural polysaccharide was used to reproduce the viscoelastic behaviour while the composition was mimicked by adding mucin which is the main glycoprotein forming mucus.

An in vitro mucosal surface was recreated by coupling the mucus model to 96-well permeable supports pre-coated with structured layers of phospholipids (PAMPA). Eventually, the permeability of a library of commercially available drugs was investigated in the absence and presence of the mucus model loaded on PAMPA plates. The mucus model not only represented a physical barrier, but it really behaved as an interactive filter. Different molecular structures were differently retained by mucus. The diffusion of the majority of the tested compounds was reduced; for some of them, the effect was less pronounced while for a few the diffusion was even enhanced. Multivariate statistical analysis was used to decipher the molecular descriptors that play a pivotal role in drug retention on mucus. Since drug development is characterized by a high rate of failure, the mucus platform could help to reduce at an early drug discovery stage the number of poor performers that reach preclinical trials. Moreover, the model is completely tunable as other mucus components (lipids, DNA, proteins) could be included during the production phase.

Session 5

Fighting resistant pathogens; new antiviral therapies

Assault, Siege or Trojan Horse Strategy: Use of Natural Products to Fight Bacterial Infections

Mark Brönstrup (Helmholtz Centre for Infectious Research)

Multidrug resistant bacterial pathogens have become a major health concern. Especially infections by gram-negative bacteria are challenging, since their complex cell membrane architecture strongly impedes the uptake of drugs. Because microbial natural products continue to be the prime source to tackle these issues, we have investigated natural products as the basis for novel antibiotic.

A broad spectrum of gram-positive and gram-negative pathogens is addressed by cystobactamids, oligo-arylamids originally isolated from Cystobacter sp.. Our efforts to optimize the antibiotic properties of the cystobactamids by medicinal chemistry will be presented.

Beyond a classic 'assault' of bacteria with such antibiotics, the conjugation of natural products to targeting functions has been beneficial to improve their drug properties. In the so-called Trojan Horse Strategy, antibiotics are conjugated to siderophores to hijack the bacterial siderophore transport system, and thereby enhance the intracellular accumulation of drugs. We synthesized novel artificial siderophores, characterized their transport and resistance mechanisms, and their efficacy when coupled to antibiotic natural products. Finally, we present a novel approach for the selective bacterial targeting and infection-triggered release of antibiotic conjugates in the alternative siege concept, using the lipopeptide colistin as the antibiotic effector.

Synthetic Heparan Sulfate Mimetics Potently Inhibit SARS-CoV-2 by Disrupting the Spike-ACE2 Interaction

Vito Ferro (University of Queensland)

The cell surface polysaccharide heparan sulfate (HS) has recently been identified as a co-receptor with the ACE2 protein for recognition of the S1 spike protein on SARS-CoV2 virus, revealing an attractive new target for therapeutic intervention. Here we show that the HS mimetic drug candidate pixatimod binds directly to the SARS-CoV-2 spike protein receptor binding domain (S1-RBD), altering its conformation and destabilizing its structure. Molecular modelling identified a binding site overlapping with the ACE2 receptor site. Consistent with this, pixatimod inhibits binding of S1-RBD to ACE2-expressing cells and displays a direct mechanism of action by inhibiting binding of S1-RBD to human ACE2. Assays with four different clinical isolates of live SARS-CoV-2 virus show that pixatimod potently inhibits infection of Vero cells at doses well within its safe therapeutic dose range. In the transgenic hACE2 mouse model, pixatimod-treated animals showed a significant reduction in viral titers in nasal turbinates and in brain.

This demonstration of potent anti-SARS-CoV-2 activity establishes that synthetic HS mimetics can target the HS-Spike protein-ACE2 axis. Together with other known activities of pixatimod our data provides a strong rationale for its further investigation as a potential multimodal therapeutic to address the COVID-19 pandemic.

Interfering with bacterial survival strategies

Sara Sattin (University of Milano)

Persistence is a bacterial bet hedging strategy that allows for temporary tolerance to antibiotic treatment. This phenotypic switch paves the way to the chronicity of certain infections and to the insurgence of genetic resistance. Here we present our work on targeting bacterial persisters via inhibition of the upstream of the stringent response, one of the working hypothesis for their formation. Our multidisciplinary approach comprises *in silico* studies on Rel proteins, synthesis of compounds designed *ad hoc* and evaluation of their biological activity.

Detecting pathogens and Reactive Oxygen Species using Chemical Biology

Boris Vauzeilles (Paris-Sud University)

The application of synthetic tools to explore biological processes, as well as the use of biological systems as a source of inspiration for the design of molecular devices, are the main focus of our research.

This lecture will present a selection of some of our work in this area, which includes the development of a new labeling strategy for the detection and identification of living bacteria, including Legionella pneumophila, a serious pathogen responsible for Legionnaires' disease, as well as the design and evaluation of new, fast-reactive hydrogen peroxide-sensitive triggers for the elaboration of probes for the detection of reactive oxygen species.

Discovery of antibacterial agents inhibiting the energy-coupling factor (ECF) transporters by structure-based virtual screening

Anna Hirsch (Helmholtz Institute for Pharmaceutical Research Saarland)

The emergence of antimicrobial resistance against important pathogens poses an ever-growing health threat. Hence, the pipeline of novel drug candidates should be filled with molecules featuring an unprecedented mode of action and a novel chemical structure. We tackle both challenges by targeting the Energy-coupling factor (ECF) transporter, an unexplored antibacterial target, mainly present in Gram-positive species. This family of transmembrane proteins is involved in the uptake of vitamins in a wide range of pathogenic bacteria (e.g., Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecium). [1,2] Because of their central role in the metabolism of bacteria and their absence in humans, ECF transporters are novel attractive antimicrobial targets. Here, we report on the structure-based virtual screening (SBVS), design, synthesis and structure–activity relationships (SARs) of the first class of selective, antibacterial agents against the energy-coupling factor (ECF) transporters.

Having identified a druggable pocket in the crystal structure of the L. delbrueckii ECF transporter, [3] which should play a key role in the unique mechanism of transport, our SBVS of the zinc library afforded a fragment-like hit with good in vitro and cell-based activity, a good in vitro ADMET profile and excellent oral bioavailability. [4] We adopted two distinct approaches, namely the design and synthesis of several derivatives according to a classical SAR approach and the screening of a focussed library of structurally related derivatives of our hit. Having established a new cell-based uptake assay in Lactobacillus casei, we identified a low-micromolar inhibitor of the ECF transporters with a broad spectrum of activity (MIC values in the single-digit micromolar range) and a lack of resistance development. [5]

Session 6

Natural compound chemistry and biology

Defining host-microbe interactions in a basal metazoan: the sponge

Laura Steindler (University of Haifa)

Laura's abstract will be provided soon.

Arctic marine biodiversity as a source for novel compounds

Jeanette H. Andersen (University of Tromsoe)

The success of natural products in drug discovery is unparalleled, in particular for anticancer therapeutics, where nearly half of the currently marketed drugs are derived from natural products. The marine environment comprises the majority of the global biodiversity. The wide-ranging genetic variation has engendered unique biosynthetic pathways that produce structurally diverse small organic molecules that extend the chemical space. In 1969, the first marine-derived anti-cancer drug, cytarabine (Cytosar-U®), was approved. This has since been followed by eight additional marketed marine derived anticancer drugs, including Lurbinectedin (Zepzelca® 2020). Currently, several other marine natural products are under clinical evaluation. As the marine environment and its organisms have become more accessible over the last decades, it is expected that the ocean will be the next great source of novel chemistry.

Our research group, Marbio, UiT explore Arctic and sub-Arctic marine organisms, searching for compounds with activities against cancer, bacteria and diabetes as well as compounds with immunomodulatory and antioxidative effects. We are screening a unique collection of cold-water invertebrates and marine microorganisms, and we have identified several novel bioactive molecules. The bioactivity testing and chemical investigation of extracts of the Arctic, marine hydrozoan Thuiaria breitfussi led to the isolation of the natural products, breitfussin A – H. Breitfussin C and D were found to selectively inhibit the survival of several cancer cell lines, with IC50 values as low as 340 nM. The results demonstrates that the Arctic marine biodiversity can provide novel chemistry. Marine natural products can provide a starting point for optimization of compounds for preclinical and clinical drug development.

Incorporation and modification of fatty acids in cyanobacterial secondary metabolism

Pedro Leao (University of Porto)

Cyanobacteria are a phylum of photosynthetic bacteria from which over 1000 secondary metabolites have been reported. Many more await discovery, as revealed by genome-wide analysis of the phylum. Adding to this, a large number of cyanobacterial natural products have potent biological activities and some are currently used in the clinic or are undergoing clinical trials. Hence, there are clear opportunities and motivation for natural product discovery in these organisms. Here, we will focus on our recent efforts towards the discovery of new cyanobacterial metabolites bearing fatty acid moieties. We will provide examples of how fatty acids are incorporated and modified by cyanobacteria in novel ways that generate highly unusual natural product scaffolds.

Morphological Profiling of Small Molecules for Mode-of-Action Prediction

Slava Ziegler (Max Planck Institute of Molecular Physiology)

Profiling approaches monitor up to hundreds of parameters and are used to explore bioactivity of small molecules in an unbiased manner. The cell painting assay (CPA) is a morphology-based profiling that employs high-content imaging and analysis of six stained cellular components and compartments to extract hundreds of morphological features.

Morphological fingerprints are used to assess bioactivity and are compared with fingerprints of annotated compounds with known target or activity. Profile similarity allows the generation of a target or mode-of-action hypothesis early on in the compound development process. We employed the cell painting assay to assess the bioactivity of our in-house compound collection. Detected activity can be mapped in the bioactivity cluster space and can be used to uncover unanticipated activity for reference compounds or to assign a mode of action to thus far unexplored small molecules .

Target-based screening strategies for the identification of novel natural products as protein-protein interaction inhibitors

Francisco Castillo (MEDINA Foundation)

Natural products of microbial origin have already demonstrated their therapeutic potential as anti-infective and anti-tumor treatments in phenotypic models. Most of the target-based screening efforts in this field, however, have explored a limited chemical space comprising synthetic small-molecule collections and repositioning libraries. One way to fill this gap is to refine protein-based methodologies for the efficient detection of natural product inhibitors. In the first example we report here, we demonstrate how the combination of computational and biochemical tools can lead to the identification of anti-COVID molecules. More specifically, we report the singular case of dual anti-COVID compounds, with the ability of blocking two different, structurally unrelated, molecular targets such as viral protease (Mpro) and Spike (S).

For the second example, we focus on the PD-1/PD-L1 protein-protein interaction, with implications on the development of precision immunotherapeutic agents. So far, small-molecule leads identified from synthetic libraries are characterized by a common molecular mechanism of action, their binding affinity for PD-L1. Despite such specificity, these PD-L1 binders have not succeeded beyond clinical phase 1, highlighting the need for novel screening strategies leading to alternative ways of blocking the interaction of interest. However, gold standard PD-1/PD-L1 protein-protein interaction methods only yield at optimal resolution when testing pure compounds and homogeneous samples. The work presented here shows how to improve the performance of such protein-protein interaction methods on activity-guided fractionations for heterogeneous natural product extracts of microbial origin. Such improvements are expected to maximize the chemical space scanned per assay point and to converge towards novel PD-1/PD-L1 inhibitors.

Metabolomics-Assisted Discovery of Bioactive Marine Natural Products

Deniz Tasdemir (GEOMAR Centre for Marine Biotechnology)

Marine natural product (MNP) discovery has had a short but very successful history, providing 14 MNP-derived drugs in the clinics, particularly against cancer. Many challenges, including the reisolation of known compounds hamper MNP discovery. Classical LC-MS dereplication approaches with low identification success partly addresses the rediscovery issue. Automated metabolomics workflows, e.g., MS/MS fragment similarity-based molecular networking (MN) and in silico machine-learning methodologies revolutionized metabolomics approaches, significantly enhancing NP annotation rates. In our search for anticancer metabolites, we applied a UPLC-MS/MS-based MN dereplication strategy to crude extract of an Antarctic deep-sea sponge, Latrunculia biformis. It that showed, for the first time, the presence of tsitsikammamine type bispyrroloiminoquinone alkaloids in this sponge genus. The combination of MN and anticancer activity-guided isolation scheme led to the targeted isolation of tsitsikammamine A and its new 16,17-dehydro analogue (1). The same strategy permitted isolation of a number of oligomeric discorhabdins from another L. biformis specimen, including the first trimeric discorhabdin alkaloid bearing a novel C–N bridge (C-1/N-13) between discorhabdin monomers (2,3).

The purified compounds exhibited significant anticancer activity against human colon cancer cells and/or showed affinities to established cancer targets, e.g., topoisomerase I-II and indoleamine 2,3-dioxygenase enzymes by molecular modeling and docking studies. Molecular networking also allows incorporation of additional information, such as biological activity. In another project, we mapped the in vitro anticancer activity and toxicity of marine microbial extracts onto their networks. The application of the so-called bioactivity-based MN (BBMN) workflow on the C18-SPE fractions obtained from the CHCl3 subextract of a marine fungus enabled us to predict the bioactivity scores of metabolites in the fractions and aided mass targeted rapid isolation of polyketides with anticancer activity. Modern metabolomics approaches are versatile assisting the prioritization, rapid and targeted purification of novel and bioactive metabolites, thereby accelerating drug discovery efforts on marine invertebrates or microorganisms.

Bacterial mannan polysaccharide: chemical structure and conformational studies

Angela Casillo (University of Naples Federico II)

Many microorganisms are known to produce extracellular polysaccharide. Bacterial EPSs usually occur as capsule and/or medium released polysaccharides.[1] EPSs are involved in several biological functions, such as bacteria adhesion to surface and biofilm formation, ion sequestering, and protection from desiccation. Furthermore, the enhanced production of a high-molecular-weight polyanionic EPS at sub-optimal incubation temperatures lends support to theories that EPS may serve as a cryoprotectant for microorganisms as well as their enzymes. The cryoprotectant role of EPS was established in the psychrophilic bacterium Colwellia psychrerythraea 34H grown at low temperatures).[2-4] The EPS from the psychrotolerant bacterium Pseudoalteromonas could enhance the stability of the cold-adapted protease secreted by the same strain by preventing its autolysis, avoiding enzyme diffusion, and helping the strain in enriching the proteinaceous particles and trace metals in the deep-sea environment.[5]

The chemical characterization of these polymers is the starting point for obtaining relationships between their structures and their various functions. Here, the chemical structure and conformational studies of a mannan exopolysaccharide from the bacterium Psychrobacter arcticus strain 273-4 isolated from permafrost is presented. The mannan from the cold-adapted bacterium was compared with its dephosphorylated derivative and the commercial product from Saccharomyces cerevisiae. Starting from the chemical structure a new approach through various physicochemical techniques to deepen the study of the structure/activity relationship was explored. Finally, the ice recrystallization inhibition activity of the polysaccharides is reported.

Evening session

Covid19 research

Compound repurposing by target based and phenotypic approaches to identify in-vitro inhibitors of SARS-CoV2 viral entry and replication

Philip Gribbon (Fraunhofer ITMP Screening Port Hamburg)

Compound repurposing is an important strategy to aid the identification of effective treatment options against SARS-CoV-2 infection and COVID-19 disease. The presentation will cover the results of several repurposing programs based on phenotypic and target based screens using a large scale library of bioactive compounds. Target studies will focus on SARS-CoV-2 main protease (3CL-Pro), also termed M-Pro, which is an attractive drug target as it plays a central role in viral replication by processing the viral polyproteins pp1a and pp1ab at multiple distinct cleavage sites. We have confirmed previously reported inhibitors of 3CL-Pro and have identified 62 additional compounds with IC50 values below 1 µM and profiled their selectivity toward chymotrypsin and 3CL-Pro from the Middle East respiratory syndrome virus (see https://doi.org/10.1021/acsptsci.0c00216 and https://doi.org/10.1021/acsptsci.0c00215). In phenotypic studies, compounds were screened by microscopy for their ability to inhibit SARS-CoV-2 cytopathicity in the human epithelial colorectal adenocarcinoma cell line, Caco-2 (see https://doi.org/10.1038/s41597-021-00848-4). These studies have been complemented by extensive structural investigations to reveal the binding characteristics of the compounds (see https://www.biorxiv.org/content/10.1101/2020.11.12.378422v1).

Acriflavine, a clinically aproved drug, inhibits SARS-CoV-2 and other betacoronaviruses

Kamyar Hadian (Helmholtz Zentrum München)

The COVID-19 pandemic caused by SARS-CoV-2 has been socially and economically devastating. Despite an unprecedented research effort and availability of vaccines, effective therapeutics are still missing to limit severe disease and mortality. Using high-throughput screening, we identified acriflavine as a potent papain-like protease (PLpro) inhibitor.

NMR titrations and a co-crystal structure confirm that acriflavine blocks the PLpro catalytic pocket in an unexpected binding mode. We show that the drug inhibits viral replication at nanomolar concentration in cellular models, as well as in vivo in mice and ex vivo in human airway epithelia, with broad range activity against SARS-CoV-2 and other betacoronaviruses. Considering that acriflavine is an inexpensive drug approved in some countries, it may be immediately tested in clinical trials and play an important role during the current pandemic and future outbreaks.

Enhanced formulations of Hydroxychloroquine as Organic Salts and Ionic Liquids to fight COVID-19

Miguel Santos (NOVA University Lisbon)

Since the beginning of the COVID-19 pandemic, SARS-CoV-2 has infected more than 134 million people worldwide, from which 2.9 million have died. Multiple advances in the pharmacological treatment of severe cases have been made over the last year such as dexamethasone to control inflammatory response and repurposed drugs such as hydroxychloroquine (HCQ) and remdesivir as antivirals. However, HCQ, and despite showing efficacy against SARS-CoV-2 [1] by putatively increasing lysosomal pH, it is no longer recommended for treatment of severe COVID-19 due to its toxicity at the required therapeutic doses. Nonetheless, it has shown to be an effective alternative in avoiding complications in milder cases, especially in a combinatorial regime [2]. Still there is a need for the development of more effective antiviral drugs against SARS-CoV-2, but a very long journey is expected in the search of novel drugs. A promising alternative is the enhancement of current drugs by associating them with chemical adjuvants. For more than a decade, the combination of Active Pharmaceutical Ingredients (APIs) with such adjuvants as Organic Salts and Ionic Liquids (OSILs) has risen in the academia, and has recently reached Pharma, as an alternative to improve the properties of current drugs, in particular bioavailability, chemical and thermal stability, safety and therapeutic efficiency.

In our lab, several antibiotics (β-lactam [3], fluoroquinolones [4]) and bone anti-resorbing agents (bisphosphonates [5]), among others, have been successfully combined as anions and/or cations with biocompatible organic counter-ions, with very interesting chemical and biological improvements being observed. Most recently, we set out to explore the benefits of the OSILs approach with hydroxychloroquine (HCQ-OSILs) as effective antivirals against SARS-CoV-2. Hence, in this communication we present the synthesis and characterization of fourteen novel mono- and dicationic HCQ-OSILs, and also a comparison of their water solubility and octanol-water partition coefficients with HCQ sulfate. Moreover, in vitro cytotoxicity data on Vero E6 cells and antiviral activity profile against the SARS-CoV-2 virus of the prepared HCQ-OSILs will be rationalized and discussed.

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Nanomechanics of nanobodies-protein S SARS-CoV-2 virus complexes revealed by a Virtual Atomic Force Microscope

Wieslaw Nowak (Nicolaus Copernicus University)

World population is to a large extent saved by development of antibodies against pathogens such as SARS-CoV-2. Despite natural antibodies induced by a viral infection or vaccination, application of nanobodies is extremely promising technology in fight with the current pandemic [1]. Nanobodies are single-domain antibodies that can be effectively produced in the lab. The main step in the research towards new tools against SARS-associated viruses is effective screening (or de novo design) of nanobodies [2].

Here we propose a computational strategy called Virtual Atomic Force Microscopy to monitor the strength of a nanobody interaction with protein S from the SARS-CoV-2 virus. Starting from recently published cryo-EM structures of Nb6 nanobody bound to closed and open SpikeS2P protein [2], we use Steered Molecular Dynamics computational modeling method to assess unbinding forces and to determine molecular mechanisms of binding-unbinding processes. Our approach allows for efficient ranking of synthetic nanobodies affinities to protein S. The proposed computational pipeline may be particularly useful for initial checking of already developed nanobodies against new variants of SARS viruses. Acknowledgement: This project is funded by IDUB N. Copernicus ANTICO and #MEMOBIT grants. ICNT UMK computer facilities are acknowledged

Session 7

Protein aggregation and self-assembly in disease

Atomic Resolution Map of the Soluble Amyloid Beta Assembly Toxic Surfaces

Giuseppe Melacini (McMaster University)

Soluble amyloid beta assemblies (Aβn) are neurotoxic and play a central role in the early phases of the pathogenesis cascade leading to Alzheimer's disease. However, the current knowledge about the molecular determinants of Aβn toxicity is at best scant. Here, we comparatively analyze Aβn prepared in the absence or presence of a catechin library that modulates cellular toxicity. By combining solution NMR with dynamic light scattering, fluorescence spectroscopy, electron microscopy, wide-angle X-ray diffraction and cell viability assays, we identify a cluster of unique molecular signatures that distinguish toxic vs. nontoxic Aβ assemblies. These include the exposure of a hydrophobic surface spanning residues 17-28 and the concurrent shielding of a highly charged N-terminus. We show that the combination of these two dichotomous structural transitions promotes the colocalization and insertion of β-sheet rich Aβn into the membrane, compromising membrane integrity. These previously elusive toxic surfaces mapped here provide an unprecedented foundation to establish structure-toxicity relationships of Aβ assemblies.

Finding new drugs for conformational diseases

Salvador Ventura (Autonomous University of Barcelona – UAB)

Conformational diseases are pathologies with a great social, economic and personal impact on our society. Some are well-known because they affect a large number of people, like Parkinson's disease, while others are rare disorders, such as familiar amyloidosis. All these diseases remain incurable. They exhibit very different symptoms and each of these disorders is associated with a different protein. However, the problem that these proteins experiment is always the same: at a given time they become insoluble and toxic and they impact the normal physiology of the tissue or organ in which they reside, independently if this is the heart or the brain. In this presentation I will explain the efforts of our research group to find new drugs that can slow down the progress of these devastating diseases.

Enlisting microfluidics to screen for inhibitors of toxic protein aggregates in Parkinson's Disease and other disruptors of protein-protein interactions

Daniel Otzen (Aarhus University)

Soluble oligomers of the protein alpha-synuclein are believed to be a major cytotoxic agent in the development of Parkinson's Disease, yet their very dynamic structure makes them difficult drug targets. I will describe our efforts to develop compounds targeting these oligomers by screening for the ability to prevent oligomer interactions with other biological species such as membranes and antibodies. Our approach is to use flow-induced dispersion analysis (FIDA) which allows us to monitor apparent oligomer size, a parameter which changes if the oligomer complexes with other large components. In parallel I will also describe our use of FIDA to identify small molecules to block binding of the SARS-CoV-2 spike protein to the human receptor ACE2.

Impact of ubiquitination on the aggregation of Tau protein, a key player in Alzheimer's disease

Francesca Munari (University of Verona)

The microtubule associated protein Tau is a cytosolic protein, mainly found in neurons axon, that promotes the assembly and stability of microtubules (MT), and contributes to the regulation of axonal stability and transport, neurite outgrowth and synaptic function (1). Tau is considered a key player in the neurodegenerative process underlying Alzheimer's disease (AD) and other tauopathies due to its capability to convert into toxic amyloidogenic species and self-assemble into straight and paired helical filaments (PHFs) that are found in the neurofibrillary tangles (NFTs), the defining pathological hallmark of AD (1). Since Tau extracted from PHFs was consistently found modified with specific post-translational modifications (PTMs) (1-3), the understanding of their role in Tau aggregation and altered activity may help to identify key mechanisms of Tau-mediated toxicity.

Tau from AD-PHFs was found ubiquitinated at several lysine residues within the microtubule binding domain (MBD) that is also the part of the protein that form the core of the fibrils (2,3). Ubiquitination would normally regulate Tau clearance by autophagic and proteasomal pathways. However, in pathological condition, ubiquitinated Tau aggregates are not efficiently removed and accumulate within neuronal cells with potential toxic effects. In our work (4,5) we have ubiquitinated the MBD of Tau protein by a semisynthetic approach, based on the chemical conjugation of proteins precursors, that allowed us to obtain homogenous ubiquitination of Tau at unique and precise sites. Specifically, we produced three ubiquitinated tau isomers with linkages in positions 254, 311 and 353 as representatives of the ubiquitinated Tau species found in AD-PHFs. By biophysical studies we have elucidated the impact of mono- and poly-ubiquitination on the mechanism of aggregation of Tau protein, revealing that the different conjugates exhibit diverse capability to form filaments and that the effect of the modification is site-dependent. Interestingly, ubiquitination at residue 311 had the strongest effect in interfering with the Tau conformational transitions that progress towards amyloid formation.

In silico modeling of small molecules as α-synuclein aggregation inhibitors

Serena Vittorio (University of Messina)

The search for a cure of Parkinson's disease (PD) represents a challenging task in the pharmaceutical research field. To date, the available therapies are addressed to restore dopamine levels thus reducing the motor symptoms related to PD, such as rest tremor, bradykinesia and muscular rigidity. Recently, the inhibition of α-synuclein (α-syn) aggregation has emerged as promising strategy to slow or halt the neurodegenerative process. The α-syn is a 140 aa presynaptic protein implicated in the regulation of neurotransmitter release from the synaptic vesicles. In PD α-syn aggregates into toxic oligomers and fibrils forming Lewi bodies that represent the hallmark of this neurological disorder (1). In order to identify new small molecules as α-syn aggregation inhibitors, we generated a ligand-based pharmacophore model to be used as filter to virtually screen two distinct chemical libraries: i) our in-house database CHIME 2.0 and ii) the MyriaScreen Diversity Library II. By means of this virtual screening we selected small molecules that were tested in vitro thus leading to the identification of the 3-(cinnamylsulfanyl)-5-(4-pyridinyl)-1,2,4-triazol-4-amine as promising hit compound for the development of new α-syn aggregation inhibitors. Therefore, few structural modifications were carried out thus obtaining a new series of small molecules that were synthesized and tested in order to investigate the biological profile. Finally, the binding mode of these new inhibitors was elucidated by molecular docking studies (2).

Session 8

Glycochemistry & -biology

Carbohydrate receptor ligands enable targeted delivery to immune cells

Christoph Rademacher (University of Vienna)

Important aspects of life such as self/non-self-differentiation, cell adhesion and migration are mediated by mammalian receptors recognizing carbohydrate structures. In particular, these receptors expressed by cells of the innate immune system have open new applications in immune cell modulation as novel adjuvants or for cell specific targeting because of their restricted expression pattern. However, chemical probes that specifically address these receptors are sparse and carbohydrates as their natural ligands only offer limited affinity and specificity. Hence, we have utilized fragment-based ligand as well as rational design approaches to identify small molecules capable of binding to carbohydrate receptors with sufficient affinity and specificity to modulate the biological function of lectins.

The challenges we are faced with originate from the inherent feature of carbohydrate recognition sites being rather flat and featureless. Moreover, these sites are often solvent exposed and highly hydrophilic, thus being less accessible for drug-like molecules. Fragment screening using several orthogonal methods such as NMR, SPR, and flow cytometry led to distinct hits followed by structure-activity relationship series. Hits were evolved into micromolar binders for targets from the mammalian as well as bacterial lectins. New compound classes were discovered to excellently suited for addressing Ca2+ coordinating lectins. Lessons learned about lectin structure and dynamics, as well as the development of chemical probes and their immune cell modulation will be covered in this presentation.

Of sugars and phosphates: synthesis and application of well-defined bacterial oligosaccharide structures

Jeroen Codee (Leiden University)

We are in a constant battle with bacteria infecting us. Bacteria protect themselves with a cell wall, decorated with various -often heterogeneous- glycan structures. To study the role of these glycans in the interaction with the host immune system, well-defined, homogeneous structures are required. Since these cannot be obtained in sufficient quantity and quality from bacterial sources, we have developed various routes of synthesis to generate bacterial glycans. Here we will describe how automated synthesis techniques can be used to generate bacterial phosphate-based glycans. Libraries of synthetic teichoic acids have been used in vaccination studies against the multi-drug resistant hospital bugs E. faecalis and S. aureus and to map antibody binding interactions at the molecular level. Stabilized analogues of N. meningitidis have been developed to generate shelf stable vaccines against this bacterium: the first carbohydrate vaccine using a glycomimetic antigen.

Using computers to understand how carbohydrates are processed in nature

Carme Rovira (University of Barcelona)

Carbohydrate-active enzymes (CAZymes), such as glycoside hydrolases and glycosyltransferases, constitute the main machinery for the degradation and synthesis of carbohydrates in nature. They have a myriad of industrial and biotechnological applications, ranging from biofuel production to drug design. In recent years, new CAZyme structures have been solved that pose mechanistic questions on how their carbohydrate substrates are processed, such as the identity of the catalytic residues, the role of enzyme conformational transitions and the distortion of the substrate at the transition state of the chemical reaction. Using state-of-art simulation techniques such as ab initio quantum mechanics/molecular mechanics (QM/MM) and metadynamics [1-3] we have contributed to answer these questions, providing an atomistic view of enzyme action that can guide inhibitor design. In this talk I will describe some of the CAZyme mechanisms that we have recently investigated, in a collaborative work with experimental groups [4-6].

Chemoenzymatic synthesis of sugar nucleotide chemical biology tools to explore the GDP-D-mannose dehydrogenase from Pseudomonas aeruginosa

Gavin Miller (School of Chemical and Physical Sciences, Manchester)

The opportunistic human pathogen Pseudomonas aeruginosa (PA) causes chronic bacterial infections in cystic fibrosis patients, contributing to a reduction in lung function and increased mortality rates.[1] The lung environment induces a switch of P. aeruginosa to its mucoid phenotype, which is characterised by an overproduction of the exopolysaccharide alginate. Composed of β-D-mannuronic acid and its C5 epimer α-L-guluronic acid, alginate is a key component in the formation of a bacterial biofilm, which increases persistence of the bacteria in the airways and retards antimicrobial treatments.

Inspection of the PA biosynthetic pathway reveals a key enzyme involved in alginate production, GDP-mannose dehydrogenase (GMD), which catalyses an NAD+-dependent oxidation of GDP-D-Man to GDP-D-ManA: the alginate feedstock monosaccharide.[2] We have designed and synthesised a series of GDP-Man probes to interact with the GMD active site, providing mechanistic insight and identifying the first sugar nucleotide inhibitor of GMD.[3-5]

GalNAc-related sp2-iminosugars as mutant lysosomal β-hexosaminidase A activity enhancers in late-onset Tay-Sachs disease patients' fibroblasts

Maria Isabel Garcia-Moreno (University of Seville)

Dysfuntion of human β-hexosaminidase A (Hex A) results in Tay-Sachs disease (TSD), an autosomal recessive lysosomal storage disorder (LSD) condition associated with phenotypic neurodegeneration, for which no effective treatment options are available. Since many of the TSD-causative mutations do not compromise the catalytic site of Hex A, the development of pharmacological chaperones (PCs) that can stabilize the native folding of the protein despite its anomalous conformation and restore activity appears attractive. Most reported PCs developed for LSDs are competitive inhibitors of the target enzyme; they however exert an effector action by dissociating from the corresponding mature enzyme:inhibitor complex in the presence of an excess of substrate in the lysosomes of patient cells [1].

A main problem is that Hex A inhibitors oftentimes also inhibit the related enzyme O-linked N-acetylglucosaminidase (GlcNAcase; OGA), which represents a serious drawback for translation into the clinics [2]. Based on structural information and the known substrate selectivity profile of HexA and OGA [3], we have addressed this problem by designing sp2-iminosugar glycomimetics [4] closely related to N-acetylgalactosamine (GalNAc). The new candidates feature either a neutral piperidine-derived thiourea or a basic piperidine-thiazolidine bicyclic core and are accessed through a structure diversity-oriented approach. Compounds behaving as selective nanomolar competitive inhibitors of human Hex A at pH 7, with high Hex A/OGA selectivity, and displaying a ten-fold lower inhibitory potency at pH 5 were identified, which should facilitate the dissociation of the Hex A-glycomimetic complex at the lysosome, were the Hex A substrate (namely GM2 ganglioside) accumulates. In agreement with this notion the selected candidates specifically increased the levels of lysosomal Hex A activity in patient fibroblasts having the G269S mutation, the one with the highest prevalence in late-onset Tay-Sachs disease.

