CHIMIA

CHIMIA 2019, Volume 73
ISSN 0009-4293
www. chimia.ch
Supplementa to Issue 7-8/2019

SCS
Swiss Chemical
Society

SCS Fall Meeting 2019
Poster Abstracts

Session of Medicinal Chemistry & Chemical Biology

September 6, 2019
Irchel Campus, University of Zürich, Switzerland
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Molecular level monitoring of long non-coding RNA self-splicing by means of smFRET

E. Ahunbay[1], S. Zelger-Paulus[1], R. Börner[1], R. K. Sigel[1]*

[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.

The dynamics of biomolecules are extensively studied by means of Förster Resonance Energy Transfer (FRET). To disentangle the ensemble average and to visualize distinct conformational states and state-transitions, their fine surveillance is achievable at the single-molecule (sm) level by smFRET [1]. In this study, we take advantage of this technique to monitor the splicing events of a long non-coding RNA, namely the group II intron ai5γ, found in the mitochondria of *Saccharomyces cerevisiae*. Recent advances in the field of splicing by *in vitro* studies under near-physiological conditions especially focus on the nature of ligation of the coding regions catalyzed by the group II intron itself [2]. In addition, these endeavors are greatly complemented with conformational state analysis by employing smFRET [3]. Nevertheless, to fully capture the complex self-catalytic mechanism, it is crucial to couple the changes in the ribozyme structure with function.

Oriented towards multi-color fluorescence imaging, here, we propose a highly precise and stable intermolecular labeling strategy, which is promising to site-specifically tag the particularly long intron, without compromising the ribozyme activity. This bioorthogonal approach involves phosphoramidate activation of the 5’ terminus of the RNA and its further functionalization with an amine modified fluorophore, as well as the periodate oxidation of the 3’ end and its subsequent modification with a hydrazide attached dye [4]. Our choice of FRET pair is Cy3 and Cy5 dyes, donor and acceptor, respectively. Thereby, dual fluorescent labeling of the flanking exons grants the visualization of the folding pathways and the self-splicing mechanism of the intron.

Financial support by the Swiss National Science Foundation (RKOS) and the University of Zurich and the Graduate School of Chemical and Molecular Sciences Zurich (CMSZH) is gratefully acknowledged.

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Looking at the biosynthetic pathway of the molybdenum cofactor in search of the missing metabolite for the Moco riboswitch

F. Amadei[1], S. Gallo[1], K. Schrader[2], G. Schwarz[2], R. O. Sigel[1]*

[1]University of Zurich, Department of Chemistry B, [2]University of Cologne, Institute of Biochemistry

Molybdenum cofactor (Moco) consists of Molybdenum (VI) coordinated to a tricyclic pyranopterin and it is an essential metabolite for almost all living organism. In fact, Moco-dependent enzymes use of the redox properties of the molybdenum centre to catalyze fundamental reactions in the metabolic cycles of carbon, nitrogen and sulphur. Moco is biosynthesized through a highly conserved 4-step pathway starting from GTP which is respectively converted in cPMP, MPT, MPT-AMP and Moco prior to the insertion of the molybdenum centre deriving from molybdate. This pathway is common in almost all living organisms and it involves a variety of different enzymes, which expression seems to be controlled by an ncRNA regulatory element, the Moco riboswitch. In fact, the use of a *Lac*Z reporter system demonstrated that the Moco riboswitch from *E.coli* is capable of gene regulation [1]. However, no evidence of the direct interaction between this RNA and Moco or any of its biosynthetic precursors could ever be observed. This is due to the scarce availability and high oxygen sensitivity of Moco and its precursors. The peculiar organic oxygen-sensitive scaffold, common in most of these molecules makes them unsynthesizable. However, protocols to isolate cPMP from bacteria and to obtain Moco inserted in a Moco carrier protein (MCP) from *Chlamydomonas reinhardtii* were established [2,3]. The main aim of this project is to determinate the identity of the metabolite that causes a conformational change in the Moco riboswitch. For this purpose, native gel electrophoresis and different footprinting assays were performed under strict oxygen-free condition. We proved that GTP, molybdate and cPMP are not specific Moco riboswitch binder. Moreover, both the apo-MCP and the Moco loaded MCP from *C.reinhardtii* cause conformational changes in the RNA structure. However, when the MCP is loaded with Moco, the protein has a higher affinity to the RNA compared to the apo-MCP. Our findings suggest that Moco plays a role in the RNA-protein interaction enhancing the affinity between the two species. These results led to the hypothesis that the MCP is not only involved in the Moco transfer but that it might have an active role in the RNA gene regulation.

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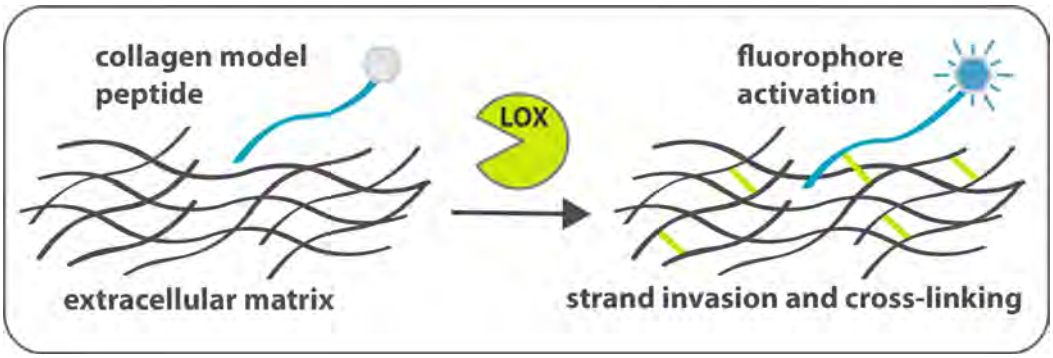
Chemical Approaches to Monitor Collagen Cross-linking in the Extracellular Matrix

M. R. Aronoff[1], N. B. Hentzen[1], L. Pompizi[1], S. Kang[1], H. Wennemers[1]*

[1]Laboratorium für Organische Chemie, D-CHAB, ETH Zürich

Collagen is an essential protein that provides mechanical strength to skin and other tissues.[1] While the characteristic PPII-like secondary structure of the proline-rich collagen peptides enables their assembly into triple helices and further fibrous structures,[2] many of the macroscopic structural qualities like tensile strength and proteolytic resistance arise from the cross-linking of collagen in the extracellular matrix (ECM).[1] This cross-linking occurs post-translationally during collagen fibril formation when lysyl oxidase (LOX) catalyzes the oxidative deamination of the ε-amine of lysine to an aldehyde that can react with adjacent strands in order to generate divalent and trivalent cross-links.[3] While LOX-mediated collagen cross-linking occurs as a normal mechanism in collagen maturation during tissue development or wound healing, perturbations in LOX activity and collagen development are correlated with a number of diseases and disorders including pulmonary and hepatic fibrosis, cardiomyopathy, and tumor metastasis.[4]

Here we present a chemical approach to monitor collagen remodeling and cross-linking with the application of synthetic collagen model peptides (CMPs). Building upon previous knowledge of the conformational effects that dictate the triple helix formation of functionalized collagen, CMPs were created that can anneal and covalently attach within the natural collagen of the ECM.[5,6] Further, in order to visualize cross-linking during the collagen maturation process, a complimentary LOX-activatable fluorescent probe was designed that enables colocalization of the collagen probes with the sites of LOX activity. Ultimately, we envision that these tools will facilitate study of collagen remodeling at the molecular level while simultaneously providing a means to perturb the development of fibrosis by anchoring therapeutics directly at the desired site of action, thus providing new avenues for the diagnosis and treatment of fibrotic disorders and metastatic cancer.



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Influence of peptide composition for the stability of their Ag(I) complexes

L. Babel[1], M. Hologne[2], O. Walker[2], K. M. Fromm[1]*

[1]Department of Chemistry, University of Fribourg, Chemin du Musée 9, 1700 Fribourg, Switzerland., [2]Université de Lyon, CNRS, UCB Lyon 1, ENS-Lyon, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, 69100 Villeurbanne, France.

Histidine, methionine and cysteine are well known to strongly bind silver(I).[1] Model tetrapeptides containing one histidine and one methionine as end groups, HisXXMet, have shown that a linear Ag(I) coordination takes place, forming a 1:1 complex (Figure 1).[2] Moreover, in those tetrapeptides, the binding constant for silver was observed to slightly vary depending on the nature of the two central amino acids. In this study, we investigated the effect of each amino acid for the coordination of silver by the peptide. Fluorescence spectroscopy with a competitive fluorescent peptide containing tryptophan was used to assess the thermodynamic parameters of each of the studied peptides. We observed that glutamic acid (E, Glu) in certain positions in the peptide can enhance the binding character of the peptide while arginine (R, Arg) decreases it. This work also investigates whether this stabilization/destabilization is due to the preorganization of the peptide or to intermolecular coordination of the silver to the glutamate residue. To that end, several NMR measurements were also conducted, and solution NMR structures were resolved.

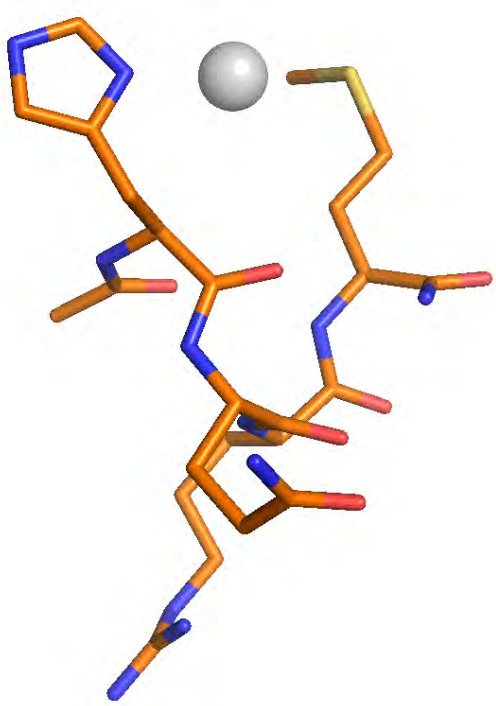

Figure 1. Model of the coordination mode of one Ag(I) by a model peptide inspired by SilE protein containing one HisXXMet unit.

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X-ray structure of a second generation peptide dendrimer as Lectin complex at 1.4 Å resolution

S. Baeriswyl[1], A. Stocker[1], S. Javor[1], T. Darbre[1], J. Reymond[1]*

[1]University of Bern, Freiestrasse 3, CH-3012 Bern

We recently showed that co-crystallization of C-fucosylated peptides with the bacterial lectin LecB from *Pseudomonas aeruginosa* was a very efficient way to obtain high quality X-ray structures of otherwise difficult to crystallize molecules.[1, 2, 3, 4] Herein we report the first complete X-ray structure of a second generation peptide dendrimer. Similarly to the X-ray structure of a first generation peptide dendrimer reported previously [4], this second generation dendrimer engages in intermolecular hydrogen bonds, suggesting that peptide dendrimers may form supramolecular aggregates.

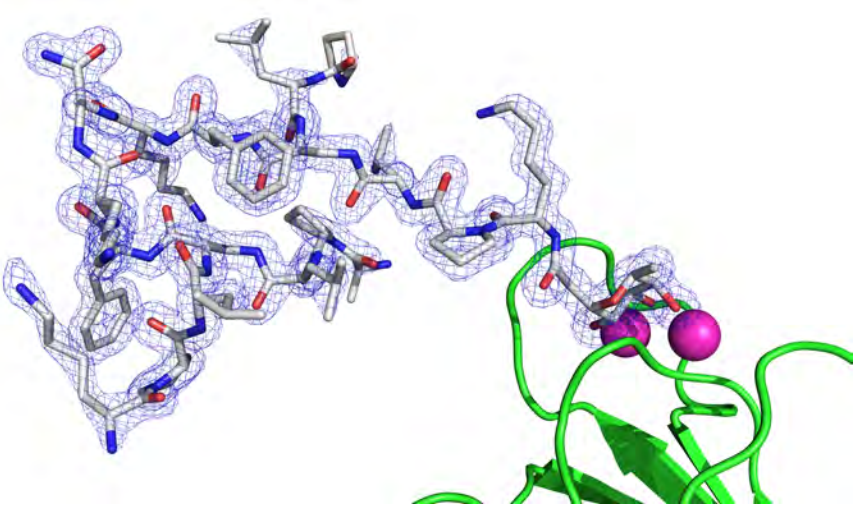

Figure 1: X-ray structure of a second generation peptide dendrimer. The peptide is shown as sticks with the electron density as a blue mesh. The protein is displayed as green cartoon with Ca^{2+} as magenta spheres.

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Factors in bacterial ergothioneine degradation

M. A. Beliaeva[1], A. R. Stampfli[1], A. Maurer[1], F. Leisinger[1], F. P. Seebeck[1]*

[1]Department of Chemistry, University of Basel

Ergothioneine is a sulfur-containing derivative of histidine that emerges from microbial biosynthesis and enters the human body via specific intestinal uptake and regulated tissue-dependent distribution. While the proteins involved in bacterial biosynthesis and uptake by animals are well-characterized [1-3], less is known about the degradative pathways of ergothioneine. We have discovered a network of bacterial proteins that mediate uptake and degradation of ergothioneine and its oxidized derivative ergothioneine sulfonic acid in a broad range of bacteria. In this presentation we describe the crystal structures and functional characterization of these proteins.

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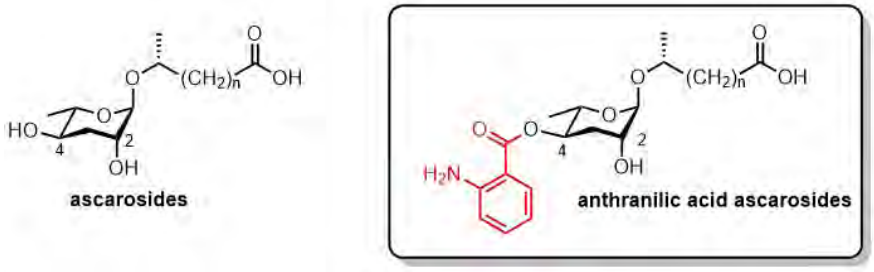
Total synthesis of a species-specific anthranilic acid ascaroside from the nematode Caenorhabditis nigoni

C. P. Bergame[1], C. Dong[2], S. H. von Reuss[1,2]*

[1]University of Neuchatel, [2]Max Planck Institute for Chemical Ecology

Chemical communication in nematodes, such as the model organism *Caenorhabditis elegans*, is regulated through the excretion of pheromones called ascarosides, a family of glycolipids based on the 3,6-dideoxysugar L-ascarylose linked to various fatty acid-like aglycones derived from the peroxisomal β-oxidation cycle [1]. These small molecules regulate nematode behaviour and developmental plasticity, as well as cross-kingdom interaction. This wide range of biological functions is paralleled by the structural diversity of these chemical cues. Indeed, small structural alterations of the side chain or the modular attachment of additional building blocks derived from primary metabolism can dramatically change their biological activity [2]. Comparative MS screening of *Caenorhabditis nigoni*, revealed some highly conserved components and highlighted the presence of species-specific ascarosides [3][4].



Here we describe the isolation, identification, and total synthesis of a novel class of modular ascarosides from *C. nigoni* that carry an anthranilic acid unit of potential L-tryptophan origin at the 4-position. To confirm the structure assignment and obtain material for functional characterization we synthesized the anthranilic acid ascaroside in 17 steps starting from commercially available L-rhamnose. In contrast to previous synthetic procedures that resulted in mixtures of 2- and 4-substituted ascaroside derivatives [2,4] and required tedious chromatographic separations, we now employed a reductive opening of a rhamnose-derived cyclic sulfate to afford a regioselectively protected 3,6-dideoxysugar [5] that facilitated the specific synthesis of the 4-linked isomer.

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Precision Drugs: A Covalent Strategy to Minimize Side Effects of PI3K Inhibitor Cancer Therapy

C. Borsari[1], E. Keles[1], I. Buslov[1], D. Rageot[1], M. P. Wymann[1]*

[1]Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel, Switzerland

Phosphoinositide-3-kinase (PI3K) signaling is a key regulator of cellular processes such as cell growth, proliferation and metabolism. Constitutively activated PI3K is frequent in tumors and drives cancer progression. PI3K is therefore broadly explored as therapeutic target, but many pan-PI3K inhibitors displayed a low response rate in clinical trials - mainly due to adverse side effects. Inhibition of PI3K using class I pan-PI3K inhibitors triggers a rapid increase in blood glucose and insulin.[1] Isoform-selective inhibition of PI3Kα might alleviate hyperglycemia and hyperinsulinemia, but the selectivity of claimed PI3Kα-specific drugs is currently limited. To date, the specific roles of the different PI3K isoforms (mainly PI3Kα and β) in insulin signaling remain controversial. A redundant physiological role of PI3Kβ in insulin action and sensitivity is under investigation.

Herein, we develop a rational approach to increase target selectivity exploiting a covalent binding of inhibitors targeting PI3K isoform-specific nucleophilic amino acid side chains. In PI3Kα, the non-conserved Cys862 is such a residue. A combination of warhead activity design, proximity and orientation allows a tight control of reversible inhibitor binding and isoform selective covalent binding. We have developed assays to characterize and select compounds *in vitro* and in cell. X-ray crystallography together with synthesis of the corresponding reversible analogs, washout experiments in SKOV-3 cells and nanoBRET experiments prove the covalent nature of the compounds. The development of isoform-selective covalent compounds represents a major step towards an increased local and temporal control of PI3K in precise and innovative cancer therapy, and has the potential to minimize metabolic side effects.