Session 9

Origin of Life & synthetic biology

On the unified chemical origins of peptides and nucleic acids

Matthew Powner (University College London)

Living organisms are highly complex chemical systems that exploit a small constellation of universally conserved metabolites. The chemical unity of these metabolites provides compelling evidence that a simple set of predisposed reactions predicated the appearance of life on Earth. Conversely, traditional prebiotic chemistry has produced highly complex mixtures that bear little resemblance to the core metabolites of life. The complexity of prebiotic chemistry until recently had suggested that elucidating life's origins was an insurmountable task, but prebiotic systems chemistry is now providing unprecedented scope to explore the origins of life and an exciting new perspective on a 4 billion-year-old problem.

At the heart of this new systems approach is an understanding that individual classes of metabolites cannot be considered in isolation if the chemical origin of life on Earth is to be successfully elucidated. In this talk several recent advances suggesting the proteinogenic peptides and canonical nucleotides are predisposed chemical structures will be presented.

Coacervates as protocellular models?

Dora Tang (Max Planck Institute of Molecular Cell Biology and Genetics)

In the 1920's Oparin hypothesized that membrane free compartments formed by coacervation would have provided a viable route to compartmentalize prebiotic reactions as a precursor to the modern cell. Studies which support this hypothesis are limited in that the precise chemical composition and conditions on prebiotic earth remain a mystery. Despite this, using bottom-up approaches allows us to generate physically relevant protocell models in the lab. This provides a means to unravel the effect of compartmentalization by coacervation can have provided a selection pressure for facilitating the transition from a chemical world to a biological world.

Here, I will present strategies for the design and synthesis of protocell models based on liquid-liquid phase separation of oppositely charged components (coacervates) and describe how these compartments can provide alternative environments compared to buffer solution to tune reaction kinetics.

Non-enzymatic metabolic reactions and life's origins

Kamila Muchowska (University of Strasbourg)

Life is governed by an intricate network of chemical reactions that make up metabolism. How the biochemistry of life as we know it came to be is studied by prebiotic chemistry. Lots of efforts in this area have focused on life's building blocks, often obtained in multi-step chemical syntheses [1]. However, focusing only on the molecular building blocks, rather than the processes that produce them, may have caused us to overlook what might be a fundamental feature of life. Why does life use the molecules, reactions, pathways, and overall organization that it does? In this talk, I will present how the chemistry that led to life could have begun as a primitive non-enzymatic version of the biochemistry we know today, initially promoted by naturally occurring catalysts, for example geologically abundant iron-rich minerals and salts [2]. If it existed, such a reaction network would have built up and broken down life's chemical building blocks in much the same way as the pathways that do it today. The knowledge of processes and mechanisms that may have led to the emergence of life's core biochemical machinery is of paramount importance not only to the origins studies of life on Earth,[3] but also to the search for life-like systems beyond our planet.

A Modular, Dynamic, DNA-based Platform for Regulating Cargo Distribution and Transport between Lipid Domains

Roger Rubio-Sánchez (University of Cambridge)

Biological membranes feature highly evolved proteo-lipid machinery able to co-localise in lipid rafts, nano-scaled assemblies believed to underpin signal transduction [1], amongst other cellular processes. Bottom-up synthetic biology aims to replicate life-like behaviours in model artificial cells [2], often using synthetic lipid bilayers as passive enclosures that lack the functional complexity associated to their biological analogues. DNA nanotechnology has emerged as a popular choice for biomimicry, coupling bio-inspired nano-devices with model membranes using amphiphilic oligonucleotides [3]. In fact, amphiphilic DNA nanostructures also undergo partitioning in lipid domains [4], evoking the affinity of proteins for raft microenvironments.

Here, we regulate the lateral distribution of DNA nanostructures in phase-separated membranes by exploiting the tendency of cholesterol and tocopherol motifs to respectively enrich liquid-ordered (Lo) and liquid-disordered (Ld) domains. By prescribing combinations of multiple anchors, changes to nanostructure topology, and size, our DNA architectures are programmed to achieve partitioning states that span the energy landscape. In addition, the functionality of our approach is showcased with a responsive biomimetic DNA device that dynamically achieves ligand-induced reconfiguration and mediates cargo transport between lipid domains. Our synergistic platform [5] paves the way for the development of next-generation biomimetic DNA-based architectures, that can achieve sensing and communication in synthetic cellular systems.

Active coacervate droplets: protocells that grow and survive

Karina Nakashima (Radboud University)

Liquid-liquid phase separation plays an important role in cellular organization, and it is an emerging alternative in compartment-first hypotheses for the origin of life. Control over phase separation by enzymatic reactions is essential in order to use droplets as organelle mimics or protocells. To elucidate the physicochemical principles that govern the nucleation, growth and coarsening of biomimetic droplets, we use ATP-based complex coacervate droplets that we control by a kinase reaction. We track the coacervates by microscopy and follow their active growth at a single-droplet level. We quantify the partitioning of all components in our system by HPLC and fluorescence labelling to support our results with a kinetic comparison.

We show that droplet size increases as a result of the chemical reaction, an active behavior that is a plausible mechanism for protocellular growth. Moreover, growth rate can be averaged over the entire droplet population, and is significantly affected by environmental conditions and droplet composition. We also find that Ostwald ripening is suppressed in complex coacervates, and therefore these compartments, although membraneless, are more stable than it is usually speculated in the literature. Our findings show that the behavior of active droplets, obtained through coupling phase separation to enzymatic reactions, can be quantified and explained in terms of chemical principles.

Additional abstract submissions

Sorted by family name

Targeting ACE2 to Restrict SARS-CoV-2 Using Triciribine

Mir Shoebulla Adil (University of Georgia)

The severe acute respiratory syndrome coronavirus 2 that causes coronavirus disease 2019 (COVID-19) binds to the angiotensin-converting enzyme 2 (ACE2) to gain cellular entry. Akt inhibitor triciribine (TCBN) has demonstrated promising results in promoting recovery from advanced-stage acute lung injury in preclinical studies. In the current study, we tested the direct effect of TCBN on ACE2 expression in human bronchial (H441) and lung alveolar (A549) epithelial cells. Treatment with TCBN resulted in the downregulation of both messenger RNA and protein levels of ACE2 in A549 cells. Since HMGB1 plays a vital role in the inflammatory response in COVID-19, and because hyperglycemia has been linked to increased COVID-19 infections, we determined if HMGB1 and hyperglycemia have any effect on ACE2 expression in lung epithelial cells and whether TCBN has any effect on reversing HMGB1- and hyperglycemia-induced ACE2 expression.

We observed increased ACE2 expression with both HMGB1 and hyperglycemia treatment in A549 as well as H441 cells, which were blunted by TCBN treatment. Our findings from this study, combined with our previous reports on the potential benefits of TCBN in the treatment of acute lung injury, generate reasonable optimism on the potential utility of TCBN in the therapeutic management of patients with COVID-19.

Fighting Flu: biological and computational profile of new small molecules targeting hemagglutinin

Mariangela Agamennone (Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara)

The outbreak of Sars-CoV-2 dramatically demonstrated the risks of a global viral spread and highlighted the key role of prevention. In this context, Influenza virus is a highly monitored pathogen; in fact, it is largely diffused in the avian population, that works as a reservoir, and its spillover to humans could represent a serious threat. Despite its global diffusion, a few drugs are on the clinic with vaccination representing the most important tool to prevent seasonal Flu. The search for an effective weapon is still an unmet goal. Among viral macromolecules, hemagglutinin (HA) represents an interesting target. HA is a large trimeric mushroom-shaped glycoprotein expressed on the viral surface and responsible for the adhesion to the host cell through the receptor binding site (RBS), and the successive viral internalization through the structural rearrangement involving the conserved stem region of HA (Figure 1). Because of its double role, it is exploited for the identification of both universal vaccines,(1) and small molecule inhibitors.(2) In this context, we recently identify new drug-like compounds with antinfluenza activity toward two H1N1 strains.

They demonstrated to block both the adhesion to the host cell and the fusion process mediated by HA.(3) In the present work, the ability of previously identified compounds to work as broad spectrum inhibitors acting against H3N2 viral strain has been evaluated in hemagglutinoinhibition (HI) assays. Two over the five tested compounds were active also toward H3N2 Influenza virus. To validate the scaffold activity, 43 compounds analogue of the two broad spectrum molecules were selected and purchased for HI testing on three viral strains. Four of them showed to be active toward all three strains in both HI and neutralization assays (Table 1). To depict the HA binding process of most active compounds a computational study has been carried out; in particular, the homology models of the three HAs were generated and a structure-based approach was applied to get insight on the binding process. Experimental data suggest a possible binding on two different sites of the HA surface. This hypothesis has been confirmed by computational studies that found possible interactions in both the RBS and the stem region of the HA. These findings can represent an interesting starting point to optimize new and useful inhibitors of Influenza virus endowed with broad-spectrum activity and promising pharmacokinetic profile.

Investigational Studies on a Hit Compound Cyclopropane–Carboxylic Acid Derivative Targeting O-Acetylserine Sulfhydrylase as a Colistin Adjuvant

Giannamaria Annunziato (University of Parma)

Antibacterial adjuvants are of great significance, since they allow the therapeutic dose of conventional antibiotics to be lowered and reduce the insurgence of antibiotic resistance (1). Recently we reported that an O-acetylserine sulfhydrylase (OASS) inhibitor can be used as a colistin adjuvant to treat infections caused by Gram-positive and Gram-negative pathogens (2). A compound that binds OASS with a nM dissociation constant (3) was tested as an adjuvant of colistin against six critical pathogens responsible for infections spreading worldwide, Escherichia coli, Salmonella enterica serovar Typhimurium, Klebisiella pneumoniae, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus, and Staphylococcus pseudintermedius.

The compound showed promising synergistic or additive activities against all of them. Knockout experiments confirmed the intracellular target engagement supporting the proposed mechanism of action. Moreover, compound toxicity was evaluated by means of its hemolytic activity against sheep defibrinated blood cells, showing a good safety profile. The 3D structure of the compound in complex with OASS was determined at 1.2 Å resolution by macromolecular crystallography, providing for the first time structural insights about the nature of the interaction between the enzyme and this class of competitive inhibitors. Our results provide a robust proof of principle supporting OASS as a potential nonessential antibacterial target to develop a new class of adjuvants and the structural basis for further structure–activity relationship studies.

Chemical Targeting of DNA and histone methylation to reprogram cells in human diseases: challenges and future perspectives

Paola B. Arimondo (Institut Pasteur)

Currently there is a large interest in the role of epigenetic changes in human diseases and great expectations from drugs bridling these changes. The epigenetic approach is a strategy of choice since it participates to gene regulation in living organisms and processes integrating the impact of the environment and contributing to cell plasticity. Indeed, these modifications have an impact on genome activity and participate to modulate gene expression without altering the DNA sequence. The main epigenetic processes are DNA methylation, histone methylation, nucleosome positioning and noncoding RNA-associated silencing.

Proteins involved in "writing", "erasing" and "reading" of the chemical chromatin modifications conveying the epigenetic regulation have been identified and studied. Some inhibitors have been designed and are in clinical trials for cancer. Most studies have been conducted till now in cancer, but recently it has emerged that these modifications play a major role in other diseases. Here we will show the limits and challenges of the chemical strategies that target these modifications (1). Then we will show examples of the design of compounds that modulate the methyltransferases of DNA (2) and histones (3) and their impact on the cancer phenotype (4) and to bypass malaria drug resistance (5). Finally, we will present the use of these compounds as chemical probes to study the molecular mechanisms responsible of the aberrant and discuss discuss the future perspectives of such tools.

Taking advantage of chitosan adhesive properties to improve gold nanoparticle effectiveness in biomedical applications

Alvaro Artiga (University of Strasbourg, Supramolecular Science and Engineering Institute)

Gold nanoparticles (AuNPs) have shown a large potential for biomedical applications such as biosensing, drug delivery, cancer therapy and photothermal therapy (PTT) until date. However, their real use in medical treatment remains unexplored due to the lack of control over their interaction with biological components and their accumulation patterns after parenteral administration. Chitosan has showed promising results for drug delivery thanks to its physicochemical properties [1]. Herein, we would like to present a set of studies developed to improve AuNPs properties and effectiveness for PTT and oral administration by the use of chitosan entrapment of the AuNPs. Nanoparticle (NP)-mediated PTT rests on the ability of NP to convert light energy into heat as a promising method for selectively destroying tumour cells. One inherent limitation to NP-mediated PTT is that the NP must arrive at the site of action to exert their function and this typically involves cellular internalization. In previous studies, we have described how gold nanorods (AuNRs) showed limited cell internalisation provoking a failure in their effectivity for PTT [2].

Here we describe the use of chitosan for the encapsulation of AuNRs inside biocompatible and cell-adhesive chitosan hydrogel matrix, presenting the ability to adhere to the cytoplasmic membranes of cells and rendering them as highly efficient PTT agents of eukaryotic cells in vitro [3]. Non-invasive oral administration may reduce many of the long-term side-effects and risks associated to parenteral administration of AuNPs. However, the low stability of AuNPs in the acidic environment of the stomach and their limited absorption in the intestine results in a poor bioavailability after oral administration. Here, we also present the entrapment of AuNPs within chitosan-coated nanocapsules as an efficient mean of protection from simulated gastric conditions and enhanced uptake by epithelial colorectal cells up to 80 fold [4]. In addition, a novel inkjet-based technology for the microencapsulation of AuNPs with high encapsulation efficiency and ease of scale-up inside biocompatible chitosan hydrogel has been developed [5]. This high throughput continuous automated inkjet technology has been employed for the production of hybrid microcapsules with cell-adhesion properties and able to resist degradation over a large range of pH, showing the ability to protect AuNPs and adhere to colon in preliminary oral delivery in vivo studies.

Anti-inflammatory and immunomodulatory properties of coffee extracts

Valentina Artusa (Università degli Studi di Milano-Bicocca)

Coffee consumption is associated with potential health benefits linked to the prevention of inflammation-related diseases. However, the roasting process affects coffee chemical composition, by lowering the content of polyphenols in favour of the formation of brown-coloured compounds generated by Maillard reaction, which can also have anti-inflammatory potential. In our study, we aimed to compare the ability of green and roasted coffee extracts to modulate the inflammatory response of stimulated THP-1-derived macrophages. After a pre-treatment with extracts, we measure immune mediators released in response to LPS stimulation. Results indicate that green and roasted coffee extracts are able to modulate the expression of pro-inflammatory mediators deriving from both the MyD88/NF-κB-dependent (IL-1β, IL-6, TNF-α) and the TRIF/IRF3-dependent (IFN-β) TLR4 pathways.

Moreover, we test high- and low-molecular weight subfractions isolated from roasted coffee extracts and chlorogenic acid (5-CQA) as the major component of green coffee extract, to assess their involvement in the anti-inflammatory effects we have observed. While 5-CQA activity reflects the properties of green coffee extract, the two subfractions in some cases are active only when concomitantly present, suggesting a synergistic effect between polymers and small molecules. These findings further increase understanding of how coffee consumption could reduce risk of conditions that share low-grade inflammation as their physiopathological basis, due to the high intake of 5-CQA and Maillard reaction products.

Effect of the introduction of pyridine-containing ligands on the anticancer activity and selectivity factor of a series of novel Re(I) complexes

Pezhman Ashoo (Departamento de Química Inorgánica, Universidad de Murcia, and Institute for Bio-Health Research of Murcia (IMIB-Arrixaca), E-30071 Murcia, Spain)

In the development of metal-based anticancer chemotherapeutic compounds, the ligand play a pivotal role in enhancing the biological activity including entering the cells as well as interaction with major organelles such as nucleus and mitochondria. Our group has previously reported the successful synthesis and application of benzimidazole cyclometalated complexes as antiproliferative agents [1, 2]. In this work, we have designed, synthesized and characterized a series of organometallics Re(I) complexes with good cytotoxicity against selected cancer cell lines. However, the primary compounds containing chloride group also demonstrated undesirable activity in normal cells.

Aiming at reducing their side effect, we have latter modified their structure by the substitution of chloride with pyridine and 4-dimethylaminopyridine (DMAP). The new compounds, noticeably, have been improved on the cytotoxic properties and selectivity factor due to presence of these ligands. Biological studies on these compounds in various types of cancer cells revealed apoptosis being produced as a mode of cell death. The results obtained in this research clearly show how the introduction of pyridine derivatives as ligands increase the cytotoxicity and biological activity of anticancer complexes.

Trehalose-based neuroprotective autophagy inducers

Giulia Assoni (University of Trento)

α,α-trehalose (O-α,-D-glucopyranosyl-[1→1]-α-D-glucopyranoside, 1, Figure 1), is a non-reducing disaccharide in which two D-glucose residues are linked through their anomeric positions. It is widespread in nature, occurring in fungi, bacteria, yeast, insects, and plants, but it is absent in vertebrates. It possesses unique physical and chemical properties, which make α,α-trehalose an attractive ingredient in food, health and beauty, and pharmaceutical products 1. Autophagy (from the Ancient Greek autóphagos, meaning "self-devouring" and kýtos, meaning "hollow"), is a self-degradative process during which double-membrane vesicular structures (autophagosomes) are formed and cytosolic components are delivered to the lysosome for breakdown. Autophagy has a crucial role in maintaining cellular homeostasis, mediating the removal of unneeded, misfolded, or aggregated proteins, damaged organelles (i.e., mitochondria, endoplasmic reticulum, and peroxisomes), as well as intracellular pathogens 2.

Abnormal autophagy was observed in several human disorders, including neurodegenerative diseases, metabolic diseases, infections, and cancer 3. Recent studies 4 have demonstrated that α,α-trehalose is able to induce autophagy in neural cells, hampering development of diseases that result from the aggregation of anomalous proteins folding, such as ALS, tauopathies, and FTD. α,α-trehalose poorly permeates biological membranes, because of its high hydrophilicity. Moreover, higher vertebrates express on their intestinal membrane trehalase enzymes to hydrolyze trehalose to two molecules of glucose, preventing its bioavailability by oral administration. Thus, extremely high dosages are needed for in vivo effects in animals and humans. We designed and synthesized novel trehalose-based chemical entities with measurable autophagy-inducing properties, and biologically active trehalose-based probes to gather information about its molecular mechanism of action 5. A small array of trehalose prodrugs (2-5, Figure 1), putative probes (6-10, Figure 1), and inorganic nanovectors (12, Figure 1) will be presented together with the evaluation of their autophagy-inducing properties, bioavailability, and molecular mechanism of action.

Deglycosylation Methods for the Preparative Scale Release of N-glycans

Anna Ballesteros (CIC BiomaGUNE)

Complex N-glycans are sought-after biomolecules for advancing biomedical applications in glycoscience. However, their synthesis is challenging, expensive and time consuming. Moreover, some N-glycan structures present in the nature cannot be synthesised due to its complexity. Isolation of these structures from natural sources via enzymatic or chemical methods is an alternative. Chemical release represents the cost-effective alternative to enzymatic methods. In the present study we compare three previously reported methods (PNGase F, ammonia[1], bleach[2]) and 2 novel methods (sodium hydroxide, barium hydroxide) for the preparative scale release of N-glycans from commercial chicken egg white glycoproteins. In our study we focused on scalability, N-glycan yield and profile and side product formations comparing 5 methods for N-glycan release.

Fatty acids-induced conformational transitions and aggregation of the repeat domain of tau.

Barracchia, Carlo Giorgio (Dep. Biotechnology - Univerisity of Verona)

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by the concomitant deposition of extracellular fibrils composed of beta-amyloid peptide and intracellular filaments of the microtubule-associated protein tau1. Although the processes underlying this structural transition are still unknown, the association of tau with diverse classes of molecules, including proteins, nucleic acids, and lipids, could promote this abnormal dysfunction2. Lipids are abundant in nature, they are available to cytosolic tau, and are also seen to associate with aggregated tau on the cell membrane3.

Because binding of tau to lipid molecules may have both functional and pathological manifestations4-6 investigating the nature of this interaction is the focus of our current work. In an attempt to elucidate the association of tau with two unsaturated fatty acids (UFAs) (arachidonic and oleic acid) at the sub-molecular level, we carried out a variety of solution NMR experiments in combination with circular dichroism and fluorescence measurements7. Our work highlighted that tau4RD, the highly basic four-repeat domain of tau, strongly interacts with UFAs, perturbing their supramolecular states and itself undergoing time-dependent structural adaptation. tau4RD and UFAs form heterotypic assemblies which could explain pathological behaviour and serve as molecular targets for diagnosis or therapy.

Bacterial Degradation of Ergothioneine

Mariia Beliaeva (Department of Chemistry, University of Basel)

Ergothioneine is a sulfur-containing derivative of histidine with antioxidant properties. Only fungi, many bacteria, some archaea, and few plants produce it, while most plants and animals rely on absorbing ergothioneine from their environment. Ergothioneine biosynthesis in aerobic and anaerobic conditions has been well studied, but much less is known about the genetic and structural basis for ergothioneine catabolism. In this report, we describe the in vitro reconstitution of a five-step enzymatic pathway in Paenibacillus sp. through which bacteria can degrade ergothioneine to L-glutamate, trimethylamine, hydrogen sulfide, carbon dioxide, and ammonia. The first two steps are catalyzed by the two enzymes ergothionase and thiourocanate hydratase.

These enzymes are closely related to the first two enzymes in histidine catabolism, histidine-ammonia lyase, and urocanate hydratase. However, the crystal structure of thiourocanate hydratase reveals specific structural features that strictly differentiate the activity of this enzyme from that of urocanate hydratases. The final two steps are catalyzed by metal-dependent hydrolases that share most homology with the last two enzymes in uracil catabolism. The early and late parts of this pathway are connected by an entirely new enzyme type that catalyzes desulfurization of a thiohydantoin intermediate. Thus, ergothioneine catabolism has combined and adopted two existing pathways of primary metabolism. Homologous enzymes are encoded in many soil-dwelling firmicutes and proteobacteria, suggesting that bacterial activity may have a significant impact on the environmental availability of ergothioneine.

Competitive effectors of alpha-synuclein

Francesco Bellia (Institute of Crystallography – CNR)

Parkinson's disease (PD) and α-synucleinopathies are characterized by the progressive loss of neuronal cells and the decline of cognitive and motor functions. Biochemical and neuropathological evidence supports the role of oxidative stress, metal dyshomeostasis and α-synuclein (αSyn, a presynaptic and intrinsically disordered protein), in the development of these disorders [1]. Mounting evidence suggests that the aggregation of αSyn is a crucial event in the pathogenesis of α-synucleinopathies. Metal-protein interactions play an important role in αSyn aggregation and might represent a link between the pathological processes of protein aggregation, oxidative damage, and neural death. High Copper concentration is detected the cerebrospinal fluid of PD patients, as well as in the Lewy bodies, the intracellular aggregates of αSyn. Moreover, Copper regulates αSyn intracellular localization and cytotoxicity [2]. Lipoxidation and carbonylation have also been observed in neurodegenerative diseases. αSyn seems to induce lipid peroxidation and, conversely, αSyn carbonylation has been found in PD. Lipoxidation leads to the formation of the so-called Reactive Carbonyl Species (RCS); in particular, acrolein (ACR) and 4-hydroxy-nonenal (HNE) have been reported to affect the aggregation process of αSyn [3,4].

We recently investigated the interplay the interplay between ACR, copper and αSyn [5]. We also explored dose- and time-dependent effects of other RCS on αSyn using an approach based on Ultra Performance Liquid Chromatography coupled with High-Resolution Mass spectrometry. Moreover, we evaluated the effects of Cu2+ ions on these chemical modifications, and the influence of His carbonylation on Cu2+-binding. Finally, we investigated the effects of ACR and Cu2+ ions on αSyn aggregation by a fluorescence assay and dynamic light scattering (DLS).

Supramolecular Engineering of Peptide-based Bioinstructive Multilayered Biomaterials for Spinal Cord Repair

João Borges (University of Aveiro)

Spinal cord injury is a severe neurodegenerative disorder arising from traumatic damages inflicted on central nervous system (CNS), often resulting in a permanent loss of motor function and sensory perception [1]. Despite the growing knowledge in understanding the lesion pathophysiology, there is not an effective therapy to promote functional recovery. Recently, the emerging field of supramolecular chemistry together with advances in nanotechnology, tissue engineering and regenerative medicine have paved the way for developing neural extracellular matrix (ECM)-mimetic biomaterials aimed at building up a pro-regenerative microenvironment at lesion site and triggering axonal repair [2-4]. However, the developed biomaterials still fail in recreating the complex composition, multiresponsive dynamic nature and mechanical robustness of native ECM. Herein, emphasis will be given to the supramolecular design of biomimetic multitactical nanobiomaterials, endowed with topographical, biomechanical, and biochemical cues, to recapitulate the diversity of signals in native CNS microenvironment and stimulate axonal regrowth and spinal cord repair (Figure 1).

The bioinstructive biomaterials rely on biocompatible hyaluronic acid and poly(L-lysine) biopolymers, and laminin-mimetic biofunctional peptide, recreating neural ECM and promoting neurite outgrowth. The biomaterials were assembled on templates denoting distinct nanotopographies by combining molecular self-assembly with the cost-effective and highly versatile Layer-by-Layer assembly technology. The biomaterials were further crosslinked to enhance cell functions. The physicochemical and morphological properties of developed biomaterials will be disclosed, and the in vitro biological performance unveiled using primary neuronal cortical cells to reveal their potential to stimulate axonal outgrowth and be used in spinal cord repair. This work was supported by Programa Operacional Regional do Centro – Centro 2020, in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of the project SUPRASORT (PTDC/QUI-OUT/30658/2017). M. Lopes, C. Sousa, M. Torrado, and J. Borges acknowledge FCT for the PhD grants (2020.05210.BD, 2020.04408.BD, SFRH/BD/146754/2019) and individual contract (2020.00758.CEECIND), respectively. This work was developed within the scope of project CICECO–Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through FCT/MCTES.

PRECISION DRUGS: A COVALENT STRATEGY TO MINIMIZE SIDE EFFECTS OF PI3K INHIBITOR CANCER THERAPY

Chiara Borsari (University of Basel)

Inhibitors of the phosphatidylinositol 3-kinase (PI3K) – protein kinase B (PKB/Akt) - mechanistic target of rapamycin (mTOR) axis are considered as valuable assets in cancer therapy. A considerable effort has been dedicated to the development of drugs targeting the PI3K-mTOR axis[1-4], and some of them are currently evaluated in preclinical and clinical studies. Herein we present a strategy to convert a phase II clinical candidate, a pan-PI3K inhibitor (PQR309, bimiralisib)[1], into highly selective PI3Kα-covalent inhibitors aiming to minimize the on-target metabolic side effects of PI3K inhibitor cancer therapy. We exploited a rational approach to increase target selectivity by covalently targeting a PI3Kα non-conserved nucleophilic amino acid side chain, namely Cys862. A reactive moiety, so called warhead, was introduced into a chemically modified bimiralisib. A combination of warhead activity design, proximity and orientation allows a tight control of reversible inhibitor binding and isoform selective covalent binding. To avoid off-target reactions, we have set up a method to quantitatively evaluate warheads' reactivity and optimize for selective Cys862 modification. An extensive Structure Activity Relationship (SAR) study was performed and a wide range of linear and restricted rotation linkers introduced. A comprehensive understanding of the kinetics of irreversible inhibition allowed to interpret SAR and identify compounds with optimal kinact (maximum potential rate of inactivation). X-ray crystallography and mass spectrometry experiments validated the covalent modification of Cys862. Our pilot compounds exceed specificity and potency over an experimental dimethyl-substituted enone, CNX-1351[5]. Moreover, they are metabolically stable in rat liver microsomes and outperform the rapidly metabolized CNX-1351. Our strategy to investigate and tune warheads' reactivity represents a major step forward in the rational design of covalent chemical tools, overcoming the serendipity in the discovery of irreversible compounds. Moreover, we provide highly selective chemical tools to dissect PI3K isoform signaling in physiology and disease. A clarification of the role of the different PI3K isoforms in insulin signaling allows to address the challenges in isoform selectivity and to develop PI3K inhibitors showing ideal isoform specificity.

Exploring the implication of DDX3X in DENV infection: discovery of the first-in-class DDX3X fluorescent inhibitor

Annalaura Brai (University of Siena)

In the absence of effective drugs or vaccines for the treatment of the five Dengue virus (DENV) serotypes, the search of novel antiviral drugs is of primary importance for the scientific community. In this context, drug repurposing represents the most used strategy, however, the study of host targets is now attracting attention since it allows to identify broad-spectrum drugs endowed with a high genetic barrier. In the last ten years, our research group identified several small molecules DDX3X inhibitors1-3 and proved their efficacy against different viruses including novel emerging ones. Herein, we focused our efforts on expanding the structure-activity relationship (SAR) around the two series of already discovered DDX3X inhibitors, concentrating our work on the search of novel promising compounds active against DENV infection. As a result, we discovered novel DDX3X helicase inhibitors with improved antiviral activity, comparable or lower than those reported for known broad-spectrum antivirals such as ribavirin or sofosbuvir. Notably, the most promising derivative was about 9-times more active than the previous hit (compound 1). In addition, we investigated the mechanism of action of our compounds in infected cells, synthesizing a novel fluorescent derivative namely 25. Immunofluorescence analysis confirms that 25, during the first hours of DENV infection, co-localized with DDX3X promoting the reduction of NS5 positive cells and recovering the cell number, over the time (until 72hrs). The low cytotoxicity of compounds, evaluated by measuring ATP concentration, indicates once again that our compounds are characterized by high cellular tolerability. Overall, our results confirm that DDX3X inhibitors represent a safe and promising class of antivirals, supporting their evaluation in an animal model of DENV infection.

Active Pharmaceutical Ionic Liquids as new platform for Tuberculosis

Luis Branco (Universidade Nova de Lisboa (FCT-NOVA))

Tuberculosis (TB) is caused by infection with the slow-growing Mycobacterium tuberculosis (MT); currently causes 1.4 million deaths every year as well as 8 to 10 million new infections have been detected [1]. Despite enormous efforts in the discovery of novel drugs, tuberculosis (TB) remains the first bacterial cause of mortality worldwide. The World Health Organization (WHO) has estimated that one-third of the total world population is latently infected with bacilli of MT (an estimated 2 billion people). The Control of the global TB epidemic has been impaired by the lack of an effective vaccine, by the emergence of drug-resistant forms of M. tuberculosis, and by the lack of sensitive and rapid diagnostics [1]. In this context, novel active pharmaceutical drugs based ionic liquids (APIs-ILs) have been developed in order to improve the drug performance in terms of its stability, solubility, permeability and delivery [2]. Recent achievements of our research team allowed the development of sustainable synthetic methodologies for combination of pharmaceutical drugs such as anti-tuberculostatic (isoniazide, ethambutol), beta-lactams antibiotics (ampicillin, meropenem) and fluoroquinolone antibiotics (ciprofloxacin and norfloxacin) and other drugs (mefloquine, ibuprofen) as cations or anions with adequate biocompatible counter-ions [3-5].

The selection of counter-ions is important to improve the bioavailability, delivery, stability and therapeutic properties of the original TB drugs. The use of amphiphilic and hydrophobic as well as more polar counter-ions elucidate the cation-anion interactions and drug delivery. Herein, the different approaches using active pharmaceutical ILs as new platform for treatment of Tuberculosis will be presented. Acknowledgements: This work was supported by the Associate Laboratory for Green Chemistry- LAQV which is financed by national funds from FCT/MCTES (UID/QUI/50006/2019) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER - 007265). The National NMR Facility is supported by FC&T (ROTEIRO/0031/2013 – PINFRA/22161/2016, co-financed by FEDER through COMPETE 2020, POCI, and PORL and FC&T through PIDDAC). The authors thank the financial support by FCT – MCTES (PTDC/QUI-QOR/32406/2017; MAR2020 (MAR02.01.01-FEAMP-0042; INOVA4AQUA project) and Solchemar company.

DESIGN OF Au-BASED HYBRID NANOMATERIALS FOR ADVANCED THERAPEUTIC APPROACHES.

GABRIELA CALDERÓ (UNIVERSITAT DE BARCELONA)

The design of nanomaterials including polymers and metals, is focusing increasing interest for their advantageous properties in the medical field [1]. On the one hand, polymeric nanoparticles are especially suited for the encapsulation of drugs, thus providing improved chemical stability, enhanced biocompatibility and controlled release of drugs [2]. On the other hand, gold nanomaterials possess shape-dependent surface plasmon resonance that can reach the near infrared region (NIR), a peculiar physical property with a great medical potential due to the optical and thermal responses which can be generated upon proper stimulation [3]. In this context, hybrid nanomaterials are expected to gather interesting properties of both, polymeric and gold nanoparticles, in a single entity providing an advanced tool for the diagnosis and treatment of a vast variety of diseases including infectious diseases, cancer, neurodegenerative diseases, etc. In this study, hybrid nanomaterials have been developed using soft chemistry approaches. Firstly, ethylcellulose or poly(lactic-co-glycolic acid)(PLGA) nanoparticles with hydrodynamic diameters between 150 and 200 nm were prepared using a low-energy emulsification approach.

The surface charge (zeta potential) of these polymeric nanoparticles can be tuned from positive (+39 mV for ethylcellulose) to negative (-40 mV for PLGA) by a proper selection of stabilizing and capping agents. In addition, gold nanomaterials of different shapes, sizes and surface charges have been successfully synthesised following procedures described in the literature and attached to the surface of the polymeric nanoparticles by electrostatic interaction. Hence, the positively charged ethylcellulose nanoparticles were decorated with citrate-coated Au nanospheres of about 33 nm and a zeta potential of -22 mV. The hybrid ethylcellulose-Au nanomaterial showed a mean hydrodynamic diameter of about 190 nm and a maximum absorption peak around 598 nm. By contrast, the negatively charged PLGA nanoparticles were successfully decorated with CTAB-coated Au nanorods with typical dimensions (length x width) of 31 nm x 4 nm (i.e. aspect ratio of 3.35 nm) and a zeta potential of about + 30 mV. The hybrid PLGA-Au nanomaterials showed a mean hydrodynamic diameter around 250 nm and a maximum absorption band at about 800 nm, within the biological window. The synthesised Au-based hybrid nanomaterials show promising features for biomedical applications.

Protein directed Dynamic Combinatorial chemistry: an efficient strategy for drug discovery

Andrea Canal Martín (CIB-CSIC)

The conventional methodology for drug discovery is to find a chemical compound for a specific target. However, an alternative approach based on the design of systems where targets direct the synthesis of their best binders has emerged as Protein-directed Dynamic Combinatorial Chemistry (PD-DCC).1 In PD-DCC, selected building blocks react with each other through reversible chemical reactions providing mixtures of oligomers (Dynamic combinatorial libraries, DCLs). Due to its ability to respond to external stimuli, proteins can change the equlibrium towards the formation of their high-affinity compounds, changing the distribution of each specie.2 This approach selects directly ligands, therefore, the drug discovery process is accelerated. Here we report the identification of a non-competitive inhibitor of Glucose oxidase, using a dynamic combinatorial approach through thiol-disulfide exchange at low temperature and physiological conditions.3

Synthesis of chlorogenic acid amides as anti-hyperglycemic agents

Nunzio Cardullo (University of Catania)

Diabetes mellitus is characterized by high blood glucose levels due to hydrolysis of starch by pancreatic α-amylase and absorption of glucose in the small intestine by α-glucosidase. Diabetes is a significant cause of blindness, kidney failure, heart attacks, stroke, and lower limb amputation. Deaths from diabetes increased by 70% globally between 2000 and 2019. (1) Thus, there is an urgent need in the search of new and effective antidiabetic drugs, besides considering certain side effects showed by commercial antidiabetic drugs. α-Glucosidase and α-amylase inhibition is an established protocol in the search for potential antidiabetic agents with hypoglycemic activity. Many natural products have been reported for their anti-hyperglycemic activity. Among these, phenolic acids, including chlorogenic acid, and some phenolic esters and amides have shown α-glucosidase inhibition. (2-4) In this scenario, the purpose of this study was to obtain new chlorogenic acid amides as potential hypoglycemic agents. The amides are synthesized by condensation of chlorogenic acid, properly activated at carboxyl group, with aliphatic- and alkyl aryl-amines.

The methodology furnishes the expected products in good yields without further protection/deprotection steps. The new compounds are evaluated as inhibitors of α-glucosidase and α-amylase activity by in vitro assays based on UV-Vis and fluorescence measurements. Some of the analyzed amides have shown higher inhibitory activity than that of the natural lead chlorogenic acid. The obtained data have given more insights onto the mechanism of interaction/inhibition of the synthesized amides toward the targeted enzymes. The hypoglycemic activity showed by chlorogenic acid amides is supported by in silico molecular docking calculations, highlighting the putative binding mode. This research is supported by University of Catania Research Program (PIA.CE.RI-2020-2022) -Starting Grant. Project: NA.DIA. (bioactive NAtural products against DIAbetes)

Multitarget, curcumin-inspired 2-pyrrolin-5-ones as neuroprotectors

Noelia Carmona (Department of Chemistry in Pharmaceutical Sciences (Organic and Medicinal Chemistry Unit), Faculty of Pharmacy, Universidad Complutense, Madrid)

Improvements in life expectancy have led to a steep increase in age-related patologies, including neurodegenerative diseases. Neurodegenerative disorders are characterized by their multifactorial etiology and involve several common hallmarks including protein misfolding and aggregation, dysregulation of ionic homeostasis (especially of Ca2+) and mitochondrial disfunction that promotes free radical formation and oxidative stress.[1][2] Curcumin has attracted much attention as a potential treatment of neurodegenerative diseases because of its good neuroprotective profile that combines antioxidant activity, metal chelation and amyloid antiaggregating ability.[3] However, curcumin is chemically and biologically unstable, mainly due to fragmentation of its beta-dicarbonyl moiety.

We present here a library of diversely substituted curcumin analogues with the dicarbonyl structure rigidified into a 2-pyrrolin-5-one moiety in order to increase their chemical and metabolic stability. Their synthesis (see the Scheme) was initiated by an in-house developed multicomponent procedure to obtain the 2-pyrrolin-5-one core from primary amines, glyoxal and beta-ketoesters.[4] This was followed by an acylation step, phosphonate generation and a final Horner-Wadsworth-Emmons reaction to introduce diversity. The library was initially characterized by studying their antioxidant properties, including their ability to induce the NRF2-dependant Phase II antioxidant response. After cell viability studies, the compounds were examined in neuroprotection models against hyperphosphorylation and oxidative stress insults.

Theoretical studies on glycine synthesis in pre-biotic conditions

Juan Francisco, Carrascoza Mayen (1- Institute of Computer Science, Poznan University of Technology. 2- European Centre for Bioinformatics and Genetics ECBiG)

Glycine is one of the molecules elementary for life, however its production in pre-biotic conditions still is the subject of debate. Here we present Car-Parrinello Molecular Dynamics and metadynamics simulations of the glycine formation in different settings of temperature, electric field and starting from simpler inorganic substances. Additionally, excited states for selected reactants were studied at TD-DFT and CASSCF levels of theory. Our results suggest spontaneous glycine formation is possible via radical reaction pathway between formic acid and formaldimine. This study can be of interest for astrobiology, organic chemistry, photochemistry and related life-sciences.

Efficient transmembrane anion transport by click-tambjamines, a versatile family of compounds

Israel Carreira-Barral (Universidad de Burgos)

Facilitated anion transport is a blossoming area of research in supramolecular chemistry.[1] Despite the important part played by anions in many biological processes (e.g. cell homeostasis), the development of anionophores, i.e., molecules capable of translocating anions across lipid membranes, is still at an early stage.[2] This, together with their potential application as anticancer therapeutics, as a result of the toxicity derived from an altered homeostasis,[3] and in the treatment of channelopathies like cystic fibrosis, as channel replacement therapies,[4] makes these compounds an attractive object of study. Anion carriers are usually equipped with hydrogen-bond donor groups, particularly N-H fragments, enabling reversible anion coordination and forming a supramolecular complex capable of diffusing throughout the lipid bilayer (Figure 1). Figure 1. Top: Click-tambjamines, a novel family of tunable and efficient anion transporters. Bottom: Structures of the studied compounds. Inspired by a family of natural compounds called "tambjamines", herein we present a novel family of anionophores, "click-tambjamines" (Figure 1).[5] In this versatile molecular design one of the tambjamines' pyrrole rings is replaced by a 1,2,3-triazole ring, which allows the obtention of a wide variety of compounds in a fast and simple way. Transmembrane anion transport assays (chloride-selective electrode, emission spectroscopy) confirm that those shown here are very active anion carriers in both POPC vesicles and living cells, and display moderate cytotoxicities in both healthy and cancerous cell lines.

Taken together, these results indicate that this class of compounds might be a good starting point for the design of a therapy aimed at the treatment of anion-transport related diseases, such as cystic fibrosis.[4] Acknowledgements: Financial support from the European Union's Horizon 2020 research and innovation programme (TAT-CF project, Grant Agreement 667079), Instituto de Salud Carlos III (Grant PI18/00441) (co-funded by the European Regional Development Fund, a way to build Europe) and "La Caixa" Foundation and Caja Burgos Foundation (CAIXA-UBU004) is gratefully acknowledged. I. C.-B. thanks Consejería de Educación de la Junta de Castilla y León and FEDER for a post-doctoral contract.

Exploring the binding spectrum (druggability) of the human ALIX-V domain as a target for the development of broad-spectrum antivirals

Raúl Carrillo (University of Granada)

Viral Late domains (L-domains) are short and conserved sequences found in multiple families of enveloped viruses, retrovirus (HIV), filovirus (Ebola, Marburg) and rhabdovirus (Rabies). L-domains are able to mediate viral budding through their interaction with several cellular targets. For this reason, the inhibition of these interactions has been established as an attractive strategy for the development of novel host-oriented and broad-spectrum antivirals. The V domain of human ALIX is one of such targets whose interaction with LYP(x)nL-type Late domains allows the subsequent recruitment of the ESCRT-III complex through its protein CHMP4, eventually ending in the release of viral particles. Also, ALIX-V is implicated in the non-ESCRT mediated budding process of other viruses, such as some coronaviruses, having been recently identified as a critical factor for SARS-CoV and SARS-CoV-2 virulence. Here, we present a thermodynamic characterization of the binding between the natural L-domains and the ALIX-V domain via Isothermal Titration Calorimetry and MicroScale Thermophoresis. Additionally, we have tested ALIX-V binding interface through the massive screening of pure compound libraries using miniaturized Thermofluor assays. A small set of positives have already been identified as ALIX-V ligands, whose ability to interfere with Late domain binding has been validated using AlphaScreen as an orthogonal technique. For the best hits, IC50 values were determined and their ability to block viral budding in vivo was assessed using Virus-Like Particle assays. Our results provide new insights of the interaction between ALIX-V with the viral proteins and open the way towards the development of novel broad-spectrum antivirals.

Grignard addition onto carbohydrate-derived nitrones as a straightforward access to alkylated azasugars for fighting Gaucher and Parkinson diseases.

Francesca Clemente (University of Florence)

Pharmacological Chaperone Therapy (PCT) is an emerging approach to Lysosomal Storage Disorders (LSDs), in particular for those forms that involve impairment of the central nervous system. The PCT constitutes nowadays a new therapeutic approach potentially applicable to the whole range of protein misfolding diseases including Parkinson disease. Thus, the identification of novel PCs of this target still represents an exciting area of research.1 Pharmacological chaperones (PCs) are small molecules able to pass the blood brain barrier and acting as competitive inhibitors of the enzyme. When used in sub-inhibitory amount, they can correct the folding and/or stabilize the enzyme's catalytic activity.2 Our recent synthetic efforts in the development of novel azasugar-based compounds performing as enhancers of β-glucocerebrosidase (GCase) activity, the deficient enzyme in Gaucher Disease, led to the synthesis of the N-octyl trihydroxypiperidine 2 (up to 1.50 fold increase of GCase activity at 100 μM concentration on human fibroblasts derived from Gaucher patients bearing the N370/RecNcil mutation)(Figure 1).3 The focus of this work is the investigation of the best position of the octyl alkyl chain at the piperidine nucleus, in order to identify a new more promising PC.

We developed a straightforward strategy that allowed to shift the alkyl chain to the C-2 position of the hit piperidine 2. Our approach employed low cost D-mannose as starting material and the Grignard reagent addition to nitrone 3 as the key step, followed by a reductive amination/ring closure step (Figure 1). Careful tuning of the reaction conditions and use of an appropriate Lewis acid allowed to obtain the target compounds 6 and 7, epimers at C-2.4 Instead, a nucleophilic addition to nitrone 11, derived from the one-pot condensation/oxidation strategy to our carbohydrate derived aldehyde 1 with octylamine, provided the two disubstituted trihydroxypiperidines 14 and 15 with both configurations at C-2, after the final intramolecular reductive amination step (Figure 1). The new C,N-dialkyl trihydroxypiperidines 14 and 15 better mimic the structure of glucosylceramide, the GCase natural substrate. In this contribution, preliminary biological evaluation of our best PC on Parkinson animal model will be presented.

A multicomponent approach to biology-oriented libraries with multitarget activity against neurodegenerative diseases.

Ángel Cores (Universidad Complutense de Madrid)

Natural products and their modified derivatives are a source of inspiration in drug discovery programs. In particular, the systematic study of the basic features that make them biologically relevant allows the design and synthesis of natural product-inspired simplified compound collections that preserve their biological relevance. The rational design and synthesis of libraries inspired in natural products is also known as "biology-oriented synthesis".[1] Here we describe the preparation of three different families of natural products analogues with neuroprotective profile in routes comprising a maximum of four steps, two which are multicomponent processes.

The 2-pyrrolin-5-one core was synthesized by a modification of the classical Hantzsch three-component pyrrole synthesis developed by our group. [2] Subsequent Knoevenagel condensation or acylation followed by a Wadsworth-Emmons reaction furnished the target cinnamic amide and curcumin analogues, respectively.

A three-component 1,3-dipolar cycloaddition reaction between the Knoevenagel adducts, isatin derivatives and α-amino acids afforded a library of dispiro compounds designed as rhynchophylline analogues. Most members of these libraries showed low cytotoxicity and were good inductors of Nrf2, the master regulator of the phase II antioxidant response. Some compounds also showed good neuroprotective properties in oxidative stress situations such as toxicity induced by treatment with rotenone/oligomycin, and also against hyperphosphorylation induced by treatment with okadaic acid, with a well-balanced multitarget profile.

A pharmacophore-based virtual screening approach to discover novel Parkinson's Disease drug candidates

Pedro Cruz-Vicente (University of Beira Interior, Portugal)

Parkinson's Disease (PD) is characterized by progressive neurodegeneration, involving a loss of dopaminergic neurons in the brain and is considered one of the main causes of disability and mortality worldwide [1]. The current PD drug therapy is only for symptomatic relief and is mainly focused on restoring dopaminergic function in the brain [1]. To date, the most effective drug for PD treatment is levodopa (L-DOPA) combined with catechol-O-methyltransferase (COMT) inhibitors to increase its bioavailability and pharmacological benefit [2]. However, the currently clinically used COMT inhibitors display several adverse side effects, for instance, hepatoxicity, poor blood-brain barrier permeability [2], thus the development of novel improved COMT inhibitors is of great interest. In this work, a pharmacophore-based virtual screening methodology was used to discover new COMT inhibitors to improve PD therapy. For this, the pharmacophore was built and aligned using the structure of known inhibitors, followed by screening against a database of 1500 decoys to obtain the GH score and the enrichment value [3]. After validation, the model was screened in the ZINC Pharmer database to discover hit molecules that had the desired pharmacophoric moieties, simultaneously the drug-likeness was used as a filter to improve the analysis and select the most promising candidates [3]. The one hundred best-ranked hits were further studied by molecular docking to determine their affinity towards the COMT (PDB#6I3C) active site [4]. After this, based on the binding energies, the atomic interactions formed with the binding site and the predicted ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity), the best-ranked compounds were purchased and further studied. Specifically, the compounds were evaluated regarding their in-vitro inhibitory effect on human recombinant COMT lysates from Komagataella pastoris cells [5] and their cytotoxicity was also determined in rat dopaminergic cells (N27) and human dermal fibroblasts (NHDF). In summary, the proposed structure-based drug design strategy allowed the discovery of novel PD drug candidates with a very promising in-vitro COMT inhibitory activity and reduced cytotoxicity. This work is part of the project "Design of new Catechol-O-methyltransferase inhibitors with therapeutic potential for diseases of the central nervous system" - Project Centro-01-0145-FEDER-000019 - C4-Centro de Competências em Cloud Computing.

Prebiotic RNA assembly: Chemistry, catalysis, and compartmentalization

Saurja Das Gupta (HHMI/Massachusetts General Hospital/Harvard Medical School)

The ability of RNA to function as a carrier of heritable information as well as enzymes (ribozymes) has made it central to the emergence of life on earth. Non-enzymatic polymerization/ligation of monomers/oligomers activated by reactive moieties like prebiotically-relevant 2-aminoimidazoles (2AI) can generate short RNAs, but these processes are inefficient. The appearance of ribozymes that catalyze RNA assembly was therefore a major transition in the chemical evolution of life. We used in vitro selection to identify ligase ribozymes that utilize the building blocks of non-enzymatic ligation as substrates, thus bridging chemical and biocatalytic RNA assembly. These ribozymes catalyze processive ligations with substrates as short as 4 nt and function under the chemical conditions of prebiotic 2AI activation of RNA oligomers, allowing in situ RNA activation chemistry to drive ribozyme-catalyzed ligation in one pot as it would have been on early earth. But how did the complex ligase ribozymes emerge from chemistry?

We demonstrated the non-enzymatic assembly of a functional ligase ribozyme from 3' aminoacylated RNA oligomers. This amino acid-bridged chimeric polymer unites the building blocks of RNA and protein presenting a potential intermediate between the RNA and protein worlds. To further connect primitive and modern biology, we evolved a pool of ligase ribozymes that use 2AI-activated RNAs as substrates to a pool of ligases that use RNA substrates activated with triphosphate, the activating group used in modern RNA building blocks (NTPs). Next, we sought to establish RNA-catalyzed RNA assembly within protocells made with fatty acids. However, these prebiotic compartments are unstable at [Mg^{2+}] required to support ribozyme function. To solve this problem, we identified a ligase sequence that functions at sub-millimolar [Mg^{2+}] which enabled us to constitute the first instance of ribozyme-catalyzed RNA assembly within fatty acid protocells. In addition, our recent discovery that the presence of prebiotic small molecules such as ethylene glycol and D-ribose stimulate catalytic ligation at low [Mg^{2+}] provides a more general 'systems' approach toward realizing catalytic RNA assembly within these protocells. Our efforts to integrate various aspects of catalytic RNA assembly have brought us closer to assembling a self-replicating chemical system, capable of inventing Darwinian evolution.

BIOLOGICAL ACTIVITY AND ANTICANCER PROPERTIES OF ARENE Ru(II) COMPOUNDS WITH N-PHENANTHROLINE GLYCOSYLAMINE LIGANDS

Elena de la Torre-Rubio (Universidad de Alcalá)

Since the discovery of the successful antitumor agent cis-platin, the discovery of new metallodrugs is an emerging research area. Due to the well-known disadvantages of cis-platin, novel metallodrugs with new mechanisms of action are required. Among the variety of metal compounds studied, ruthenium derivatives have proved a great potential. In this context, polypyridyl compounds such as 1,10-phenanthroline are powerful bidentate metal chelating ligands with a demonstrated ability to act as DNA intercalators and groove binders (1). Furthermore, they have served as scaffolds for the design of several potent stabilizers' DNA G-quadruplexes, which are secondary DNA structures investigated as potential targets for anticancer drug development (2). Glycoconjugation is a recognized anticancer synthetic strategy that can help to enhance selectivity and biodistribution of metallodrugs (3) due to the Warburg effect, one of the hallmarks of cancer (4). Furthermore, carbohydrates are known to be generally good binders for nucleic acids (4), favoring a possible interaction between the complexes and the DNA. Herein, we report the synthesis, characterization, and biological studies of a new family of water-soluble glycoconjugates arene Ru(II) compounds. Metal compounds-DNA interaction studies have been also carried out by different assays, together with a comparative cytotoxic analysis of N-phenanthroline glycosylamine organic derivatives (5) and newly synthesized compounds against the PC-3 cell line of human prostate cancer, including an antimetastatic evaluation.

Interstrand triazole bridge as chemical platform for the conformational and metabolic stabilization of β-hairpin peptides: application to a VEGF-mimicking proangiogenic peptide

Lucia De Rosa (Istituto di Biostrutture e Bioimmagini – CNR)

Peptide based pharmaceuticals represent an innovative class of drugs showing several advantages over the traditional small organic molecules and recombinant protein therapeutics [1]. Peptides are considered to be high efficacious and selective drugs, exerting their biological effects through the targeting and modulation of biomolecular interactions. However, the translational potential of peptide therapeutics is limited by their low metabolic stability, which shorten their circulation half-life and in vivo efficacy. Therefore, molecular tools useful to metabolically stabilize bioactive peptides are strongly needed. Recently, the interstrand triazole bridge emerged as an effective chemical tool to structurally constrain and thus stabilize β-hairpin peptides that would be otherwise flexible in solution and, hence, metabolically unstable [2-4].

We introduced such chemical tool into a previously reported bioactive β-hairpin peptide, named HPLW, in order to obtain an improved analog endowed of a higher metabolic stability (Fig. 1). HPLW is a Vascular Endothelial Growth Factor (VEGF)-mimicking β-hairpin peptide endowed of proangiogenic effect and showing promising application in the proangiogenic therapy [5]. HPLW was cyclized by installing an interstrand 1,4-disubstitued 1,2,3-triazole bridge using a chemo-selective click-chemistry reaction, the Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) reaction. The modified peptide was demonstrated to retain the structural and biological properties of the original peptide, resulting organized into a β-hairpin conformation end exerting a proliferative and proangiogenic effects, but showed a remarkable metabolic stability in human serum. Besides providing a novel and promising peptide drug candidate with potential biomedical application, our work highlights the excellent utility of the triazole bridge as chemical platform for the conformational and metabolic stabilization of β-hairpin bioactive peptides.

TARGETING AXL RECEPTOR IN DRUG DISCOVERY

Rossella Di Stasi (Istituto di biostrutture e bioimmagini (IBB)-CNR)

Axl is a tyrosine kinase receptor playing key role in several biological processes, as its activation promotes cell proliferation, survival, migration and angiogenesis.1 Axl receptor exhibits a cytoplasmic TK domain, a transmembrane region and an extracellular portion, harboring two N-terminal immunoglobulin (Ig)-like domains and two fibronectin type III (FNIII) repeats. The growth arrest specific protein-6 (Gas6) is the natural ligand of Axl.2 Gas6 binds to the extracellular portion of Axl, leading to receptor dimerization and activation. The deregulation of Axl signaling has been associated to several high impact diseases, such as cancer, multiple sclerosis and viral infections.3,4 Thus, Axl is emerging as a novel and interesting molecular target in drug discovery. Molecules that selectively bind to Axl receptor appear useful in biomedicine to develop new therapeutic agents or diagnostic tools. We aim at developing novel peptide molecules targeting Axl receptor and Gas6 using structure based rational design and the phage display library screening technique.

To this aim, we prepared by recombinant means the extracellular Ig-like (Ig1-2) and fibronectin type III domains (FNIII-1 and FNIII-2) of Axl to be used as bait in phage display experiments. We also set-up the synthetic procedure for the synthesis by native chemical ligation of Ig2, FNIII-1 and FN-III-2 domains in their D-enantio form, successfully obtaining D-Ig2 Axl5 labeled with a biotin at the N-terminal position to allow its strepavidin-guided immobilization in library screening experiments. Finally, D-Ig2 Axl domain was purified and refolded to be employed in the selection of metabolically stable D-peptide binders of Axl using the innovative screening technique called "mirror-image phage display" (Fig. 1). At the end of phage library biopanning procedure, we obtained 12 different L-peptide binders selectively targeting D-Ig2 Axl domain, successively synthesized by means of D-amino acids in their metabolically stable D-form. These D-peptides are currently being used in binding studies through ELISA assays with recombinant Axl ectodomain, in order to identify the best Axl binders.

De Novo Design of Selective Quadruplex–Duplex Junction Ligands and Structural Characterisation of Their Binding Mode: Targeting the G4 Hot-Spot

Laura Díaz Casado (Instituto de Química Orgánica (IQOG) - Centro Superior de Investigaciones Científicas (CSIC))

Targeting the interface between DNA quadruplex and duplex regions by small molecules holds significant promise in both therapeutics and nanotechnology. Herein, a new pharmacophore is reported, which selectively binds with high affinity to quadruplex–duplex junctions, while presenting a poorer affinity for G-quadruplex or duplex DNA alone. Ligands complying with the reported pharmacophore exhibit a significant affinity and selectivity for quadruplex–duplex junctions, including the one observed in the HIV-1 LTR-III sequence. The structure of the complex between a quadruplex–duplex junction with a ligand of this family has been determined by NMR methods. According to these data, the remarkable selectivity of this structural motif for quadruplex–duplex junctions is achieved through an unprecedented interaction mode so far unexploited in medicinal and biological chemistry: the insertion of a benzylic ammonium moiety into the centre of the partially exposed G-tetrad at the interface with the duplex. Further decoration of the described scaffolds with additional fragments opens up the road to the development of selective ligands for G-quadruplex-forming regions of the genome.

Biological study of transferrin-conjugated formulations of metallodrug-functionalized nanomaterials

Diana Díaz García (Rey Juan Carlos University)

The use of nanomaterials in medicine is a current alternative to classical treatments. In particular, mesoporous silica nanoparticles (MSN) are a promising option due to the advantages they offer in drug transport, namely, their high loading capacity, the control of pharmacokinetics and the combination of simultaneous functionalities. In this context, molecular vectors can be easily incorporated in nanomaterials in order to increase cellular internalization and, therefore, induce a greater accumulation of the drug (active targeting) in the affected area. With this in mind, our research group has successfully synthesized a variety of silica systems with tin derivatives as the cytotoxic agent and folic acid as the targeting molecule, observing an improvement in in the selectivity and therapeutic properties of the synthesized materials.1-3 In this communication, our strategy has been focused on the design of multifunctional systems based on MSN functionalized with a cytotoxic organotin(IV) fragment, transferrin (Tf) as a new targeting molecule and the fluorophore FITC as an imaging molecule. Similarly, an analogous system based on a cytotoxic agent of titanium (titanocene) has been designed in order to evaluate if the Ti-Tf interaction improves the activity. All the synthesized materials showed potent cytotoxicity against the A2780 ovarian cancer cell line, as well as a significant improvement in cell internalization, even with only 1% incorporation of transferrin in the nanostructured system. The study of growth factors VEGF-A, FGF-2 and NF-κβ transcription factor in the treated cancer cells showed that the MSN-based materials were able to modulate and downregulate these factors. To summarize, these novel systems based on the formulation with transferrin are promising agents for the treatment of cancer due to their potent antiproliferative activity and their antiangiogenic capacity.

Developing an Accurate and Sensitive Liquid Chromatography-Mass Spectrometry Method Using a Powerful Prediction Platform

Snežana Đorđević (Polymer Therapeutics Lab., Prince Felipe Research Institute)

Before moving to clinical studies, all promising therapies, including advanced therapeutics such as drug delivery systems, must be characterized by robust, simple, and cost-effective approaches to improve batch-to-batch consistency of identity, efficacy, and safety [1]. Method sensitivity and accuracy remain two crucial points in this context, especially for those employed in determining free drug content, drug loading, stability, and release kinetics. Therefore, the development of analytical methods for related studies requires special consideration, and now, we underscore the importance of a "design of experiments" approach (DoE) in the development of liquid chromatography-mass spectrometry (LC-MS) methods for the quantification of four anti-cancer drugs. In the methods developed using the DoE approach, six LC parameters (gradient time, column temperature, % formic acid, flow, % acetonitrile at the i) beginning and ii) end of a gradient and) were selected as factors with a potentially significant effect on the four instrument responses: peak width, tailing factor, capacity factor and peak area for every analyzed compound. Moreover, the optimization of five MS factors (ion spray voltage, temperature of ion source, curtain gas pressure, nebulizer gas pressure, and auxiliary gas pressure) with potentially significant effects on ionization efficiency were also optimized by DoE. Using the desirability function, we discovered optimal LC-MS conditions that provide accepted values for all 16 instrument responses. Specifically, we minimized peak width, maximize peak area, and obtain the tailing and capacity factors in the accepted range (<2 and 2-8, respectively [2]). To validate the DoE model prediction, we performed a confirmation analysis and obtained accuracy in the range from 84.5% to 116.5%. These findings highlight the importance of DoE in securing method sensitivity and accuracy by optimizing peak width, tailing factor, peak area, and MS parameters. Furthermore, DoE allowed us to set a capacity factor value sufficient for the separation and detection of a drug and its possible metabolites in drug release studies. We believe that the implementation of DoE in method development will foster higher confidence in preclinical results and improve preclinical studies' efficacy and reproducibility with regards to advanced therapeutics. Acknowledgments: Fundació La Caixa Health Research Grant (LCF/PR/HR19/52160021- NanoPanTher)

Unexpected similarity between unrelated protein binding sites revealed by a novel 3D computer vision-inspired method.

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The increasing number of druggable pockets in protein structures enables structure-based drug design via pocket similarity assessment. Identifying pocket similarity between unrelated targets across the proteome is valuable to drug design [1] but still is a challenge to binding site comparison methods, notably local similarities arising from cavity microenvironment. We therefore developed ProCare [2], a novel computational method to compare protein pockets using a 3D point cloud registration algorithm. In computer vision, point cloud registration is a fundamental problem of finding the best transformation (rotation, translation, scaling) to match two clouds of points. A protein pocket is here represented as an ensemble of 3D points annotated by atomic coordinates and microenvironment specific pharmacophoric properties. Following the characterization of each point with a hybrid shape-chemical descriptor (c-FPFH) [2-3], two pockets are aligned by superimposing their corresponding points sharing the most similar patterns. The alignments are evaluated by estimating the proportion of aligned points sharing the same pharmacophoric properties. Out of a large-scale comparison where subpockets from the sc-PDB database were compared to the Tumor Necrosis Factor alpha (TNF-α) [4], ProCare suggested a similarity with the non-nucleoside binding site of HIV-1 reverse transcriptase (HIVRT) [2]. Extensive binding site comparisons using different structures of TNF-α [4] and HIVRT reinforced this similarity hypothesis, which was later confirmed by microscale thermophoresis assay where two out of three tested HIVRT approved drugs were found to bind to TNF-α (KD in the 20-40 µM range).

Remarkably, other state of the art binding site comparison methods as well as ligand 2D fingerprints and 3D shape methods were not able to detect that similarity. ProCare allows local comparison of protein pockets of different sizes while yielding visually interpretable results. The method is ideally suited to identify local and unobvious similarities among totally unrelated targets, and appears as a promising idea generator for fragment-based ligand design, able to pick starting fragments at a proteomic scale, not necessarily influenced by existing ligand or cavity neighborhoods.

Fighting viral infections by targeting host pathogen interactions

Bernhard Ellinger (Fraunhofer ITMP)

Viral infections are a complex, multi-step process involving not only viral, but also multiple cellular factors. To date, drug discovery methods have primarily focused on the inhibition of single viral proteins making the process prone to select resistance mutations. In two recent projects on HIV-1 and SARS-CoV-2 we built assays to identify small molecules inhibiting viral replication by targeting cellular factors. This approach promises long lasting drug efficiency as viral mutations are most likely not effective in restoring the function of the cellular proteins. In the case of HIV-1 an unbiased phenotypic approach was used in combination with a diversity focused library to identify new pharmacophores for HIV-1 treatment. In total 26,048 molecules were screened and we were able to identify 93 highly active and diverse small molecules. These hits were enriched with 279 similar compounds from the in-house library to identify promising structural features and the most active compounds were validated using orthogonal assay formats (1). In the case of SARS-CoV-2 we used a drug repurposing library to facilitate a fast translation into in vivo and clinical trials.

Next to an unbiased phenotypic cytopathicity assay we also identified a cellular factor critical for viral replication which was validated in a bioinformatics approach. The phenotypic screening resulted in 258 active molecules from a 5632 compounds containing repurposing library. From these hits 67 were subjected to detailed cytopathicity and cytotoxicity studies (2). The cellular factor, guanylate kinase, which was identified by a partner as critical for viral replication was used in a biochemical assay and active molecules were evaluated using a viral replication assay.