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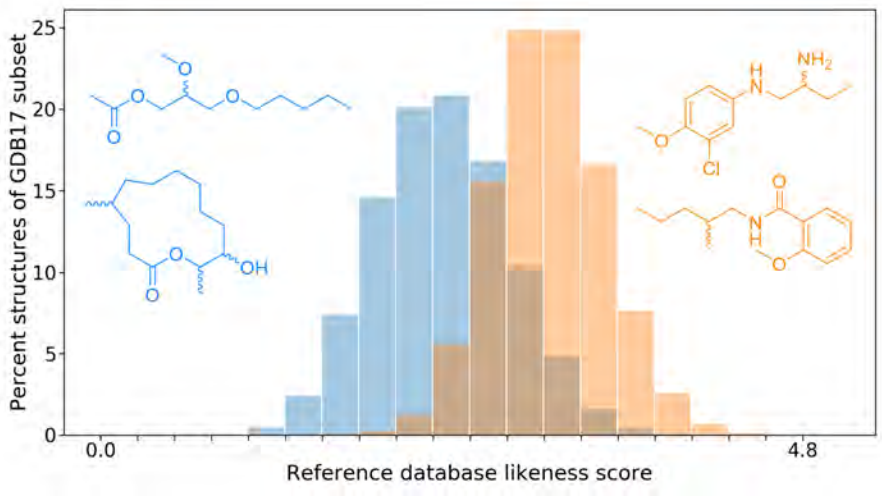
Synthetic Accessibility and Natural Product Likeness of GDB Molecules

S. O. Bühlmann[1], J. Reymond[1]*

[1]University of Bern, Freiestrasse 3, CH-3012 Bern

The generated databases GDB list all possible organic molecules up to a given size obeying simple rules of chemical stability and feasibility.[1] With 166 billion structures up to 17 atoms of C, N, O, S, and halogen, GDB17 represents to date the largest small molecule database.[2] Although our enumeration rules restrict ring strain and functional groups, most GDB17 molecules contain feature combinations which would be difficult to realize in the laboratory. To address this problem we recently reported two subsets of GDB17 with reduced complexity following either fragment rules (FDB17),[3] or medicinal chemistry rule (GDBMedChem).[4] Here we propose an alternative approach based on filtering GDB17 according to synthetic accessibility and natural product likeness scores based on the occurrence of substructures in known molecules using an approach inspired by Ertl et al.[5]

Scoring of GDB17 compounds using the two databases ChEMBL (orange) and UNPD (blue):



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Luminescent glycoclusters: towards lectin detection as a diagnostic strategy

J. P. Byrne[1]

[1]School of Chemistry, National University of Ireland Galway, Ireland

Bacterial infections and contamination are significant causes of illness and death. Novel rapid point-of-care diagnostic strategies are of societal and economic value, particularly in the context of growing antibiotic resistance and hospital-acquired infections (*e.g. Pseudomonas aeruginosa*, a leading cause-of-death in cystic fibrosis patients), where standard lab-based diagnosis usually takes 2–5 days.[1]

Many pathogenic bacteria (*e.g. E. coli, P. aeruginosa*) produce characteristic carbohydrate-binding proteins, such as the important class of proteins called lectins. While much recent research has focussed on the use of glycoclusters and glycomimetics as inhibitors for these proteins, as part of antimicrobial therapeutic strategies,[2] these interactions also offer a potentially valuable pathway towards detection and diagnosis of pathogens. These often-specific interactions have been probed by use of fluorescently-tagged proteins and glycan arrays[3], however, tagging of the carbohydrate-derivatives allows detection of the unmodified proteins.[4]

With this goal in mind, this work presents novel glycocluster molecules, which have luminescent properties (Figure 1). These multivalent systems offer the potential to bind with lectins in solution, functioning as sensors with applicability for diagnosis of bacteria in clinical settings.

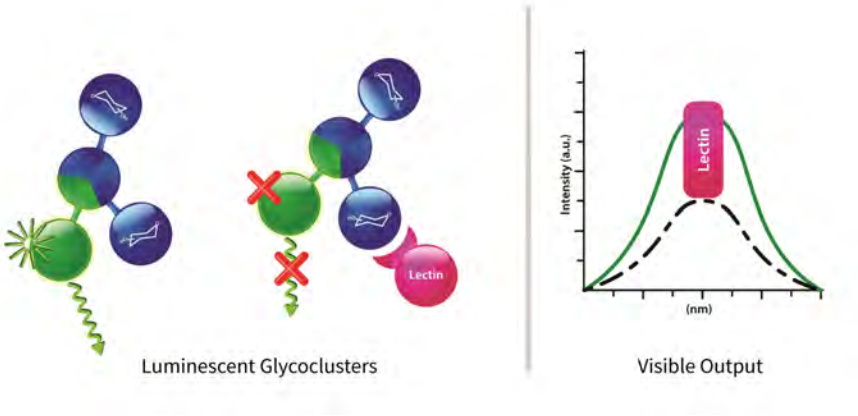

Figure 1. Schematic representation of luminescent glycocluster sensing strategy

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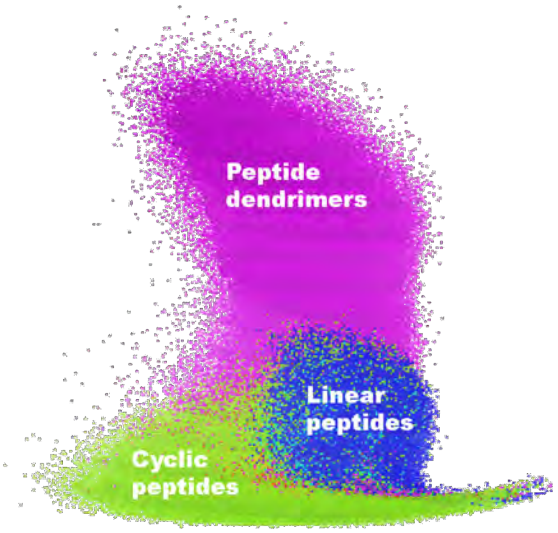
A Genetic Algorithm to Explore the Peptide Chemical Space

A. Capecchi[1], J. Reymond[1]*

[1]University of Bern, Freiestrasse 3, CH-3012 Bern

We recently used the concept of molecular shape and pharmacophores to describe and explore the chemical space of large molecules such as protein from the Protein Databank (Xian Jin et al., 2015), bicyclic peptides (Dibonaventura et al. 2017 and 2018), peptide dendrimers (Siriwardena et al. Angew. 2018), and non-Lipinski molecules from PubChem and ChEMBL (Capecchi et al. 2019). Here we used the same concept in combination with a genetic algorithm (GA) to explore the chemical space of linear, (poly)cyclic and dendritic peptides. Our GA optimizes random peptides for shape and pharmacophore similarity to any target molecule by iterative cycles of mutations and selection, and thereby allows us to explore an extremely vast chemical space of at least 10^{25} peptides without the need for exhaustive enumeration.



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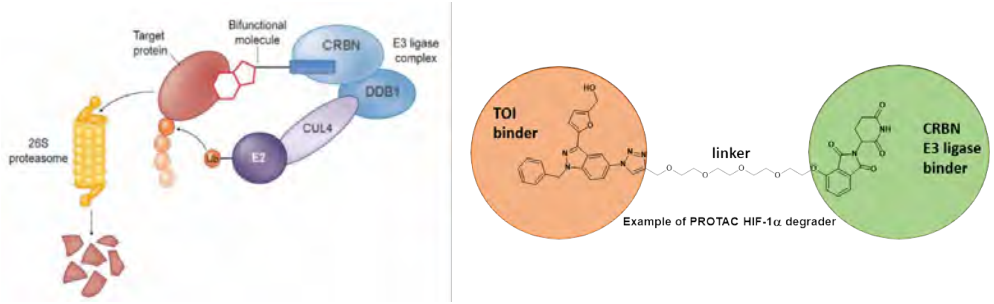
Design and synthesis of PROTACs HIF-1α and -2α degraders

C. Cecchini[1], C. Terenzi[1], V. Ceserani[2], J. Theurillat[2], S. Tardy[1], L. Scapozza[1]*

[1]School of Pharmaceutical Sciences, University of Geneva, Rue Michel-Servet 1, 1206 Genève, [2] Functional Cancer Genomics Group, Institute of Oncology Research, Via Vela 6, 6500 Bellinzona

The classical approach of drug discovery focuses on the development of high selective small molecules able to bind and to inhibit enzymatic active sites. This strategy faces limitations in the context of "undruggable" proteins, which are challenging to target. The discovery of Proteolysis Targeting Chimeras (PROTACs) as an alternative strategy to induce selective protein degradation enabled the development of a new approach against different diseases including cancer.(1) Generally speaking, a PROTAC acts as a bi-functional molecule which can bind a target protein and induces its degradation through a specific ubiquitin ligase. In the context of dysregulated ubiquitination, the E3 ubiquitin ligase adaptor Von Hippel-Lindau (VHL) is recurrently mutated in clear renal cell (RCC) cancer. Typically, the protein function of VHL is entirely lost by gene deletion of one allele and deleterious point mutations on the other allele.(2) The absence of VHL promotes tumorigenesis by accumulation of HIF-2α and HIF-1α that normally are ubiquitinated by VHL and then degraded through the proteasome.(3) In this project, we aim at generating PROTACs which can induce selective protein degradation of HIF-1α and -2α in order to counteract their accumulation in RCC cancer lacking VHL ubiquitin ligase. More specifically, PROTACs can recruit other types of E3 ubiquitin ligases, such as cereblon (CRBN) and can lead to substrate degradation by forming a ternary complex.



PROTACs may be synthetized combining binders for ligases and binders for the target of interest (TOI). VHL and cereblon binders have been already reported in the literature (4), as well as HIF-1α and -2α inhibitors.(5,6) To date, five different PROTACs have been synthetized in good yield (31-81%) using a click reaction approach.(4) Western-blot analysis of cells treated by PROTACs showed that two of them increase degradation of HIF-1α in VHL-deficient RCC cells, while another one enhances degradation of HIF-2α. The biological evaluation of the fifth PROTAC is still ongoing.

These results prove that, in the absence of VHL, cereblon ubiquitin ligase (CRBN) may induce selective degradation of HIF-1α and -2α. Additional work is ongoing in order to characterize further the PROTACs and establish structure-activity relations (SAR). This will allow the optimization of their structure towards more potent and selective HIF-α degraders.

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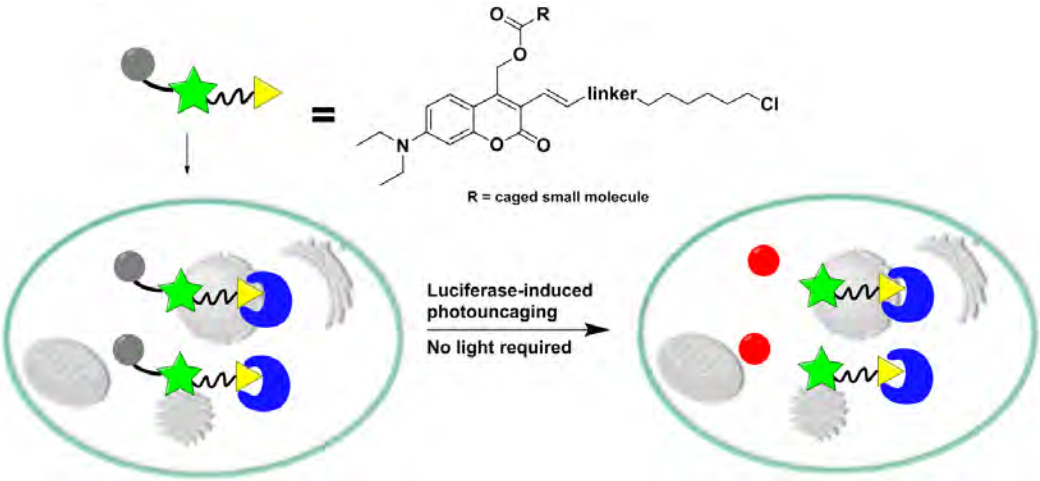
Luciferase-induced photouncaging: Bioluminolysis

D. Chang[1], N. Winssinger[1]*

[1]Department of Organic Chemistry, University of Geneva

Bioluminescence resonance energy transfer (BRET) has been widely used for studying dynamic process in biologic systems such as protein-protein interactions and other signaling events[1]. The vast majority of BRET applications have been focused on using the protein/energy generated by the luciferase as a signal to report an event. More recently there has been a shift, where BRET is utilized as a switcher to perform a function, such as controlling neuronal activity, gene expression, and a photo switchable fluorescent protein[2-5]. Herein, we report the application of BRET to perform a bioorthogonal reaction: coumarin photouncaging to release small functional molecules in living cells, referred to as bioluminolysis. Coumarin caged small molecules are modified with halotag linker to bind with halotag fused Nanoluc protein (H-Luc) covalently. The efficient BRET from H-Luc to coumarin would induce the excited state of coumarin, hydrolysis to release small molecules. We demonstrated the successful release of a fluorescence probe coumarin from H-Luc protein in Hela cells with fast kinetics, breaking the interaction between streptavidin and H-Luc. Moreover, we applied this BRET induced photouncaging system to release inhibitor ibrutinib in SKBR3 cells.



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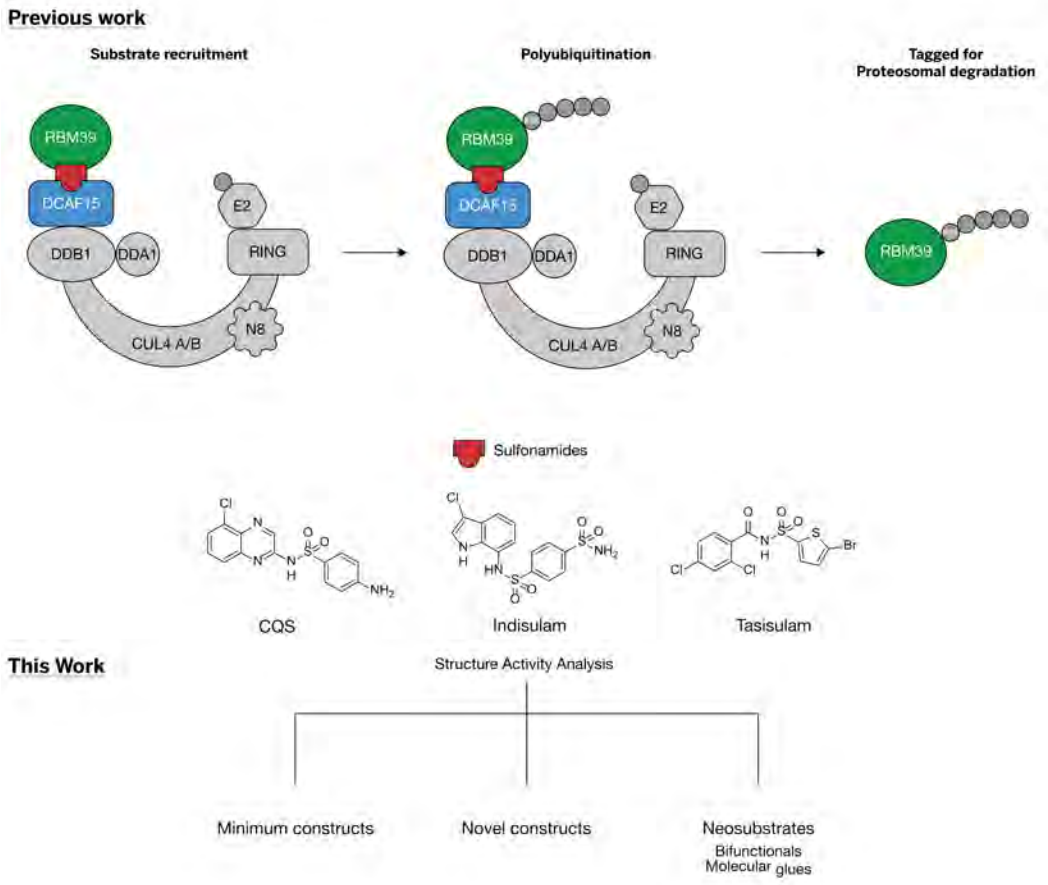
Exploring DCAF15 for reprogrammable targeted protein degradation

S. Coomar[1], D. Gillingham[1]*

[1]Department of Chemistry, University of Basel

Targeted proten degradation by reprogramming E3 ligases with bifunctional small molecules such as PROTACs is an emerging topic in chemical biology. To date only a few E3 ligases have been shown to be amenable for such a purpose. Recently various arylsulfonamides were shown to induce degradation of the splicing factor RBM39 via the RING type E3 ligase CRL4DCAF15. Here we identify the arylsulfonamide most suited for chemical modifications, make a structure activity analysis and demonstrate its behaviour in bifunctional reprogramming.



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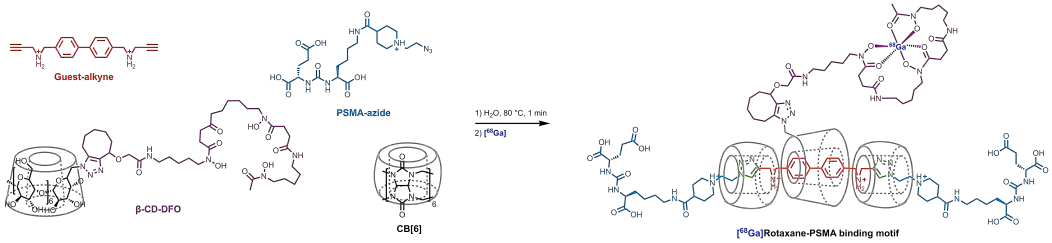
Radiolabelled ^{68}Ga-rotaxanes as non-covalent platforms for supramolecular drug delivery

F. d'Orchymont[1], J. P. Holland[1]*

[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Supramolecular system, such as rotaxanes have shown great potential as carriers for drug delivery systems due to excellent biocompatibility. In particular, host molecules such as β-cyclodextrins (β-CD) and cucurbituril CB[6] have the ability to trap molecules through molecular recognition in their unique hydrophobic cavities. Rotaxanes can be synthesised using the CB[6] alkyne-azide cycloaddition reaction (CB-AAC) [1]. The cycloaddition is catalysed inside of the CB[6] cavity by an efficient preorganisation and stabilisation of the transition state through a hydrogen bonding network between the substrates, CB[6] and β-CD utilised as a co-catalyst [2]. Using this ability of CB[6] and β-CD, we aim to develop an efficient and modular synthetic tool for the synthesis of non-covalently bound targeted radiotracers in high radiochemical yield and purity. Due to the symmetry of the reaction, we can introduce the targeting vector, such as the prostate membrane specific antigen (PSMA) binding motif, Gly-NH-C(O)-NH-Lys twice. To introduce the metal ion to the system, β-CD is monofunctionalised with the Desferrioxamine B chelate (DFO). We thus successfully synthesised a rotaxane within one minute in H_2O by mixing the four components in an ideal stoichiometric ratio at 80 °C. Further optimisation of the one-pot cooperative capture and radiolabelling method as a rapid approach to construct supramolecular radiotracers is advancing and the biological properties of targeted [^{68}Ga]rotaxanes are under evaluation in cellular assays and *in vivo*.