Artificial metalloenzymes based on Vancomycin for stereoselective synthesis

Giorgio Facchetti (University of Milan)

Artificial metalloenzymes, stemming from the combination of transition metal catalysts embedded within a biological environment, have recently risen up as a promising approach to merge the reactivity of metal-based catalysis and the specificity of biocatalysis[1]. Dalbapeptides, such as vancomycin, teicoplanin and ristocetin are variously substituted heptapeptides whose antibiotic activity depends on their binding to the D-Ala-D-Ala dimer of peptidoglycan precursors thus resulting in the inhibition of cell wall biosynthesis. In this system, indeed, the source of chirality is due to the presence not only of the aminoacidic chain, but also from the atropoisomerism of their structure. This interaction results stabilised by an array of hydrophobic interactions and five key hydrogen bonds and it is marked by such a low dissociation constant (KD = ~10-17 M) that it makes dalbapeptides an innovative option to the classical biotin/(strept)avidin second sphere coordination system[2,3]. In this context, aminoethylbenzensulfonamide ligands decorated with the D-Ala-D-Ala dimer at different positions of the phenyl ring were employed for the synthesis of the hybrid catalysts bearing an iridium active centre. In the presence of vancomycin, a new class of artificial reductases was obtained and applied to the stereoselective synthesis of chiral cyclic amines through an asymmetric transfer hydrogenation reaction in different aqueous media. An encouraging 48% (S) e.e. was obtained in the asymmetric reduction of the salsolidine precursor in NaOAc 0.1 M buffer at pH 5 whereas in the case of the most demanding isoquinoline substrates, the meta-artificial metalloenzyme afforded the product in an outstanding 70% (S) e.e. when applied to quinaldine. Indeed, the system shows remarkable potential for application as a practical method for the synthesis chiral sultam precursor.

Synthesis and antimicrobial evaluation of novel fluoroquinolone-based antibacterial agents bearing a quaternary ammonium moiety

Joanna Fedorowicz (Department of Chemical Technology of Drugs, Medical University of Gdansk, Poland)

The WHO identified antimicrobial resistance as one of the three greatest threats to human health, a particularly serious problem for patients whose immune systems are compromised. Persistent pathogens lead to higher health care costs because they often require more expensive drugs and extended hospital stays. Since a single drug is not always able to adequately control the illness, the combination of drugs with different pharmacotherapeutic profiles may be needed. Hybrid drugs are molecules intended to act at multiple targets. Interestingly, the fluoroquinolone (FQ) chemotherapies linked to another antibacterial agent represent the most comprehensively described hybrid compounds [1]. Recently, we have reported the synthesis and biological activity of a series of fluorescent FQ hybrid compounds featuring fused quaternary quinolone-triazolinium moiety [2-4]. Novel derivatives exhibited antibacterial activity against various pathogens, including biofilm-forming Pseudomonas aeruginosa, featured delayed antibiotic resistance development, caused a defect in DNA decatenation, and were potent DNA gyrase inhibitors comparable to the reference drug, ciprofloxacin. This project aimed to evaluate biological activity of new antibacterials incorporating a FQ drug and a quaternary ammonium compound to confirm the hypothesis that a new class of hybrid agents exhibits a unique dual mechanism of action: destabilization of bacterial membrane structures due to the presence of quaternary nitrogen atom and inhibition of bacterial type II topoisomerases elicited by FQ portion. FQ derivatives were design and synthesized by exhaustive alkylation to give compounds incorporating permanent positive charge on the nitrogen atom. The products were characterized by NMR, IR, MS, X-ray crystallography, and elemental analysis. Subsequently, the obtained derivatives were screened in vitro for antimicrobial activity against a panel of Gram-positive and Gram-negative bacterial strains. The most active compounds exhibited promising antibacterial action in the low micro- and nanomolar range, especially towards pathogens from the ESKAPE group. Molecular docking experiments revealed that all the synthesized compounds can interact in the FQ-binding mode at bacterial type II topoisomerases active sites. The Project was financed by the Polish National Agency for Academic Exchange as part of the Bekker Scholarship Programme and Medical University of Gdansk subsidies.

Synthesis and Evaluation of Glycomimetics as potential Siglec-10 Immunomodulators

Andrea Fernández Martínez (CICbiomaGUNE)

The sialic acid-binding immunoglobulin-like lectins (Siglecs) are important regulators of the immune system1. High affinity ligands derived from sialic acid that target specific Siglec can provide valuable therapeutic tools. A question of interest is how to design ligands that bind to these regulatory receptors with sufficient avidity and specificity to manipulate the immune system. In this project we focus on the design of potential ligands based on a trisaccharide scaffold that combines modifications in sialic acid and galactose; the second residue next to sialic acid known to make contacts to Siglec binding site (Figure 1) 2. With these tools we seek to study Siglec-10 receptor binding mode to natural ligands and use this information to rationally synthesize high affinity glycomimetics with an increased inhibitory potential and a greater selectivity for Siglec-10 or other Siglecs. By this way we could use these compounds as potential leads for the development of glycan based immune therapies.

Lipid A-Dependent Morphology and its Effect on Antimicrobial Resistance of ESKAPE Gram-(-) Bacteria

J. Felipe Franco-Gonzalez (Department of Structural and Chemical Biology, Centro de Investigaciones Biológicas Margarita Salas, CIB-CSIC)

AntiMicrobial Resistance (AMR) has dramatically increased over the past three decades becoming a worldwide health emergency.[1] Strains of particular concern are known by the acronym ESKAPE, which groups Gram-(+) bacteria: Enterococcus faecium, Staphylococcus aureus; and, Gram-(-) bacterias: Klebsiella pneumoniae (K.p.), Acinetobacter baumannii (A.b.), Pseudomonas aeruginosa (P.a.), and Enterobacter species (e.g., Escherichia coli, E.c.). In particular, Gram-(-) bacteria are highlighted due to their cell envelope complexity which exhibits strong resistance to antimicrobials. Their cell wall contains the periplasm membrane and a thin peptidoglycan layer encased by the outer membrane (OM). The OM acts as protective and selective barrier to most small drugs. Its mechanisms of resistance involve chemical alterations of lipopolysaccharides (LPS), which face the external environment and are indispensable for the bacterial survival.[2] The LPS structure is composed by three main chemical units: a lipid A, a core oligosaccharide and an O-antigen. A key element for AMR is the chemical structure of the lipid A moiety whose changes (e.g., acyl chain length, saturation, branching, and charged head groups) induce physico-chemical property modifications such as fluidity, charge balance, and permeability to antibiotics.3 Liposomes are used as biological membrane models which by tuning their lipid composition can mimic membrane vesicles of pathogenic bacteria, and thus, help to understand AMR mechanisms and to promote drug discovery. Nevertheless, although great progress has been made on the development of computational membrane models to understand resistance mechanisms at molecular level, computational studies with liposomes are scarce.[3] In this work, coarse-grained (CG) molecular dynamics (MD) simulations were used to model liposomes from ESKAPE Gram-(-) bacteria (K.p., A.b., P.a., E.c.). We captured the role of lipid A and cholesterol on liposome morphology and physico-chemical properties. Additionally, three antimicrobial peptides (AMPs) were used to unveil their implications on membrane disruption and their effect on the physico-chemical properties of bacterial liposomes. This novel study opens promising avenues to understand the hidden molecular keys of bacterial membranes and to develop new AMPs to overcome AMR.

Melanin-like film@glass as Smart Adsorbent Biomaterial for Water-Remediation from Dye-Pollutants

Massimiliano Gaeta (Università degli Studi di Catania - Dipartimento di Scienze Chimiche)

Water pollution, today, represents one of the most severe environmental issues. The rapid global population growth, the use of pesticides and fertilizers, the untreated human and industrial wastewater, have been determining a fast reduction of usable freshwater, which in turn implicates a forthcoming water-scarcity by 2050 [1]. Several efforts have been devoted to developing high-efficiency, low-cost, and eco-friendly materials for water remediation [2]. Inspired by adhesive proteins secreted by mussels for attachment to wet surfaces [3], melanin-like polymers have been successfully employed to provide highly resistant and adhesive biomaterials for the deposition of multifunctional films for water-remediation [2,4]. The robust adhesion to surfaces is related to an extensive network of covalent and noncovalent interactions due to phenolic hydroxyl/quinone groups based on DOPA. Indeed, the natural occurrence of melanin arises from enzymatic oxidation, operated by tyrosinase enzyme, of L-3,4-dihydroxyphenylalanine (L-DOPA) which leads to the deposition of melanin polymers [5]. Therefore, by exploiting the well-known adhesive and adsorbent properties of melanin-like coatings, herein it is reported the preparation of a smart device apt to remove a common dye-pollutant, the methylene blue (MB), from water (Fig.1). A small-scale commercial glass substrate - surface area about 3 square centimetre- was dipped in a not-stirred and aerated 0.5mM L-DOPA pbs solution for some days. After one-week dipping, the glass substrate was coated by a quasi-homogeneous and porous dark melanin-like film also evidenced from AFM surface morphology studies. The functionalised substrate was employed to remove MB from contaminated water revealing a high adsorption rate -more than 90%- in few hours of treatment. Afterwards, the adsorbed MB was merely photodegraded by simulated solar irradiation bringing back the quasi-pristine melanin film. The restored composite substrate was reclaimed as dye-adsorbent showing an exceptional re-usability, that is, more than 60% of adsorption rate. These promising results illustrate the chance to realise a composite biomaterial for water-remediation with multi-purpose advantages: i) low-cost energy, self-assembled and biodegradable material; ii) high efficacy as dye-pollutant adsorbent; iii) recyclability; iv) potential scalability for real and practical application overcoming the expensive filtration process based on the most common adsorbent materials.

PhenQE8, a novel 1,10-phenanthroline G4 ligand

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G-quadruplexes are DNA structures formed from G-rich sequences that are built around tetrads of hydrogen-bonded guanine bases. These structures have been found in several genomic regions with relevant biological functions, such as the telomeres and several oncogenes. Indirect inhibition of telomerase by stabilization of telomeric G-quadruplexes has anticancer effects.1,2 Based on this discovery, a wide variety of compounds designed to selectively interact with this structure have been developed.3,4 On the other hand, natural and synthetic guanidine derivatives are of great interest due to their pronounced biological activity.5 Consequently, we have focused on the synthesis of a novel ligand derived from 1,10-phenanthroline modified with guanidinium groups to improve the non-covalent interactions with G-quadruplexes. Additionally, we have studied its preferential capacity to selectively recognize G-quadruplex versus double stranded DNA structures.5 We report herein an improved synthesis of this novel quadruplex ligand and its preliminary biological testing. Specifically, we have evaluated the interactions with telomeric G-quadruplex DNA and duplex DNA by different techniques (DNA FRET melting, without and with a dsDNA competitor sequence, CD, equilibrium dialysis, etc.) along with its cytotoxic activity in tumor cultured cells. Acknowledgements Financial support from Spanish MINECO and MICINN (AEI, Agencia Estatal de Investigación, grants CTQ2015-72625-EXP, PID2019-108251RB-I00, RED2018-102574-T), from Comunidad de Madrid (PEJ-2017-AI/SAL-6160, PEJ-2018-TL/SAL-11409) and from Universidad de Alcalá (CCG19/CC-009, CCG2018/EXP-024, UAH-AE-2017-2) is gratefully acknowledged.

Use of chimeric fibrin/filaggrin homocitrullinated peptide for the detection of anti-CarP in palindromic rheumatism patients

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Anti-citrullinated peptide/protein antibodies (ACPAs) are the most relevant serological markers in rheumatoid arthritis (RA), making it a hallmark of the disease (1). However, other post-translational modifications have been described in the context of RA; acetylated proteins, proteins containing MAA (malondialdehyde-acetaldehyde-adducts) and homocitrullinated proteins are the most relevant (2). Specifically, antibodies against homocitrullinated (carbamylated) peptides/proteins (Figure 1), known as anti-CarP, have been found in 10-20% of patients ACPA negative. Palindromic rheumatism (PR) is an intermittent form of arthritis that may evolve to chronic rheumatic disease, mainly RA. The nature of PR is unclear, as it may be considered a disease in itself, an abortive form of RA, or just a pre-RA stage. It is established that a significant number of patients with PR show a similar serum autoantibody profile to that of RA (3). Recently, an analysis by our group of the antibody immune response to post-translational citrullinated antibodies showed a more restricted response in PR than in established RA, with less use of the IgM or IgG isotypes (4). With the aim to further characterize the immune response present in PR, we have analysed the presence of two anti-CarP specificities (homocitrullinated peptide and protein) and measured anti-IgG, IgA, and IgM isotypes in sera from PR patients and compared to RA patients.

For this purpose, we designed and synthesized by solid-phase synthesis a chimeric homocitrullinated peptide derived from the fibrin and filaggrin proteins (CFFHP) as well as a non-homocitrullinated version that was used as a specificity control, and determined anti-CarP specificities by home-made ELISA assays. We also worked with a protein antigen, fetal calf serum (FCS), using both the carbamylated and non-modified forms with the same goal. Our results show the presence of anti-CarP in PR patients, nevertheless in fewer cases (24% of positivity vs 64% in RA), with a smaller proportion of isotypes and lower titers of antibodies (5).

Discovery of natural product antifoulants to prevent fouling of oil and gas structures

Jessica Gomez-Banderas (National Decommissioning Centre at the University of Aberdeen)

Biofouling - the aggregation of microbial cells, forming biofilms, and the sequential attachment of hard macro-foulers such as barnacles and mussels - is a prevalent issue in the oil and gas and maritime industries. Marine fouling causes increased drag on vessels, resulting in increased fuel consumption, and heavy maintenance costs are associated with removing marine growth from offshore installations on a routine basis. Currently, antifouling solutions such as copper-based paints and algaecides are in use, however, recent studies are showing that some of these coatings accumulate in the marine environment [1] and have harmful effects on marine life [2], highlighting the need for an environmentally friendly and non-toxic solution to marine fouling. Marine natural products are a potential source of new antifouling compounds due to the variety of active compounds previously discovered from marine organisms. To date, there are over 198 antifouling active metabolites which have been isolated from sponges, gorgonians and soft corals [3] and there are inevitably many more to be discovered. This project aims to study the metabolites and chemical defence compounds produced by marine invertebrates which do not show fouling on their outer surfaces, to find potent antifouling active compounds. This will be achieved using a bioassay-guided fractionation approach, using an in-house biofilm growth inhibition assay which uses key bacteria found in marine biofilms.

Flavanone-derived hybrid compounds as multitarget-directed ligands for the potential treatment of neurodegenerative diseases

Jorge Gómez-Carpintero (Universidad Complutense de Madrid)

Alzheimer´s disease (AD) is the leading cause of dementia, with a steadily growing incidence rate, and represents an immense challenge for healthcare systems throughout the world. In the past decades, many efforts to develop effective drugs against this disease have been made, but the few therapeutic treatments that have been approved provide only temporary symptomatic relief, none of them being disease-modifying. These poor results can be due to the multifactorial nature of the disease and the large number of biological pathways implicated in its pathogeny. In this context, the development of multitarget-directed ligands (MTDLs) is an emergent approach to the treatment of AD.1 The enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are a validated targets for the treatment of AD.2,3 and oxidative stress is an important cause of neurotoxicity that underlies the onset and progression of AD and other neurological disorders.4 Herein, we describe the design, synthesis and pharmacological study of two new series of hybrid compounds behaving as multitarget-directed ligands for the potential treatment of AD. These compounds were designed by merging the key structural elements of well-known AChE and BChE inhibitors tacrine and donepezil with a flavanone framework (naringenin or hesperitin) that is expected to provide antioxidant and β-amyloid aggregation inhibitory properties.

Recognition of Sialic Acid: 3D Perspective of the Complement Deregulation Activity of FHR-1 and Mutants

Elena Gómez-Rubio (Margarita Salas Center for Biological Research (CIB-CSIC))

The complement system (CS) plays a critical role in innate immunity, and its activation yields a cascade of proteins involved in homeostasis, inflammation and defense against pathogenic agents (1). The CS alternative pathway has a basal activation and therefore it requires regulators to prevent self-damage in host tissues. Factor H (FH) is the main regulator of the alternative pathway upon distinguishing between host and non-host cells via sialic acid recognition and binding C3b protein, an opsonin generated by the CS cascade that binds unspecifically self and non-self surfaces, to prevent the damage in host surfaces (1). However, the Factor H-related proteins (FHR) also bind C3b but lack the capacity of distinguishing between self and non-self surfaces due to the inability to recognize cell surfaces decorated with sialic acid glycans. Furthermore, mutations in FHR-1 can lead to CS dysregulation causing chronic diseases like atypical hemolytic uremic syndrome (aHUS)(2). The understanding of sialic acid recognition and the identification of selective modulators of FHR-1 and mutants could be helpful to identify novel drugs for the treatment of these pathologies. We have combined computational approaches, such as protein-protein docking, protein-ligand docking, and molecular dynamics simulation, with biochemical, immunological and NMR approaches, to shed light into the molecular recognition processes of sialic acids by FH, FHR-1 and aHUS-related mutants, and their binding to C3b (3).

These studies have shown that the development of aHUS associated to these mutants could be due to the acquisition of the ability to bind sialic acid, and therefore compete with FH, and to the ability of FHR-1 mutants to bind C3b (Fig.1)(3). Furthermore, by means of virtual screening, we have searched for small molecules able to selectively modulate FHR-1 and its mutants, and we have identified a collection of sialyl derivatives as possible selective binders. Figure 1. Mutant FHR-1 L290V (green) in complex with 6'-sialyl-lactose (blue) from docking calculations. Bottom right shows the electrostatic potential surface of the sialic acid binding site in mutant FHR-1 L290V.

Theranostic fibrous silica nanoparticles containing chlorambucil and organotin(IV) metallodrugs: An effective synergistic therapeutic approach to triple negative breast cancer

Santiago Gómez-Ruiz (COMET-NANO Group. Department of Biology and Geology, Physics and Inorganic Chemistry, ESCET, Universidad Rey Juan Carlos.)

Triple-negative breast cancer (TNBC) constitutes approximately 20% of breast cancers and it is characterized by a very negative prognosis and poorer survival rates than other breast cancer subtypes. TNBC therapy usually expects drugs combination and, in this context, the simultaneous action of both DNA alkylating agents, such as chlorambucil (N,N-bis(2-chloroethyl)-p-aminophenylbutyric acid),[1] and highly cytotoxic metallodrugs such as organotin(IV) derivatives[2] may be an attracting alternative for its effective therapeutic approach. Thus, we have designed and synthesized a multifunctional theranostic nanoplatform based on fibrous silica nanoparticles (of the KCC-type)[3] containing those two therapeutic compounds together with a molecular imaging agent (NIRF dye) and a TNBC-targeting fragment (folic acid). After their full physico-chemical characterization, the synthesized materials were assessed in vitro against TNBC cancer cells and an in vivo using orthotopic TNBC murine models. The achieved results confirmed both the selective diagnosis of the tumor area and, thanks to the synergistic effect of chlorambucil and the organotin (IV) metallodrug, an enhanced therapeutic activity against human breast adenocarcinoma. In this communication the most recent results of this project will be discussed. Acknowledgements We would like to thank the Ministerio de Ciencia e Innovación (Agencia Estatal de Investigación) for the research grant RTI2018-094322-B-I00, Comunidad de Madrid and Universidad Complutense de Madrid for research project no. 2017-T1/BIO-4992 and Microscopy & Dynamic Imaging Unit of CNIC, Madrid, Spain (as a part of the ReDiB-ICTS also supported by FEDER).

Trifaceted trehalose cyclooligosaccharides for organ-selective gene delivery

Manuel González-Cuesta (University of Seville)

Instilling discriminated domains in nanostructured platforms allows constructing anisotropic (patchy) nanoparticles displaying directional interactions that emulate those controlling self-assembling processes in biological systems. Molecular nanometric entities (molecular nanoparticles, MNPs) exhibiting defined symmetry and persistent shape and volume, in combination with precision synthesis methodologies, offer unique opportunities towards this end [1]. The synthesis of gene delivery systems (vectors) based on monodisperse cyclodextrin (especially β-cyclodextrin; βCD) derivatives is a paradigmatic example: both the self-assembling properties and the abilities to form nanocomplexes with nucleic acids (CDplexes) are tightly dependent on the anisotropic disposition of clusterized cationizable and lipophilic groups at opposite rims of the macrocyclic core in a Janus-like architecture. Increasing the number of cationic or lipophilic patches is expected to broaden the opportunities to regulate directional recognition phenomena in search for artificial viruses. However, neither cyclodextrins nor any of the commonly used MNP platforms allows breaking the two-face Janus boundary. Following previous work on DNA glycocarriers [2, 3], here we disclose patchy molecular nanoparticles (MNPs) with a Mickey Mouse architecture, built on a trehalose-based trifaceted macrocyclic scaffold, purposely designed to condense plasmid DNA (pDNA) into transfectious nanocomplexes. The new vectors encompass segregated cationic and lipophilic clusters, in either 2:1 or 1:2 ration, with an angular disposition; they can thus electrostatically interact with pDNA and further engage in hydrophobic contacts to promote condensation. Notably, the size, shape and internal structure of the co-assemblies can be molded by fine-tuning the characteristics of the patches, which strongly influences the transfection efficacy in vitro and in vivo. Outstanding organ selectivities can then be programmed with no need of including a biorecognizable motif in the formulation, emphasizing the compelling effect of both the cyclooligosaccharide architecture and the nanocomplex topology on the biological activity. The results provide a versatile strategy for the construction of fully synthetic and perfectly monodisperse nonviral gene delivery systems based on carbohydrates uniquely suited for optimization schemes by making vector patchiness the focus.

Prebiotic Photochemical Link Between RNA and DNA

Nicholas Green (MRC Laboratory of Molecular Biology, Cambridge)

The advent of life requires informational inheritance mediated by a suitable polymer - the identity of which is debated - that can undergo replication in the absence of enzymes. The common RNA world hypothesis invokes RNA as this polymer,1,2 but other evidence implies that life may have started with a heterogeneous nucleic acid genetic system including both RNA and DNA. Such a theory streamlines the eventual 'genetic takeover' of homogeneous DNA from RNA as the principal information storage molecule in the central dogma, but requires a selective abiotic synthesis of both RNA and DNA building blocks in the same local primordial geochemical scenario. Herein our recent work demonstrating efficient and selective prebiotic syntheses of DNA molecules, alongside those of RNA, is discussed.3,4 These results support the notion that purine deoxyribonucleosides and pyrimidine ribonucleosides may have coexisted before the emergence of life.

Post-translational Modifications of Cytochrome c: A Key Between Health and Diseases

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Mitochondria are the powerhouses of the cell, whilst their malfunction is related to several human pathologies, including neurodegenerative diseases, cardiovascular diseases, and various types of cancer. A key protein in mitochondrial metabolism and control of redox signaling is cytochrome c, a small soluble heme protein that acts as a redox carrier in the respiratory electron transport chain [1]. However, cytochrome c is likewise an essential protein in the cytoplasm acting as an activator of programmed cell death [2]. Such a dual role of cytochrome c in cell life and death is indeed fine-regulated by a wide variety of protein post-translational modifications [3-4]. The objective of this work is to carry out an in-depth analysis of how cytochrome c modifications emerge as a control mechanism of cell metabolism but also as a key element in the development and prevention of pathologies [5].

Unveiling new roles for FUR (Ferric Uptake Regulator) proteins in Anabaena sp. PCC7120: FurA as a putative carbon/nitrogen balance sensor via 2-oxoglutarate

Jorge Guío (University of Zaragoza)

In cyanobacteria, 2-oxoglutarate (2-OG) is a metabolite whose function is to provide carbon skeletons that allow the incorporation of ammonium through the GS-GOGAT cycle, connecting both carbon and nitrogen metabolism. It has also been described as a signal molecule that reflects the cellular carbon/nitrogen balance, modulating the DNA-binding activity of the key regulator of nitrogen metabolism NtcA (1). FurA (Ferric Uptake Regulator) from the cyanobacteria Anabaena sp. PCC7120 is a transcriptional regulator that has been proposed to act as a redox sensor protein, due to its disulfide reductase activity (2) and its ability to interact with heme (3). As previous studies showed that FurA was able to control nitrogen metabolism (4) we wondered if this transcriptional regulator could also act as a sensor of carbon/nitrogen balance via 2-OG. In order to predict whether 2-OG was able to interact with FurA, a model of FurA tridimensional structure was built and docked in silico with 2-OG. Results revealed that this metabolite was able to bind to FurA, a fact that was confirmed by Isothermal Titration Calorimetry (ITC) assays. Furthermore, Electrophoretic Mobility Shift Assays (EMSA) of FurA were carried out in presence of 2-OG, showing that FurA binding activity to the ntcA gene promoter region was modulated by this metabolite. Finally, as docking simulations suggested two putative binding sites, we constructed two mutants of FurA in which the residues that were predicted to interact with 2-OG were replaced by alanines. These FurA mutants were analyzed by ITC and EMSA assays, showing that Arg70 was involved in 2-OG binding.

Finding lacking pieces of the complex jigsaw puzzle of nitrogen metabolism in cyanobacteria: FurC (PerR) as a key regulator of nitrogen fixation and heterocyst differentiation in Anabaena PCC 7120

Jorge Guío (University of Zaragoza)

Nitrogen metabolism in cyanobacteria is of great importance, since it allows the incorporation of this element into the trophic chains. The form of nitrogen preferred by cyanobacteria is ammonium, as it is the metabolite that is incorporated into carbon skeletons. Ammonium can be incorporated through specific permeases but generally comes from the metabolism of other forms of nitrogen, mainly from the assimilation of nitrate and nitrite. Furthermore, in the absence of a combined nitrogen source, some species of cyanobacteria are capable of fixing atmospheric nitrogen. Nitrogen fixation is carried out by a multienzymatic complex called nitrogenase, which is inhibited by oxygen. Consequently, in filamentous cyanobacteria such as Anabaena sp. PCC7120 nitrogen fixation takes place in specialized cells called heterocysts, which have anaerobic conditions. The formation of heterocysts from vegetative cells involves a series of important morphological and metabolic changes based on a process of differential gene expression which allows the creation of an anaerobic environment (1). The identification of transcriptional regulators that control nitrogen metabolism is of interest in order to enhance the biotechnological applications of cyanobacteria in biofertilization or biofuel production. However, nitrogen metabolism and heterocyst differentiation are rather complex and several key aspects are yet to be discovered. FUR (Ferric uptake regulator) proteins are a family of transcriptional regulators which have traditionally been associated with the regulation of metal homeostasis. Nevertheless in cyanobacteria they have a global role, controlling various cellular processes. FurC, one of the FUR paralogs of Anabaena PCC7120, seemed to be involved in nitrogen metabolism, since its expression is controlled by the global nitrogen regulator NtcA (2). Consequently we sought to investigate its role in the genetic control of nitrogen metabolism. In this work, we have analysed the transcriptomic profile of a FurC overexpression strain in the absence of nitrogen and we have studied its morphology and physiology under these conditions. Our results show that the overexpression of this transcriptional regulator impairs heterocyst development and reveal that FurC directly regulates the expression of genes involved in the assimilation of nitrogen sources and the differentiation of heterocysts, suggesting that FurC is a key regulator of nitrogen metabolism in cyanobacteria.

Application of Ribosomally Synthesized and Posttranslationally-Modified Peptides (RiPPs) as Epitope Grafting Scaffolds for Drug Development

Julian Hegemann (Technische Universität Berlin)

RiPPs are often associated with interesting biological properties; e.g. antimicrobial, antifungal, or antiviral activities. Their biosynthesis follows a common principle: A genetically-encoded precursor peptide is matured by corresponding processing enzymes. In the precursor, an N-terminal leader region mediates enzyme interactions and modifications are introduced in the C-terminal core peptide. Eventually, the leader peptide gets removed by proteolysis and the mature RiPP is released. Lanthipeptides are RiPPs, which are defined by the presence of characteristic beta-thioether crosslinks.[1] Amongst the many known lanthipeptides are the prochlorosins (Pcn) from Prochloroccocus MIT9313.[1,2] This cyanobacterium encodes 30 different precursor peptides (ProcAs) combining highly conserved leader regions with highly diverse core peptides. All ProcAs are matured by a single processing enzyme, ProcM. A thorough mutational analysis was performed on the ProcA2.8 precursor, which yields the bicyclic Pcn2.8 lanthipeptide, exchanging single residues and altering structural elements to investigate how these changes affect processing by ProcM.[2] Furthermore, Pcn2.8 was tested as scaffold for epitope grafting and the RGD integrin binding epitope was introduced at different positions. Amongst the generated peptides, one high affinity (Ki = 1.6 nM) binder of the avb3 integrin was identified.[2] This integrin is important for angiogenesis and therefore a promising target to inhibit the growth of certain tumor types. Lasso peptides comprise another RiPP class known to be highly amenable for changes of their amino acid composition.[3-5] Their defining structural feature is a constrained lariat knot-like topology that confers high proteolytic stability. Incorporation of the RGD epitope into the antimicrobial lasso peptide microcin J25 also yielded an avb3 integrin binder, which was further optimized by introduction of hydrophobic residues adjacent to the binding motif.[4,5] In this way, a lasso peptide with high affinity (Kd = 4.1 nM) for the avb3 integrin was generated. [4,5] The work presented here demonstrates the potential of using RiPPs to generate highly potent receptor binders by epitope grafting. The well-studied avb3 integrin was used for proof-of-concept studies employing RiPPs from the lasso peptide and lanthipeptide families. Our results suggest that through the established strategies, these RiPPs could also be utilized to display other small peptide epitopes.

Network-based Bioinformatic Drug Discovery to identify novel therapeutic targets in mucinous ovarian cancer

Jessica Holien (RMIT University)

Multiple lines of evidence have found mucinous ovarian cancer (MOC) to be different from other ovarian cancers. However therapeutically they are treated the same. To that end, although this is a rare disease, when detected at a late stage or upon recurrence, MOC is almost always fatal due to resistance to conventional chemotherapy. Thus, there is an urgent need to develop novel therapies for this tumour type. We have amassed the largest multi-platform genomics data for MOC, however traditional bioinformatic analysis showed only the "undruggable" KRAS was statistically upregulated [1]. Advances in computational systems and network biology have meant that, for the first time, we can begin to understand how single protein-protein interactions cooperate in the cell to form complex protein-protein networks comprised of numerous protein-protein interactions. These approaches are cheap, quick, and can often find important regulators of proteins that would otherwise not been considered in these in vitro screens, due to low coverage. Furthermore, networks-based analyses can demonstrate the dynamic nature of genetic interactions and predict the consistent re-wiring events that occur in response to changing conditions (i.e. chemotherapy etc). Therefore, these in silico bioinformatics approaches are a useful way to prioritize potential protein pairs and/or combinations of proteins for functional studies. Described here is a unique in silico pipeline that we have developed and applied to our transcriptomic data, comparing MOC to benign mucinous tumours (in the absence of a known normal tissue of origin). In short, this pipeline mapped the protein-protein network in MOC and then by utilising structural bioinformatics, prioritised the proteins in the MOC network for their "druggability" i.e. ability to be readily disrupted by small molecules. Using this protein-protein interaction modelling, we identified the strongest 16 candidates that are structurally tractable to therapeutic targeting by small molecules. siRNA knockdown of these candidates in MOC cell lines and tumour organoids has identified at least three proteins which are now undergoing a structure-based drug discovery program.

Recombinant synthesis of human trefoil factor family 2 (hTFF2) protein

Kirtikumar B Jadhav (University of Vienna)

Human trefoil factor 2 (hTFF2) belongs to an important family of peptides containing a well-structured trefoil domain[1]. hTFF2 is secreted into the gastrointestinal tract where it plays important role in protecting and repairing the mucosa; it thus holds therapeutic promise for the treatment of chronic gastrointestinal disorders[2]. hTFF2 contains 106 amino acid residues (15Asn glycosylated) and displays two trefoil domains formed by 7 disulfide bonds. Its 3D-structure, mode of action and target receptor remain unknown as only limited amounts of hTFF2 can be obtained from human tissue extraction. Here, we describe a yeast expression system designed to produce hTFF2 and its glycosylated and 15N-enriched analogues for physiological, biochemical and spectroscopic studies. We designed the hTFF2 gene encoding a fusion protein and constructed recombinant plasmids and optimized conditions for protein expression. The secreted hTFF2 was found in a glycosylated and non-glycosylated form S. cerevisiae. We also produced a 15N-enriched analogue of hTFF2 to facilitate NMR structure determination. Furthermore, we also describe our semi-synthesis approach to synthesize hTFF2 protein by expressed protein ligation using E. Coli based expression system. Access to large quantities and 3D structure of hTFF2 will help to elucidate its mode of action in gastrointestinal protection and wound healing. Figure 1: hTFF2: a) sequence, b) structure modelled after homologous porcine TFF2.