We thank the Swiss Government Excellence Scholarship scheme for a student award (FdO), the Swiss National Science Foundation (SNSF Professorship PP00P2_163683), the Swiss Cancer League (Krebsliga Schweiz; KLS-4257-08-2017), and the University of Zurich (UZH) for financial support.



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Targeted lung cancer bioimaging using multifunctional harmonic nanoparticles

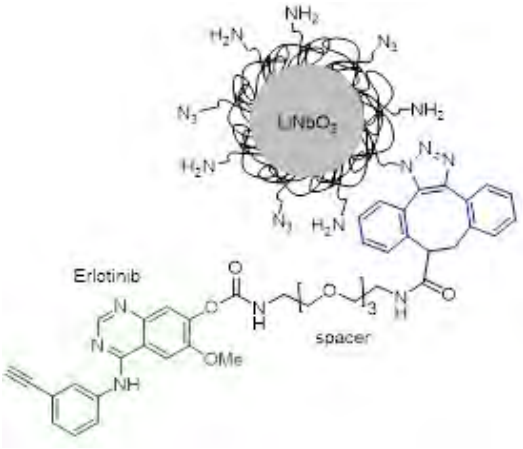
R. de Matos[1], J. Vuilleumier[1], S. Passemard[1], D. Staedler[2], S. Constant[3], L. Bonacina[4], S. Gerber-Lemaire[5]*

[1]Ecole Polytechnique Fédérale de Lausanne, [2]TIBIO Sagl, [3]Epithelix, [4]University of Geneva, GAP-Biophotonics, [5]Institute of Chemical Sciences and Engineering, EPFL Lausanne

The recent and rapid progress in nanotechnologies has paved the way for the investigation of nanomaterials in clinical settings for early detection, diagnosis and targeted treatment of cancer, which represents a major health burden in developed countries.[1] The ability to produce inorganic nanoparticles of tunable size and composition, combined with their surface properties suitable for chemical functionalization have generated intense efforts to develop novel theranostic tools based on multifunctional nanomaterials.[2]

Lung cancer is the most frequently diagnosed cancer in men and women and represents the most common cause of cancer-related deaths, both in the United States and in Europe, with a significant rate of 27% and 21% of total cancer deaths.[3] Epithelial growth factor receptor (EGFR) was reported as a valuable biomarker for lung cancer targeting due to its overexpression in non-small cell lung cancer.[4]

In this context, we present the design, synthesis and evaluation of an analogue of the EGFR tyrosine kinase inhibitor Erlotinib. This derivative was further conjugated to the surface of poly(ethyleneglycol) (PEG) coated harmonic LiNbO3 nanoparticles for targeted multiphoton imaging of lung cancer cells expressing EGFR.



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Synthesis and Photophysical Properties of BODIPY-Tethered Trithiolato-Bridged Dinuclear Ruthenium(II)-Arene Compounds

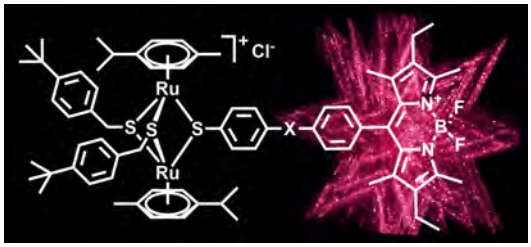
O. Desiatkina[1], E. Păunescu[1], J. Furrer[1]*

[1]University of Bern, Department of Chemistry and Biochemistry, Freiestrasse 3 CH-3012 Bern, Switzerland

Cationic trithiolato-bridged dinuclear ruthenium(II)-arene compounds bearing one ($[(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-SR})_3]^+$) or, two ($[(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-SR1})_2(\mu_2\text{-SR}_2)]^+$) types of thiol ligands, exhibit interesting anticancer[1] and antiparasitic activity.[2] Ascertaining and quantifying their intracellular uptake and fate, and identifying their mechanism of action has become a prerogative for entering clinical trials in a near future. One of the methodologies enabling the investigation of sub-cellular localization of metal complexes is the confocal fluorescence microscopy of fluorophore-tagged conjugates.

BODIPYs have attracted a considerable interest, due to their advantageous photophysical properties, which can be tuned *via* chemical modifications. These dyes have found numerous applications in optical imaging, biological labeling, and fluorescence sensing.[3] There are only few reports describing the tagging of bioactive Ru(II)-arene compounds with BODIPY dyes and the use of the resulting hybrid molecules in imaging.[4,5]

In this context, we have synthesized a series of new BODIPY-tethered trithiolato-bridged dinuclear ruthenium(II)-arene conjugates by anchoring the dye as pendant arm on one of the bridged thiol presenting a suitable functional group. Three types of covalent linkage (ester, amide and amine bond) between the organometallic moiety and the fluorophore were explored. In addition, we have measured their photophysical properties and compared them to those of the free dyes and of previously reported ruthenium analogues presenting different BODIPYs fastened on the binuclear core on flexible linkers.



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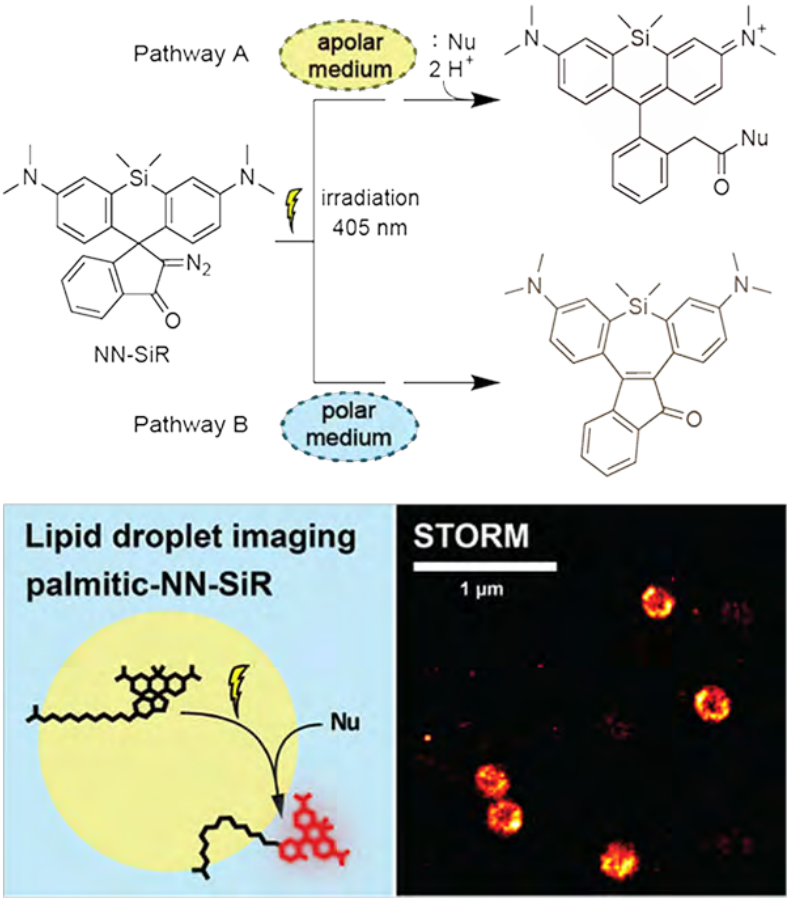
Single-Molecule Imaging of Lipid Droplets with Environment-Sensitive Photoactivatable Probes

A. Eordogh[1,4], C. Paganini[2], D. Pinotsi[3], P. Arosio[2], P. Rivera-Fuentes[1,4]*

[1]Laboratory of Organic Chemistry, ETH Zurich, [2]Biochemical Engineering Laboratory, ETH Zurich, [3] Scientific Center for Optical and Electron Microscopy, [4]Institute of Chemical Sciences and Engineering, EPF Lausanne

Stochastic optical reconstruction microscopy (STORM) is a superresolution microscopy technique which relies on bright fluorophore labels that allow optical control of the fraction of emitting molecules.[1] Photoactivatable, diazoindanone-masked silicon rhodamine (NN-SiR) is well suited for this application.[2] The photoactivation of NN-SiR, displays two main pathways: A) formation of fluorescent photoproduct, via Wolff-rearrangement with subsequent reaction with a nucleophile, and B) non-fluorescent photoproduct formation via an alternative intramolecular carbene insertion. Pathway A was found to take place in apolar environments, so we chose lipid droplets to showcase the use of environment dependent photoactivation in bioimaging.
Photoactivation experiments were carried out in oil droplet model systems, generated in a microfluidic device. To achieve stable labeling of phase boundary, a palmitic acid derivative was synthetized, which was used to visualize lipid droplets in live and fixed cells. Live cell STORM imaging was accomplished using lipid droplet induced HeLa cells, and 3D-STORM was used on fixed cells to display shell-like labeling of lipid droplets.



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Sortase mediated conjugation of triglycine functionalised chelates to Her2 targeting DARPins

R. Fay[1], J. Schaefer[2], B. Dreier[2], A. Plückthun[2], J. P. Lamb[1]*

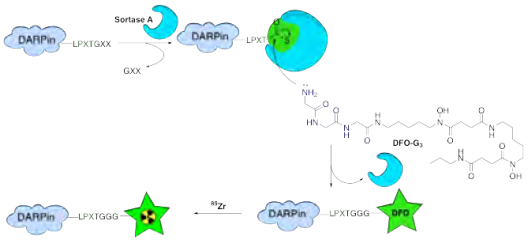
[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland, [2]Department of Biochemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland.

DARPins (Designed Ankyrin Repeat Proteins) are highly stable genetically engineered antibody mimics. The G_3-DARPin possesses picomolar affinity for Her2 (Human epidermal growth factor receptor) which is overexpressed in many breast and ovarian cancers. The G_3-DARPin therefore has potential in diagnostic imaging of Her2 positive cancers.

The Sortase-A enzyme enables site-specific transpeptidations to occur between biomolecules containing a N-terminus LPXTG amino acid motif and triglycine functionalised probes. The Sortase-A enzyme converts the threonine residue present into an activated thioester which can undergo nucleophilic attack by a triglyine probe in theactive site of the sortase enzyme. N-terminus His_6tags are installed on both the Sortase-A enzyme and the DARPin substrate. Successful conjugation to the DARPin results in His_6tag cleavage which enables the product to easily be separated from the starting materials by Ni-NTA affinity chromatography.

The synthesis of novel triglycine modified chelates, HBED-CC-G_3, and DFO-G_3, which can coordinate the radionuclides $^{68}Ga^{3+}$and $^{89}Zr^{4+}$was a prerequisite to utilise Sortase-A bioconjugation to form novel immunoPET agents. DFO-$G_3$was synthesised in two steps from DFO mesylate in an overall yield of 29%. HBED-CC-G_3 was synthesised in seven linear steps from 3-(4-hydroxyphenyl)propionic acid in an overall yield of 15%. DFO-G_3 and HBED-CC-G_3 were conjugated to the G_3-DARPin using a 25 fold or 50 fold excess of ligand. The purified DFO-G_3-DARPin could be radiolabelled with a radiochemical conversion (RCC) >95% (radio-TLC), a radiochemical purity >90% (NAP-10) and a molar activity of around 6.3 MBq/nmol. The immunoreactivity of [^{68}Ga]GaHBED-CC-G_3 was determined *in vitro* in SK-OV-3 (Her2 +ve) cells was determined to be 75±8%.

We have been able to successfully utilise Sortase-A conjugation of two different triglycine modified chelates to synthesis new DARPin conjugates which can be labelled with $^{68}Ga^{3+}$and $^{89}Zr^{4+}$. These conjugates may be further studied as PET tracers to image Her2 positive tumours *in vivo*.



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A novel Post-Treatment process of medical and pharmaceutical material using $scCO_2$

M. Furlan[1], P. Widmer[1]*

[1] eCO2, Via Brüsighell 6, CH-6807 Taverne, Switzerland

The paramount advantage of using $scCO_2$ over other extraction solvents such as alcohol and hydrocarbons resides on its unique tunable solvation properties, based on temperature and pressure, combined with its low toxicity and negligible residual. Since CO_2 effectively inactivates microorganism it can be applied as final sterilization step, thus in combination with the post treatment process the overall process time can be significantly reduced. Furthermore, to reach the supercritical state, CO_2 require temperature close to the physiological value thus allowing the treatment of temperature sensitive materials, these can be damaged by solvent-based process and by classical sterilization methods.

The proposed process developed by eCO_2 face al those challenges, that have to be overcome in order to get reproducible product characteristics and cGMP compliant product purity between the production batches. The innovative nature of the process lies in the horizontally placed process chamber equipped with a special rotatable basket. The rotation of the basket, which enhances the mass transfer and thus the dissolution of the impurities to be removed, lead to homogeneous conditions through the entire process chamber volume while the ordered placement of the material inside the basket increases the contact area and allows a homogeneous exposure with the $scCO_2$. These conditions turn to be essential for an even purification effect through the entire volume of the process chamber which is of paramount importance for the quality of the final product and thus for the reproducibility of the process. We compared the proposed post treatment process with a well-established solvent-based purification process for the purification of a commercial bio-material. Several campaigns have been conducted by stacking the materials to be treated at the top, middle and bottom of the basket and analyzing their final impurity residual content. The analysis showed that no difference in purity could be detected between the different positions, suggesting that the exact location of the material (at the periphery, in the middle, or at the center of the basket) has no influence, thus obtaining homogeneous composition among the charge. No difference in purity could be detected also when the samples were packed in contact with each other. Furthermore, the proposed process is 10-times faster compared with the traditional one, which lasts for more than 10 days, and it also decrease the raw materials expenses by a factor 10.

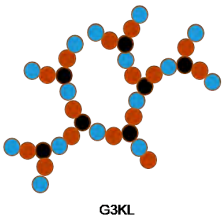
The new proposed post-treatment process for impurities removal through $scCO_2$ has proved very promising as substitute of standard solvent-based processes allowing to reduce by a factor 10 both the process time and the raw material expenses. The tunability of the solvation properties by mild temperature and pressure conditions and the low toxicity of CO_2 coupled with the horizontal design of the process chamber with the rotating basket lead to an even impurity removal. The treated materials do not show any composition difference related to the position in the basket. Due to the homogeneous conditions and the batch to batch reproducibility the proposed process complies with the cGMP standards and thus can be applied in the pharmaceutical-, medical-, and food-industries.

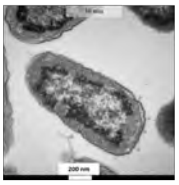
Killing mechanism of antimicrobial peptide dendrimer G3KL investigated by fluorescence microscopy

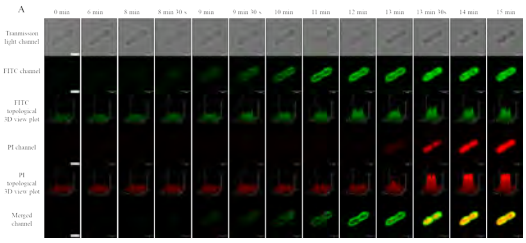
B. Gan[1], T. Darbre[1], J. Reymond[2]*

[1]University of Bern, [2]University of Bern, Freiestrasse 3, CH-3012 Bern

We recently discovered that peptide dendrimers such as **G3KL**, consisting of three successive generations of lysine-leucine dipeptides connected by lysine branching residues, efficiently kill Gram-negative bacteria including multidrug resistant clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, opening a new avenue to address this public health threat.[1] Here we report two fluorescent analogs of **G3KL** bearing either fluorescein (**G3KL-Fluo**) or dansyl (**G3KL-Dansyl**) that show a similar bioactivity profile to **G3KL**. We combined biophysical and imaging techniques to study their mechanism of action. Super-resolution stimulated emission depletion (STED) microscopy and time-lapse imaging revealed that fluorescein-labelled **G3KL** localize at the bacterial membrane and traverse the bacterial cell wall to accumulate in *P. aeruginosa*. Shortly after the addition of the compound, the bacterial membrane is permeabilized as demonstrated by the strong influx of propidium iodide into the cell. Quantification of bacterial uptake shows that **G3KL-Fluo** can accumulate in the bacteria up to millimolar range. Transmission electron microscopy images together with LPS competition assays show that **G3KL** completely destroys the outer leaflet and can bind to the released LPS. As it crosses the bacterial cell wall, it disintegrates the inner membrane and form large aggregates together with the compound and DNA in the bacteria.







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Carboxymethylated and methylated DNA damage as a basis for prediction of colon cancer risk

S. M. Geisen[1], T. Kostka[2], M. T. Empl[2], P. Steinberg[3], S. J. Sturla[1]*

[1]Department of Health Sciences and Technology, ETH, [2]Institute for Food Toxicology, TiHo Hannover, [3]Federal Research Institute of Nutrition and Food, MRI

N-Nitroso compounds (NOCs) are known human carcinogens and a diet high in red or processed meat stimulates their formation in the human gut. The molecular basis of NOC carcinogenesis involves DNA alkylation, or formation of DNA adducts, leading to replication-associated mutagenesis. Among the DNA adducts formed from NOC exposure, those arising from alkylation of the oxygen in the DNA base guanine include the pro-mutagenic lesions O6-methyldeoxyguanine (O6-MedG) and O6-carboxymethyldeoxyguanine (O6-CMdG). Although O6-MedG gives rise to both GC-AT and GC-TA transition mutations, the protein O6-methylguanine DNA methyltransferase (MGMT) repairs O6-MedG and thereby offering protection from genotoxic effects. Whether MGMT can also repair O6-CMdG however, thereby mitigating additional effects of this damage in target cells, is not yet known. To understand the potentially protective role of MGMT in O6-CMdG-induced mutagenesis, we established a nanoflow liquid chromatography tandem mass spectrometry approach for simultaneously quantifying O6-MedG and O6-CMdG in biological samples. We applied this approach to test whether expression of MGMT reduced the O6-CMdG burden of human colon epithelial cells (HCEC) exposed to a carboxymethylating agent and whether these persisted by treating cells with the MGMT-inhibitor O6-Benzylguanine. The data suggest MGMT may offer protection from O6-CMdG mutagenicity, but less effectively than for O6-MedG. These results are anticipated to help identify individuals or populations susceptible toward persistent DNA damage derived from (carboxy-) methylating agents and understand factors that mitigate its adverse effects.