Selected Fungal Natural Products with Antimicrobial Properties

Dorota Jakubczyk (Institute of Bioorganic Chemistry, Polish Academy of Sciences)

Natural products are an invaluable source of new drugs as they constitute more than two-thirds of clinically used antibiotics and 50% of anticancer drugs.1,2 Plant pathogenic fungi produce many secondary metabolites which display high bioactivity. The ergot alkaloids are a structurally diverse group of alkaloids derived from tryptophan and dimethylallyl pyrophosphate (DMAPP) and are produced by so-called ergot fungi such as Claviceps purpurea, Aspergillus japonicus, Aspergillus fumigatus or Neotyphodium lolii.3,4 The potent bioactivity of ergot alkaloids have resulted in their use in many applications throughout human history. Ramularia collo-cygni (Rcc), plant pathogenic fungus is another rich source of secondary metabolites such as rubellins.5 Rcc is responsible for the important barley disease Ramularia leaf spot (RLS). Rubellins might be responsible for the leaf necrosis in RLS and consequently barley yield losses between 20 to 70% all over the world. Despite the pathogenic properties of rubellins these compounds may have beneficial applications in medicine. Miethbauer et al. have observed activities against gram-positive bacteria, even multidrug-resistant (MDR) strains but more extensive screening of the whole bacterial and fungal libraries is desired. I will present chemistry and role of fungal natural products basing on examples of ergot alkaloids and rubellins biosyntheses.

Development of state-of-the-art protein labeling tools to probe the local microenvironment at the surface of macromolecules.

Michał Jakubczyk (Institute of Bioorganic Chemistry Polish Academy of Sciences)

Studies of the inside of the cells are conducted using more and more advanced techniques and instruments giving rise to shifts in our understanding of the way cellular processes take place. It is known that the local microenvironment of functional proteins is not the same as in the bulk of intracellular media and can be indicative of protein state.1 Therefore, methods able to distinguish between a few molecular layers distance from the protein are highly desired. Moreover, for the monitoring of protein functionality to be accurate it must be done with minimum interference to the protein activity. In celluo use of small-molecule probes to get insight into intracellular processes in real time has been realized using different physical properties of the reporter molecules (fluorescence, luminescence, NMR, etc.). The probe must also include in its structure a POI-specific ligand in order to be target selective. The present work focuses on the development of fluorogenic probes and protein labeling tools that comprise of elements with optimal properties for chemical biology applications. The technology utilizes the ligand-directed covalent labeling strategy2 that allows for covalent protein tagging at the same time eliminating the interference to its functionality by cleaving off the warhead - leaving the active site unoccupied. For the in celluo studies of protein microenvironment (viscosity, pH, metal ion concentration, etc.) the probes can be equipped with Si-rhodamines for their optimal optical properties (absorption maximum, extinction coefficient, fluorescence emission maximum, fluorescence quantum yield, fluorogenicity) and controllable membrane permeability (lactone/zwitterion equilibrium).3 Crucial for the performance of the probes are the length and polar character of the linkers connecting the mentioned elements.4 Those properties have to be adjusted by trial-and-error for a specific protein in order to achieve the maximal labeling efficiency. In this work carbonic anhydrase (CA) was selected as a model protein for its previous successful tagging by the ligand-directed labeling strategy.5 CAs are also very attractive targets as they are involved in a large number of processes and their abnormal expression and activity have been associated with different human diseases. Acknowledgements The presented work was done within OPUS 15 project funded by the National Science Centre - Grant 2018/29/B/ST4/01498.

Chemical diversity of native Australian fruits: the unique volatile chemotypes of five finger lime (Citrus australasica) cultivars

Joel Johnson (CQUniversity Australia)

The finger lime (Citrus australasica F.Muell.) is one of six Citrus species endemic to Australia and has attracted recent interest as a potential commercial crop (Delort & Yuan, 2018). In contrast to most established crops, which show very low levels of intraspecific genetic diversity as an inadvertent result of selective breeding efforts, finger limes retain high levels of morphological diversity in the fruit size and colour (Agrifutures Australia, 2017). However, the extent of chemical diversity within this species is unknown. To fill this knowledge gap and aid in future commercialisation efforts for this species, we conducted a comparative analysis of the volatile constituents in five Australian finger lime cultivars. Gas chromatography coupled with single quadrupole mass spectrometry (GC-MS) revealed the presence of at least 113 volatile constituents in the peel extracts. Seventy five of these compounds were positively identified, including at least 10 compounds not previously reported from this species. A further 31 volatile compounds were tentatively identified. All finger lime cultivars had limonene as the most abundant volatile compound (ranging from 61.4-87.5% of the total volatile content), with the predominant chemotypes including limonene/γ-terpinene/citronellal, limonene/β-citronellol/citronellal, limonene/bicyclogermacrene/γ-terpinene and limonene/bicyclogermacrene/β-myrcene. Most of these chemotypes have not been reported in previous work on finger lime volatiles (Delort, et al. 2015). In addition to displaying highly distinctive chemotypes compared to the commercial Tahitian lime (Citrus × latifolia), there was a high level of inter-varietal differences, indicative of extensive chemodiversity within Citrus australasica as a species. Similarly, profiling of the phenolic acids via high-performance liquid chromatography (HPLC) revealed that this chemical diversity extended to the type and abundance of various phenolic acids and flavonoids in both the pulp and peel. Due to the unique organoleptic properties stemming from their volatile constituents, finger limes show promise for commercial development as a boutique food.

In silico Approaches for Targeting an Essential Mycobacterial Protein, PrsA

Alexander Kingdon (University of Birmingham)

Mycobacterium tuberculosis is a leading cause of death worldwide and with increasing cases of drug-resistance, new treatments options are required. PrsA is an essential protein in mycobacteria and is necessary for arabinogalactan production and nucleotide biosynthesis. This protein is not currently targeted by any drugs in clinical development and represents a potential drug development avenue. Currently, no crystal structure of M. tuberculosis PrsA (MtPrsA) is available, hence the SWISS-MODEL structure, based on M. smegmatis (93% similarity) was utilised. Molecular dynamic simulations were performed on the modelled structure, to gain information on the protein's predicted flexibility. The output trajectory was clustered, and representatives of each major cluster were used in downstream analysis. Ensemble docking, molecular docking on the ensemble of MtPrsA structures, of the GSK-177 prioritised compounds (Ballell et al 2013) was undertaken. The docking predictions were also re-evaluated using machine learning based programmes, including RfScore (Wójcikowski et al. 2017).

This approach yielded several compounds which could be taken forward for further computational analysis and experimental validation. The compound rankings were also retroactively compared to experimental data studying the GSK-177 compounds' interactions with mycobacteria (Wójcikowski et al. 2017). This research represents a potential workflow for future in silico drug development efforts against M. tuberculosis and further work may allow the identified compounds to be used for the treatment of TB.

An integrated biophysical and in silico approach for discovery of Spire2-FMN2 interaction inhibitors

Radoslaw Kitel (Jagiellonian University)

Actin nucleators, which include proteins such as Arp2/3, formins and Spire1/2, represent an important subfamily of actin-binding proteins (ABPs) that facilitates the formation of actin filaments in cells. Interestingly, Spire1/2 and formin FMN2 interacts with each other, forming a complex that is involved in nuclear actin filament assembly upon DNA damage, thereby promoting efficient DNA repair. The number of functions of Spire-FMN2 complex is likely to grow and its contribution to cell biology is still far from well-understood. While compounds targeting Arp2/3 complex and formins are available, no chemical probes disrupting the interaction between Spire and FMN2 have been discovered so far.

To fill this gap, we undertook a fragment-based screen using differential scanning fluorimerty and fluorescence polarization assays followed by ultimate hit validation with NMR spectroscopy (15N-HSQC). This led us to the discovery of several potent fragments that binds selectively to Spire2, but not Spire1, leading to inhibition of its interaction with formin FMN2. Furthermore, we used in silico techniques including molecular docking and electrostatic complementarity measurements that form solid foundation for successful elaboration of fragments into potent inhibitors.

Organoruthenated nitroxoline derivatives exert potent antimetastatic action through cathepsin B inhibition

Jakob Kljun (University of Ljubljana, Faculty of Chemistry and Chemical Technology)

The proteolytic activity of the lysosomal cysteine peptidase cathepsin B (catB) plays a critical role in the molecular mechanisms of cancer progression. In 2011, the antibacterial agent nitroxoline (nxH), a member of the 8-hydroxyquinolone family, was shown to be a potent reversible inhibitor of catB .[1] Its 7-substituted derivatives potently impair tumor progression in both in vitro and in vivo models and these effects correlate with catB inhibition (Figure 1).[2] We continued to explore the chemical space of the 5-nitroquinoline scaffold by organoruthenation with [η6-p-cymene)Ru(X)] species (where X = Cl, Br, I or N3) at positions 1 and 8 of the nitroxoline ring (Figure 2).[3] By synthesizing 11 ruthenium compounds bearing either the clinical drug nxH or its potent cathepsin B (catB) inihibiting derivatives, we have shown that organoruthenation of the nxH lead scaffold is a viable strategy to obtain highly potent and specific inhibitors of catB endo- and exopeptidase activity, as demonstrated by enzyme kinetics and microscale thermophoresis. Moreover, we demonstrated that the novel metallodrugs significantly impair processes of tumor progression by catB inhibition in in vitro cell-based functional assays at low non-cytotoxic concentrations.

The present study provides us with several insights into the influence of chemical structure on the pharmacological properties of this class of compounds. In general, we observed an improvement in catB inhibition, a reduction in extracellular matrix degradation and tumor cell invasion compared to free ligands, and a correlation with the reactivity of the monodentate halide leaving ligand (Cl > Br > I ≈ azide). These results are the culmination of our research on biological properties of ruthenium-hydroxyquinoline complexes.[3,4,5]

Structure-Based Design of Anti-Adhesive Glycomimetic Inhibitors of Virulence Factor of Drug Resistant Burkholderia Cenocepacia

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Burkholderia cenocepacia is a gram-negative bacterium responsible for deadly lung infections in immunocompromised or cystic fibrosis patients, which presents extreme resistance to almost all clinically used antibiotics.[1] B. cenocepacia produces a lectin called BC2L-C which acts as a virulence factor involved in adhesion to host cell and subsequent infection process.[2] The N-terminal domain (BC2L-C-nt) of the lectin has been characterized as a novel fucose-binding domain with a TNF-α-like architecture while the C-terminal domain specifically binds to the mannose.[2] Therefore, BC2L-C-nt is an interesting target for designing molecules for anti-adhesive therapy which can prevent lectin mediated bacterial adhesion to the host epithelium. Structure-based virtual screening of a small fragment library identified potential hits predicted to bind in a new region (site X, Fig.1) in the vicinity of the fucose binding site.[3] The interaction of the fragments with the protein domain was confirmed using several biophysical techniques including STD-NMR. The X-ray structure of BC2L-C-nt complexed with one of the identified fragments confirmed binding at the expected location and therefore the druggability of site X. Connecting the fragments to the fucose core was first performed in silico, resulting in the design of several fucose-derivatives. Experimental validation through chemical synthesis, bioassays and structural characterisation is in progress.

Emergence of homochirality in large molecular systems

Gabin Laurent (Gulliver UMR CNRS 7083, ESPCI Paris, Université PSL, 75005 Paris, France)

The selection of a single molecular handedness, or homochirality across all living matter, is a mystery in the origin of life. Frank's seminal model showed in the fifties how chiral symmetry breaking can occur in non-equilibrium chemical networks. However, an important shortcoming in this classic model is that it considers a small number of species, while there is no reason for the prebiotic system, in which homochirality first appeared, to have had such a simple composition. In this work, using random matrix theory, we show that large non-equilibrium reaction networks undergo a generic and robust phase transition towards a homochiral state as a consequence of the fact that they contain a large number of chiral species. We also quantify how abundant are chiral species in the chemical universe of all possible molecules of a given length by data analysis, to determine the caracteristic molecule size beyond which this symmetry breaking could occur easily. Finally, we propose that Frank's model should be extended to include a large number of species, in order to possess the transition towards homochirality as confirmed by numerical simulations.

Ultra-sensitive luminescence characterization as a novel approach in medical sensing and fingerprint recognition

Aneta Lewkowicz (1 University of Gdańsk, Faculty of Mathematics, Physics and Informatics)

Luminescence probes analysis is a significant area of fluorescence spectroscopy. In this project we propose fluorogenic probes e.g. 1,8-diazafluoren-9-one (DFO) for amino acids detection [1,2]. DFO itself is weakly fluorescent, but upon complex formation with α-amino acids its fluorescence intensity dramatically increases. The fluorogenic probes have been used for determination of protein concentration and for detection of low molecular weight amino acids in chromatography. Moreover, such probes can be also successfully used as FRET labels. The right fluorescent properties of the DFO probes make them a perfect candidate for sensitive and specific biomarkers. In the literature, we can find information about proposed putative urogenital tract cancer markers [3]. Nevertheless, highly specific, sensitive and universal biomarkers in cancerogenesis have not been found yet. The α-amino acids are important as potential biomarkers of urogenital tract cancer.

In this work, we demonstrate the innovative approach which combines analytical and spectroscopic methods for selection of potential biomarkers. Such materials may in the future be an essential tool in clinical diagnosis of cancer's presence based on analysis of biological samples or amino acids detection. Additionally, we explore the possibilities of application of innovative forms of materials in analysis of amino acids in latent fingerprint residue[4].

Phenotypic and machine learning approaches to hit to lead optimization of broad spectrum non-toxic antiparasitic agents

Pasquale Linciano (Department of Drug Sciences, University of Pavia)

Machine learning approaches are being used increasingly in pharmaceutical drug discovery (1) and have been used by several groups including our own for virtual screening of neglected tropical diseases such as Ebola, Chagas and against Mycobacterium tuberculosis (Mtb) datasets with Bayesian models (2). We have identified broad-spectrum anti-infective chemotherapy agents with activity against Trypanosomes, Leishmania and Mtb species from a phenotypic screening program using the 456 compounds composing the TydockPharma library (Ty-Box). The compound library was screened in whole cell based HTS campaigns against the mentioned pathogen species. Moreover, since the potential liability and toxicity represent a limiting bottleneck in the progression of the compounds in the preclinical phase, the entire library was evaluated at an early stage for drug-drug interactions (CYP inhibition) and human toxicity adopting in-vitro HTS technologies (3,4).

The prioritization of compounds was guided by chemoinformatic approaches to identify the best primary hits for antiparasitic potency and reduced/null human toxicity. In addition, Bayesian models were generated to identify the structural features of each chemotype accounting for activity and toxicity to guide the design and preparation of a second library of optimized hits to provide a quality lead compound, Ty-40, with a potential for further refinement toward a preclinical candidate. Subsequent in-vitro characterization confirmed the predictive models for the promising and innovative chemical scaffold with low micromolar activity against the parasites, with acceptable half-life after IV administration. The pharmacokinetic results warrant a lead optimization program and/or a formulation strategy to ameliorate the PK profile of the compound.

Combination of natural product ring distortion and pseudo-natural product design yields stereochemically and biologically diverse pseudo sesquiterpenoid alkaloids

Jie Liu (Max-Planck Institute of Molecular Physiology)

Structurally complex and diverse natural products (NPs) display diverse biological activities and inspire the synthesis of new bioactive compound classes. We describe the synthesis and biological evaluation of a new natural product-inspired compound class obtained by combination of the conceptually complementary complexity-to-diversity ring distortion-[1] and pseudo natural product (pseudo NP)[2] design strategies. Fragment-sized α-methylene-sesquiterpene lactones (SLs) whose different scaffolds can formally be viewed as related to each other or are obtained by ring distortion were combined with alkaloid-derived pyrrolidine fragments by means of highly selective stereocomplementary 1,3-dipolar cycloaddition reactions[3].

Cheminformatic analysis and morphological profiling in the cell painting assay revealed that the resulting pseudo sesquiterpenoid alkaloids are both chemically and biologically diverse, and that biological performance distinctly depends on both the structure of the sesquiterpene lactone-derived scaffolds and the stereochemistry of the pyrrolidine fragment. Biological investigation of the compound collection led to the discovery of a novel chemotype inhibiting Hedgehog dependent differentiation of multipotent murine mesenchymal progenitor stem cells into osteoblasts.

Paper-based biosensors with rare-earth-doped nanoparticles for the quantification of glucose concentration.

Gabriel López-Peña (Universidad Autónoma de Madrid, Applied Physics Department)

Over 59 million people in the EU suffer from diabetes, and the number continues to rise [1]. The control of diabetes is largely based on the use of glucose sensors. Currently, the typical procedure for glucose control involves extracting a drop of blood. With an increase of sensors glucose-sensitivity it will be possible to measure the concentration of glucose from other body fluids like tear drops. We propose the use of paper-based biosensors in combination with rare-earth-doped nanoparticles (NPs) for the detection of glucose. Due to the positive surface of the NPs, sugars can easily bind to them by electrostatic interaction. The detection method takes advantage of the changes in the emission suffered by the NPs when there is OH in the surrounding medium, which is an indicator of the presence of glucose. Er3+ ions are used as a probe because of the changes in the green-to-red intensity ratio when there is glucose in the medium[2]. These NPs are deposited in paper strips. Then the strips are introduced in a glucose-rich medium in and the green-to-red ratio is measured for the quantification of the concentration of glucose. With this strategy, we propose a first step towards a more reliable, cheap and accessible paper-based biosensors for the detection of glucose.

Vibrational, structural and kinetic features of Cobalamins: an ESI FT-ICR MS study.

Alessandro Maccelli (Università degli studi di Roma, La Sapienza)

The most relevant Co-based biomolecule in human body is Cobalamin (Cbl), a water-soluble cofactor involved in radical-based rearrangements and methyl cation transfers, with notably radical scavenger skills[1]. It is described as a corrin ring containing a Co metal ion in a formal Co(III) oxidation state in an octahedral geometry. A 5,6-dimethylbenzimidazole (DMBI) represents a built-in axial ligand at the end of a peripheric long chain pendant bound to the metallic center either in a base-on or base-off form. Scavenging properties of Cbl towards small inorganic ligands are well documented. Among them, nitric oxide (NO) is known to form a Cbl derivative, nitrosylcobalamin (NOCbl)[2]. This intermediate has been reported as an antitumor agent, utilizing the cofactor transport protein as specific cell receptor acting as a 'Trojan Horse'[3]. On the other hand, Cbl works as efficient NO scavenger reversing the muscle relaxation and the NO-induced cellular growth response. Despite the biological significance of this interaction, only few studies have been reported on this topic. In addition, Cbl derivates (Cbls) show therapeutic activity against high NO levels, like in septic shock. A relevant question concerns the formal Co oxidation state in NOCbls, either Co(III) or Co(II). Herein, an integrated approach based on FT-ICR MS (Fourier-transform ion cyclotron resonance mass spectrometry), IRMPD (Infrared multiple photon dissociation), and ion-molecule reactions with NOx (x=1, 2) has been applied. In the gas phase, selected families of Cbls ions have been submitted to structural and reactivity investigation. Vibrational features of [Cbl(II)+Na+]+ have revealed a possible co-presence of base-off and base-on forms. A different behavior applies to [Cbl(III)+]+, for which the exclusive existence of a base-off conformer has emerged. All the species studied undergo addition reactions with NO and NO2 neutrals, although the latter neutral show greater affinity for Cbls ions. Interestingly, hydrogen atom transfer (HAT) reactions have emerged for [Cbl(III)+]+ species, paving the way to further in depth studies on these cofactors.

A quantitative study of cAMP signaling in urinary bladder smooth muscle towards the overactive bladder pathophysiology

Chitaranjan Mahapatra (University of California San Francisco)

Urinary bladder smooth muscle (UBSM) exhibits increased spontaneous phasic contractions under pathophysiological conditions such as the overactive bladder (OAB) [1]. Enhanced spontaneous electrical activities in the form of action potentials (sAP), depolarization and hyperpolarization are associated with the underlying spontaneous contractions [1, 2]. The large-conductance voltage- and Ca2+-activated K+ (BK) ion channels are key regulators of UBSM cell excitability and it has been predicted that activation of nanodomain cAMP signaling pathways modulates contractility by altering the BK channel activity [3, 4]. This quantitative study investigated the hypothesis of whether the rise of internal cAMP levels can modulate the generation of sAPs to regulate the UBSM excitability by coupling BK channel activity. A cAMP signaling pathway is established using the internal cAMP concentration. Then the cAMP model is integrated into a whole cell UBSM model [5], which consists of nine ion channels including BK channels and independent calcium dynamics.

We investigated the modulating effects of cAMP signaling pathway on BK ion channel current, resting membrane potential (RMP), AP and internal Ca2+ concentration. BK currents were evoked by applying a 200-ms depolarizing pulse every 5 s in 20-mV increments from a holding potential of 20 mV to− 120 mV. The RMP of the set to− 50 mV. The AP is generated after injecting a current of 10 nA for 10 ms. It is observed that the rise of internal cAMP hyperpolarized the RMP to− 55 mV. The cAMP-induced a left-shift to the BK ion channel's steady-state activation curve, and then the outward BK ion channel current increased. It also reduced the internal Ca2+ concentration and as a consequence, it does not evoke the AP for the above-mentioned current injection due to less excitability. In conclusion, this study reveals that the endogenous phosphodiesterases, which hydrolyze cAMP may lead to novel therapeutics for overactive bladder.

Innovative chemistry to illuminate biology: Novel fluorescent ligands to study GPCRS binding and signaling

Maria Majellaro (Celtarys Research)

For a long time, radioactivity has been the "gold standard" in high throughput screening and other pharmacological assays to study GPCRs. Due to its specific requirements, environmental concerns, and the costs associated to these experiments, fluorescence-based assays started to widespread and gradually replaced radioactivity. Among the remarkable advantages, fluorescent ligands demonstrated to be suitable for different type of experiments such as fluorescence microscopy, high content screening, FRET, HTRF and BRET in both pre-transfected and living cells(1). However, fluorescent ligand employment has not yet been adopted widely due to the challenging development process and the consequent lack of optimal fluorescent probes available for the majority of the targets of interest. In Celtarys Research we have developed a rational and versatile synthetic strategy enabling to rapidly identify optimal fluorescent probes to tag diverse molecular targets. The relevant role of GPCRs in drug discovery (2) led us to start applying our technology in this field, developing fluorescent probes for different receptor families and applications.

Herein we document some representative case studies, such as the applicability of fluorescent probes developed by us in High Content Screening, Fluorescence Polarization, Flow Cytometry and Fluorescence Microscopy in living cells so evidencing the potential and robustness of this core technology in the context of GPCR research.

Design of Biocompatible Carborane Carriers for Boron Neutron Capture Therapy

Tainah Dorina Marforio (University of Bologna - Chemistry Department "G. Ciamician")

Boron Neutron Capture Therapy (BNCT) is a non-invasive, therapeutic approach for the treatment of different types of cancers (Figure 1). The effectiveness of BNCT is based on adequate and selective accumulation of 10B in the tumor tissue (approximately 109 atoms/cell), which is still the major problem in drug development for BNCT. Blood-brain barrier permeability, low toxicity to healthy cells and persistence in the malignant tissues are the "golden rules" for effective BNCT agents.3 Nowadays only two drugs, BPA (boronphenylalanine) and BSH (sodium borocaptate), are clinically used in BNCT. Icosahedral closo-carboranes ($C_2B_{10}H_{12}$) promise to be good candidates in BNCT because of the presence of 10 boron atoms (Figure 2) and a lipophilic character that eases the cross of hydrophobic membranes. Nevertheless, their insolubility in water requires the design of delivery vehicles able to carry closo-carboranes to the target (drug delivery system). Peptides and proteins2 are the best candidates for boron delivery, since they are biocompatible, water soluble and naturally recognized by cells. The biological performances of these biomolecules as carriers depends upon the affinity of the carborane with the amino acid sequence of the host. By means of in silico methods we ranked the interaction of each amino acid towards three carborane isomers: ortho-carborane (1,2-$C_2B_{10}H_{12}$), meta-carborane (1,7-$C_2B_{10}H_{12}$) and para-carborane (1,12-$C_2B_{10}H_{12}$). The affinity of the amino acids with the carboranes is calculated by Molecular Dynamic (MD) simulations followed by Molecular-Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) analysis.3 This protocol quantifies the binding affinity of each complex, defining the non-covalent interactions (hydrogen bonding, hydrophobic, aromatic, cation-π, anion-π, surfactant-like, electrostatic) that govern the binding.

Our results suggest that charged residues display the highest affinity towards the carborane cages, and that negatively charged sidechains (aspartate and glutamate) strongly bind ortho-, meta- and para-carboranes (Figure 3). These results are essential for i) the design of linear or cyclic peptides ii) the identification of protein hosts, able to disperse carboranes in water and to deliver them to the target cell. The results reported here are part of a computational-experimental project funded by AIRC (Italian Association for Cancer Research) and supported by PRACE-ICEI aimed to identify new vehicles for boron delivery in BNCT.

Design of a novel generation of glycan mimetics to inhibit MGL-mediated immune tolerance

Nuria Martinez-Saez (Glycotechnology Group. CIC biomaGUNE)

The appearance of aberrant glycans on the tumor cell surface is one of the emerging hallmarks of cancer. Tumor growth is accompanied by tumor evasion of the immune system which limits the efficacy of cancer vaccines. However, the role of tumor glycosylation in immune evasion has mostly been overlooked, despite the fact that aberrant tumor glycosylation alters how the immune system perceives the tumor and can also induce immunosuppressive signaling through glycan-binding receptors. New strategies to avoid the immune escape mechanisms generated by tumor cells are required. The interaction between the immune system and Tumour-Associated Carbohydrates Antigens (TACAs) is facilitated by a diverse set of carbohydrate-binding receptors, as the C-type lectin receptors (CLRs) which mediate specific interactions with TACAs controlling many features of the immune response. Human macrophage galactose-type lectin (hMGL) is a CLR that recognizes terminal GalNAc moieties, and is, therefore, a prime receptor for the aberrant glycans in cancer. It is upregulated in immature dendritic cells (DCs) and macrophages interacting with terminal GalNAc of the CD45 protein of effector T cells, resulting in reduced proliferation, cytokine secretion and induction of T-cell apoptosis.

Given the important role that MGL plays to evade the immune system, we designed and synthesized a library of multivalent MGL ligands mimetics that could serve as selective inhibitors to reverse GalNAc-mediated immune suppression for cancer immunotherapy. Antigens as LacdiNAc (LDN; GalNAcβ1-4GlcNAc) and the fucosylated LacdiNAc (LDNF; GalNAc(Fucα1-3)β1-4GlcNAc) are natural substrates of MGL. We synthesized LDN and LDNF containing oligosaccharides mimetics, introducing structural modifications at relevant positions of these molecules to improve their affinity with hMGL. For their obtaining, N-azidoacetylgalactosamine (GalNAz) and 6-azidofucose (FucAz) were enzymatically incorporated into the G0 oligosaccharide. The azido group was employed to incorporate different prosthetic groups by click chemistry. The evaluation of the binding affinity between our glycomimetic library and hMGL was performed by microarrays, showing promising results in the design of a novel generation of glycan mimetics that could act as inhibitors of MGL interaction with cancer cells, blocking the downstream signaling that culminates in immune suppression.

Hypoxia-activated nitroaromatic compounds supported nanostructured silica-based systems for cancer therapy

José M. Méndez-Arriaga (COMET-NANO Group, Departamento de Biología y Geología, Física y Química Inorgánica, ESCET, Universidad Rey Juan Carlos)

Many malignant solid tumours induce an imbalanced oxygen demand by cancer cells that are rapidly dividing, creating hypoxia conditions [1]. Nitro containing compounds, including nitroaromatics, have been demonstrated bioreductive activity that can be selectively activated in the hypoxic tumour environment [2]. One of the most interesting approaches for obtaining more selective metallodrug-functionalized systems is the use of nanostructured silica-based materials, which have recently been considered very interesting scaffolds for the development of drug delivery systems [3]. Several nitrobenzoic acids and nitroaromatics compounds were selected and supported in two different nanostructured silica-based systems (SBA-15 and MSN) to use them as therapeutic agents. They also have been further modified with fluorescent agents to add a diagnostic application to the nanomaterials. In vitro tests are carried out at present time to determine the best materials to be promoted to the next step of anticancer analysis.

Development of a covalent binding tool: Furan Crosslink Technology

Laia Miret Casals (Ghent Univeristy)

Our research group has developed a novel furan crosslink technology for oligonucleotides, further applicable to the investigation of peptide-protein interactions (1-2). Furan, a small aromatic compound, is incorporated in peptides using 2-furyl-L-alanine. The furan moiety can be selectively oxidized to a reactive aldehyde upon generation of singlet oxygen. A site-selective crosslink reaction occurs between the oxidized furan moiety and sulfhydryl and/or amine groups present in the protein if sufficiently proximal. As the furan moiety is isosteric with histidine and isoelectronic with tyrosine, the incorporation in peptides is well tolerated. Here, we have developed a fast and highly efficient furan-oxidation mediated technology for protein-protein and peptide-protein interactions in two different systems. Initially, we studied the interaction between actin, the major cytoskeletal protein of the cell that forms filaments, and Tβ4 that regulates the polymerization of actin and keeps the actin in the monomeric form. The furan-modified Tβ4 analogues were still able to sequester monomeric actin with comparable capacity as wildtype Tβ4, and moreover, to efficiently crosslink to monomeric actin by singlet oxygen generation by irradiation with a white light source in the presence of a photosensitizer. The peptide-receptor complex was submitted to in-gel tryptic digestion and later by AspN or chymotrypsin digestion in solution. The isolated peptides were analyzed by mass spectrometry (MS) and searches for crosslinked peptides were performed using xiSEARCH v1.7.6.1. We have been able to identify the crosslinked peptide and also to pinpoint the exact crosslinked amino acid of the actin protein to the furan-modified Tβ4 analogue. The furan crosslink technology was further optimized to enable crosslinking of furan-modified peptide ligands to GPCR proteins on live cells with spontaneous endogenous oxidation of the furan moiety. We studied the neuropeptide kisspeptin-10 and its G-protein coupled receptor GPR54, which play a role in breast cancer and in the regulation of mammalian reproduction. We have described selective crosslinking of a furan-modified kisspeptin-10 analogue to its membrane receptor GPR54 in live cells, with no toxicity and high efficiency (1). In addition, we have been able to pull-down the peptide-receptor complex using a Biotin-furan-modified kisspeptin-10 and it is being characterized by MS analysis.

Combining Rational and High-Throughput Strategies for the Identification of Tsg101-UEV Ligands of Interest as Broad-Spectrum Antivirals.

Fernando Jesus Montero Segovia (University of Granada)

PTAP viral Late domains are short and conserved sequences found in multiple viruses, such as Ebola, HIV or HTLV, that mediate viral budding through the interaction with the UEV domain of human TSG101 (TSG101-UEV)(1=. Blocking TSG101-UEV/Late domain interactions has been shown to inhibit viral egress (2). Thus, searching for inhibitors of these interactions is an attractive strategy for generating novel broad-spectrum antivirals. Tsg101-UEV is a challenging target for rational design due to the low affinity of its natural interactions and its flat and featureless binding interface. To overcome these difficulties we have implemented a multidisciplinary approach combining biophysical studies and rational approaches with high-throughput screening methodologies.

We present here the results of a detailed structural and thermodynamic analysis of the binding of TSG101-UEV with peptide ligands derived from the Late domains of HIV, Ebola and HTLV and the screening of small compound libraries. Using an in vitro thermal shift assay a set of small-molecule ligands have been identified that bind Tsg101-UEV with microM binding affinities. The ability of these compounds to block the interaction between full-length Tsg101-UEV and HIV-p6 has been validated through high-content cellular assays and the inhibitory activity of viral budding has been tested using Virus-like particles and live virus assays.

The results of these two complementary approaches have produced valuable information about the molecular determinants of binding affinity in this system, of interest for the development of broad-spectrum antivirals.

Near-Infra Red Organic Photosensitizers for Photodynamic Therapy

Maria Jesus Moran Plata (University of Torino, Department of Chemistry, NIS Interdepartmental and INSTM Reference Centre.)

Photodynamic therapy (PDT) is a clinical approach adopted worldwide to treat pre-cancerous and cancerous diseases based on a photochemical reaction between a light activatable molecule or photosensitizer (PS), light, and molecular oxygen. When the PS molecule is irradiated by light at a specific wavelength, highly cytotoxic singlet oxygen and reactive oxygen species (ROS) are formed, causing damage to targeted cancer cells[1]. An ideal PS should fulfill specific clinically relevant requirements: intense absorption in "the biological tissues" transparency window (600-900 nm), high molar absorption coefficients, remarkable brightness and photostability, especially in the biological environment. Among numerous PS already proposed, NIR polymethine dyes (PMD), such as cyanines (CY) and squaraines (SQ), have been extensively studied for many biomedical applications[2][3]. However, despite their excellent photodynamic activity, their chemical instability and self-aggregation properties when in contact with biological media still limit their effective clinical application. To overcome these drawbacks, the incorporation of these dyes in nanoparticles (NPs) is extremely important to prevent the formation of dye aggregates in aqueous environment. Here we present new series of CY and SQ dyes synthetized by microwave irradiation and photophysically characterized by UV-Vis and fluorescence spectroscopy. Afterwards, the ROS production ability of the CY and SQ samples has been evaluated using the 1,3-diphenylisobenzofuran (DPBF) assay. In presence of ROS, the decrease of the UV-Vis absorbance intensity of DPBF was recorded and compared with the results already optimized in presence of other well-known PS reported in literature, i.e.: methylene blue and rose Bengal. In addition, the in vitro biological assessments were examined by exposing the cells to increasing concentration of each PSs to select the maximum non-cytotoxic concentration suitable for performing the in vitro PDT. Finally, the potential photocytotoxicity of each squaraine and cyanine dyes was evaluated by performing an in vitro photodynamic treatment, showing low cytotoxicity in dark, but promoting phototoxic effect upon irradiation. On all the series of the tested PMD an unexpected structure-activity relationship was evidenced.

Pattern-Generating Fluorescent Molecular Probes for Chemical Biology

Leila Motiei (Weizmann Institute of Science)

Fluorescent molecular probes have become a powerful tool in protein research. However, these probes are less suitable for analyzing specific populations of proteins in their native environment. In this talk, I will give an overview of a new class of fluorescent molecular probes [1-5] recently developed in our group, and show how they can be used to detect individual proteins, protein combinations, as well as binding interactions and dynamic changes that occur on their surfaces. In the second part of this talk, I will present a new class of fluorescent molecular sensors that combines the properties of small molecule-based probes and cross-reactive sensor arrays (the so-called chemical nose/tongue') and explain how these pattern-generating probes could expand the fluorescent toolbox currently used to detect and image proteins. [5]

Specifically, I will show how such systems can be used to identify combinations of specific protein families within complex mixtures and to discriminate among protein isoforms in living cells, where macroscopic arrays cannot access.[5]

Ebselen-inspired multitarget compounds as potential neuroprotective agents.

Miguel Muñoz Silva (Department of Chemistry in Pharmaceutical Sciences (Organic and Medicinal Chemistry Unit), Universidad Complutense de Madrid)

Neurodegeneration of the central nervous system is characterized by a progressive loss of neuronal structure and function resulting in physical and mental impairments. Some of the most common and prevalent neurodegenerative diseases include Alzheimer´s disease, Parkinson´s disease, Huntington´s disease and amyotrophic lateral sclerosis. Historically, the "one molecule – one target – one disease" paradigm has been the basis of drug discovery. This approach has not been successful for neurodegenerative diseases, probably due to the complex and multifactorial nature of their pathological mechanisms and thus there is much current interest in the multi-target drug approach in connection with these diseases.(1) Many of them share pathological mechanisms including alterations in redox homeostasis, mitochondrial dysfunction and neuroinflammation, among others. In particular, the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the subsequent oxidative damage plays an important role in the progression of neurodegenerative diseases. Ebselen (EBS, 2-phenyl-1,2-benzisoselenazol-3(2H)-one), is a non-toxic organo-selenium compound that has attracted considerable interest in medicinal chemistry in view of its ability to mimic glutathione peroxidase (GPx), an antioxidant enzyme that catalyzes the reduction of hydroperoxide molecules at the expense of glutathione (GSH).(2) In this context, we describe the design, synthesis and chacterization of a library of hybrid compounds that combine the core of ebselen with structural elements designed to achieve additional antioxidant and anti-inflammatory properties by induction of the NRF2-ARE Phase II antioxidant response. These derivatives were obtained using as the key step a thermal copper-promoted C-Se bond formation with KSeCN as a source of selenium.(3) Moreover, using a conventionally applied nuclear magnetic resonance spectroscopy assay, all derivates were tested as antioxidants by application of a GPx-like catalyst model. (4)

Nanohybrid cerium-enzyme nanogels as fluorescent biosensor

Pablo Muñumer (Universidad del País Vasco - Euskal Herriko Unibertsitatea (UPV-EHU))

Recent research shows that encapsulating single enzymes within a thin polymeric layer gives raise to biomaterials with higher stability against denaturation [4]. The resulting material, so-called single enzyme nanogel (SEN), also serve as scaffold for the design of new functional nanomaterials with outstanding downstream applications [1]. Glucose oxidase (GOx) is an enzyme that has been broadly used in glucose sensing technologies as it triggers redox response in the presence of glucose by releasing hydrogen peroxide [2]. Herein, lanthanide-bearing glucose oxidase (GOx) SENs are employed as fluorescent glucose sensors (Fig. 1). The SENs were synthetized by encapsulating GOx in-situ into a polyacrylamide-based nanogel with phosphate moieties, which were subsequently coordinated with Ce(3+) cations. The fluorescent emission of the Ce(3+) increased when coordinating to the phosphate groups, as was previously reported [3] (Fig 2). This Ce(3+) oxidized to Ce(4+) by the hydrogen peroxide produced by GOx, hence quenching the emission in response to the glucose concentration (Fig. 3). This sensor showed trace-level sensitivity, having a LOD of 168.9 nM. The sensitivity of this system is comparable with other high-sensitivity fluorescent glucose sensors [3]. The successful design of this glucose integrated nanosensor enlightens new routes on biochemical sensing.

How to keep mosquitoes from biting: Modeling conformational changes of muscarinic acetylcholine receptors in response to ligand binding.

Beata Niklas (Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University)

Malaria is disease spread by mosquitoes that affects over 220 million people annually resulting in about half a million deaths each year. Unfortunately, the most commonly used repellents (i.e. DEET) lose their activity as mosquitoes become resistant and there are many concerns regarding their safety. Therefore, there is a high need for new generation of mosquito repellents. Muscarinic acetylcholine receptors (mAChRs), which belong to the G protein-coupled receptors family, play a crucial role in both human and insect neuronal system. Recent studies on D. melanogaster showed that they modulate olfactory responses and food-seeking behavior [1] thus being a potential target for a new generation of repellents. By using molecular dynamics (NAMD, CHARMM, 200 ns) we investigated the conformational changes of M1 mAChR receptor induced by docking of insect repellents (DEET, IR3535), M1 agonists, antagonists and modulators.

Knowing the molecular basis of these ligand induced changes is the key to finding compounds that would serve as selective malaria vectors repellents, hopefully having no side effects in human.

Elucidating the mechanism for the biosynthesis of N-acetyl desferrioxamine siderophores

Kate Nolan (School of Medical Sciences (Pharmacology), The University of Sydney)

Desferrioxamine B (DFOB) is a tri-hydroxamic acid siderophore biosynthesised by Streptomyces pilosus and other bacteria within the Actinomycete phylum. It is used clinically to treat secondary iron overload that occurs from the treatment of transfusion-dependent blood disorders such as beta thalassaemia. DFOB biosynthesis occurs via a nonribosomal peptide synthetase independent pathway, involving a cascade of four enzymes, the DesABCD cluster [1,2]. DesC is an acyl transferase that catalyses the N-acetylation or N-succinylation of N-hydroxy-1,5-diaminopentane (HDP). This generates N-acetyl-N-hydroxy-1,5-diaminopentane (AHDP) or N-succinyl-N-hydroxy-1,5-diaminopentane (SHDP), respectively. DesD then condenses one SHDP monomer to an AHDP-SHDP dimer to produce the trimeric siderophore. [3]. DFOD1 is an N-acetylated variant of DFOB, identified in culture supernatant of S. pilosus and other bacteria such as Salinispora tropica [4]. N-Acetyl fluorinated analogues of DFOB were detected in a recent study which prompted the current study on the biosynthesis of these constructs. This work is focused on delineating the steps involved in the biosynthesis of N-acetylated DFOD1 and precursors. DesC and DesD have been recombinantly expressed from S. tropica and overproduced in Escherichia coli [5], with the purified protein used for functional studies with synthetic precursors of the siderophore assembly.

Functional studies will focus on whether DesC is capable of iterative cycles of acylation. Studies will also address whether DesD is capable of condensing the acetylated monomers. This work will report results that provide new knowledge on the biosynthesis of this subclass of siderophore. These mechanisms could have wider implications for the N-acetylation pathway for other natural products.

Reversible Control of DNA Binding with Cucurbit[8]uril-Induced Supramolecular 4,4'-Bipyridinium–Peptide Dimers

Paula Novo Valencia (Departamento de Química, Facultade de Ciencias and Centro de Investigacións Científicas Avanzadas, Universidade de Coruña)

Regulation of many cellular processes in living organisms, such as gene expression, is interestingly performed by diverse noncovalent protein-based complexes conforming intricate regulatory networks, normally by converting between homo- and heterooligomeric assemblies or mono- and bivalent states (1). Inspired by these natural complexes, chemists have developed supramolecular systems that have shown potential for the regulation of protein assemblies, modulating their function by means of host–guest interactions (2). In this regard, the macrocyclic host cucurbit[8]uril (CB[8]) stands out due to its unique ability to form 1:2 hetero- or homodimeric inclusion complexes with a variety of guests. Particularly, the CB[8]-bipyridinium pair constitutes a supramolecular switch that allows for further implementation of dynamic behaviour due its sensitivity to external stimuli (3). In this context, to the best of our knowledge, the use of the CB[8]-bipyridinium pair to reversibly control the dimerization and the biological activity of functional peptides has barely been explored. For this purpose, we have developed a 4,4′-bipyridinium-peptide conjugate (P1)(4), based on the basic region of the Basic Leucine Zipper transcription factor GCN4, which binds to its target DNA (ATF/CREB) upon forming a supramolecular homoternary complex with CB[8](5). UV spectroscopy allowed us to study the P1-CB[8] interaction, obtaining a good quality fitting for a 2:1 sequential binding model for the supramolecular complex. Subsequent studies of the DNA binding capabilities of the supramolecular dimer P12:CB[8] by circular dichroism spectroscopy, allowed us to demonstrate that P1 only binds to ATF/CREB as a supramolecular dimer in the presence of CB[8] with an apparent dissociation constant of 75 nM. Importantly, we have also demonstrated that the P12:CB[8]:ATF/CREB complex can be conveniently disrupted in a reversible fashion by disassembly of the host–guest complex upon changing the pH or the addition of a specific competing guest.

This work constitutes a new approach to the supramolecular control of peptide assemblies, offering the possibility of designing new peptide conjugates that can be implemented into complex protein-based networks, and could be reversibly controlled in a straightforward fashion by using the robust and well-established host–guest chemistry of CB[8].

A new phosphorescent iridium based drug with potent in vitro and in vivo anticancer activity

Enrique Ortega-Forte (University of Murcia)

The interest in the development of metal complexes as anticancer agents is emerging spurred by the success of platinum drugs such as cisplatin. Despite its wide use in the clinic, chemotherapeutic regimen with cisplatin can cause general toxicity, undesirable side-effects and drug resistance phenomena. In order to overcome these limitations, a new iridium(III) complex has been synthetized, and its physico-chemical properties characterized. Its red phosphorescence allowed us to track the location inside tumor cells. The complex was not only able to exert potent in vitro antiproliferative activity towards cisplatin-resistant cancer cells, but also displayed low toxicity to normal dividing cells. Flow cytometry and fluorescence-based assays confirmed an apoptosis-independent mode of cell death in ovarian cancer cells. In addition, a C. elegans tumoral model has been developed to test the antitumor activity, thereby revealing high effectiveness in reducing tumor growth in vivo.

The results obtained in this work provide insights into the combination of both in vitro cell-based experiments and in vivo evaluation using C. elegans for the identification of new metal-based anticancer compounds.

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ISOXAZOLE/ISOXAZOLINE-SUBSTITUTED BENZAMIDE DERIVATIVES AS POTENTIAL PARP INHIBITORS

Konstantinos Ouzounthanasis (Laboratory of Organic Chemistry, Department of Chemistry, Aristotle University of Thessaloniki)

ISOXAZOLE/ISOXAZOLINE-SUBSTITUTED BENZAMIDE DERIVATIVES AS POTENTIAL PARP INHIBITORS Konstantinos A. Ouzounthanasis, Stergios R. Rizos, Alexandros E. Koumbis* Laboratory of Organic Chemistry, Department of Chemistry Aristotle University of Thessaloniki, 54124, Thessaloniki, email: akoumbis@chem.auth.gr Poly-ADP ribose polymerases (PARP) are mostly known for their contribution in the repair of single strand breaks of the DNA and as a result, their biological activity is quite important and valuable. PARP-1 plays an important role in the mechanism of DNA reparation and thus is referred as molecular sensor and "first responder" for single strand breaks in DNA. Inhibition of PARP can be used as a tool primarily against cancer cells in combination with other methods such as radiotherapy and chemotherapy, although PARP inhibitors can be used as single agents [1]. Benzamide derivatives were early recognized as PARP inhibitors [2], whereas Olaparib® is the first PARP inhibitor drug to be approved for use as a single agent both by EMA and FDA [3] (Image 1). (Place Image 1 here) Image 1: Structures of known benzamide PARP inhibitors: 3-aminobenzamide (I) and Olaparib (II). Herein, we describe a novel versatile synthetic plan for compounds that could potentially act as PARP inhibitors (Image 2). Based on the known pharmacophore, our design incorporates the structural feature of benzamide and further introduces an isoxazole/isoxazoline ring. For the construction of the heterocyclic moiety, we initially utilize key 1,3-dipolar cycloaddition reactions between in situ prepared nitrile oxides and appropriate alkene dipolarophiles [4]. Then, the targeted isoxazole or isoxazoline benzamide derivatives are obtained upon simple amidation (non-cyclic III, IV, V) or a rearrangement reaction (locked lactamic VI) [5]. Based on the possible variation of selected substituents (A, B and X) on these frameworks, we expect to obtain a series of libraries for further biological evaluation. (Place Image 2 here) Image 2: Preparation of isoxazole/isoxazoline benzamide libraries.

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Targeting Besides the Peptidoglycan, a Novel Approach for the Development of New Antimicrobial Agents

João Paquete Ferreira (UCIBIO-NOVA)

The ability of Bacteria to grow in sessile communities known as biofilms, together with the rise in antibiotic resistant strains, represents a serious threat, as it gets more difficult to eliminate infections. Development of strategies and therapeutics that can address these challenges is of pivotal importance. Most commonly, therapeutics tend to target the bacterial cell wall, more precisely the peptidoglycan. However, despite this polymer being the main constituent of the bacterial cell wall, there are other equally important components crucial for bacterial survival and adherence to surfaces. One of those biomolecules are the wall teichoic acids (WTAs). Depletion of bacteria from WTAs impairs their growth and division leading to much more susceptible individuals, that have more difficulty causing infection. The biosynthesis pathway of WTAs is quite complex, yet the last step in this pathway occurs outside the cell and is accomplished by the LytR-CpsA-Psr (LCP) family of proteins, making this step a very interesting one to target. In this work we were able to heterologous overexpress the LytR protein from Streptococcus dysgalactiae subsp. dysgalactiae (SDSD), in E. coli BL21(DE3), purify, using affinity and size exclusion chromatography, obtain crystals and solve, for the first time, the protein structure at a 2.80 Å resolution.

Analysis of the structure revealed a high structural homology with other LCP proteins deposited in the PDB and allowed to understand that the conserved molecular determinants important for activity are also present in the determined structure. Characterization of the protein in solution was also performed using SAXS technique. Preliminary results suggest that the protein might adopt a different conformation in solution when compared to the crystal form. Further characterization of the protein is necessary to understand if the protein really has two distinct conformations, and if the presence of a ligand can induce this conformational change in solution. Also, we tried to characterize the interaction of the protein with small molecules that might act as inhibitors, however no interaction was observed. Further characterization might help improve what is known about the mechanism on how these proteins transfer the WTAs to the peptidoglycan, aiding in the development of inhibitors.

Synthesis of enantiopure benzothiazepines and benzodiazepines for their study as calcium channel blockers and neuroprotectors.

José Miguel Pérez (Department of Chemistry in Pharmaceutical Sciences (Organic and Medicinal Chemistry Unit), Faculty of Pharmacy, Universidad Complutense Madrid.)

The improvement in lifespan promotes an increase in age-related diseases, such as neurodegenerative diseases. Neurodegenerative diseases (NNDs) are characterized by the loss of neurons in the brain and / or spinal cord and have become one of the most important health problems worldwide. These diseases are multifactorial pathologies with different etiologies sharing and many mechanistic pathways, namely: protein misfolding, neuroinflammation, impaired mitochondrial function, increased oxidative stress, mitochondrial dysfunction, alterations in calcium homeostasis, among others.1 Dysregulation of intracellular calcium is one of the most striking hallmarks of NDDs. Furthermore, Ca2+ concentration is connected with other dysfunctions, including oxidative stress, energy impairment, and inadequate proteostasis, by a complex biochemical network. NNDs are characterized by abnormally high cytoplasmic calcium levels in neurons. Among many relevant calcium transporters, the mNCX protein is a mitochondrial sodium-calcium exchanger that has an important role in the control of intraneuronal Ca+2 homeostasis.2 For this reason, it is a potentially important target against NNDs, in spite of which there is a notorious lack of mNCX agonists. The chiral benzothiazepine derivative known as CGP-37157, the first compound reported as a selective mNCX antagonist, has become a milestone in this area and has been shown to be neuroprotective in various cell models.3 One of the main limitations found in the use of CGP-37157 is the fact that it has always been tested as a racemic mixture because its pure enantiomers have not been obtained so far.

Therefore, the preparation of both CGP-37157 enantiomers in pure form is an important goal, and is likely to lead to a compound with improved selectivity and potency as an antagonist of mNCX. We describe in this communication our progress towards this goal, using a resolution strategy based on the use of chiral auxiliaries. Another significant problem posed by CGP-37157 is its short half-life in animal models due to rapid oxidative metabolism of its sulfide moiety. These metabolites have not been pharmacologically characterized and they may well be partly responsible for the neuroprotective effects of CGP-37157. We also describe our work towards the synthesis of both diastereomers of the CGP-37157 sulfoxide and the corresponding sulfone.

Design and Synthesis of N-glycomimetics with high affinity for DC-SIGN

Damián Pérez (University of Basque Country - CIC BiomaGUNE)

C-type lectin receptors (CLRs) are a super family of around 1000 carbohydrate binding proteins found on the surface of many cells, and in particular antigen presenting cell (APCs) where they are involved in self/non-self-differentiation by the immune system. Certain CLRs can therefore be considered a form of glycol-immune checkpoint inhibitors. The carbohydrate Lewis antigens that commonly detected on cancer cells, bind to the C-type lectin DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule 3-Grabbing Nonintegrin), driving to innate immune suppression. release of the immune cells from their dormant state by interfering with the pertinent carbohydrate-protein interactions could become a complementary immune therapy approach the treatment of cancer. Synthetic carbohydrate chemistry has been and continues to be the most reliable, scalable and common source for glycans. Even so, the synthesis of very complex such a multiantennial N-glycans is a challenge because of its high cost in time and resources. Herein, egg yolk powder has been used as a gram-scale source of sialilglycopeptide (SGP).

Through organic chemistry and chemoenzymatic truncation/elongation we were able to modify the extracted SGP to prepare a scaffold for two different types of bi-antennary N-glycan mimetics, modifying the natural Lewis X domain: LDNF-type and LeX-Type (Figure 1). In order to obtain a preliminary library of N-glycan mimetics, click reactions with a series of alkynes were performed directly on microchip array and screened against DC-SIGN. This initial screen allowed us to evaluate the potential of the resulting mimetics.

An integrated approach towards the design of new tubulin targeting agents

Helena Pérez-Peña (1), Zlata Boiarska (2), Anne-Catherine Abel (3)

(1,2 - University of Milan, Italy 3 - Paul Scherrer Institute, Switzerland)

Microtubules (MTs) are highly dynamic protein polymers that are part of the cytoskeleton and thus are essential to various cellular processes; including maintenance of cell shape, cell migration, and intracellular transport. For many years the crucial function of MTs in cell division has been exploited for cancer treatment, leading to the discovery of numerous, structurally diverse small molecules able to bind to tubulin, commonly known as microtubule targeting agents (MTAs) [1]. Despite extensive research, many aspects of tubulin dynamics and its regulation remain elusive. In order to further investigate the effect of small molecules on tubulin structure and dynamics, we have established an integrated approach to produce and evaluate new tubulin binders. Here we present our first series of ligands based on maytansine, a highly potent beta-tubulin binding agent [2]. Our integrated approach relies on the combination of computer-aided modelling, synthesis and structural biology working in parallel to solve real-time problems and achieve a common objective. Docking experiments were performed to evaluate the ability of initially designed molecules to bind to the maytansine site on tubulin. Selected molecules were synthesized by the acylation of maytansinol, and their activity on tubulin was assessed biochemically by determining the effect on tubulin polymerization dynamics, on cell viability and by determining their respective tubulin-binding constants. The crystal structures of the most active tubulin-ligand complexes were determined by X-Ray crystallography.

Our results provide the foundation for the development of next-generation MTAs to act as molecular probes for the direct modulation of microtubule stability, which will yield the opportunity to exploit the microtubule breakdown associated to neurodegenerative diseases.

Two for the price of one: Bivalent venom peptide design for modulation of human voltage-gated sodium channels.

Alicia Peschel (Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna)

Voltage-gated sodium (NaV) channels are pore-forming transmembrane proteins that play essential roles in excitable cells, and they are key targets for antiepileptic, antiarrhythmic, and analgesic drugs. Selective modulation of NaV channels is crucial to dissect their role in health and disease, and animal venoms are an established source of pharmacological probes that have been used to target these channels. We implemented a heterobivalent design strategy to modulate the potency, selectivity, and binding kinetics of NaV channel ligands. We conjugated the cone snail venom peptide μ-conotoxin KIIIA which occludes the pore of the channel and prevents sodium entry, to an analogue of huwentoxin-IV, a spider-venom peptide that allosterically modulates channel gating by binding to one of the voltage sensor domains. Orthogonal hydrazide and copper-assisted azide- alkyne cycloaddition conjugation chemistries were employed to generate a series of bivalent ligands using heterobifunctional polyethylene glycol linkers spanning 40–120 Å. The bivalent ligand with an 80 Å linker had the most pronounced bivalent effects, with a significant slower dissociation rate and 4–24-fold higher potency when compared to the monomer peptides for the human NaV1.4 channel.

This study highlights the power of bivalent ligand design in modulating potency and binding kinetics and expands the repertoire of pharmacological probes for exploring the function of NaV channels.

Organic Salts and Ionic Liquids as Ideal Platform in Combination Therapy Against Resistant Bacteria

Zeljko Petrovski (Departamento de Química Faculdade de Ciências e Tecnologia - Universidade Nova de Lisboa)

Organic salts and ionic liquids based on active pharmaceutical ingredients and their derivates (OSIL-APIs): ampicillin (Amp), penicillin G hydrolysate (seco-Pen), amoxicillin hydrolysate (seco-Amx) and fluoroquinulones were prepared in moderate to high yield using buffer neutralization procedure [1]. The prepared OSIL-APIs were tested against sensitive and resistant gram-positive and gram-negative bacteria [2-4]. They show good significant increase in solubility (up to 246 times), partition properties and significant increase of antibiotic activity particularly against resistant species with relative decrease of inhibitory concentration in respect to parent antibiotics (RDICs) up to >1000 and MIC values of 5nM for [C16Pyr][Amp] against E. coli TEM CTX M9 and [C16Pyr][seco-Amx] against MRSA ATCC 43300.

The gathered data suggest that the adequate ionic pairing is vital to enhance or promote antibiotic activity, with possible alterations in their mechanism of action according to the selected counter-ion. Recently combination of antimicrobials with non-active compounds was suggested as a promising strategy to address bacterial resistance and in this respect OSIL-APIs can provide ideal platform for drug modification and studies [5].

Chemical assembly and immunological evaluation of fully-synthetic multivalent anticancer vaccines based on a Tn antigen analogue

Carlo Pifferi (CIC bioGUNE)

Tumor associated carbohydrate antigens (TACAs), such as the Tn antigen, have emerged as key targets for the development of synthetic anticancer vaccines.[1] However, the induction of potent and functional immune responses has been challenging and, in most cases, unsuccessful. Indeed, while subunit vaccines based on homogeneous antigens offer more precise targeting and improved safety compared with traditional whole-cell vaccines, they are also less immunogenic and require an adjuvant to increase antigen's immunogenicity and potentiate the immune response.[2] Multivalency represents a hallmark of carbohydrate-protein interactions; given the importance of antigen-mediated crosslinking of B-cell receptors in triggering the preliminary events that lead to effective antibody production, multivalent glycoconjugates are tools of choice in carbohydrate-based vaccine design (Fig. 1).[3] Herein, we report the design, synthesis and immunological evaluation in mice of Tn-based vaccine candidates with multivalent presentation of the Tn antigen (up to 16 copies), both in its native serine-linked display (Tn-Ser) and as an oxime-linked Tn analogue (Tn-oxime).[4] Such modular constructs included cyclopeptide scaffolds which allowed for a multivalent carbohydrate antigen display, as well as functionalization with helper T-cell (CD4+) and cytotoxic T-lymphocyte (CD8+) peptide epitopes.[5] The high-valency vaccine prototypes were synthesized through a late-stage convergent assembly (Tn-Ser construct), and a versatile divergent strategy (Tn-oxime analogue), using chemoselective click-type chemistry. In combination with the saponin QS-21 as an adjuvant, the 16-valent Tn-oxime construct induced robust, Tn-specific humoral and CD4+/CD8+ cellular responses, with antibodies able to bind the Tn antigen on the MCF7 cancer cell surface (Fig. 2). The superior synthetic accessibility and immunological properties of this fully-synthetic vaccine prototype makes it a valid candidate for further advancement towards safe and effective synthetic anticancer vaccines.

LigAdvisor: a web platform designed for charting novel polypharmacology and drug repurposing routes from crystallographic ligands and known drugs.

Luca Pinzi (University of Modena and Reggio Emilia (IT))

The design of drugs able to simultaneously modulate the activity of multiple targets (polypharmacology) and the repositioning of known drugs towards novel therapeutic needs (drug repurposing) have raised overt interest in drug discovery. Indeed, polypharmacology and drug repurposing tasks, which are now mainly pursued on rational grounds, allows to achieve improved therapeutic effects compared to single-target and combined therapies, and to quickly improve the therapeutic arsenal available for the treatment of particular clinical conditions, respectively.[1-3] Several computational tools and databases specifically providing structural, biological or clinical information on targets and ligands are currently available to facilitate drug design. However, the information provided by these databases often cannot be easily integrated, or requires intense computational processing to be fully exploited in in silico drug design contexts. To help overcome such limitations, we developed LigAdvisor (https://ligadvisor.unimore.it/),[4] a data-driven web platform integrating information from DrugBank (DB), Protein Data Bank (PDB), UniProt and clinical trials, with similarity data provided by chemoinformatic analyses. In particular, data mining and chemoinformatic analyses were firstly performed to curate high-quality datasets of ligands from PDB and DB. The analyses were restricted to these databases of ligands, as they are less populated by potential false positive records, and could benefit of the available crystal structures and activity data, which are of central interest in target-driven drug discovery.[5] Then, their structures were compared through different 2D similarity approaches (i.e., MACCS and ECFP4 fingerprints) and associated to their respective molecular targets and clinical data, retrieved from UniProt and clinical trials, respectively. Finally, the collected data was framed into a web server, accessible through an intuitive interface, to enable its efficient integration and exploitation in different drug discovery contexts. As designed, LigAdvisor aims at facilitating the integration of similarity results on already known PDB and DB ligands (or even, user custom molecules), with data on targets and clinical trials by means of a user-friendly interface, both in ligand- and target-focused terms.

PRMT1 Inhibition Induces Differentiation of Colon Cancer Cells

Alexander Plotnikov (Weizmann Institute of Science)

Differentiation therapy has been recently revisited as a prospective approach in cancer therapy by targeting the aberrant growth, and repairing the differentiation and cell death programs of cancer cells. However, differentiation therapy of solid tumors is a challenging issue and progress in this field is limited. We performed High Throughput Screening (HTS) using a novel dual multiplex assay to discover compounds, which induce differentiation of human colon cancer cells. Here we show that the protein arginine methyl transferase (PRMT) type 1 inhibitor, MS023, is a potent inducer of colon cancer cell differentiation with a large therapeutic window. Differentiation changes in the highly aggressive human colon cancer cell line (HT-29) were proved by proteomic and genomic approaches. Growth of HT-29 xenograft in nude mice was significantly delayed upon MS023 treatment and immunohistochemistry of tumor indicated differentiation changes. These findings may lead to development of clinically effective anti-cancer drugs based on the mechanism of cancer cell differentiation.

Antiproliferative Activity of Nitric Oxide-Donor Largazole Prodrugs

Federica Poggialini (University of Siena)

Largazole is a natural product isolated in 2008 from marine cyanobacteria Symploca sp., identified as the most potent and selective Class-I deacetylase (HDAC) inhibitor, that showed a broad-spectrum growth-inhibitory activity against epithelial and fibroblastic tumor cell lines and a low cytotoxicity profile.1-3 The structure of Largazole is characterized by the presence of a planar 16-membered depsipeptide core bearing a metabolically labile thioester side-chain, which, upon hydrolytic cleavage, liberates Largazole-thiol, the bioactive HDAC inhibitor species. Over the last decades, dual nitric oxide (NO) donors/HDAC inhibitors have been developed as novel anticancer chemical entities, potentially more efficacious than selective HDAC inhibitors, owing to the capability of NO to specifically modulate the function of some HDAC isoforms and to overcome tumor cell resistance to conventional treatments.

Herein, we report two-hybrid analogues of Largazole (Figure 1), as dual HDAC inhibitor and nitric oxide (NO) donors potentially useful as anticancer agents and we discuss their in vitro biological profile. In particular, the in vitro release of NO, the metabolic stability of the modified thioester moiety of Largazole, bearing the NO-donor function/s, and the antiproliferative activity in Osteosarcoma (U-2OS), Neuroblastoma (IMR-32), and Colorectal adenocarcinoma (Caco-2) cell lines are presented.4

Combinatorial discovery of synthetic biohybrid ligands for RNA-hairpins and for the SARS-CoV-2-spike protein

Sebastian Pomplun (Massachusetts Institute of Technology (MIT))

The de novo discovery of ligands for challenging and novel drug targets often requires the cumbersome screening of individual compounds from large libraries. Here we present a fully chemistry based affinity selection - mass spectrometry (AS-MS) platform: within days synthetic polyamide compound libraries with > 100 million members can be produced, screened against targets of interest and originate hits with nanomolar affinity for their targets. We use AS-MS for the rapid discovery of synthetic high-affinity peptide binders for the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. The peptides display excellent selectivity for RBD over human serum proteins and can detect picomolar RBD concentrations in a biological matrix. We further expanded the AS-MS platform for the discovery of compounds targeting oncogenic pre-miRNA hairpins. In nature nucleic acids are often controlled by large supramolecular protein/oligonucleotide complexes as in the case of ribosomal protein synthesis.

Rather than forming large complexes to coordinate the role of different biopolymers, we dovetail protein amino acids and nucleobases into a single low molecular weight precision polyamide polymer. We established efficient chemical synthesis and de novo sequencing procedures and prepared combinatorial libraries with up to 100 million biohybrid molecules. This biohybrid material has a higher bulk affinity to oligonucleotides than peptides composed exclusively of canonical amino acids. Using affinity selection mass spectrometry, we discovered variants with a high-affinity for pre-microRNA hairpins. Our platform points toward the development of high throughput discovery of sequence defined polymers with designer properties, such as oligonucleotide binding.