Developing the production of radiolanthanide ^{161}Tb and its characterization towards clinical application

P. V. Grundler[1], N. Gracheva[1], Z. Talip[1], J. R. Zeevaart[2], U. Köster[3], C. Bailat[4], Y. Nedjadi[4], T. Duran[4], R. Schibli[1,5], C. Müller[1]*, N. P. van der Meulen[1,6]*

[1]Center for Radiopharmaceutical Sciences ETH-PSI-USZ, Paul Scherrer Institute, Villigen-PSI, Switzerland, [2]Radiochemistry, South African Nuclear Energy Corporation (Necsa), Brits, South Africa, [3]Institut Laue-Langevin, Grenoble, France, [4]Institute of Radiation Physics (IRA), Lausanne, Switzerland, [5]Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland, [6]Laboratory of Radiochemistry, Paul Scherrer Institute, Villigen-PSI, Switzerland

The radioisotope ^{177}Lu is currently regarded as the “gold standard” of radionuclide therapy. However, the cancer does not tend to remain in remission. From this perspective, ^{161}Tb ($T_{1/2}$ = 6.89 d), a therapeutic radiolanthanide which shows similar decay characteristics and chemical behaviour to that of ^{177}Lu, may be superior as a result of its co-emission of conversion and Auger electrons which can kill small metastases [1]. Stringent quality requirements must be met to allow in vitro and in vivo experiments to be performed reliably. Efforts were put into development and improvement of the ^{161}Tb purification process based on the production method reported previously [2].

Enriched ^{160}Gd oxide targets were irradiated either at the SAFARI-1 (South Africa) reactor, the high flux reactor of ILL (France), or the spallation-induced neutron source (SINQ) at PSI (Switzerland) using the ^{160}Gd(n,γ)^{161}Gd→^{161}Tb nuclear reaction to yield a no-carrier-added (n.c.a.) product. ^{161}Tb is separated from ^{160}Gd using cation exchange chromatography and concentrated using extraction chromatography before elution of the final product at high yields (8-20 GBq) in a small volume.

The radionuclidic purity of $^{161}TbCl_3$ was ≥99.9% at End of Separation (EOS). Using this quality of product, it was possible to achieve labelling of DOTANOC with ^{161}Tb at 180 MBq/nmol specific activity at a labelling efficiency of ≥99%. The radiolabelling yield of DOTA with ^{161}Tb was comparable to n.c.a. ^{177}Lu over a two-week period. With the aim of future instrument calibration, the nuclide is also being assessed metrologically with ongoing standardization and half-life measurements of ^{161}Tb.

In conclusion, high yields of $^{161}TbCl_3$ in a quantity and quality suitable for high-specific radiolabelling, useful for preclinical and potential clinical application, was produced using a variety of irradiation sources and an innovative chemical separation method.

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Structure-activity studies on the photoradiosynthesis of ^{89}Zr-DFO-labelled MetMAb for molecular imaging of gastric cancer

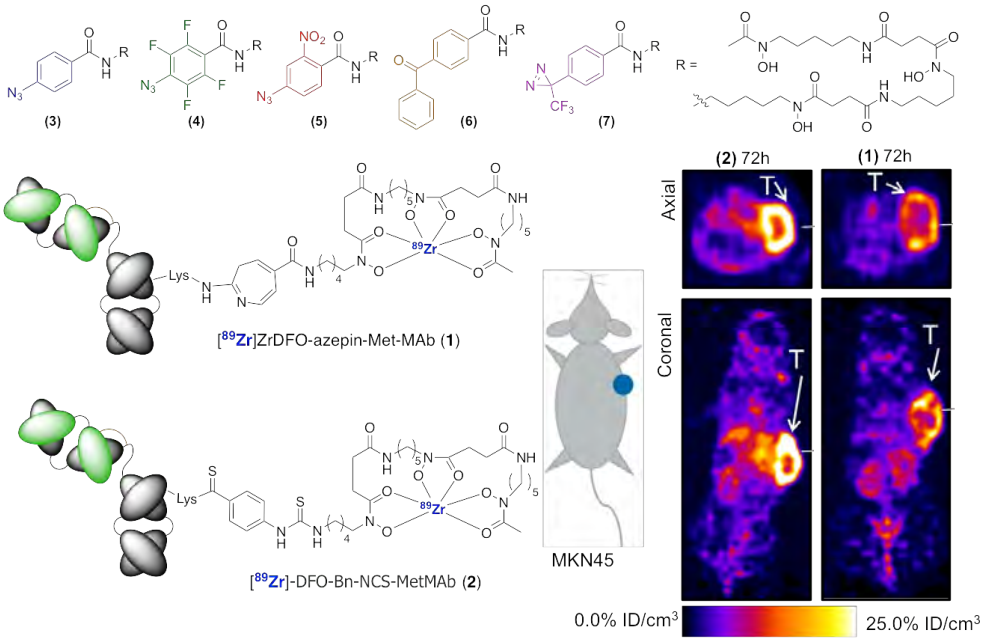
M. Gut[1], S. Klingler[1], R. Fay[1], L. Eichenberger[1], J. P. Holland[1]*

[1]Department of Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich

Classic methods to radiolabel proteins are typically multi-step procedures. First the protein is functionalised and purified, then in a second step, the protein-conjugate is radiolabelled. Our group recently developed a method to radiolabel antibodies using a photoactive aryl azide in a one-pot procedure in

In this study, structure-activity relationships were explored by coupling different photoreactive groups to the chelate deferoxamine (DFO) and evaluating their photochemical conjugation properties using the engineered antibody fragment, MetMAb (onartuzumab). Experiments showed that under our experimental conditions, the non-substituted aryl azide (**3**) is the most reactive (~53% photoradiochemical conversion [PCC]), followed by the tetra-fluoro aryl azide (**4**) with PCC ~35%. Photoradiochemical labelling using the *meta*-nitro arylazide (**5**) was successful but PCC reduced to ~16%. By changing the nature of the photoactive group we also showed the benzophenone derivative (**6**) and the diazirine (**7**) led to no protein radiolabelling.

The most successful photoreactive chelate was then used for *in vitro*and *in vivo*studies. ^{89}ZrDFO-azepin-MetMAb (**1**) was synthesized using a one-pot approach and the biochemical properties were compared to ^{89}Zr-DFO-Bn-NCS-MetMAb (**2**) produced via standard bioconjugation methods. Similar specific tumour uptakes were observed in PET images in mice bearing MKN-45 xenografts, with ex vivo biodistribution indicating that tumour-associated radioactivity reached 15.4±5.2 (*n*= 4) for **1** and 21.4±11.6 (*n*= 4) %ID/g for **2** at 72 h post-injection. Blocking studies confirmed that tumour-uptake of the radiotracers was specific. Overall, experiments showed that photoradiochemical labelling of proteins with chelates derivatised with non-substituted aryl azides is a viable approach for radiopharmaceutical synthesis. Further studies are underway to address the scope of photoradiosynthesis with different radionuclides, chelates, photoactive groups and biological vectors.



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Development of a therapeutic collectin-11 inhibitor to minimize ischemia reperfusion injuries

R. Hevey[1], D. Berta[1], M. Maraj Martinez[1], T. Wichers[1], T. Brunner[1,2], M. Smieško[2], S. Rabbani[1], D. Ricklin[1]*

[1]Molecular Pharmacy, Dept. Pharmaceutical Sciences, University of Basel, [2]Molecular Modeling, Dept. Pharmaceutical Sciences, University of Basel

The complement system is a complex pathway which plays an important role in innate immunity and as a first line of defense against invading organisms, damaged tissues, and maintenance of healthy cell populations. Despite its association with host defense, complement can turn against host cells when dysregulated or excessively triggered. Complement is considered a major contributor to various inflammatory diseases, and its lectin pathway has been implicated in transplantation- and ischemia-triggered clinical conditions. Specific inhibition of this initiation pathway may therefore pave the way for novel therapeutic approaches.

Among the potential targets are collectins, a family of C-type lectins which bind pathogen- or damage-associated molecular patterns (PAMPs/DAMPs), thereupon enhancing phagocytosis and/or inducing surface opsonization. Collectin-11 (CL-11, CL-K1) is broadly expressed in multiple tissue types and has been described as an activator of complement via the lectin pathway. It has been previously demonstrated that locally-produced CL-11 plays an important role in both acute kidney injury and late-stage/chronic renal inflammation, and that preventing CL-11 binding to the renal cell surface can reduce neutrophil filtration, C3d deposition, and tubular injury [1,2].

Given the inherently weak affinities of native carbohydrates for lectins, we have been focused on developing glycomimetic inhibitors of CL-11 with enhanced affinities and improved pharmacokinetic properties. Based on insights from *in silico* molecular dynamics simulations, we have constructed several glycomimetic libraries and evaluated them using biochemical and biophysical methods in efforts to eventually develop a therapeutic entity, as well as to further evaluate the biological role of CL-11.

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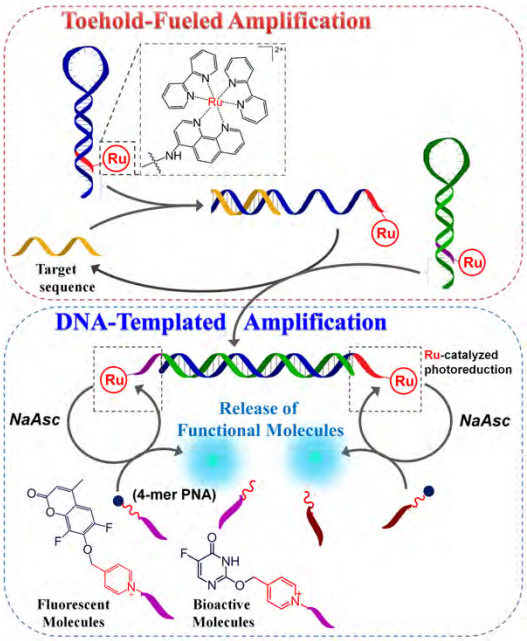
Robust DNA Circuit for the Release of Functional Molecular Outputs

K. T. Kim[1], S. Angerani[1], D. Chang[1], N. Winssinger[1]*

[1]Department of Organic Chemistry, NCCR Chemical Biology, Faculty of Science, University of Geneva, 30 quai Ernest Ansermet, 1205 Geneva, Switzerland

DNA circuits have been used as computational devices that convert an oligonucleotide input into a specific output. However, canonical DNA circuits suffer from low yield and limited output scope, typically oligonucleotides. Herein, we demonstrate the coupling of a DNA circuit to templated reactions in order to achieve high level of amplification in the output of a small functional molecule in response to a nucleic acid input. The system features fast reaction kinetics regardless of the target length, high output based on quadratic signal amplification, and also target-specificity at single-nucleotide level, which goes beyond the ability of the canonical DNA circuit and the templated reaction.



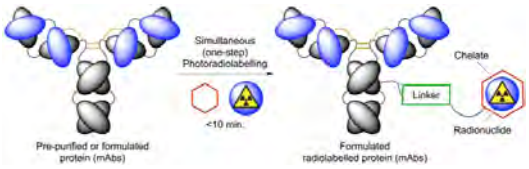
Simultaneous photochemical conjugation and ^{89}Zr-radiolabelling of antibodies for immuno-PET

S. Klingler[1], L. Eichenberger[1], J. P. Holland[1]*

[1]Department of Chemistry, University of Zurich, Win

A method for the simultaneous, one-step photochemical conjugation and ^{89}Zr-radiolabelling of antibodies was developed. The aim of this work was to leverage our recent identification of a photochemically activated pathway for rapid, one-step conjugation and radiolabelling of antibodies for immuno-PET (Figure 1).[1-4]

A photoactivatable chelate based on desferrioxamine B functionalised with an arylazide moiety (DFO-ArN_3 [**1**]) was synthesised in one step and purified by semi-preparative high-performance liquid chromatography with a purity >95%. Density functional theory (DFT) calculations were used to investigate the mechanism of arylazide activation and photochemical conjugation to primary amines. Time-dependent DFT calculations were also used to probe the nature of the electronic excited states involved in the photochemical reactivity of the arylazide group. The radiolabelled complex, ^{89}Zr-**1**$^{+}$ was produced and characterised *via* standard radiochemical methods. ^{89}Zr-radiolabelling experiments were used to determine the efficiency of photochemical conjugation *via* a conventional two-step approach and by a simultaneous one-step method. A two-step approach involving photochemical coupling then ^{89}Zr-radiolabelling of the intermediate DFO-azepin-trastuzumab gave a measured photochemical conjugation efficiency of 3.5 ± 0.4%. In contrast, the simultaneous one-step process gave a higher conjugation (and photoradiolabelling) efficiency of ~76% making the photoactivation process competitive with many existing thermally-mediated bioconjugation methods. Stability measurements, cellular-based saturation binding assays, PET imaging and biodistribution studies in mice bearing SK-OV-3 tumours were performed. Experimentals in cells and *in vivo* confirmed the biochemical viability of photoradiolabelled [^{89}Zr]ZrDFO-azepin-trastuzumab. PET imaging revealed high tumour uptake. Comparison of the biodistribution data between the normal and blocking groups showed a specific accumulation of radioactivity in SK-OV-3 tumours (65.8 ± 14.2 %ID g^{-1} in the normal group *versus* 12.1 ± 4.1 %ID g^{-1} in the blocking group, *P*-value = 0.0006).



Experimental data support the conclusion that the combination of photochemistry and radiochemistry is a viable strategy for producing radiolabelled antibodies, immunoglobulin fragments and other proteins for imaging and therapy. This alternative technology has many attractive features and work is ongoing to explore the full potential of our photoradiochemical approach for labelling other proteins, peptides, small-molecules and nanoparticles with various chelates and radionuclides.

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Exploring the nature of UV induced RNA-protein cross-linking with the help of CLIR-MS

A. Knörlein[1], C. Sarnowski[2], T. de Vries[3], M. Götze[2], F. Allain[3], R. Aebersold[2], J. Hall[1]*

[1]Institute of Pharmaceutical Sciences, ETH Zürich, [2]Institute of Molecular Systems Biology, ETH Zürich, [3]Institute of Molecular Biology and Biophyiscs, ETH Zürich

RNA-protein interactions play a fundamental role in many biological processes. Perturbations of these interactions can lead to cellular dysfunction and diseases including neurodegeneration and cancer. [1] Therefore, identifying the RNA targets of RNA binding proteins (RBPs) is crucial to understand the molecular mechanisms of RBP-mediated diseases.[2] State-of-the-art methods to reveal the positing of a protein on RNA are UV crosslinking and immunoprecipitation (CLIP) and its related protocols.[3] All these technics depend on the inherent reactivity of ribonucleotides under 254 nm UV irradiation to induce an irreversible, covalent cross-link with interacting proteins in close proximity. Even though UV crosslinking is widely applied due to its simple procedure, the standard UV-crosslinking reaction remains poorly understood. A preference in crosslinking efficiency for specific nucleotides and amino acid residues is suggested, but to our knowledge never investigated in a systematic manner.[4]

We applied a new method called CLIR-MS (Cross linking of isotope labelled RNA and tandem mass Spectrometry) to study the RNA protein in a systematic manner.[5] Stable isotope labelling of specific RNA nucleotides produces distinct mass shifts in the resulting mass spectra of a peptide-RNA adduct. Their presence enables retention of both RNA site positional information as well as peptide site localization by traditional MS 2 peptide sequencing by a single experiment. The isotopic labelling of a single nucleotide in the RNA sequence by solid phase RNA chemistry allowed us to gain a single nucleotide resolution for the first time. This represents an improvement over previous applications where only peptide or RNA site information is retained.

FOX 1 recognition motif (RRM) in complex with the FOX binding element RNA (FBE, 7 nucleotides UGCAUGU) provides a robust model for CLIR MS studies. In addition, the structure of RBFOX RRM and UGCAUGU was first solved using nuclear magnetic resonance (NMR) spectroscopy.[6] By synthesizing a library of FBE mutants, testing their binding towards FOX RRM and investigating their cross linking behavior, we envisioned to understand the specificity of the cross linking reaction.

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Structure-activity study and molecular insights in the mode of action of complement C3 inhibitor Cp40

C. Lamers[1], B. Wagner[1], P. Gros[2], J. D. Lambris[3], D. Ricklin[1]*

[1]Molecular Pharmacy, Pharmazentrum, Klingelbergstr. 50, University of Basel, 4056 Basel, Switzerland, [2]Crystal and Structural Chemistry, Bijvoet Center for Biomolecular Research, Department of Chemistry, Faculty of Sciences, Utrecht University, 3584 CH Utrecht, The Netherlands, [3]Department of Pathology and Laboratory Medicine, School of Medicine, University of Pennsylvania, 422 Curie Blvd., Philadelphia, PA 19104, USA

The complement system serves in blood circulation as “first line of defense” against injurious stimuli and invaders. Upon activation, a series of cascading enzymatic reactions leads to an amplification of the response and to pathogen clearance and opsonic cell killing. Yet complement has also gained increasing interest as a potential drug target, since it may be inadvertently triggered on human cells or biomaterial surfaces, thereby contributing to clinical complications in the pathogenesis of various autoimmune, inflammatory and age-related diseases as well as transplant rejection. While the involvement of dysregulated complement activation in inflammatory and autoimmune diseases is now widely recognized[1], so far only one complement-specific drug has reached the market.