When bitter is better: extraction optimization and promising biological activity of Humulus lupulus L. bitter extract

Maria Ponticelli (University of Basilicata)

Nowadays, several active molecules from plants are considered interesting alternatives able to prevent or treat chronic diseases. Among them, the female inflorescences (hop cones or "hops") of Humulus lupulus L. have attracted attention for their health-promoting properties. Besides, hops, which are rich in polyphenolic compounds and acyl phloroglucinol derivatives are widely used for beer preservation and for the characteristic aroma and bitter flavour [1]. Recent studies have reported that hop humulones (α-acids) and lupulones (β-acids) are the strongest antioxidant source [2] thus, the optimal conditions for their extraction are a crucial point to be issued and in this work, we have analyzed their yield using the response surface methodology (RSM). Particularly, maceration and digestion technique were used for the extraction and the influence of different EtOH/H2O proportions (X1:5-50-95%), extraction times (X2:60-90-120min), and temperatures (X3:25-50-79°C) was investigated. Each extract was analyzed spectrophotometrically by using the ASBC method and it was found that the percentage of α-acid extracted is directly proportional to ethanol concentration. The best results were indeed obtained using 95% EtOH/H2O, in agreement with those reported by Anioł and Żołnierczyk [3]. In particular, the percentage of α-acid ranged from 2.80 ± 0.02% w/w to 15.91 ± 0.01% w/w for 5% EtOH/H2O and 95% EtOH/H2O, respectively. In line with the data obtained for α-acids, the percentage of β-acids varied between 2.43 ± 0.04% w/w and 11.72 ± 0.50% w/w for 5% EtOH/H2O and 95% EtOH/H2O, respectively. Hence, based on these results the RSM was carried out showing that the optimal conditions of extraction procedures were X1:95%, X2: 60min, and X3: 25°C. This macerated hydroalcoholic extract with a higher percentage of hop bitter acids was tested for the evaluation of antioxidant activity by using HepG2 as a model cell line. The extract showed a remarkable antioxidant activity, assessed by flow cytometry, both in normal and t-BuOOH stress-induced conditions at all the tested concentrations (200-100-50-10 μg/mL) comparable to that of the well-known antioxidant N-acetyl-L-cysteine. Mitochondrial membrane potential was also evaluated. These results suggest that hop bitter acids' implication in the prevention of ROS generation could exert their potential application in oxidative stress-linked illnesses like cardiovascular, cancer, and neurological disorders.

Mining the Leishmania Kinome

Exequiel Porta (Department of Chemistry, Durham University)

With >12M of the world's poorest people infected and an economic cost best estimated by >3M disability adjusted life years, the global health challenge represented by leishmaniasis is huge [1]. This challenge is heightened by the fact that the few efficacious drugs available are difficult to administer and often exhibit serious, potentially fatal, side-effects and are also subject to growing resistance. Consequently, the discovery and validation of well-characterised targets to underpin future drug discovery programmes is essential. Activity-based protein profiling (ABPP) has become a powerful tool for exploring the proteome based on protein chemical reactivity [2]. In the simplest description, this involves the synthesis of molecules containing reactive functional groups (warheads) that have the ability of separating and purifying proteins. In this work, we aimed to develop and explore options to target Leishmania kinases through the design, synthesis, and application of ten different kinase targeting probes. Kinases are responsible for the regulation of many biological functions and have been targeted for many other indications notably cancer. Consequently, there may be existing drugs with proven efficacy and safety profiles that we can repurpose to radically shorten the time and cost required to bring new drugs to the clinic. Robust workflows have been established to elucidate the Leishmania kinome. Details of this, together with studies exploring protein identification and quantification, competitive ABPP experiments and quantitative proteomics mass spectrometry (MS) analyses will be described. These approaches will allow us to establish a landscape of the chemically modifiable Leishmania kinome to enable comparison with genetic and proteomic studies undertaken elsewhere.

Site-specific Protein Functionalization Using Activated Cysteine Based Chemical Biology Methods

Yuchen Qiao (Texas A&M University)

Proteins, as one of the most essential biomacromolecules, involve in various cellular processes. To obtain enough biological tools that assist scientists to study the cellular functions of human proteins, protein functionalization has become more and more important and well-developed. Proteins containing a C-terminal modification are critical to the protein synthesis via expressed protein ligation. They are usually made by recombinant fusion to intein. Although powerful, the intein fusion approach suffers from premature hydrolysis and low compatibility with denatured conditions. To totally bypass the involvement of an enzyme for expressed protein ligation, we developed an activated-cysteine directed protein ligation (ACPL) technique using 2-nitro-5-thiocyanatobenzoic acid (NTCB) as cysteine cyanylating reagent for undergoing nucleophilic acyl substitution with amines including a number of L- and D-amino acids and hydrazine. The afforded protein hydrazides could be used further for expressed protein ligation. We demonstrated the versatility of this approach with the successful synthesis of ubiquitin conjugates, ubiquitin-like protein conjugates, histone H2A with a C-terminal posttranslational modification, RNAse H that actively hydrolyzed RNA, and exenatide which is a commercial therapeutic peptide. The technique, which is exceedingly simple but highly useful, expands to a great extent the synthetic capacity of protein chemistry and will therefore make a large avenue of new research possible. Dehydroalanine exists natively in certain proteins and can also be chemically made from a protein cysteine. As a strong Michael acceptor, dehydroalanine in proteins has been explored to undergo reactions with different thiolate reagents for making close analogues of posttranslational modifications (PTMs) including a variety of lysine PTMs. We explored an NTCB-triggered dehydroalanine formation which is highly efficient when cysteine is at the flexible C-terminal end of the protein. We produced ubiquitin (Ub) and ubiquitin-like proteins (Ubl) containing a C-terminal dehydroalanine residue with high yields. Although this method was found not effective when cysteine is at an internal region of a protein, we believe this method will find broad applications in studying Ub and Ubl pathways and functional annotation of many PTMs in proteins such as histones.

Tunable Methacrylamides for Covalent Ligand Directed Release Chemistry

Rambabu Reddi (Weizmann Institute of Science)

Targeted covalent inhibitors are an important class of drugs and chemical probes. However, relatively few electrophiles meet the criteria for successful covalent inhibitor design.1 Here we describe α-substituted methacrylamides as a new class of electrophiles suitable for targeted covalent inhibitors. While typically α-substitutions inactivate acrylamides,2 we show that hetero α-substituted methacrylamides have higher thiol reactivity and undergo a conjugated addition–elimination reaction ultimately releasing the substituent. Their reactivity toward thiols is tunable and correlates with the pKa/pKb of the leaving group. In the context of the BTK inhibitor ibrutinib, these electrophiles showed lower intrinsic thiol reactivity than the unsubstituted ibrutinib acrylamide. This translated to comparable potency in protein labeling, in vitro kinase assays, and functional cellular assays, with improved selectivity. The conjugate addition–elimination reaction upon covalent binding to their target cysteine allows functionalizing α-substituted methacrylamides as turn-on probes. To demonstrate this, we prepared covalent ligand directed release (CoLDR) turn-on fluorescent probes for BTK, EGFR, and K-RasG12C. We further demonstrate a BTK CoLDR chemiluminescent probe that enabled a high-throughput screen for BTK inhibitors. Altogether we show that α-substituted methacrylamides represent a new and versatile addition to the toolbox of targeted covalent inhibitor design.

Development of a cyanobacterial natural products library for anticancer drug discovery

Mariana Reis (Interdisciplinary Center of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto)

Cancer continues to be an ailment with a tremendous negative socio-economic impact worldwide. Therefore, discovery of new and more effective anticancer drugs is still a priority to ensure healthy lives. Natural products constitute a privileged source for the discovery of anticancer compounds[1]. In this way, cyanobacteria are considered to be one of the most promising groups of organisms capable of producing secondary metabolites with biotechnological and pharmaceutical interest [2]. Dolastatin 10 that inspired 4 FDA approved anticancer drugs is a successful example of this [3]. The Culture Collection of cyanobacteria (LEGEcc - CIIMAR) is a valuable and underexplored natural resource that can underpin the discovery of promising bioactive compounds. LEGEcc comprises more than 600 different cyanobacterial strains, collected in different ecosystems and locations [4].

In order to explore this chemo-diversity for the discovery of anticancer compounds we developed a semi-automated method for prefractionation of crude extracts that was associated with anti-cancer assays preformed in 2D and 3D cell models. Herein, we will present the expedite constitution of the cyanobacterial Natural Products Library (LEGE-NPL) with 512 fractions, which were screened for their cytotoxic effect in the colon carcinoma cells (2D HCT 116, 3D HCT 116) and in the non-carcinogenic cell line hCMEC/D3. The combination of relevant cancer models led to the selection of 13 active fractions. Their chemical composition was investigated by mass spectrometry for molecule annotation and dereplication through the Global Natural Products Social Molecular Networking. This work led to the selection of 5 cyanobacterial strains as producers of novel promising anticancer compounds.

FIRST TOTAL SYNTHESIS OF CYTOTOXIC CHABROLONAPHTHOQUINONE B – DESIGNING OF BIOACTIVE ANALOGUES

Stergios Rizos (Laboratory of Organic Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece)

Stergios R. Rizos, Konstantinos A. Ouzounthanasis, Alexandros E. Koumbis* Laboratory of Organic Chemistry, Department of Chemistry Aristotle University of Thessaloniki, 54124, Thessaloniki, email: akoumbis@chem.auth.gr Several secondary metabolites possessing mainly cytotoxic activity have been isolated from the organic extract of the soft coral Nephthea chabrolli in South Taiwan. However, chabrolonaphthoquinone B (1, Image 1) is the sole chiral naphthoquinone related meroditerpenoid which was isolated from this marine invertebrate and shows very interesting biological activity [1-2]. (Place Image 1 here) Image 1: Picture of soft coral Nephthea chabrolii (photo taken from doi:10.3390/md10061288) and structure of chabrolonaphthoquinone B (1). Compound 1 exhibits cytotoxic activity against a series of human cancer cell lines (MDA-MB-231, Hep-G2 and A549 with IC50s 4.7, 12.4 and 33.9 μM, respectively) [2]. Prompted by its intriguing structure and interesting activity we embarked in a project dealing with the preparation of synthetic 1 and its analogues. Herein, we initially describe our results towards the first enantiospecific total synthesis of 1 employing a chiral pool approach (Image 2). Our synthetic route involves as key-step a modified Julia olefination (a) [3] of the sulfone bearing aliphatic fragment 2 and the aromatic aldehyde 3, in order to construct the E-trisubstituted double bond. For the introduction of the dimethyl side-chain a two steps addition-elimination approach was adopted (b). In turn, fragments 2 and 3 were prepared from isopropylidene- D-erythronolactone (4) (via a stereoselective aldol reaction [4]) and from Danishefsky's diene 5 and bromoquinone 6 (via a regioselective Diels-Alder reaction [5]). (Place Image 2 here) Image 2: Retrosynthesis of chabrolonaphthoquinone B (1). Our flexible synthetic strategy, appropriately modified, allows for the targeted preparation of designed analogues of the natural product (Image 3), as well. This is expected to facilitate the determination of key structural features for the activity and the identification of potential new derivatives with better biological profile. (Place Image 3 here) Image 3: Modification sites for the preparation of analogues of compound 1. Acknowledgements: Part of this work was realized using the facilities of OPENSCREEN-GR (Hellenic Small Molecules Center, Aristotle University of Thessaloniki)

Targeting Brain Metastasis with Rationally Designed Polypeptide-based Combination Conjugates

Fernanda Rodriguez-Otormin (Polymer Therapeutics Laboratory, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain.)

The brain is one of the most well-protected organs in the body, guarded from external insults by selectively permeable biological barriers, such as the blood-brain barrier (BBB). This neurovascular barrier hampers drugs' biological activity by impeding their accumulation in the brain within their therapeutic window. Receptor-mediated transcytosis, an active transcellular pathway followed by hormones, growth factors, lipoproteins, and other macromolecules, represents one of the most investigated routes for brain drug delivery due to the promotion of transcytosis across the BBB. "Trojan horse"-based strategies employ conjugation of high-affinity and low-avidity ligands of specific BBB receptors to nanomedicines to enhance BBB crossing by RMT (1). In this project, we developed a novel nanomedicinal approach to treat triple negative breast cancer (TNBC)-associated brain metastasis, using star-shaped polyglutamic acid (St-PGA) vehicles carrying a synergistic combination of drugs and Angiopep-2 as targeting moiety to promote BBB crossing and brain accumulation.

By reiterative design cycles using well-established in vitro and in vivo models and state-of-the-art physico-chemical characterization techniques, we obtained a St-PGA combination conjugate that efficiently reduced tumor growth and metastatic progression in an orthotopic TNBC mouse model (2) without causing animal weight loss. In parallel, we optimized the conjugation and density of Angiopep-2 for efficient BBB crossing. We synthesized fluorescently labeled St-PGA conjugates with various Angiopep-2 loadings and evaluated permeability using an in vitro BBB model (3). In vivo biodistribution analysis confirmed that St-PGA-Angiopep-2 crossed the BBB and accumulated in the brains of healthy mice.

Finally, thanks to the orthogonal chemistries used for drug and Angiopep-2 conjugation, we combined both synthetic protocols to yield an Angiopep-2 bearing St-PGA combination conjugate. We evaluated this novel nanomedicine's efficacy in a well-established in vivo breast cancer brain metastasis model (4,5), obtaining promising preliminary results.
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A Rationally Designed Polypeptide-based Combination Conjugate for the Treatment of Metastatic Triple Negative Breast Cancer

Fernanda Rodriguez-Otormin (Polymer Therapeutics Laboratory, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain.)

While breast cancer represents the leading cause of death from cancer in females, survival rates have improved over the last decade thanks to early diagnosis and therapeutic advancements derived from the development of endocrine- and human epidermal growth factor receptor 2 (HER2)-targeting therapies. Unfortunately, triple negative breast cancer (TNBC) patients cannot benefit from said improvements given the lack of progesterone and estrogen receptor and HER2 expression (1). Thus, there exists an urgent need to develop novel therapies for TNBC and associated metastases. We developed and in vivo-evaluated star-shaped polyglutamates (St-PGA) as a first step, discovering improved pharmacokinetics compared to linear PGAs (2). The biodegradability, versatility, and multivalency of St-PGA encouraged the development of a family of St-PGA-based combination conjugates bearing synergistic drugs as an efficient therapy for metastatic TNBC. Reiterative design cycles using well-established in vitro and in vivo models and state-of-the-art physico-chemical characterization techniques fostered the optimization of drug linking chemistry and ratios. We synthesized St-PGA through controlled polymerization techniques via ring-opening polymerization of N-carboxy anhydrides (2,3). We produced single and combination St-PGA-drug conjugates using various drug ratios and linkers and evaluated their cytotoxic activity in vitro in human breast cancer cells (MDA-MB-231-Luc). Combination conjugates displayed IC50 values similar to the free drugs combined at the same ratio, suggesting that covalent conjugation failed to affect drug activity. We evaluated the anti-tumor activity of St-PGA combination conjugates in a well-established orthotopic metastatic TNBC model (4). In vivo data identified the optimal St-PGA combination conjugate, which significantly reduced primary tumor and metastatic progression compared to other conjugates and free drugs administered at equivalent doses. Furthermore, the conjugate improved the drug combination's toxicity profile, preventing the animal weight loss associated with free drug administration. These data confirm the safety of these novel nanomedicines and suggest their potential in the treatment of solid tumors. Acknowledgements Junta Provincial de Valencia of the Spanish Association Against Cancer (AECC predoctoral grants), European Research Council (Grant ERC-CoG-2014-648831 MyNano), and FEDER (PO FEDER Valencian Community-2014-2020).

Design, synthesis and Biological evaluation of New, glycolipid-based Toll-Like Receptor 4 (TLR4) Modulators

Alessio Romerio (Università degli studi di Milano-Bicocca)

Innate Immunity is the first defense line in multicellular organisms against internal of external threats. It acts through inflammation, triggered by the recognition of specific Pathogen or Damage Associated Molecular Patterns (PAMPs or DAMPs) by specific pattern-recognition receptors (PRRs). Toll-Like Receptor 4 (TLR4) is one of the most important PRRs, and it responds to gram-negative bacteria lipopolysaccharide (LPS). TLR4 modulation is emerging as an important therapeutic approach in several clinical settings: TLR4 inhibition has a potent anti-inflammatory effect; on the other hand, TLR4 mild activation can be used to stimulate immunity in vaccine adjuvants or to develop cancer immunotherapeutic drugs. We present here rationally designed lipid A analogues based on a monosaccharide structure that are active in binding MD-2/TLR4, thus activating or inhibiting LPS/TLR4 or DAMP/TLR4 signalling. We also present synthesis optimization of TLR4 modulators, with the aim of producing versatile synthetic intermediates and reducing the number of synthetic steps to efficiently scale the synthesis up for industrial purposes.

Thermal oscillations enable reshuffling of genetic material in a primitive cell cycle

Roger Rubio-Sánchez (Biological and Soft Systems, Cavendish Laboratory, University of Cambridge)

Single-chain fatty acids are plausible prebiotic building blocks that could have facilitated the self-assembly of membranes to compartmentalise primitive protocells [1]. In spite of their ability to host prebiotically-relevant processes such as non-enzymatic nucleic acid activation [2] and replication [1, 3], their limited stability, for instance, to changes in temperature [4] and pH suggests that primitive cellular forms could only sustain (bio)chemical pathways in a narrow range of conditions. Here, we propose a novel primitive cell cycle driven by environmental fluctuations, likely to have occurred in early Earth scenarios, that enables the generation of potentially-superior daughter protocells with reshuffled parental content. Our mechanism is reliant on temperature-induced pH fluctuations, which in turn facilitate reversible bilayer-to-oil phase transitions that fully account for membrane disassembly and reassembly, while enabling content release, and re-encapsulation.

Using a range of spectroscopic, imaging, and modelling approaches, we show that thermal oscillations provide a platform for recursive content mixing in the context of both membrane and cytosolic material. Finally, we demonstrate that such a re-shuffling platform supports the emergence of next-generation protocell cohorts displaying enhanced traits, which feature functional nucleic acids yet start from inactive parental genetic content. Our results [5] provide evidence of an environmentally-mediated cell cycle with implications that, in the absence of highly-evolved biological machinery, hint at a plausible avenue to initiate Darwinian evolution in early proto-cellular systems.

Substrate Dissipation Energy Regulates Cell Adhesion and Spreading

Pasquale Sacco (1) AREA Science Park (Trieste); 2) University of Trieste, Department of Life Sciences)

Recent evidences have led to hypothesize that dissipation of energy through viscoelastic extracellular matrix (ECM) could play a cardinal role in directing cell-fate decisions, but whether and how it correlates with specific cell response has remained unclear up to date. In this talk I will introduce substrate dissipation energy as novel cell-fate controller.[1] Specifically, I will illustrate recent findings about viscoelastic and plastic chitosan-based substrates endowed with different dissipative energies capable of modulating cell behavior in terms of adhesion and spreading. While keeping constant stress relaxation and systematically decoupling overall stiffness from linear elongation, we have introduced an energy dissipation term (J/mol), that is the molar energy required to deviate from linear stress-strain regime and enter into plastic region. Strikingly, we have unveiled an inverse relationship between substrate energy dissipation and cell response, with high adhesion/high spreading and low adhesion/no spreading detected for substrates at low and high dissipation energy, respectively.

We concluded that cells decide how to react depending on the effective energy they can earmark for their functions. Of note, I will show how combinations of facing 5-consecutive sugars (pentads) composing substrates are essential in damping shear stress, thus behaving as cell traction forces dampers. Collectively, in this talk I will illustrate how the crosstalk between cells and ECM can be considered as energetic in origin.

The PDK1 Conformational Landscape

Mariana Sacerdoti (Instituto de Investigación en Biomedicina de Buenos Aires- CONICET- MPSP)

Phosphoinositide-dependent protein kinase 1 (PDK1) is a master kinase of the PI3-kinase signaling pathway that phosphorylates at least 23 other evolutionary related AGC kinases. It has an N-terminal kinase domain (KD), a linker region and a C-terminal PH domain. Most work on PDK1 regulation has focused on the KD, but recent work suggests the existence of PDK1 dimers(1). Over the years, our laboratory has used a chemical and structural biology approach to characterize the bidirectional allosteric regulation between the PIF-pocket, a regulatory site located on the small lobe of the KD, and the ATP-Binding site of PDK1(2). Phosphorylation by PDK1 is required for the activity of all substrates and most, like S6K, SGK, PKC, rely on a docking interaction where a C-terminal hydrophobic motif interacts with the PIF-pocket of PDK1(3). Interestingly, this interaction is not a requirement for the phosphorylation of PKB/Akt after PI3-kinase activation, but both proteins have a PH domain that binds PIP3 at the cell membrane and colocalize there. Recent discoveries show that PKB/Akt can also be phosphorylated by PDK1 in a PIP3 independent context(4), suggesting the existence of alternative mechanisms of interaction. Inositol polyphosphorylated molecules derived from InsP5 inhibit PDK1 activity in vitro (5). However, we found that 2-O-Bz-InsP5 (HYG8) does not inhibit the phosphorylation of a peptide substrate termed T308tide. Moreover, we show that HYG8 can selectively inhibit the phosphorylation and activation of PKB/Akt by PDK1 in vitro, but does not inhibit the phosphorylation and activation of SGK. HYG8 disrupts PDK1 dimers, measured by AlphaScreen and corroborated by single molecule fluorescence microscopy. H/D exchange experiments indicate that HYG8 stabilizes a monomeric conformation of PDK1, where the linker-PH domain region protects the "back" of the KD (monomeric conformation I).

We performed a small scale screening to find additional small molecules that regulate the formation of PDK1 dimers. Results suggest the existence of a second monomer (monomeric conformation II). We conclude that PDK1 could exist as a dynamic equilibrium of conformations, which appear physiologically relevant because of the impact on the selective interactions and substrate phosphorylation. Such regulatory mechanisms could lead to novel approaches for the design of protein kinase inhibitors, targeting protein interactions to regulate the phosphorylation of a subset of substrates.

Supramolecular amphiphilic peptides for atorvastatin loading as a novel neuroprotective strategy

Elena Sánchez López (Department of Pharmacy, Pharmaceutical Technology and Physical Chemistry, Faculty of Pharmacy, University of Barcelona)

Neurodegenerative diseases constitute one the most common causes of death in developed countries1. According to the WHO around 6.8 million people die every year as a result of neurological disorders. These diseases constitute a great concern because of aging worldwide population. Despite this high incidence, effective therapeutic treatments are still limited. Several statins such as Atorvastatin (ATV) are currently in use to treat hyperlipidaemias, but in addition, it has been described they exert beneficial effects in some neurodegenerative diseases. It is well documented that their administration improves neurological functional responses in patients2. ATV has low aqueous solubility resulting in low oral bioavailability and thus presents a challenge to obtain a suitable dosage form. In a previous work, we demonstrated that the entrapment of ATV in PLGA-PEG nanoparticles derivatized with a cell penetrating peptide (HIV-TAT) could be used as alternative neuroprotective strategy to deliver ATV to neurons3. In the present work, we have explored other nanotechnological approaches, such as peptide amphiphiles (PAs) that constitute one of the novel drug delivery carriers able to entrap different types of active compounds and deliver them in a sustained manner to a specific target site4. Therefore, amphiphilic cell penetrating peptides derived from HIV-TAT have been designed in order to entrap ATV aiming to increase its bioavailability. Three PAs were obtained (mC16-TAT47-57, dC16-TAT47-57 and qC16-TAT47-57) and their interaction with ATV was studied. PAs were synthesized using standard 9-fluorenylmethoxycarbonyl-based Solid Phase Peptide Synthesis. The structures were characterised in terms of ATV loading percentage, average size and transmission electron microscopy (TEM) after negative staining. As can be observed in Figure 1, among the three peptide-based structures, qC16-TAT47-57 loaded with ATV showed the formation of nanofibers around 11,5 nm of diameter and length of 142 nm and a loading efficiency around 40%. Interestingly, the presence of ATV increases the formation of more defined supramolecular structures.

These studies confirm that PAs and specially qC16-TAT47-57, constitute a promising drug delivery system able to encapsulate lipophilic neurodegenerative drugs such as ATV. Figure 1. TEM images of nanostructures formed by amphiphilic peptides entrapping atorvastatin. A) mC16-TAT47-57, B) dC16-TAT47-57, C) qC16-TAT47-57.

The dodecylsulfoxide sp2-iminosugar glycolipid (Ss)-DS-ONJ induces an anti-inflammatory response in a diabetic retinopathy context

Elena Matilde Sánchez-Fernández (University of Seville (Spain))

Diabetic retinopathy (DR) is one of the most common complications of Diabetes Mellitus (DM) and is directly associated with inflammatory processes [1]. Indeed, inflammation and apoptosis are important pathological processes of DR that often lead to necrosis of retinal cells. The pro-inflammatory environment in the course of DR is associated with altered immune response in the retina and, therefore, their modulation offers appealing opportunities to slow DR progression, and prevent the loss of visual function. We have previously observed that some sulfur-linked glycolipid mimetics incorporating a sugar-like 5N,6O-oxomethylidenenojirimycin moiety, namely sp2-iminosugar glycolipids (sp2-IGLs) [2], efficiently promoted microglia polarization from the pro-inflammatory (M1) towards to the anti-inflammatory (M2) state. Biochemical and computational data support a mechanism involving non-canonical auto-phosphorylation of p38α mitogen-activated protein kinase (MAPK), a key player in the inflammatory cascade. Contrary to other immuno-regulatory glycolipids, the sp2-IGLs are metabolically stable and can be prepared in the laboratory in pure anomeric form with total stereo-selectivity, thereby providing a suitable platform for glycodrug design. Here we report the beneficial effect of (SS,1R)-1-docecylsulfiny-5N,6O-oxomethylidenenojirimycin ((Ss)-DS-ONJ), a member of the sp2-IGL family, by decreasing iNOS and inflammasome activation in Bv.2 microglial cells exposed to pro-inflammatory stimuli. Moreover, pretreatment with (Ss)-DS-ONJ increased heme-oxygenase (HO)-1 as well as interleukin 10 (IL10) expression in LPS-stimulated microglial cells, thereby promoting M2 (anti-inflammatory) response by the induction of arginase-1. The results strongly suggest that this is the likely molecular mechanism involved in the anti-inflammatory effects of (SS)-DS-ONJ in microglia. (SS)-DS-ONJ further reduced gliosis in retinal explants from type 1 diabetic BB rats, which is consistent with the enhanced M2 response [3]. In conclusion, biasing microglia polarization dynamics towards M2 status by sp2-IGLs with anti-inflammatory activities offers promising therapeutic interventions at early stages of DR.

Using structural tools to unreveal the interactions between vanadium-based complexes and proteins

Marino F. A. Santos (UCIBIO, FCT-NOVA)

Vanadium (V) is a biologically important element and, in the last years, its therapeutic use –inorganic and complexed with small organic ligands –has been also investigated (namely as insulin-enhancer agents) as widely reported.1,2,3Here, we report a biophysical and structural study on the interactions of inorganic (V(IV)OSO4 and NaV(V)O3) and organic (bis(acetylacetonato)oxidovanadium(IV), V(IV)O(acac)2) vanadium-based compounds with two proteins: human serum transferrin (HTF) and Hen egg white lysozyme (HEWL).HTF, animportant metal ion blood carrier, was firstly used to confirm the protein-ligand binding using Small Angle X-ray Scattering (SAXS). Four datasets –native apoHTF, apoHTF-V(IV)OSO4, apoHTF-NaV(V)O3 and apoHTF-V(IV)O(acac)2 –were collected at beamline BM29 (ESRF, Grenoble, France). The data was properly analyzed and significantly different parameters –namely P(r) function and Dmax –have been determined suggesting the compounds binding to the protein.

In fact, the results indicate a conformation modification by the partial closing of apo-HTF upon binding which is less pronounced that the one caused by the FeIII ion.4,5To further characterization, soaking experiments with HEWL and inorganic and organic V-based compounds were performed. Different high-resolution structures were obtained revealingmetal adducts with interesting features to be properly explored in a near future.6In conclusion, this work proves that V-based compounds may be transported in blood by HTF. The obtained structural insights are important to properly evaluate the pharmacokinetics of the compounds contributing for their putative use as safe drugs

Styrylquinoline derivatives with embedded dicarbonyl boron complexes as potential multitarget theranostic compounds against Alzheimer´s disease

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Neurodegenerative diseases are some of the most prevalent maladies worldwide. Even if they are caused by different types of disorders, protein misfolding is commonly found in several of them. Alterations in protein structure lead to activity failures, producing dysfunctions and anomalies. One of the most important neurodegenerative disease is Alzheimer´s Disease (AD), and since its development is directly correlated to ageing, it is expected to affect a rising amount of people in the future(1). Even if its aetiology remains incompletely understood, it is known that protein misfolding plays a key role in it. These processes involve deposition of amyloid beta-peptide and hyperphosphorylated tau protein, but are not the only alterations present; oxidative stress, neuroinflammation, mitochondrial dysfunction and an imbalanced glutamatergic and cholinergic tone are known to have influence in the development of AD. Currently, there is no treatment for AD. Some drugs achieve a temporary amelioration of symptoms, but none of them addresses the cause nor cures the disease. For this reason, new alternatives are needed for the treatment of AD. One approach includes multitarget directed ligands which bind to different targets and regulate several pathways at the same time. This approach is particularly interesting for multifactorial diseases like AD. Additionally, theranostic compounds provide therapy and diagnostic information simultaneously. This allows assessment of the molecule activity, the organism response and the pharmacokinetics, and makes these compounds promising for personalized medicine. Due to all of this, our research group is interested in combining both characteristics in new multitarget theranostic compounds against AD. On the basis of a common scaffold of styrylquinoline for its promising properties disclosed in our group(2), a novel family of molecules were found to exhibit remarkable properties, including inhibition of tau protein aggregation, neuroprotective and antioxidant activity(3). Furthermore, beta amyloid detection was possible due to their fluorescence emission in the near-infrared range. In continuation of the push and pull strategy that worked in previous attempts, we describe the synthesis and characterization of new styrylquinoline derivatives bearing dicarbonyl moieities at the quinoline C-6 position as well as their boron complexes.

Synthesis and structural characterization of a library of nitrogen analogues derived from honokiol

Claudia Sciacca (University of Catania- Department of Chemical Sciences)

Since ancient times the use of medicinal plants has been considered the only treatment for several diseases. For centuries extracts from bark and roots of Magnolia's tree have been used in traditional Japanese and Chinese medicine as treatments for gastrointestinal disorders, anxiety, allergies, inflammation and other diseases due to their multiple therapeutic properties. Magnolol and honokiol, two neolignans with bisphenolic structure, have been identified as the main bioactive constituents from the bark of Magnolia tree. The two neolignans' biological properties have been the subject of many studies that have provided a non-exaustive list of activities including antitumor, antiangiogenic, anti-inflammatory, antimicrobial, antiviral, antioxidant and neuroprotective (1). These properties suggest the synthesis of magnolol and honokiol analogues as potential bioactive molecules. Recently, some analogues and derivatives showed an antitumor (2) and an hypoglycemic (3) activity higher than those of the natural lead.

The main goal of this work is the synthesis and structural characterization of a library of nitrogen analogues of honokiol. This objective has been pursued with an efficient synthetic strategy based on three steps: Suzuki-Miyaura cross-coupling reaction from phenols and/or aromatic amines to obtain bisphenols, followed by allylation reaction and a subsequent transposition to insert the allylic chains on the two aromatic rings. (Fig.1) The new compounds will be subjected to biological and biochemical studies.