In this presentation we reflect on the development of the picomolar complement inhibitor Cp40, a next-generation derivative of the compstatin peptide. The C3 inhibitor compstatin was originally identified using a phage display approach[2] and several of its derivatives are currently in clinical development. In particular, the presented study is focused on recent structure-activity relationship investigations. Based on a novel co-crystal structure of Cp40 in complex with its complement target C3b, we used site-specific modifications/deletions, nonproteinogenic amino acids and tailor-made building blocks to elucidate the SAR in detail and identify key interaction determinants. By employing functional SPR experiments, we were able to further elucidate the molecular mode of action of Cp40. Building on this insight, we follow various strategies (e.g. prodrug approaches or bioconjugation) to improve affinity and pharmacokinetic properties for a use of this promising inhibitor class in a broad range of disease models.

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Discovery of a New Acetylated Lysine Mimic and Optimization of CBP/P300 Bromodomain Inhibitors

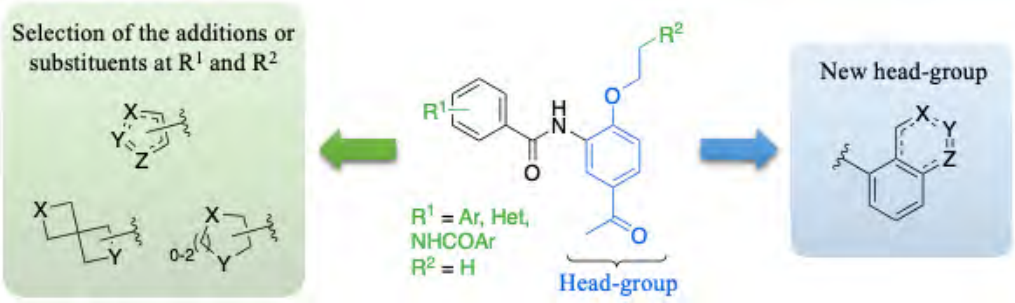
E. Laul[1], V. Pascanu[1], M. S. Kirillova[1], A. Dolbois[1], P. Sledz[2], A. Caflisch[2], C. Nevado[1]*

[1]Department of Chemistry, University of Zürich, [2]Department of Biochemistry, University of Zürich

Bromodomains play a key role in the complex epigenetic regulation processes as “reader” modules of acetylated lysine residues on histone tails. This leads to a signaling cascade to activate the transcription and expression of genes.[1] Highly homologous CBP and P300 bromodomains have recently received significant interest due to their important role in the development of various human cancers.[2] However, their specific biological functions remain elusive. Orthosteric inhibitors of CBP/P300 bromodomains could further clarify the roles of these proteins in promoting disease.

Previously we reported CBP/P300 inhibiting chemical probes, bearing a *p*-ethoxy-acetophenone head-group that mimics the endogenous acetylated lysine.[3,4] Extensive optimization efforts afforded lead compounds with low-nanomolar potency, exquisite selectivity over related proteins and adequate metabolic stability and toxicity profiles. However, critical solubility issues and lack of cellular target engagement prevented further *in vivo* development of these molecules.

In this work, we examined various strategies to address these challenges, including the addition of solubilizing moieties, substitution patterns prone to disrupt planar conformations and an increase in the amount of sp^3 carbon atoms. Significant improvements could be achieved without sacrificing the potency of our probes. In addition, via *in silico* high-throughput fragment-docking, a new head-group has been discovered, furnishing an improved potency and solubility compared to the previously reported *p*-ethoxy-acetophenones.



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Cross-linkable polymeric contrast agent for high-resolution imaging of the vascular system with X-ray microCT

N. Le[1], W. Kuo[1], V. Kurtcuoglu[1], B. Spingler[1]*

[1]University of Zurich

X-ray microcomputed tomography (microCT) is a powerful tool for 3D reconstruction of the vascular systems in small animals, which is crucial for understanding many physiological and pathological processes.[1] It is able to provide micrometer scale resolution on whole mouse organs in intact, native hydrated state. For capillary resolution *ex vivo* X-ray microCT however, the currently available contrast agents have different drawbacks.[2] Although vascular casting with hydrophobic plastic resins can provide high X-ray attenuation coefficients, it suffers from several limitations. Gas and water inclusions can create small hole artifacts in larger vessels and high viscosity requires higher pressures to reliably fill the smallest capillaries.[3,4] Small molecule angiography contrast agents pass the blood vessel walls within minutes, preventing any high-resolution imaging. While nanoparticle-based blood pool contrast agents are used for *in vivo* vascular imaging providing resolutions up to 10 µm, they tend to sediment and aggregate in an *ex vivo* setting and can diffuse more slowly through the tissue as well, making them unsuitable for the longer scan times required in high resolution imaging.

To overcome all these drawbacks, we have developed a water-soluble low-viscosity polymeric contrast agent, which is cross-linkable with glutaraldehyde. All of these properties allow the contrast agent to combine the reliable filling of hydrophilic contrast agents with the permanent retention of vascular casting. This allows for easier sample preparation, longer scan times associated with high-resolution imaging and multiple acquisitions of the same sample, such as for hierarchical imaging or dual-energy X-ray microCT applications.

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Exploration of chlorin e6 derivatives for photodynamic therapy

N. Le[1], M. Kalt[1], B. Spingler[1]*

[1]University of Zurich

Photodynamic therapy is an emerging method for treatment of different types of cancer including skin cancer, lung tumours and some of non-cancerous diseases like: various skin conditions as acne, warts and actinic keratosis.[1,2] Chlorin e_6, which is the degradation product of chlorophyll a or pheophytin a, recently attracted great attention due to its considerably fast and selective accumulation in tumor sites as well as its low dark toxicity. Furthermore, with three free carboxylic groups, chlorin e_6 can be functionalized with various side chains to give novel chlorin derivatives. The most prominent examples among them should be Talaporfin and Photolon. Talaporfin is the conjugation product of chlorin e_6 with L-aspartic acid, which was approved in Japan for the treatment of early stage lung cancer.[3,4]

Here, we report the synthesis and biological evaluation of novel platinated derivatives of chlorin e_6 and its decarboxylated product- chlorin e_4, which might combine the phototoxicity of chlorins and chemotoxicity of platinum derivatives.

Acknowledgements: We thank University of Zurich for financial support of our research.

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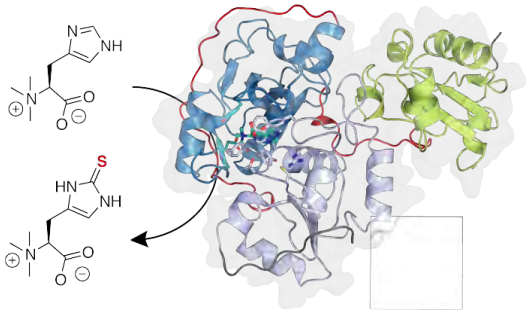
Structural and mechanistic basis for anaerobic ergothioneine biosynthesis

F. Leisinger[1], R. Burn[1], M. Meury[1], P. Lukat[2], F. P. Seebeck[1]*

[1]Department of Chemistry, University of Basel, [2]Structure and function of Proteins, Helmholtz centre for infection research

Ergothioneine, a sulfur metabolite that occurs in microorganisms, fungi and animals[1], was suggested to be essential to mitigate inflammatory or cardiovascular disease, dementia and other epiphenomena of aging when actively accumulated[1a,1f, 2]. In recent years broad scientific consensus has formed around the idea that ergothioneine protects the cells from reactive oxygen species. However, it was recently discovered that ergothioneine can also be made by strictly anaerobic microorganisms using oxygen independent chemistry[3]. This finding further indicates that the previous focus on the role of ergothioneine in oxidative stress protection may be to narrow and that this sulfur metabolite is also important for oxygen-independent life. Based on the crystal structure and kinetic observations of anaerobic ergothioneine biosynthetic enzyme (EanB) from the green sulfur bacterium *Chlorobium limicola* we describe the catalytic mechanism of an unprecedented C-S bond forming reaction[4]. Structural insights enabled the assignment of specific function to all crucial active site residues. Significant active site similarity among EanB homologs suggests that oxidative sulfurization of heterocyclic substrates may apply to several different biosynthetic pathway occurring in proteobacteria, spirochaetes and firmicutes.



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Development of a Multifunctional Cyclopentadienyl based Linker for the Design of Novel Cancer Theranostics

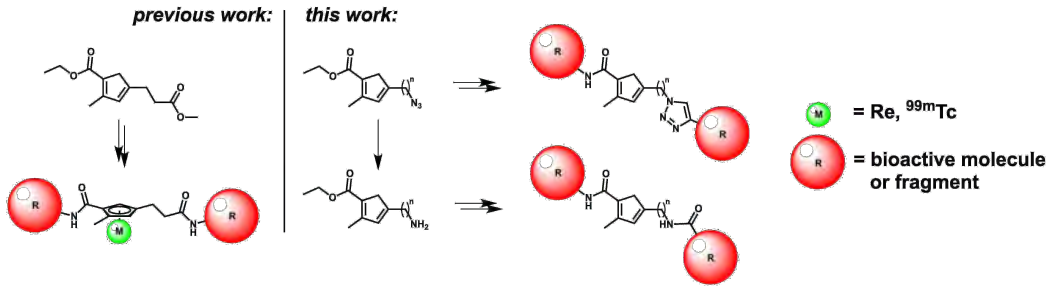
R. Lengacher[1], R. Alberto[2]*

[1]University of Zurich, Department of Chemistry, Win, [2]Department of Chemistry, University of Zurich, Win

For the creation of novel radiopharmaceuticals, a suitable radioisotope needs to be introduced into a biologically active lead-system. ^{99m}Tc can be applied in such a fashion, since it is already widely used for the diagnostics of various targets.[1] Cyclopentadiene (Cp) offers an interesting way to introduce Tc into lead structures, as evident by the Re-analogues of ferrocifen.[2]

Recently, our group developed a multifunctional Cp, bearing two ester functionalities, that can be employed in such a way. The two ester functionalities could serve as linkers between two bioactive targeting moieties, while the Cp acts as a chelator for Re and ^{99m}Tc.[3]

In this presentation, new derivatives of the previously reported Cp are showcased, where one of the ester groups is replaced by a novel functional group. Using an azide in this position not only increases the orthogonality of the two binding sites, but also opens up new synthetic routes to couple the Cp to bioactive molecules, such as “Click”-reactions that are already widely used in bio-, medicinal-, and radiochemistry.[4] Furthermore, the azide can be reduced to the corresponding amine, resulting in a Cp bearing amine and carboxylic acid moieties and thereby mimicking an amino acid, which can be easily radiolabelled with ^{99m}Tc.



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Synthesis of Fluorescent Tools to Better Understand the Canalicular Lipid Transporter System

M. Leuenberger[1], S. Bernhard[2], P. Grossenbacher[1], B. Stieger[2], M. Lochner[1]*

[1]Institute of Biochemistry and Molecular Medicine, [2]Department of Clinical Pharmacology and Toxicology

Canalicular bile formation is regulated by an elaborate array of ABC-transporters i.e. BSEP (Bile Salt Export Pump or ABCB11), MDR3 (or ABCB4) and ABCG5/G8. Bile salts are biosynthetically complex molecules. Nature developed an efficient pathway to recycle them. In the small intestine, the Apical Sodium-Bile salt Transporter (ASBT) is crucial for the efficient reabsorption of bile salts (BS^-). Bile salts returning to the liver by the portal vein are taken up at the basolateral membrane by OATPs (Organic Anion Transporting Polypeptides) and NTCP (Na^+-Taurocholate Co-transporting Polypeptide) and are pumped into the canaliculus by BSEP.

The goal of our research is the synthesis of novel fluorescent substrates and primary antibodies as probes to investigate the mechanism of bile salt secretion into the canaliculus and localisation of the various transporters.

Three fluorescent dyes were coupled to the side chains of cholic acid (CA) and chenodeoxycholic acid (CDCA) to mimic the known transport substrates glycocholate (GC) and glycochenodeoxycholate (GCDC): Nitrobenzofurazan (NBD), dansyl and a coumarin dye (Pacific Blue). The activity of the synthetic fluorescent bile salts was evaluated in CHO cells expressing the different transporters, as well as in Sf9 cell vesicles expressing BSEP, resulting in a quite complete profile of the BS^--transporter interactions.

Functionalized Proline-Rich Peptides Bind the Bacterial Second Messenger c-di-GMP

S. Loosli[1], C. A. Foletti[1], H. Wennemers[2]*

[1]ETH Zurich, Laboratory of Organic Chemistry, [2]ETH Zurich

C-di-GMP is an attractive target in the fight against bacterial infections since it is a near ubiquitous second messenger that regulates important cellular processes of pathogens, including biofilm formation and virulence.[1-3] Screening of a combinatorial peptide library enabled the identification of the proline-rich tetrapeptide Gup-Gup-Nap-Arg, which binds c-di-GMP selectively over other nucleotides in water. Computational and CD spectroscopic studies provided a possible binding mode of the complex and enabled the design of a pentapeptide with even higher binding strength towards c-di-GMP. Biological studies showed that the tetrapeptide inhibits biofilm growth by the opportunistic pathogen *P. aeruginosa*.

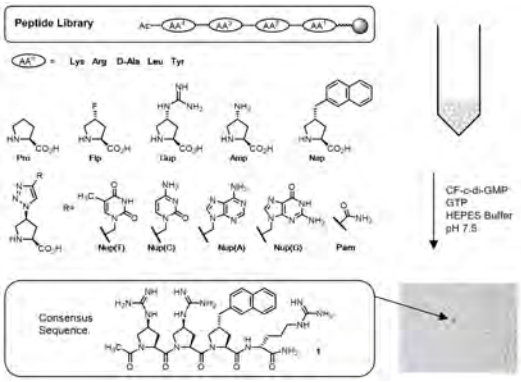

Table 1. Binding affinities of peptides **1-4**, **1a-1f**, **5** and **6** with c-di-GMP, c-di-AMP and pGpG.[a]

Peptide		ΔG [kcal mol^{-1}]		
		c-di-GMP	c-di-AMP	pGpG
1	Ac-GupGupNapArg-NH_2	-6.2	-4.7	-4.7
2	Ac-NapArgArgArg-NH_2	-6.3	-5	-5.1
3	Ac-GupNapArgArg-NH_2	-6.2	-5.1	-5.1
4	Ac-NapGupGupArg-NH_2	w.b.	w.b.	w.b.
1a	Ac-ProGupNapArg-NH_2	w.b.	w.b.	w.b.
1b	Ac-GupProNapArg-NH_2	w.b.	w.b.	w.b.
1c	Ac-GupGupProArg-NH_2	-5.1	-5.9	w.b.
1d	Ac-GupGupNapAla-NH_2	n.b.	-4.1	w.b.
1e	Ac-GupGupNapDArg-NH_2	-5.4	-5	-5.6
1f	Ac-GupGupNap-NH_2	w.b.	w.b.	w.b.
5	Ac-NapGupGupNapArg-NH_2	-7.9	-6.5	-6.5
6	Ac-NapGupGupNapDArg-NH_2	-7.5	-6.4	-6.8

[a] Binding affinities at 25°C in deionized water, error ± 0.1 kcal mol^{-1}. w.b. = weak binding, indicates that ΔG is higher than -4– -4.5 kcal mol^{-1}, the lowest ΔG values detectable under the experimental conditions.

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Improved design of miR-CLIP probes towards elucidation of miRNAs functions

A. L. Malinowska[1], Y. Wang[1], J. Hall[1]*

[1]Institute of Pharmaceutical Sciences, ETH Zürich

MicroRNAs (miRNAs) constitute a class of small, endogenous, noncoding RNAs (ncRNAs) and have a great influence on various processes within the cell, including cell growth and differentiation [1]. By base-pairing to their specific, partially-complementary sites located predominantly in the 3’ untranslated region (3’-UTR) of the target messenger RNAs (mRNAs), miRNAs participate in the post-transcriptional regulation of gene expression. MiRNAs are responsible for controlling the expression of up to 60% of human protein-coding genes [2] and their dysregulation has been related to many pathological processes and diseases such as cancer [3]. MiRNAs can be considered as either therapeutic agents or therapeutic targets [4]. In order to use microRNAs for therapeutic purpose, in-depth understanding of mechanisms governing the miRNAs activity is of great importance. One of the key challenges is the elucidation of microRNAs’ targets and the sites of canonical and non-canonical interactions. Imperfect pairing between miRNA and target mRNA in animals [5], as well as high false positive and false negative rates for current prediction algorithms generate a need for the experimental validation in addition to theoretically predicted sites of interaction.

For this purpose, miRNA analogues bearing various cross-linkers can be applied. In the presence of cross-linkers, a covalent bond between the miRNA and the mRNA target is formed, enabling elucidation and/or validation of the target site. The microRNA cross-linking and immunoprecipitation (miR-CLIP) approach developed in our group allows capturing predicted and unpredicted miRNAs targets in cells by employing bis-modified pre-miRNAs containing biotin and trioxsalen [6]. As shown by Imig et al., the bis-modified miR-106a probe is able to cross-link to complementary regions present in mRNA targets, but the exact site of cross-linking has not been determined yet. In order to develop universal miR-CLIP probes, more knowledge about the functioning of the aforementioned probes is needed.
Herein, we present the results of the *in vitro* cross-linking assays with a set of synthesized trioxsalen-labelled miR-106a analogues. The results suggest that the cross-linking is taking place with the uridine positioned 2-3 base pairs upstream from the desired cross-linking site. This knowledge is used to prepare optimized miR-CLIP probes, with the ability to cross-link with the target mRNA independently of the miRNA sequence.

This work was supported by a grant from the Swiss National Science Foundation (205321_169612) and NCCR RNA and Disease (D51NF40-141735).

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NMR structure determination of the CPEB3 ribozyme.