In silico modelling of prebiotic reaction networks to understand autocatalysis and origins of life

Siddhant Sharma (Blue Marble Space Institute of Science)

Complex chemical reaction networks can grow exponentially in terms of the chemical diversity they generate. It is unknown whether such networks easily discover or shuttle fluxes through autocatalytic sub-networks. Such sub-networks may be common or rare or anywhere in between in organic chemistry in general. In our study, we aim to provide a map for experimental chemists studying complex organic reactions using an automated rule-based reaction generation to simulate the reactions involved in various plausible abiotic reactions proposed to account for the organic diversity observed in carbonaceous meteorites, thus providing ample data for ground-truthing. We applied graph transformation rules [1] based on well-documented reaction mechanisms and chemical intuition and applied various constraints to the outputs, such as disallowed output structural motifs, thereby restricting them. We used isomorphism tests to match the output molecular structures to experimentally reported structures as a test of the completeness of our methods. The monoisotopic exact masses of the molecules in the computed reaction network product set were calculated and used to match peaks identified in high-resolution FT-ICR-MS data of the same reaction. We modeled the alkaline degradation of glucose using our workflow and found that our model was able to explain 96% of the structures reported in analytical studies (e.g., Yang and Montgomery, 1996). When the same workflow was applied to simulate formose chemistry, we were able to match all the structures reported by Decker & Schweer (1982) and Omran et. al. (2020). The reaction network was further assessed for the existence of potentially autocatalytic loops by loading the network topology into a graph database where pattern matching queries could be executed to search for patterns of interest. This work demonstrates some efficient methods for finding reaction pathways and autocatalysis in in silico modeled reaction networks [2]. This kind of in silico modeling enables the comprehensive study of chemical reaction pathways and knowledge of possible compounds involved in such reaction networks can guide future untargeted searches for the analysis of organics in the cometary, meteorite, and extraterrestrial planetary samples and provide new insights into carbohydrate chemistry, organic geochemistry, metabolomics and the mysteries of the origin of life.

New N-heterocyclic Carbene Complexes of Silver and Gold with promising activity against breast cancer cell lines

Marco Sirignano (University of Salerno Department of Chemistry and Biology)

Since the serendipity determination of anticancer activity of cis-platin in 1969 by Rosenberg, the development of new organometallic drugs and diagnostic agents have gained a huge interest by scientist1. In the past 50 years, other platinum complexes as oxaliplatin, nedaplatin, lobaplatin have been agreed for the treatment of several tumours. Oxaliplatin was the first organo-platinum drugs able to overcome the pharmaco-resistance of cis-platin in the treatment of colon carcinoma2. However, the use in chemotherapy of these compounds is not exempt of limitations. In the first instance, they have poor activity against some tumors due to intrinsic or acquired resistance and their clinical use leads many non-desirable effects such us nephrotoxity, ototoxicity and neurotoxicity. All these unresolved problems stimulate the researcher to synthesize new organometallic compounds with a desirable specificity aimed to minimizing the unwanted effects. Today, non-platinum complexes are not used for clinical application, but some transition metal compounds are screened in clinical trials: complexes of ruthenium (e.g. NAMI-A and KP1019), titanium (e.g. budotitane and titanocene dichloride), or gallium (e.g. gallium nitrate and gallium maltolate) have already been tested in clinical phase studies3. Recently metal N-heterocyclic carbene (M-NHC) complexes have attracted the attention of scientific community, because most of them have shown higher cytotoxicity than cis-platin4. NHCs have product an important impact in organometallic chemistry for the accessible synthetic route, the facile modulation of steric and electronic properties and their high stability, by modifying the steric and electronic properties of substituents on the backbone and/or on the nitrogen atoms at the NHC ligand4. In the last years, silver and gold complexes bearing N-heterocyclic carbene ligand have shown an interesting biological activity toward breast cancer cell lines5. The breast cancer is the primary cause of death of young women. Some synthesized complexes showed an interesting activity against MCF-7 and MDA-MD 231 cell lines. Based on these observations, we have synthesized new complexes of silver and gold, bearing NHCs ligands, to improve their solubility in biological media. Moreover, in vitro and in silico studies have revealed the ability, of these complexes, to inhibit the human topoisomerase I and II, which are two essential enzymes involved in replication of DNA and its metabolism

New cyanobacterial phenolate-type siderophores discovered by activity-guided isolation using 3D cancer spheroid cultures

Maria Lígia Sousa (CIIMAR-Interdisciplinary Centre of Marine and Environmental Research)

The use of advanced in vitro models, such as multicellular spheroid cultures (MSC) is gaining attention for cancer research and screening of bioactive compounds. Cyanobacteria have been recognized on the past decades as fruitful producers of unknown metabolites, as dolastatin 10, a compound that inspired 4 approved anticancer drugs currently in use. The current work aims to use MSC model in bio-guided screening of cyanobacterial extracts, in order to identify new potential metabolites with anti-tumoral activity. Viability effects on MSC were assessed by acid phosphatase (AP) and general morphology was monitored under fluorescence microscopy using fluorescent dyes (propidium iodide for dead cells, and calcein AM for cytoplasmic esterases). Crude organic extracts of 27 cyanobacterial strains from Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) were selected to assess their potential as producers of antitumoral metabolites. MSC were incubated with the selected cyanobacterial extracts over 48h, followed by viability assessment using AP and fluorescence microscopy. Crude extracts of the strain Nodularia sp. LEGE 06071, producer of the compound nocuolin A, which exhibit cytotoxic effects on both monolayer and MSC, was used as a control of this methodology. Among 27 strains we identified one promising cyanobacterial strain with consistent cytotoxic effects reducing significatively the viability of MSC. Moreover, dereplication analysis using the Global Natural Product Social Molecular Networking (GNPS) indicated that unknown compounds were the main content of the active fractions. Therefore, using bioactivity verification using MSC between chromatographic fractionation steps and aided by spectroscopic techniques (HRLC-MS and NMR), four new compounds with phenolate-type siderophore structure were isolated. Their full structure elucidation and bioactivity as well as biosynthetic cluster characterization is currently under investigation.

Using Robot Mechanics to Improve Ensemble Docking Procedures. Alzheimer and HCV Targets Case Studies.

Laurentiu Spiridon (Institute of Biochemistry of the Romanian Academy)

Virtual drug screening remains an important filtering step in the drug design protocols as it can reduce dramatically the chemical search space for subsequent high-throughput screening. Ensemble docking methods (Carlson et. al, 1999) are more becoming due to generally better results compared to rigid receptor docking. However, they come with additional computational cost used for receptor sampling, the reduction of which is the main objective of the study described here. Recently, applied mechanics algorithms were successfully applied for biomolecule sampling with considerable increase in efficiency (Spiridon et. al, 2020). This work incorporates aforementioned developments for two targets: apelin receptor (APJ) for Alzheimer's disease and NS2-NS3 for Hepatitis C.

This study tries to enhance the conformational space sampling of the receptors and ultimately get a better estimation of the docking scores. Ensemble docking procedures are highly dependent on the accuracy of the receptor conformation distribution, which is usually distorted by regular molecular dynamics or too narrow for the majority of Markov Chain Monte Carlo methods. Hamiltonian Monte Carlo (HMC) coupled with Gibbs sampling were used in this work. Gibbs blocks were defined as degrees of freedom mapped onto joints between rigid bodies. Kinematics and dynamics algorithms borrowed from robot mechanics render the method as fast as regular molecular simulation programs. Ergodicity is achieved by including a block for the joint probability as Cartesian HMC. NS2-NS3 and APJ rigid body HMC, combined with detailed atomistic simulation, were used to recover their free energy landscape.

Geometric features convergence and cluster count analysis showed increased sampling efficiency. In the case of NS2-NS3, a possible way of action is inferred for a new chemical scaffold obtained by a high-throuput screening approach. APJ cluster representatives are further used for stratified sampling ensemble docking procedures (Xie et al, 2018). Molecular conformational sampling based on rigid body dynamics presents itself as a promising method to collect receptor conformations and possible exposure of novel binding sites in large biomolecules. FUNDING: National Programme P3 - European and international cooperation, PN-III-P3-3.5-EUK-2017-02-0030

Total Synthesis Towards Desferrioxamine B Analogues

Athavan Sresutharsan (School of Medical Sciences (Molecular Biomedicine), University of Sydney, Camperdown, NSW 2006, Australia)

Desferrioxamine B (DFOB) is a linear siderophore which forms stable hexacoordinate, octahedral complexes with Fe(III) through three bidentate hydroxamic acid functional groups. The exquisite affinity for Fe(III) indicated the clinical use of DFOB as a scavenger for excess iron in patients with transfusion-dependent haemoglobin disorders. The potential clinical utility of DFOB is under scrutiny, which warrants further study into new DFOB analogues with new properties and function. Studies have used biological and semisynthetic approaches to produce analogues of DFOB, with other work focusing on total synthesis. The proposed synthesis in the current work is predicated on the trimeric structure of DFOB consisting of one monomeric unit A linked to two monomeric unit Bs. Synthesis of native and ether containing monomeric units A and B allowed for the production of a suite of eight analogues of DFOB, including native DFOB, with ether subunits inserted into positions 1 and/or 2 and/or 3. These constitutional isomers are predicted to co-elute with the compounds generated in our group using biosynthetic methods.

The benefit of this synthetic approach lies in allowing access to greater quantities of material compound when compared to the current biosynthetic method. The proposed synthetic scheme is also highly flexible and can be adapted to produce various structural analogues. The synthetic route provides access to trihydroxamic acid adducts as well as dihydroxamic acid and other analogues. Improved access to structural variety may reveal nuances in the relationship between DFOB structure and properties that may inspire further therapeutic use.

A novel β-hairpin peptide derived from the ARC repressor selectively interacts with the major groove of B-DNA

Azzurra Stefanucci (Department of Pharmacy, University G. d'Annunzio Chieti)

Transcription factors (TFs) have a remarkable role in the homeostasis of the organisms and there is a growing interest in how they recognize and interact with specific DNA sequences. TFs recognize DNA using a variety of structural motifs. Among those, the ribbon-helix-helix (RHH) proteins, exemplified by the MetJ and ARC repressors, form dimers that insert antiparallel β-sheets into the major groove of DNA (1,2). A great chemical challenge consists of using the principles of DNA recognition by TFs to design minimized peptides that maintain the DNA affinity and specificity characteristics of the natural counterparts. In this context, a peptide mimic of an antiparallel β-sheet is very attractive since it can be obtained by a single peptide chain folding in a β-hairpin structure and can be as short as 14 amino acids or less (3). Herein, we designed eight linear and two cyclic dodeca-peptides endowed with β-hairpins. Their DNA binding properties have been investigated using fluorescence spectroscopy together with the conformational analysis through circular dichroism and solution NMR.

We found that one of our peptides, peptide 6, is able to bind DNA, albeit without sequence selectivity. Notably, it shows a topological selectivity for the major groove of the DNA which is the interaction site of ARC and many other DNA-binding proteins. Moreover, we found that a type I' β-hairpin folding pattern is a favorite peptide structure for interaction with the B-DNA major groove. Peptide 6 is a valuable lead compound for the development of novel analogs with sequence selectivity.

Shedding UV light on the common origins of RNA and DNA

Rafal Szabla (EaStCHEM, School of Chemistry, University of Edinburgh, Edinburgh, UK)

Ribonucleic acid was likely the first informational polymer on Earth that was responsible for storing genetic information as well as performing enzymatic activity. However, despite numerous efforts, prebiotic syntheses of RNA nucleotides either suffered from missing elements (e.g. lack of prebiotic sources of pure ribose) [1] or given chemical selectivity, were successful for only two out of four of the canonical building blocks [2]. However, recent results suggest that all key components of genetic alphabet could have been delivered prebiotically on Earth as a mixture of RNA pyrimidine and DNA purine nucleosides [3]. In this reaction sequence, UV light offers remarkable selectivity by destroying biologically irrelevant stereoisomers and driving the key chemical transformations.

In this talk, I will demonstrate the key aspects of these photochemically driven reactions. I will also show how the oligomers of these building blocks could have protected themselves from the effects of photodamage formation in UV-rich prebiotic environments [4,5].

Evolution from simple peptides toward RNA polymerase

Shunsuke Tagami (RIKEN)

One of the greatest mysteries in life science is how the central dogma of molecular biology, the elaborate partnership between nucleic acids and proteins, was established on the ancient earth. RNA polymerase, the pivotal protein in the central dogma, is a gigantic enzyme that catalyzes and regulates RNA synthesis in the cell. To understand how such an elaborate protein emerged, we have been trying to experimentally reconstruct the evolutional pathway from primitive peptides to RNA polymerase. Recently, we have demonstrated cationic-hydrophobic peptides can form insoluble beta-aggregates, accrete RNA on their surfaces in a size-dependent manner, and thus enhance the activities of various ribozymes. Furthermore, we have reconstructed the ancient beta-barrel conserved at the core of RNA polymerase by a homodimeric peptide only with seven amino acid types. The seven amino acids are encoded on a clearly defined area in the standard codon table (GNN and ARR). These discoveries describe the evolutional origin of the RNA-peptide partnership, RNA polymerase, and the genetic code.

Microcosamine A, Microgrewiapine A and Microgrewiapine B: Three Homochiral Alkaloids?

Cameron Taylor (University of Oxford)

An asymmetric synthesis of microcosamine A, microgrewiapine A and microgrewiapine B (Microcos paniculata alkaloids) is reported. Conjugate addition of lithium (S)-N-benzyl-N-(α-methylbenzyl)amide to tert-butyl crotonate followed by enolate oxidation generates the C(2) and C(3) stereogenic centres of the target, with subsequent diastereoselective intramolecular reductive amination being used to form the piperidine ring, simultaneously producing the C(6) stereogenic centre. 1H NMR 3J coupling constant and nOe analyses allows unambiguous assignment of relative configuration of the three common stereogenic centres within the alkaloids. Comparison of specific rotation data for microcosamine A and microgrewiapine B is consistent with both possessing the absolute (2S,3R,6S)-configuration. For microgrewiapine A, conflicting data regarding the absolute configuration are revealed: in the isolation study a Mosher's analysis concludes a (2S,3R,6S)-configuration, but comparison of specific rotation data suggests a (2R,3S,6R)-configuration. Thus it is promulgated it is imprudent to suggest the absolute configuration of this natural product; its re-isolation is required for resolution of this problem.

Synthesis of stable isotope-labeled O-glycan standards and their application in clinical diagnosis

Inés Teruel Llinares (CIC biomagune https://www.cicbiomagune.es/)

Gastric cancer is among the top 5 most malignant diseases worldwide killing yearly more than 700000 patients. Due to a lack of efficient methods for its early diagnosis the disease currently has a poor prognosis, although it can be managed efficiently via multimodal treatment including chemotherapy, radiotherapy and surgery if detected at an early stage. For this reason, this project focuses on the discovery and validation of new N- and O-glycans as serum biomarkers for early detection and for the discrimination between healthy and diseased state [1]. Innovative glycan quantification methods based on the use of isotopically labeled glycan standards will be applied. This will allow an absolute quantification of glycan levels in serum in a given clinical state, using by mass spectrometry techniques. Opting for glycans instead on glycopeptides as analytes holds the promise of potentially higher method sensitivity as these glycan moieties are present on many glycoproteins and glycolipids and their enzymatic or chemical cleavage will pool analytes to produce a single entity for each antigen.

Towards this aim we are trying to develop a capture surface of specifically functionalized graphene (Figure 1) to retrieve glycans from the complex matrix and quantify selected biomarkers with the help of 13C labelled internal glycan standards by MALDI-TOF-MS. Target compounds include sialylated Lewis X and Tn derivate antigens (Figure 2), two widely expressed pan-cancer biomarkers, as synthetic targets for the preparation of 13C labelled internal glycan standards. The synthesis of these antigens is performed via various chemical and enzymatic approaches.

Modulation of tau aggregation by bioactive coffee components.

Roberto Tira (Department of Biotechnology of University of Verona)

Neurodegenerative diseases (NDs) are an ever-increasing threat to human life. A primary event in NDs is the misfolding, aggregation, and accumulation of specific proteins in neuronal cells, leading to cellular dysfunction, loss of synaptic connections, and brain damage. In Alzheimer's disease (AD), one of the pathological hallmarks is the presence of intracellular neurofibrillary tangles (NFTs) composed of "paired helical filaments" (PHFs) of hyper-phosphorylated tau [1]. Tau is an intrinsically disordered protein, which transitions between multiple conformations. The in vitro aggregation kinetics profile of tau is well represented by a sigmoidal curve in which three phases are commonly observable: the lag-phase, the exponential-phase and the steady phase. Each of these stages of the aggregation process is characterized by the prevalence of structurally different intermediates, ranging from monomers and small oligomers to active nuclei, protofibrils and elongated fibrils [2]. Mounting evidence indicates that it is possible to perturb the dynamic interconversion of tau among conformational states upon exposure to small molecules, macromolecules, and nanoparticles to redirect the formation of neurotoxic aggregates. Coffee and coffee compounds are attracting interest in the field of neuro-inflammation and neuro-protection against oxidative-stress thanks to their broad availability and ability to cross the Blood Brain Barrier [3]. Recent works demonstrate that some of these molecules such as phenylindanes and other flavonoids have the additional ability to inhibit Aβ and tau protein aggregation [4]. Moreover, they suggested that coffee components might have synergistic effects to produce the overall neuroprotective effect [5]. Relying on these attractive perspectives, with this work, we aim to investigate the potential role of different molecules (trigonelline, theobromine, genistein) in the modulation of tau aggregation. We are confident that this approach based on natural and readily available molecules is fundamental to give further possibilities in the Alzheimer's disease treatment.

CdSe Quantum Dots in human models derived from ALS patients: characterization and multiplexing studies

Carlota Tosat-Bitrián (CIB Margarita Salas - CSIC)

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.[2] Currently, a wide-number of QDs linked to different biomolecules of interest including, antibodies, DNA, peptides, streptavidin, are commercially available enhancing their use as fluorescent probes[3] Using this technology, a broad spectrum of ALS targets, like TDP-43 (TAR DNA-binding protein), phospho-TDP-43, GSK-3β will be analyzed at the single-cell level in human cell models of ALS patients such as lymphoblasts induced from patient derived lymphocytes. The scientific aim of this project is to further contribute to the molecular unravelling 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 with a molecular pathology modulation.

There is high interest in developing NDs sensors for detection, profiling and specially for drug efficacy assessment. Figure 1. Methodology for the multiplexed molecular profile with QDs and specific labelling of TDP-43, p-TDP43 and tubulin with QD565, QD605 and QD655 labelled with secondary antibodies in lymphoblast derived from ALS patients. Nuclei (blue) were stained with HCS Nuclear Mask.

Crystal structures of an unusual transcriptional activator from bacteriophage 186

Jia Truong (University of Adelaide)

The temperate coliphage 186, after infecting its host bacterium Escherichia coli, can follow either the lytic or the lysogenic developmental pathways. Crucial to this developmental decision is the lysogeny promoting factor CII. This potent transcriptional activator activates the early lysogenic promoter pE at least 400 fold, to build up sufficient immunity repressor levels for a portion of infections to commit to lysogeny. Its potency and its unusual property of binding to half-sites separated by 20 base pairs, center-to-center, suggests it may activate the pE promoter by a novel mechanism. Three crystal structures of the CII protein were solved to 2-3Å. The structures reveal that a tetrameric arrangement of CII is necessary for DNA binding, which was subsequently validated by mutational analysis and native mass-spectrometry. CII is degraded in vivo into a specific transcriptionally inactive product.

The crystal structures explain the altered self-association of the degradation product and its loss of activity. The structures combined with mutagenesis data provide a basis for modelling the CII-RNA polymerase complex at the promoter to aid in understanding the promoter activation mechanism. In the field of synthetic biology, CII could be employed to provide strong inducible gene expression in bacterial gene circuits.

DOR and pain: a new interpretative key for persistent pain management.

Rita Turnaturi (Department of Drug Sciences and Health - University of Catania)

Opioid receptors mu, delta and kappa (MOR, DOR and KOR) play a central role in pain management. They are expressed along the nociceptive pathways from primary first-order afferent neurons to the inhibitory descending system. Each opioid receptor represents a distinct target in pain treatment and differently controls nociceptive transmission.Although MOR agonists are considered as milestones in the treatment of acute pain, their efficacy in chronic pain is controversial. In fact, MOR activation produces not only analgesia but also side effects including constipation, nausea and sedation as well as tolerance and dependence development [1]. Recently, due to the availability of highly selective non-peptide agonists, DOR has become an attractive target in persistent pain treatment. In fact, unlike MOR, DOR weakly modulates acute nociceptive pain, but effectively decreases persistent pain. DOR knockout animals showed an increased nociceptive response in neuropathic and inflammatory pain, suggesting the existence of an endogenous DOR tone in these pathological conditions [1]. In this context, Pasquinucci et al. synthesized a series of derivatives of the benzomorphan- based compound LP2, a dual-target biased MOR/DOR agonist whose pharmacological fingerprint has been extensively explored in vitro and in vivo [3-5].Regarding the lead compound LP2, featured by an N-2-methoxyphenylethyl substituent, the new benzomorphan-based compounds have different substituents at the basic nitrogen.

Specifically, derivatives with N-substituents lacking the phenyl group with one or two methylene groups longer chains were synthesized. Moreover, the methoxyl group of LP2 was replaced by an ester or carboxylic group to evaluate the influence of hydrophilic and electron-withdrawing groups for selective DOR binding. The effects of N-substituent modifications on the functional profile of opioid receptors were assessed in vitro. Radioligand binding experiments were performed on brain membranes from rat and guinea pig ileum for MOR and DOR and KOR [6]. Moreover, the efficacy profile of the new compounds was detected by measuring the cAMP accumulation in cells expressing these receptors upon administration with different concentrations of tested compounds. Collectively, our results demonstrate that the N-substituent of newly synthesized benzomorphan derivatives upon binding to the MOR, DOR and KOR plays a pivotal role influencing the affinity, selectivity and efficacy of these receptors.

Synthesis, interaction and biological evaluation of DNA-targeted (2,2'-bipyridine) or (1,10-phenanthroline) p-cymene Ruthenium(II) complexes

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There has been a huge focus on DNA-binding metal complexes over the past two decades due to their high biological activity and their potential as tools for the development of novel and efficient anti-cancer metal drugs [1],[2]. Within the plethora of metal complexes that are currently studied in the field of cancer chemotherapeutics, ruthenium complexes are considered among the most promising compounds because of their in vivo and in vitro antitumor activities, and significantly lower side-effects and toxicity than other metallodrugs commonly used in therapy, such as cisplatin, carboplatin and oxaliplatin [3],[4],[5]. The main goal of the present work is to synthesize metallodrugs derived from Ru(p-cymene)(bpy)Cl2 and Ru(p-cymene)(phen)Cl2 and study their in vitro interactions with DNA secondary structures (G-quadruplex DNA or dsDNA) and cell growth suppression in cultured tumor cell lines (PC3, HeLa), (Figure 1). We report herein the preparation of these novel derivatives and the preliminary study of their interactions with telomeric G-quadruplex and double stranded DNA, by several biochemical and biophysical techniques. Recent data of biological activity will be presented. (Figure 1. Selected biologically active Ru(p-cymene)(bpy)Cl2 and Ru(p-cymene)(phen)Cl2 complexes.) Acknowledgements: Financial support from Spanish MICINN (AEI, Agencia Estatal de Investigación, grant PID2019-108251RB-I00) and from Universidad de Alcalá (CCG20/CC-026, UAH-AE-2017-2) is gratefully acknowledged.

Adaptation of Enzymes in the Valine Pathway for the Production of Methacrylate Intermediates

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Methacrylic acid (MAA) and methacrylate esters (MAs) are important precursors for the synthesis of many useful products, most notably for the manufacture of polymers [1]. The world production capacity of MAA has almost doubled in the past 15 years and reached about 2.2 million tons per year [2]. Currently MAA and derivatives are prepared synthetically by using toxic reagents, such as extremely hazardous acetone cyanohydrin (ACH) and hydrogen cyanide. In addition to this, the ACH process generates significant amounts of ammonium sulphate as by-product [3]. Consequently, there is a pressing need to discover safer and greener synthetic strategies towards the industrial production of MAA and MAs.

In this respect, microbial fermentation and synthetic biology approaches are attractive alternatives. In this poster, we will show how we have engineered an E. coli chassis by assembling key genes into bespoke gene clusters to produce methacrylate precursors such as 2- hydroxyisobutyric acid (2-HIBA).

Accelerating Antibiotic Discovery with Collaborative Technology

Mariana Vaschetto (Collaborative Drug Discovery)

Title: Accelerating Antibiotic Discovery with Collaborative Technology. Susana Tomasio, Kellan Gregory, Mariana Vaschetto Collaborative Drug Discovery, Nine Hills Road, Cambridge, CB2 1JT, United Kingdom email:info@collaborativedrug.com Abstract: The COVID-19 pandemic has brought into focus the need to be prepared for emerging threats. Arguably, one of the most significant concerns in the area is antibiotics resistance. There is a vast amount of data in the Antibiotic field that has been collected over the years and can be used as the springboard to new Drug Discovery projects. Literature data is noisy and need to be curated and trusted to be useful. In addition, successful projects such as the COVID Moonshot, with a worldwide approach to crowd sourcing science ideas, has shown that collaborative efforts are key to accelerate the delivery of new therapies1. We present an application of the CDD Vault research data management platform combining the benefits of using curated antibacterial (Gram-negative) research data in combination with machine learning methods in a collaborative platform to tackle resistance. We will demonstrate that the discovery of antibiotic and infectious diseases drug candidates can be potentiated via effective scientific-community based collaborative research. We use CDD Vault®, a hosted platform designed to archive, mine, and securely share research data across multiple sites and verticals, to accelerate the discovery process by sharing knowledge and build models together based on the past and collective know-how. We will present a few application examples of this technology including the identification/repurposing of Aditeren as a potential good candidate for crossing Gram-negative Bacteria membranes to the Cytosol. In this case, a 55 compounds training set has been used2. A Bayesian statistical model was built using the FCFP6 fingerprints, and was then used to rank compounds available in the CMC dataset. All these data are curated and publicly available freely in the CDD Vault public access section3. 1. https://www.biorxiv.org/content/10.1101/2020.10.29.339317v1 2. Challenges of Antibacterial Discovery (Silver, L. Clin Microbiol Rev. 2011) 3. https://www.collaborativedrug.com/public-access/

Total flavonoid content in edible parts of selected Allium species

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Allium species are a popular and very common vegetable in the human diet. In addition to giving the smell and taste of food, alliums are well known as plants with potential healing effects. Flavonoids from alliums have been demonstrated to have numerous phytotherapeutic effects on human health. In this paper, the content of total flavonoid in the edible parts of selected Allium species (Allium sativum var. saggitatum L. – bulb; A. fistulosum L. – whole plant; A. ampeloprasum var. ampeloprasum L. – bulb; A. nutans L. - leaves; A. odorum L - leaves.; A. schoenoprasum L. - leaves) was investigated, with the aim of assessing the quality of these species. For this purpose 6 samples of fresh plant material were prepared and extracted with 80% methanol (MeOH). The content of total flavonoid (TFC) was determined by the standard spectrophotometric aluminum chloride method, and the obtained results were expressed as mg of quercetin equivalent (QE) per gram of fresh weight. TFC was in range from 0.14 to 0.79 mg/g QE. The lowest content of TFC was determined in A. fistulosum, while the highest content of TFC was obtained in A. schoenoprasum. In A. sativum var. saggitatum L. and A. ampeloprasum var. ampeloprasum L. the presence of flavonoids was not determined. In this study, it was found that the flavonoid content is higher in alliums in which the aboveground parts are used in the diet. In agricultural practice, research is aimed at examining the impact of various agrotechnical measures, especially fertilization, in order to increase the content of secondary metabolites with therapeutic effects, in edible parts of plants.

Utilisation of Porcine Liver Esterase in the Asymmetric Ester Hydrolysis of Radioimmunochemical Ligands

James Wood (The University of Sydney)

Chemical synthesis of organic ligands used in radioimmunochemistry typically requires multiple protection and deprotection steps, which increases the synthetic effort and negatively impacts yield. Enzymes can prove useful in replacing these complex multi-step syntheses with chemical transformations that can be conducted under mild conditions. One chemical reaction that can be conducted under enzyme-mediation conditions is asymmetrical ester hydrolysis. Porcine Liver Esterase (PLE) is a carboxylesterase expressed primarily in pig liver that has an activity with high enantio- and stereoselectivity. PLE can selectively hydrolyse a single ester in a symmetrical ligand to produce an asymmetric product. In this project, a linear ligand designed from an EGTA scaffold was required as a synthetic intermediate. One of the 4 pendent carboxylic acids of EGTA was targeted for selective functionalisation in the final product, which directed the need to protect the remaining 3 carboxylic acid groups. Traditional chemical synthetic methods using stoichiometric control failed to deliver the intermediate, which prompted the revised pathway of global protection of all 4 carboxylic acids as ethyl esters, with the use of PLE for downstream hydrolysis. As EGTA possesses 2 planes of symmetry, PLE was utilised to facilitate the asymmetric ester hydrolysis of a single ethyl ester. When the globally protected EGTA construct was introduced to PLE, a single ethyl ester was hydrolysed to produce a deprotected carboxylic acid. Despite the potential to hydrolyse a second ethyl ester, this was not observed under the reaction conditions. This could be due to slow reaction kinetics associated with the second ester hydrolysis, or the generation of an anionic charge during the initial hydrolysis leading to a compromised PLE-substrate complex. The application of PLE in this synthetic scheme was innovative and provided increased reaction efficiency and yield by streamlining the synthetic process.

The success of this reaction has led to interest in utilisation of this enzyme in other symmetric ligand design. The PLE system was ineffective at hydrolysing DOTA, globally protected in the same manner as the EGTA construct, to be used as a macrocyclic analogue. This difference in substrate specificity of PLE gives insight into active site structure and sheds light on the structure-activity relationship for this enzyme. Overall, this work highlights the merit of enzyme use in organic synthesis.

Covalent labeling of proteins with responsive probes to study protein microenvironment

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The necessity of a balanced pH level in different segments of biological object was proved to be crucial for the functioning of a whole organism, individual cells or even single proteins. In case of proteins, changes in overall pH of molecular microenvironment affect the attractions and reactivities between side chain groups. As a result, changes in protein conformation and/or activity can be observed [1] affecting the affinity of ligands, protein-protein interactions and enzymatic functions [2], which influence physiological and pathological processes. [3] In our work we aim at development of chemical tools to look into particular pH changes in protein nanoenvironment (near or at the surface of it).

These tools are fluorescent probes, covalently labeled to a chosen endogenous protein of interest (POI) [4]. With use of probes responsive to pH changes [5] and super-resolution microscopy techniques, information about local pH differences at a single protein may be obtained. This opens up a possibility of analyzing physiological and pathological processes at currently inaccessible level and can be extended to other analytes beyond pH. This work is funded by National Science Centre in Poland through OPUS grant scheme (grant no. 2018/29/B/ST4/01498).

Interpretable models for mitochondrial toxicity prediction

Hongbin Yang (University of Cambridge)

Machine learning (ML) methods, including deep learning algorithms, can fit complex functions but can be difficult to interpret and be more prone to overfitting. Given its importance in regulatory toxicology and drug discovery, in this study, we aim to develop three methods, namely global substructure occurrence (GSO), k-nearest neighbour (KNN) and local decision trees (LDT), to generate interpretable QSAR models for mitochondrial toxicity. Mitochondrial toxicity assessed in a mitochondrial membrane potential assay (AID720637) was selected as a case study to demonstrate the utility of the models, which comprised 827 positive and 4787 negative unique compounds. In the GSO model, the occurrence in toxic and non-toxic compounds was used to rank the importance of each substructure, and compounds having any of the top-ranked substructures are predicted as toxic. The KNN model used Euclidean distance to obtain the 5 nearest neighbours for majority voting. The LDT models were built with the k nearest neighbours as the training data, and the k was optimised by the validation set. We obtained a final prediction using a combined model by majority vote from the three interpretable models and compared these to a random forest (RF) baseline. The RF model performed the best (Figure 1), with an F1-score (F1) of 0.62 and a balanced accuracy (BA) of 0.81, while the interpretable models were also acceptable (F1=0.56, BA=0.74 for the combined model).

To our surprise, the simplest GSO model also showed good performance (F1=0.51, BA=0.78); however, we found that the key substructures identified by the GSO model did not show sufficient generalisation as we initially expected. We then visually analysed some specific predictions to exemplify the interpretability of the models. Figure 2 shows the prediction of a macrocycle that has mitochondrial toxicity. Compounds containing the specific substructure used for the prediction were also macrocycles and were similar to the query compound (Figure 2C). Therefore, we infer that both the simple models and the complex ML models such as RF just learn the distribution of some specific substructures in the dataset rather than the mode of actions of the toxicity. And hence for decision making, we still need to validate the predictions made using the supporting compounds flagged by the models such as the most similar ones or those with key substructures, which is where interpretable models developed would provide a distinct advantage.