I. Markova[1], S. Johannsen[1], R. O. Sigel[1]*

[1]University of Zurich

This work is focused on the investigation of the solution structure and folding mechanism of the cytoplasmic polyadenylation element binding protein 3 (CPEB3) ribozyme by nuclear magnetic resonance (NMR). Ribozymes are RNA molecules that act as chemical catalysts in cells. The discovery of ribozymes was a milestone in RNA research and revealed the unique role of RNA in a multitude of cellular reactions. The CPEB3 ribozyme is until now the only confirmed small ribozyme in mammals and its role remains still unknown.[1] As RNA function is directly linked to structure, structural studies are the basis to understand RNA function.

The CPEB3 ribozyme belongs to the Hepatitis Delta Virus (HDV)-like family of self-cleaving ribozymes that have a nested double pseudoknot fold and a 5'-end cleavage activity in common.[2] The best studied ribozyme in this family is the HDV ribozyme itself, whose three dimensional structure was solved by X-ray crystallography.[3] Even though, the number of newly discovered HDV-like ribozyme is continuously increasing, the HDV ribozyme is still the only one with a known structure. Therefore, solving the structure of the CPEB3 ribozyme will not only help to enlighten its biological role but also to expand the knowledge on the HDV-like ribozyme family in general.

NMR structure determination of such a large RNA is a challenging task due to heavy spectral overlap and large line widths of the proton resonances. To overcome this issue, we use different labeling schemes. For example, we use partially deuterated nucleotides, ^{13}C, ^{15}N labeling techniques and apply up-to-date multinuclear and multidimensional NMR spectroscopy.

Financial support by the University of Zurich and the Swiss National Science Foundation is gratefully acknowledged.

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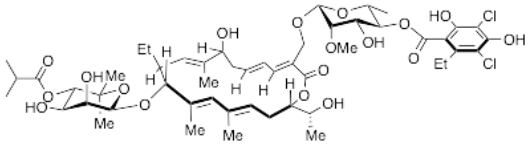
Semisynthetic Approaches for the Modification of the Glycosylated Macrolide Antibiotic Fidaxomicin

A. Meier[1], R. Berg[1], C. Gertzen[2], K. Zerbe[1], M. Gwerder[1], D. Schäfle[3], P. Sander[3], H. Gohlke[2], K. Gademann[1]*

[1]University of Zurich, Department of Chemistry, [2]Heinrich-Heine-University Düsseldorf, [3]University of Zurich, Institute of Medical Microbiology

Fidaxomicin (**1**, tiacumicin B, lipiarmycin A3) is a marketed drug for the treatment of intestine infections caused by *Clostridium difficile*. Fidaxomicin is a glycosylated macrolide and was isolated from various strains of soil bacteria.[1] It shows good activity against many Gram-positive bacteria, including some resistant strains of *Staphylococcus aureus* and *Mycobacterium tuberculosis*.[2] Investigations of the mechanism of action revealed that Fidaxomicin binds to the RNA-polymerase (RNAP) and consequently inhibits the transcription.[3]



Fidaxomicin (1, tiacumicin B, lipiarmycin A3)

In 2015, our research group accomplished the first total synthesis[4] of this complex natural product which does not possess ideal pharmacokinetic properties. Due to its low water-solubility and, as a consequence, its minimal systemic absorption the application for the treatment of infections outside the gut is limited. Therefore, semisynthetic modifications present a promising strategy to improve its pharmacokinetic properties and also circumvent resistance development. We have developed synthetic strategies that allow selective and versatile modifications of this complex natural product and herein we present the synthesis of novel derivatives of fidaxomicin (**1**) and their antibiotic activities.

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Triazole-linked organometallic architectures - Adding diversity to trithiolato-bridged dinuclear ruthenium(II)-arene compounds via CuAAC click chemistry

M. Mösching[1], O. Desiatkina[1], E. Păunescu[1], J. Furrer[1]*

[1]University of Bern, Department of Chemistry and Biochemistry, Freiestrasse 3, 3012 Bern, Switzerland

The high cytotoxicity of cationic trithiolato-bridged dinuclear ruthenium(II)-arene complexes (general formula $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}SR)_3]^+$) against human cancer cells[1] has encouraged us to pursue the development of this scaffold for obtaining biologically active compounds.

The functionalization of organometallic complexes after coordination (also termed “chemistry-on-the-complex”) provides an elegant approach to new libraries of compounds. Nevertheless, this type of modification is often hampered by the poor thermal stability of the metalorganic complexes and/or undesired ligand exchange in the presence of certain reagents and solvents. Amidation and esterification reactions catalyzed by specific coupling agents, as well as CuAAC click cycloadditions require ‘softer’ reaction conditions and are thus often considered for the synthesis of conjugates from organometallic units.[2]

The controlled synthesis of ‘mixed’ cationic trithiolato precursors allowing further modification (general formula $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-}S\text{-}CH_2\text{-}C_6H_4\text{-}p\text{-}Bu^t)_2(\mu_2\text{-}S\text{-}C_6H_4\text{-}XH)]Cl$, where -XH = -OH, $-NH_2$, $-CH_2CO_2H$)[3] enabled the obtainment of a large variety of conjugates for tailored applications.[4,5] However, in some cases this type of derivatization could not be applied and alternative synthetic pathways needed to be explored.

The facile tuning afforded by the copper(I)-catalyzed azide-alkyne (CuAAC) 1,3-cycloaddition synthesis can provide a useful and versatile access route to new molecular architectures. As proof-of-concept, we report some of our first results in the use of click chemistry for the development of various conjugates based on trithiolato-bridged dinuclear ruthenium(II)-arene moieties. Different ruthenium-based intermediates were used for the triazole-linkage with specific functional units (metabolites, fluorophores) judiciously substituted. This first insight into applying the CuAAC cycloaddition to our dinuclear Ru(II)-arene building blocks sketches the potential of this reaction to prepare *à la carte* other conjugates in the near future.

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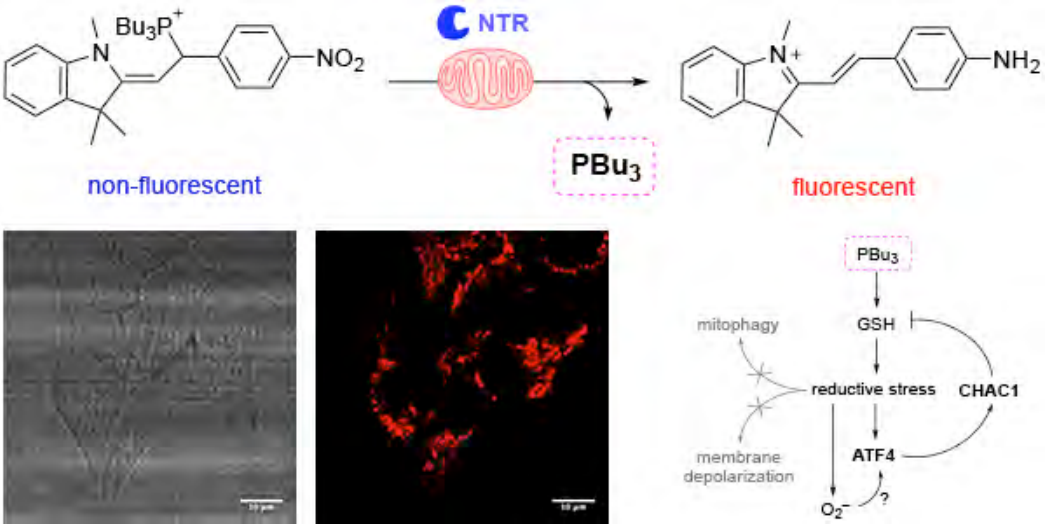
Induction of Reductive Stress in Mitochondria by Enzymatic Activation of a Trialkylphosphine Probe

J. Nguyen[1,2], A. Tirla[1], P. Rivera-Fuentes[1,2]*

[1]Laboratory of Organic Chemistry, ETH Zurich, [2]Institute of Chemical Sciences and Engineering, EPF Lausanne

Redox homeostasis is essential for organelle function in eukaryotes and its disruption is associated with cancer[1], metabolic[2] and neurodegenerative diseases[3]. We previously demonstrated that a photoactivatable trialkylphosphine can mimic certain aspects of reductive stress in living cells by increasing concentration of free thiols, protein misfolding and aggregation.[4] Redox balance however, is different in each cell compartment and disruption of mitochondrial redox state has been specifically linked to pathologies such as insulin resistance, obesity and type II diabetes.[5] In this study, we propose an organelle-targeted reducing agent based on tributylphosphine that shifts this redox balance in mitochondria towards reductive stress. We design the probe to target endogeneous nitroreductases and subsequently release tributylphosphine, as well as a fluorescent reporter within the organelle. Confocal imaging, biological assays and transcriptomics analysis on mammalian cells allowed us to quantify this redox imbalance. These results show activation of transcription factor ATF4, that influences mitochondrial redox homeostasis through modulation of genes involved in glutathione metabolism.[6]



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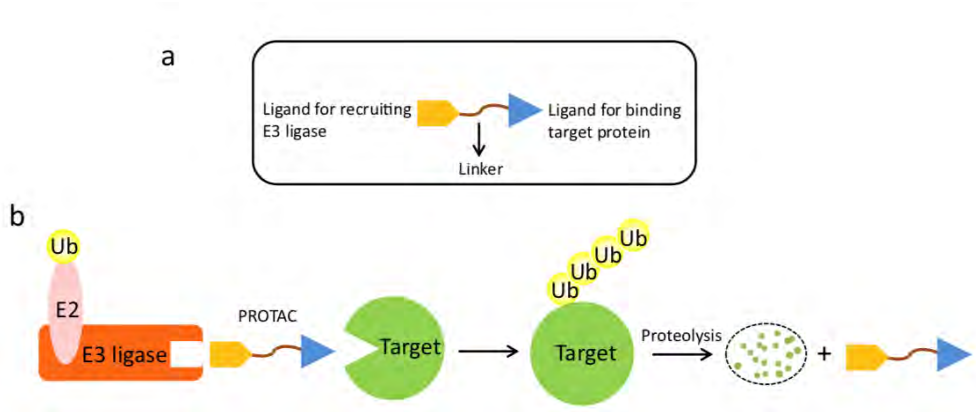
Synthesis and in vitro cell-penetration characterization of PROTAC-based platforms

S. Pannilunghi[1], S. Tardy[1], A. Gouiller[1], L. Galibert[2], T. De Smedt[2], L. Scapozza[1]*

[1]School of Pharmaceutical Sciences, University of Geneva, Rue Michel-Servet 1, 1206 Genève, [2] Alpine Institute for Drug Discovery, EPFL Innovation Park Building C, 1015 Lausanne

Proteolysis-Targeting Chimeras (PROTACs) represent a novel and emerging tool in targeted protein degradation that is currently receiving much attention for its therapeutic potential. The mechanism of action relies on PROTACs ability to recruit and hijack the ubiquitin-proteasome system (UPS) in order to activate proteasomal destruction of undruggable targets.(1) PROTACs are bispecific molecules containing an E3 ligase binder, a linker of various lengths and a target protein of interest (TOI) binder. In this sense, PROTACs work as a useful platform able to create a selective bridge between the E3 ligase and the TOI, promoting polyubiquitination and subsequent proteasomal degradation of desired proteins. Considering that engineered E3 ligases can knock out disease-causing proteins that are not physiological substrates of the UPS machinery, a vast array of different PROTACs combinations is envisaged. Nowadays, a variety of small molecules, such as Thalidomide and its derivatives and ligands for Von-Hippel Lindau (VHL), have been proven to bind E3 ligases belonging to the CRL complex, allowing the development of respectively Cereblon (CRBN)-based and VHL-based PROTACs.(2)



Despite those preliminary promising results, application of PROTAC technology is sometimes affected by poor cellular permeability and the mechanism of cell-penetration itself is still partially unclear. The aim of this project is to provide evidence and evaluate the ability of tetraethylene glycol-conjugated E3 ligase binders beyond the known ones to promote transport across the cell membrane, regardless of TOI binder nature and structural characteristics.

For this work we firstly chose 4-hydroxylthalidomide and VHL ligands to synthetize the widely used CRBN-based and VHL-based PROTACs. In a second time, a novel strategy was explored, using two brand new Nedd-4 ligands (3,4) in order to exploit this HECT ubiquitin E3 ligase as a putative degrader tool for membrane proteins. *Click chemistry* strategy was selected for in parallel coupling of ligase ligand-containing moiety and fluorescein, leading to a fluorescein-based PROTAC assembly. Further in vitro studies will be carried out to evaluate and quantify cell penetration thanks to the use of the fluorescent tracer.

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Disecting the Role of the CBP Bromodomain in Development and Hematologic Malignancies

V. Pascanu[1], A. Dolbois[1], E. Laul[1], K. Rollins[1], K. Prummel[2], C. Mosimann[2], P. Sledz[3], A. Caflisch[3], C. Nevado[1]*

[1]Department of Chemistry, University of Zurich, [2]Institute of Molecular Life Sciences, University of Zurich, [3]Department of Biochemistry, University of Zurich

The ε-*N*-acetylation of lysine residues on histone tails is one of the most prevalent post-translational modifications influencing gene expression. Bromodomains are protein modules that specifically recognize these acetylated marks, mediating protein-protein interactions and downstream signaling cascades.[1]

CBP is a bromodomain-containing transcription co-regulator with essential roles in embryonic development. Defective CBP function has been extensively associated with numerous hematological malignancies. Despite sustained earlier efforts, the specific role of the bromodomain (BRD) in relation to the other CBP domains remains poorly understood. To dissect the role of this individual domain, highly selective CBP_BRD inhibitors have been developed, with desirable physicochemical properties.[2] The most recent generation of CBP_BRD inhibitors displays single-digit nanomolar potency and excellent solubility.

The effect of our lead compounds was investigated side-by-side with a series of first-in-class CBP-degraders developed in our group. An extensive comparative study of inhibitors *vs.* degraders was performed using relevant cellular disease models. These results were complemented by *in vivo* studies using zebrafish embryos.

[1] Panagis Filippakopoulos, Sarah Picaud, Maria Mangos, Tracy Keates, Jean-Philippe Lambert, Dalia Barsyte-Lovejoy, Ildiko Felletar, Rudolf Volkmer, Susanne Muller, Tony Pawson, Anne-Claude Gingras, Cheryl H. Arrowsmith, Stefan Knapp, *Cell*, **2012**, 149, 214-231.
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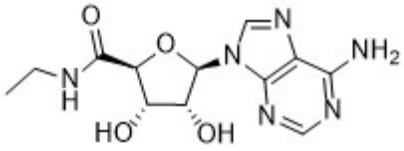
Development of Molecular Tools for the Cellular Study of Adenosine A_1 Receptors

B. Preti[1], M. Leuenberger[2], M. Lochner[2], B. G. Frenguelli[3], G. Ladds[4]

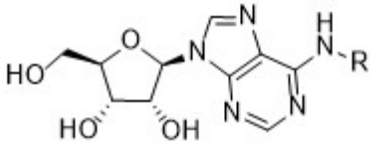
[1]Department of Chemistry , [2]Institute of Biochemistry and Molecular Medicine, [3]School of Life Sciences, [4]Department of Pharmacology

The nucleoside adenosine acts as an important signalling molecule by exerting its agonist activity at all subtypes (A_1, A_{2A}, A_{2B}, A_3) of the adenosine receptors (ARs), which belong to the family of GPCRs. These subtypes have a wide tissue distribution and are implicated in cardiovascular, respiratory, inflammatory[1] and neurological disorders. Because their common ligands have diametrically opposed effects and have overlapping tissue distribution, the ARs have been object of extensive research to discover subtype selective ligands. We are developing novel potent small molecular probes that are selective agonists of adenosine A_1R and can be used to study this G protein-coupled receptor in mammalian cells, in tissue or in *vivo*.

We have previously discovered several potent and A_1R-selective agonists that are based on the adenosine and NECA structures, which are known potent non-selective ARs agonists. Subsequently, we used a molecular modeling approach to dock our synthetic adenosine derivatives into a homology model of the human A_1R, which revealed an agonist-like binding mode with residues that are typical of adenosine and NECA agonist. Based on this model, we hypothesized that the introduction of hydrogen donor-acceptor bonds and/or hydrophobic residues in para position in the phenyl ring of the (Benzyloxy)cyclopentyl **1** derivative could increase the potency at A_1R and retain the selectivity. Newly synthesized analogues were analyzed by yeast-based screen and by a mammalian cells assay to assess the potency and selectivity. Here we report the improved synthesis of a series of these A_1R-selective agonists. In particular, we have optimized two key steps: the S_NAr-reaction to introduce substituents at the purine C-6 position by using microwave chemistry and the O-alkylation reaction on the cyclopentyl moiety of derivative **1** for the introduction of new para-substituted phenyl rings.



NECA



1: R = 2-(Benzyloxy)cyclopentyl
2: R = 2-Cylopentanol

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The HDV-like family: crystal structure of the CPEB3 ribozyme

A. I. Przytula-Mally[1], S. Johannsen[1], V. Olieric[2], E. Freisinger[1], R. K. Sigel[1]*

[1]University of Zurich, [2]Paul Scherrer Institute, Swiss Light Source

Our goal is to determine the three-dimensional structure of a catalytic RNA that occurs on the CPEB3 gene in mammals and belongs to the large family of *Hepatitis delta virus* (HDV)-like ribozymes. So far, it is the only confirmed small ribozyme in mammals and its biological role is not yet understood. Most of our knowledge is limited to the comparison with the HDV ribozyme, although the two ribozymes have only 34 % of sequence similarity. Recent NMR studies of our group confirmed the proposed complex secondary structure into a nested double pseudoknot [1]. However, the structure of the core region, dynamics and catalytic mechanism remain elusive.

Here we will focus on the structure determination by X-ray crystallography of the *Homo sapiens* (human) and the *Pan troglodytes* (chimpanzee) CPEB3 ribozymes as their sequences are identical, except for one nucleotide [2]. This one nucleotide has an enormous influence on the catalytic activity as the cleavage-rate of the chimpanzee ribozyme is almost one order of magnitude higher compared to the human variant. The three-dimensional structure of the CPEB3 ribozyme will shed important light on the structure of the catalytic core of the ribozyme. It will be also the first structure from the HDV-like ribozyme family.

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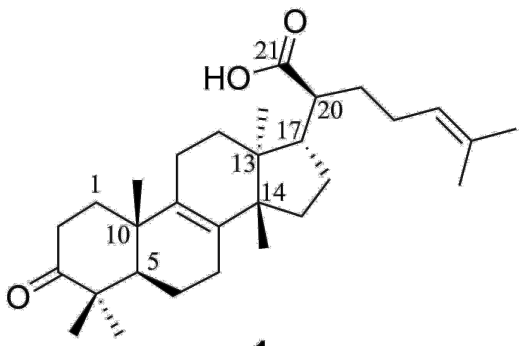
Tirucallic Acid Derivatives: Determination of their Absolute Configuration by Vibrational Circular Dichroism

J. K. Reinhardt[1], T. Nilsu[2], A. M. Klemd[3], O. Danton[1], M. Smieško[1], R. Huber[3], T. Bürgi[4], C. Gründemann[3], M. Hamburger[1]*

[1]University of Basel, Department of Pharmaceutical Sciences, [2]Kamnoetvidya Science Academy, [3] University Hospital Freiburg, Institute for Infection Prevention and Hospital Epidemiology, [4] University of Geneva, Department of Physical Chemistry

Autoimmune diseases like rheumatoid arthritis or multiple sclerosis are characterized by an increased T cell proliferation.[1] Available therapies typically exhibit severe side effects calling for better tolerated treatment options.[2] To discover new lead compounds for the treatment of autoimmune diseases, a library of 435 extracts from plants used in Traditional Chinese Medicine (TCM) was screened for inhibition of T lymphocyte proliferation. A dichloromethane extract of "Frankincense" (resin from *Boswellia thurifera*) was found active. From the active extract, a total of ten triterpenoids were isolated, among them four lanostane and a tirucallane triterpene. Both C-20 epimers of these scaffolds have been reported.[3] However, the absolute configuration of compounds bearing a carboxylic moiety at C-20 as in **1** has not been established unambiguosly. Vibrational circular dichroism (VCD) spectra of **1** were recorded and compared to the *ab initio* calculated spectra. Comparison was performed by visual comparison of the individual bands, and by calculating the similarity indices (*Sim*VCD) between the spectra.[4] In addition, findings were compared with the structure established by X-ray diffraction analysis.

Visual and computational analyses confirmed the absolute configuration of **1** as (9*R*,10*S*,13*S*,14*S*,17*S*,20*S*), which was in accord with the X-ray diffraction data. This shows the potential of VCD as a non-destructive method for establishing the absolute configuration in conformationally flexible side chains.



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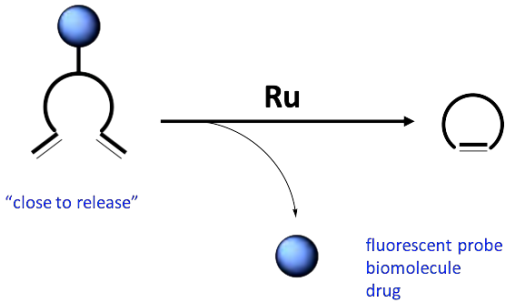
“Close to Release”: A Bioorthogonal Uncaging Reaction Based On Ring-Closing Metathesis

V. Sabatino[1], T. Ward[1]*

[1]Department of Chemistry, University of Basel

Bioorthogonal uncaging reactions are highly desirable in the field of chemical biology. In the past few years, several strategies have been adapted to work under physiological conditions. We present herein a novel uncaging reaction that results from a ring-closing metathesis event. A small molecule tethered to a diolefin substrate, is released via spontaneous 1,4-elimination following a ring-closing metathesis. This strategy, which we term “close to release”, allowed us to uncage biomolecules, drugs and fluorescent probes in aqueous media and under physiological conditions. We envision this strategy may find us in chemical biology, bioengineering and medicine.



Diverse effects of halogenated 2-aminoethoxydiphenyl borate derivatives on store-operated calcium entry in breast cancer cells

A. E. Schild[1], R. Bhardwaj[2], M. Hediger[2]*, M. Lochner[1]*

[1]Institute of Biochemistry and Molecular Medicine, University of Bern, [2]Department for BioMedical Research, University of Bern

Calcium signaling regulates a variety of cellular functions such as cell differentiation, proliferation, muscle contraction, neurotransmission and fertilization. Store-operated calcium entry (SOCE) is the ubiquitous pathway in metazoans to refill the calcium stored in the endo-/sarcoplasmic reticulum (ER and SR, respectively). Calcium stores are a crucial component of the calcium signaling machinery that maintains the calcium homeostasis. Elevated expression levels of the proteins facilitating SOCE (e.g. Oari/STIM) have been associated with various types of cancer such as breast, prostate and cervical cancer, as well as lung adenocarcinoma and esophageal squamous cell carcinoma.

2-Aminoethoxydiphenyl borate (2-APB) is the most studied modulator of SOCE to date. It has the ability to either block or enhance the calcium entry depending on its concentration. We have synthesized 2-APB derivatives substituted with halogens on one of the phenyl groups. These derivatives were screened for their ability to inhibit SOCE in its active state as well as screened for any effects on cells where SOCE components are in their inactive state. The two breast cancer cell lines MDA-MB-231 and MCF-7 were used to conduct SOCE experiments. Both of these cell lines exhibit a robust calcium entry, making them suitable studying SOCE. We found that changing the position of the halogen on the phenyl group of 2-APB gives rise to distinctly different calcium mobilization responses.

Exocyclic Metallated Tetrapyridinoporphyrazine as a Potential Photosensitizer for Photodynamic Therapy

L. Schneider[1], M. Larocca[1], W. Wu[1], V. Babu[1], C. König[1], S. Ferrari[1], B. Spingler[1]*

[1]University of Zurich

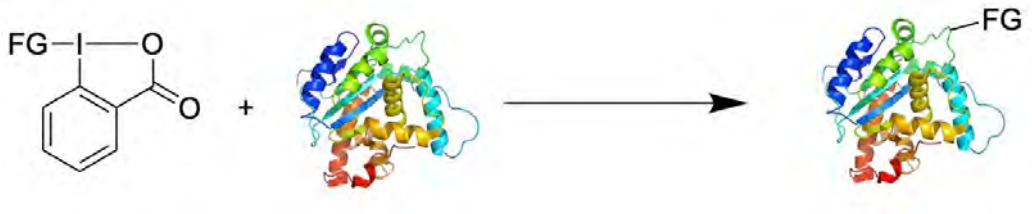
The first exocyclic metallated tetrapyridinoporphyrazine, [tetrakis-(*trans*-$Pt(NH_3)_2Cl$)-tetrapyridinoporphyrazine-zinc(II)]$(NO_3)_4$ was successfully synthesized in a multistep synthesis starting from 3,4-pyridinedicarbonitrile. The synthesis involved a platination of the intermediate tetrapyridinoporphyrazine-zinc(II) with transplatin and afforded the product in an overall yield of 31%. The final product and the intermediate were characterized, the singlet oxygen quantum yields (Φ_Δ) were determined and the cytotoxicity was investigated *in vitro* in the dark as well as under light irradiation. Additionally, the DNA binding ability of the final product was investigated.

"Doubly Orthogonal" Labelling of Peptides and Proteins

R. Simonet-Davin[1], R. Tessier[1], J. de Ceballos[1], N. Guidotti[2], J. Waser[3]*, B. Fierz[4]*

[1]Laboratory of Catalysis and Organic Synthesis, EPF, [2]Laboratory of Biophysical Chemistry of Macromolecu, [3]Laboratory of Catalysis and Organic Synthesis, [4]Institut des Sciences et Ingénierie Chimiques, EPF

Hypervalent iodine reagents are versatile reagents in synthetic chemistry.[1] For example, Ethynyl BenziodoXolone (EBX) reagents were used by our group in the ethynylation of keto, cyano and nitro ester, as well as thiols.[2] However, applications of hypervalent iodine reagents towards biomolecule labelling remain scarce. During a collaboration between our group and the group of Prof. Adibekian, the alkynylation of the thiol group of cysteine in proteins was recently achieved.[3] Herein, we present new functionalization methods of peptides and proteins using benziodoxolone reagents, leading to the first double labelling of biomolecules using hypervalent iodine reagents.[4]



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Antimicrobial Peptide Dendrimer Chimera active against Multidrug-resistant Bacteria

S. Siriwardena[1], J. L. Reymond[1]*

[1]University of Bern, Department of Chemistry and Biochemistry, Freiestrasse 3, CH-3012 Bern

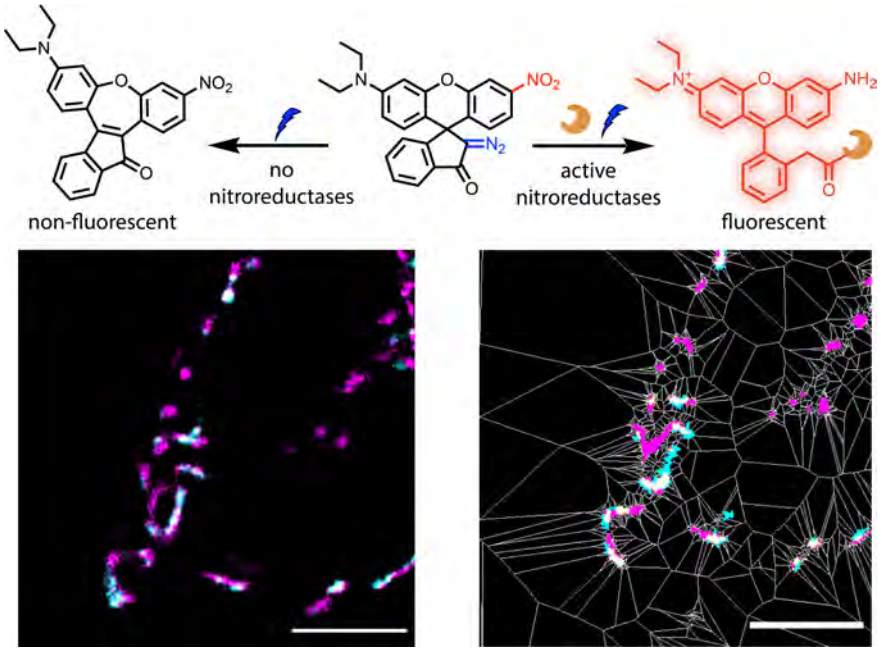
G3KL is a 3rd generation (G3) antimicrobial peptide dendrimer (AMPD) with high activity against *Pseudomonas aeruginosa* and other Gram-negative bacteria including MDR clinical isolates.[1] Recently we reported **T7**, which is an optimized analog of **G3KL** by chemical space exploration method, additionally showed good activity against *Klebsiella pneumoniae*.[2] **TNS18** is lipidated second generation peptide dendrimer which is active as **G3KL** on Gram-negative strains, but additionally showed good activity on the Gram-positive bacterium *Staphylococcus aureus* including the methicillin resistant strain MRSA.[3] Here we combining sequence elements from two different AMPDs might produce chimeric dendrimers displaying activity features of both parent compounds. We combined sequence elements from **TNS18** with those of **T7** or **G3KL** might produce a chimeric AMPD displaying activity against both *K. pneumoniae* and *S. aureus*. Synthesis and testing revealed **DC5**, a chimeric AMPD combining the branches of **TNS18** with the core of **T7** displays good activity against a broad range of multidrug resistant Gram-negative bacteria including *K. pneumoniae* as observed for **T7** and significant activity against MRSA similarly to **TNS18**.[4]

Single-Molecule Imaging of Active Mitochondrial Nitroreductases using a Photo-Crosslinking Fluorescent Sensor

Z. Thiel[1], P. Rivera-Fuentes[1]*

[1]Laboratory of Organic Chemistry, ETH Zurich, Switzerland

Many proteins are known to localize in clusters organized in specific subcellular domains. Whether the activity of certain enzymes is also restricted to microdomains is difficult to assess due to the lack of probes that allow microscopy with resolutions beyond the diffraction limit. We developed a photoactivatable fluorophore to map nitroreductase activity in live cells with single-molecule resolution. The mechanism of photoactivation and generation of fluorescence signal upon interaction with the target enzymes is based on a study that we reported previously.[1] The diazoindanone-based probe undergoes a Wolff-rearrangement following irradiation with 405 nm light. Depending on the substituents on the xanthene core, this photoreaction yields two diverging products. The nitro group favors formation of non-fluorescent products, whereas an amino substituent favors formation of a fluorescent signal. Enzymatic reduction of the nitro group prior to photoactivation is thus required in order to obtain a fluorescent signal. Experiments in solution and in live cells confirm high selectivity towards nitroreductases. Furthermore, we showed that the fluorescent photoproduct crosslinks to nucleophilic residues of surrounding enzymes, which prevents the diffusion of the probe away from the location of activation. Two-color, three-dimensional single molecule localization microscopy was used to reconstruct super-resolved images of the intracellular distribution of nitroreductase activity. Performing cluster analysis on the obtained images, we could show that nitroreductase activity is restricted to sub-mitochondrial microdomains.[2]



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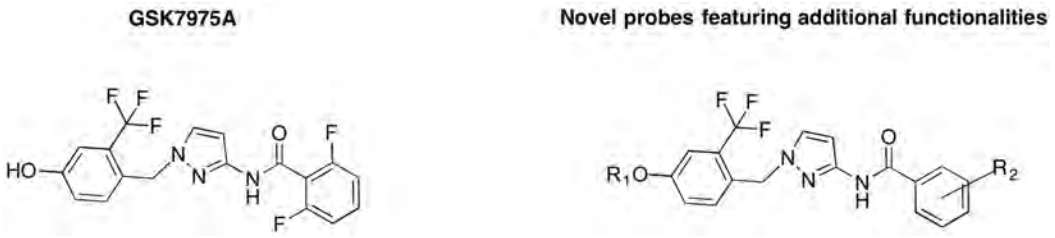
[2] Z. Thiel, P. Rivera-Fuentes, *Angew. Chem. Int. Ed.* **2019**, *in revision*.

Synthesis of functionalized probes based on GSK7975A to study SOCE

D. Tscherrig[1], R. Bhardwaj[2], J. Dernic[2], M. Hediger[2]*, M. Lochner[1]*

[1]Institute of Biochemistry and Molecular Medicine, [2]Department for BioMedical Research

A wide array of physiological functions are regulated bycalcium cations. Ca^{2+}signaling pathways that induce endoplasmic reticulum store depletion trigger a refilling process known as store operated Ca^{2+} entry (SOCE). Deviation of SOCE activity has been associated with numerous diseases such as immunodeficiency, autoimmunity and cancer, making this process an interesting target for drug development (i.e. modulators). Some SOCE modulators have been disclosed, however, their mode of action and binding site(s) are poorly understood.[1]



To elucidate the binding pocket(s) in the SOCE proteins, we are following a strategy incorporating covalent target modification followed by proteomics analysis with tandem mass spectroscopy. Hence, we synthesized novel probes based on the known SOCE-inhibitor GSK7975A featuring photo-crosslinking moieties. To facilitate the analysis of the complex proteomics results, we followed a mixed isotope strategy according to Lamos et al.[2] with a deuterated (d4) version of the GSK analogue. To make this strategy generally available we developed a deuterated diazirine building block in a new straightforward synthesis starting from cheap and easily accessible starting material. Herein, we present the synthesis of these novel probes and their preliminary biological assessment.

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Screening and repositioning the candidate drugs for retinoblastoma

P. Tseng[1], P. J. Dyson[1]*

[1]École Polytechnique Féderale de Lausanne

The combination of chemotherapy with thermotherapy (termed thermochemotherapy) is extensively used in the clinic for the treatment of retinoblastoma [1]. However, the limitation of thermochemotherapy is that the chemotherapeutic agents used were not been designed for such an application. Moreover none of these drugs are specifically activated at the heated tumor site and all of them lead to systemic toxicity. New potential chemotherapeutics for the treatment of problematic malignancies can be identified by screening libraries containing drugs already approved for other applications[2]. This so-called repurposing approach of existing drugs has the advantage that through preceding research and development efforts, the pharmacology, formulation and toxicology of these agents in humans is established, which would reduce the time and cost of approving those compounds for clinical use in cancer therapy[3]. To improve the retinoblastoma treatment, we designed a cell-based high-throughput screen (HTS) to identify novel chemotherapeutic agents that interact synergistically with a hyperthermia to induce cancer cell killing. We selected the Prestwick chemical library of known drugs (1280 compounds) and a cancer drugs collection library (80 compounds). Heat is applied at 42℃ over an hour when combined with chemotherapy. The thermoresponsive properties of the candidate drugs have been studied and the screening results not only verified that carboplatin is the best heat-activated drug among traditional drugs, but also the new discovered of chosen candidates (e.g. Gemcitabine) showed the high potential thermochemotheray efficacy. Consequently, we believe that there is considerable room for improving thermochemotherapy by screening the drug libraries to be used in combination with the heat treatment.

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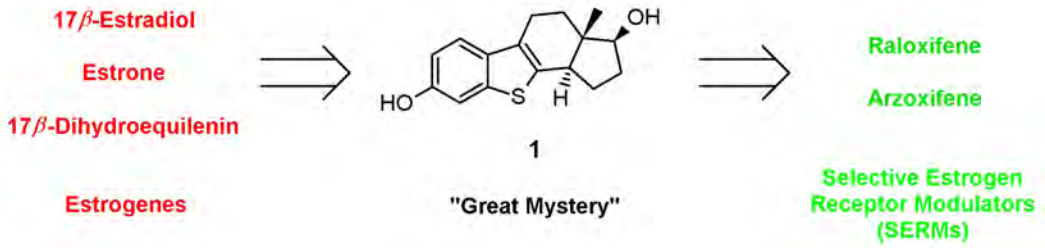
On the Hunt for the Missing Data: 6-Thia-B-norestrogens as Logic, but Neglected Intermediates between Estrogens and Benzothiophene-type SERMs

C. A. Vock[1,2]

[1]Faculty of Chemistry, University of Vienna, Währinger Str. 38-42, A-1090 Vienna, Austria, [2] Department of Food Sciences, Faculty of Life Sciences, University of Vienna, Althanstr. 14 (UZA II), A-1090 Vienna, Austria

Steroidal hormones represent a very important subclass of steroid-type natural products. In particular, steroidal sex hormones based on the estrane backbone (*e.g.* 17*β*-estradiol, 17*β*-dihydroequilenin) have attracted enormous interest due to their multiple effects in human organisms. On the search for new structural analogues with improved biological properties, synthetic 6-thia-B-norestradiol derivative **1** has been prepared already in 1969 in racemic form[1] and been reinvestigated as pure enantiomer together with all possible stereoisomers in 2003.[2]



In a recent presentation, we have shown the lack of biological data for the long-time known, but somehow forgotten 6-thia-B-norestrogens like **1**.[3] Here, we want to demonstrate the high importance of these compounds as logic bridge between the natural estrogen hormones and the later developed well-known benzothiophene-based Selective Estrogen Receptor Modulators (SERMs) like *e.g.* raloxifene or arzoxifene.[4]

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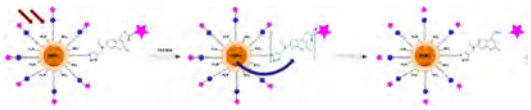
Two-photon triggered photorelease of caged molecular cargos from multifunctional harmonic nanoparticles

J. Vuilleumier[1], R. De Matos[1], G. Gaulier[2], C. Mas[3], S. Constant[4], L. Bonacina[2], S. Gerber-Lemaire[5]*

[1]Ecole Polytechnique Fédérale de Lausanne, [2]University of Geneva, GAP-Biophotonics, [3]Oncotheis, 18 chemin des Aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland, [4]Epithelix, 18 chemin des Aulx, CH-1228 Plan-les-Ouates, Geneva, Switzerland, [5]Institute of Chemical Sciences and Engineering, Gr

Nowadays, cancer is one of the leading cause of death in developed countries. The design of multimodal nanodevices for *in vivo* imaging offers the perspective of cancer detection at a very early stage. [1] The recent progress in the development of stimuli-responsive nanocarriers allows to achieve higher concentration and reduces the side effects of active molecules. The combination of controlled release of therapeutics and imaging properties in a single nanocarrier has a great potential for theranostic applications. [2] In this context, harmonic nanoparticles (HNPs), which are composed by non-centrosymmetric materials, can be easily imaged by their second harmonic generation signal in multiphoton imaging platforms.[3]

We disclosed efficient protocols for the biocompatible coating [4] and post-functionalization of bismuth ferrite ($BiFeO_3$, BFO) HNPs as well as their favorable properties for targeted imaging of human cancer cells and tissue [5-7]. Herein, we report the design, preparation and evaluation of multifunctional HNPs as novel light-activable NIR nanocarriers. Excitation of these functionalized HNPs in the near IR region generated second harmonic UV emission and subsequent selective release of the caged compounds. We recently demonstrated the uncaging process using tryptophan as cargo model upon femtosecond pulsed irradiation at 790 nm [8]. Furthermore, this strategy was applied to selective delivery of anticancer drugs (erlotinib and doxorubicin) *in vitro*.



The suitability of this methodology for the decoupled imaging of cancer cells and therapeutic intervention through exposure to uncaged molecular cargos was investigated *in vitro* by tuning the excitation wavelength in a multiphoton imaging setup.

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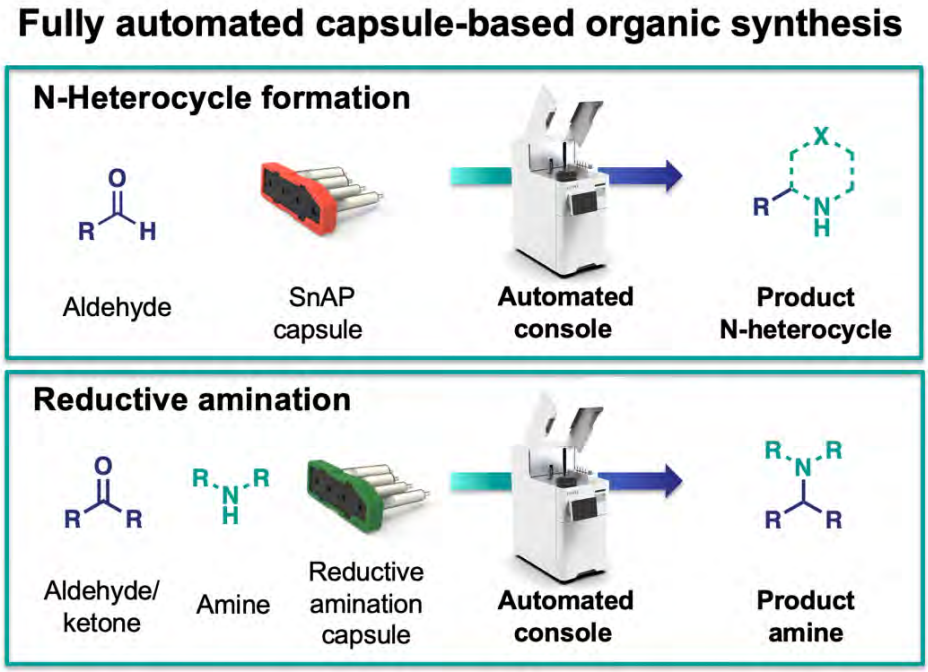
Capsule Chemistry at the Touch of a Button – a New Tool for Medicinal Chemistry

T. Jiang[1,2], B. M. Wanner[1,2], P. L. Nichols[1,2], K. Chen[2], A. McMillan[2], S. Bordi[2], J. W. Bode[2]*

[1]Synple Chem AG, [2]Laboratory for Organic Chemistry, Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland

Automation in synthetic organic chemistry is highly desirable due to improved safety, reproducibility and the productivity enhancing benefits. However, despite significant advances in the field, very few truly “plug and play” systems exist that combine both convenience and reliability with ease of use. With this goal in mind, an innovative, integrated, capsule-based, fully automated console has been developed for executing organic synthesis.



The utility of this automated technology is demonstrated for the formation of saturated N-heterocycles, reductive aminations, and multistep combinations, which enable rapid and highly efficient the preparation of complex drug-like molecules.

CHIMIA

CHIMIA 2019, Volume 73
ISSN 0009-4293
www. chimia.ch
Supplementa to Issue 7-8/2019

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Swiss Chemical
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Photoactivation of silicon rhodamines via a light-induced protonation

M. S. Frei[1,2], L. Reymond[3,4]*, K. Johnsson[1,2]*

[1]Institute of Chemical Sciences and Engineering (ISIC), École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland, [2]Department of Chemical Biology, Max Planck Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany, [3]Biomolecular Screening Facility, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland., [4]National Centre of Competence in Research (NCCR) in Chemical Biology, 1015 Lausanne, Switzerland

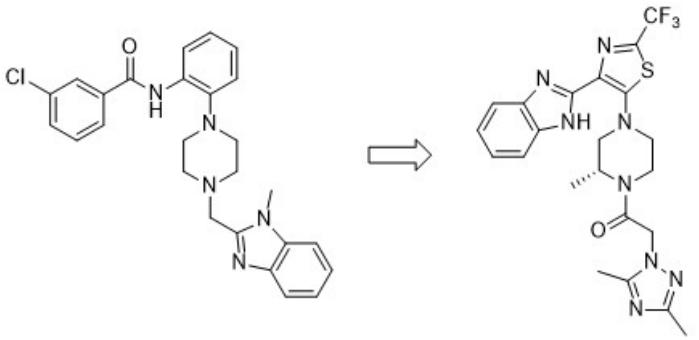
Photoactivatable fluorophores are important tools to investigate dynamic processes in cells. With the advent of super-resolution microscopy techniques based on single-molecule localization, these fluorophores have found even more applications. However, to make these techniques available for routine live-cell imaging, brighter, cell-permeable fluorophores are required. In our research, we develop new photoactivatable synthetic fluorophores based on the silicon rhodamine scaffold. This class of fluorophores has ideal properties for live-cell imaging: excitation and emission maxima in the far-red, high extinction coefficient, high quantum yield, photostability and cell-permeability. Instead of using bulky photolabile groups, we made use of photochemical concepts that require smaller structural modifications and generated a far-red photoactivatable fluorophore. The unusual mechanism of photoactivation and the fluorophore’s outstanding spectroscopic properties make it a powerful tool for live-cell super-resolution microscopy. We showed that this fluorophore can be used not only in fixed-cells, but also for following the fast dynamics of mitochondria by single-molecule localization microscopy in live-cells. Most excitingly, we could distinguish the unlabeled interior of the mitochondria from their labeled outer membrane in several cases, showcasing the power of this probe in combination with super-resolution microscopy.

Discovery of potent CXCR3 antagonists with therapeutic potential in autoimmune diseases

E. Caroff[1], E. Meyer[1], H. Siendt[2], T. Kimmerlin[3], P. Äanismaa[4], N. Schmitz[5], M. Keller[6]*

[1]Chemistry Immunology, Idorsia, [2]Chemistry Oncology, Idorsia, [3]Hit to Lead Chemistry, Idorsia, [4] Preclinical DMPK, Idorsia, [5]Medical Solution Immunology, Central Europe, UCB, [6]Biology Immunology, Idorsia

Chemokines are a family of signaling proteins secreted by cells. Their major role is to act as chemoattractants to guide the migration of immune cells through a mechanism called chemotaxis. Differences in chemokine concentrations in blood and tissues are sensed by cells expressing on their surface the cognate receptor of the chemokine, resulting in directed migration along the chemokine gradient. The CXCR3 receptor, a class A G protein-coupled receptor (GPCR), binds to the three pro-inflammatory chemokines CXCL9, CXCL10 and CXCL11 and is a key component of the adaptive immune response. We and others[1] started programs aiming at the discovery of small molecules to block the CXCR3 axis. Encouraging data recently disclosed with the CXCL10 monoclonal antibody eldelumab[2] in combination with methotrexate in a phase II trial for rheumatoid arthritis further strengthened our interest in this target. Herein we present the identification and optimization of thiazolo-piperazine analogs as potent CXCR3 antagonists and share in vivo efficacy data in a proof-of-mechanism mouse model of lung inflammation.



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Structural insights into molecular oxygen binding and activation by formylglycine generating enzyme

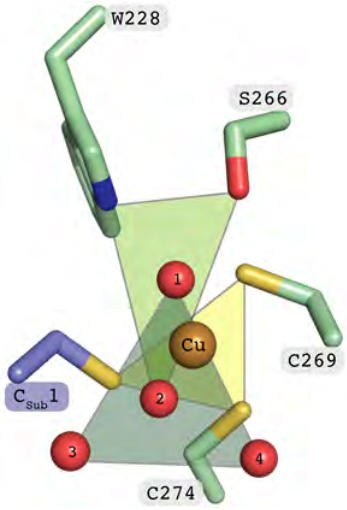
D. Miarzlou[1], F. Leisinger[1], D. Joss[1], D. Häussinger[1]*, F. P. Seebeck[1]*

[1]Department of Chemistry, University of Basel

In biological systems copper plays essential role in redox catalysis. Protein-bound can cycle between the oxidative states I, II and possibly III and thereby facilitate binding and activation of substrates. [1, 2]

The formylglycine generating enzyme (FGE) is a novel copper dependent oxidase, which participates in the activation of sulfatases in bacteria and animals. FGE catalases an O_2-dependent conversion of specific cysteine residue to formylglycine through the abstraction of the peptidyl-cysteine pro-(R)-β-hydrogen [3]. According to the recent structural investigations, FGE in the resting state has a single Cu(I), coordinated only with two cysteines in the linear geometry. The analogous sulfur rich coordination is common for copper trafficking proteins and transcription factors, but unprecedented among copper-dependent oxidases, which predominantly bind the metal in the histidine-rich tetrahedral or square planar coordination geometry. In contrast to the copper trafficking enzymes, FGE active site can bind and activate O_2 to abstract H atom from C-H bond. [4-6]

In this presentation we will describe the crystal structure of FGE from *T. curvata* in the complex with Cu(I) and a substrate peptide. This high-resolution structure, together with NMR spectroscopy and kinetic data, revealed an acidic O_2 binding pocket, closely located to the copper center. This FGE active site feature plays key role in O_2 activation.



The schematic representation of Cu(I) coordination sphere in the crystal structure of *tc*FGE in the complex with substrate analog.

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Discovery and optimization of novel, potent mGlu4 NAM compounds

F. O'Hara[1], L. Gobbi[1], M. Honer[1], L. Lindemann[1], D. Muri[1], S. Pichereu[1], J.-M. Plancher[1], B. Biemans[1]*, G. Jaeschke[1]*

[1]F. Hoffmann-La Roche Ltd, Roche Innovation Center, Basel

Allosteric modulation of the metabotropic glutamate receptors is a field receiving significant focus, both in basic science and in drug development. Nevertheless, there is a notable absence of suitable, potent NAM tool compounds for the Group III mGlus 4,6 and 8. [1] A number of recent publications have highlighted the need for selective mGlu4 NAMs as pharmacological tools to help elucidate the biology and probe therapeutic implications of this receptor. [2a, 2b, 3] The only two published mGlu4 NAMs - a small molecule [3] and a photoswitchable tool compound [4] suffer from low potency (5-10 uM). Our efforts towards the identification of mGlu4 negative allosteric modulators as tool compounds suitable for investigation of mGlu4 biology will be presented.

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Polymerase Amplification-Mass Spectrometry Coupled Quantitation of a Mutagenic DNA Adduct.

C. M. Aloisi[1], A. Nilforoushan[1], N. Ziegler[1], S. J. Sturla[1]*

[1]Department of Health Sciences and Technology, ETH Zürich, Schmelzbergstrasse 9, 8092 Zurich, Switzerland.

The formation of DNA adducts can initiate the process of mutagenesis. The lack of methods to determine low abundance DNA adducts within particular DNA sequences limits our understanding of the contributions of alkylation to mutagenesis. The use of artificial nucleotides incorporated opposite DNA adducts by engineered polymerases is a potential strategy for site-specific detection of DNA adducts. In previous studies, we created artificial nucleotides that were incorporated specifically opposite O^6-alkyl-guanine (G) adducts by a Taq polymerase mutant.[1] In this work, we designed and synthetized a new artificial nucleotide with an extended ρ-surface base that enables stronger enzymatic interactions, while retaining high and selective hybridization opposite O^6-alkylG. We made significant advances by combining the incorporation of the new artificial nucleotide opposite a biologically relevant O^6-alkylG lesion, i.e. O^6-carboxymethylG(CMG), with analytical readouts. We measured and characterized the enzymatic process, and we established a mass spectrometric method for quantifying the incorporated artificial nucleotide as a marker for the presence and sequence location of O^6-CMG. The results of this work advance chemical and biochemical tools for linking DNA alkylation to mutagenesis and for evaluating DNA adducts as potential diagnostic biomarkers.

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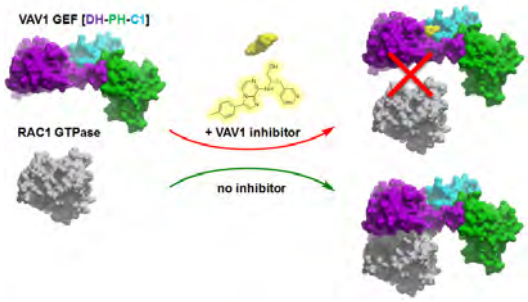
LMW VAV1 Inhibitors: Discovery, Optimization, & Mode of Action Studies

P. R. Skaanderup[1]

[1]Novartis Institutes for Biomedical Research, Chemical Biology and Therapeutics

Guanine nucleotide exchange factors (GEFs) are highly dynamic proteins and their function has been challenging to modulate with low molecular weight (LMW) compounds. GEFs activate distinct small GTPases relevant for human disease and hold promising therapeutic potential as novel drug targets. The first allosteric LMW inhibitors of VAV1 will be presented. Using biochemical screening and ligand-based hit expansion, we discovered a class of imidazopyrazines that potently and selectively inhibit VAV1-GEF dependent activation of RAC1. Initially, photo-affinity labeling was used to locate the binding site, which later was shown with high resolution co-crystal structures. The inhibitor-VAV1 complex is incompatible with binding to RAC1 leading to inhibition of guanine nucleotide exchange. Optimization led to a compound with an IC_{50}-value of 61 nM, a first in class allosteric VAV1 GEF inhibitor.

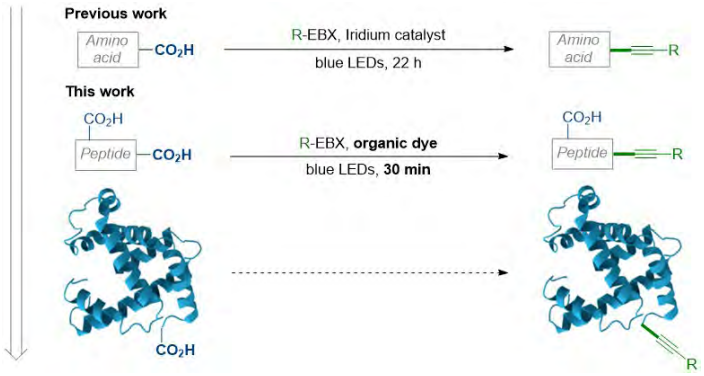


C-terminal Bioconjugation Through Photoredox Catalyzed Decarboxylative Alkynylation

M. Garreau[1], F. Le Vaillant[1], J. Waser[1]*

[1]Laboratory of Catalysis and Organic Synthesis, EPF

Targeting the C-terminal position of peptides is of high interest, both for designing novel scaffold for drug discovery and for further applications in protein labelling. Due to the selectivity challenge between carboxylic acids on the side-chains and this position, efficient methods are still very rare. As the extension of the decarboxylative alkynylation on amino acids developed previously in our group,[1] we report herein a decarboxylative alkynylation on peptides C-terminal position.[2] Using photoredox catalysis and hypervalent iodine EBX reagents, functionalized peptides are obtained in 30 min. Novel organic dyes were designed to enable high selectivity. Various functional groups were tolerated, both on peptides and EBX reagents, leading to the introduction of diverse functionalities on native peptides up to hexamers. Our efforts now target larger peptides and proteins, with the development of conditions in aqueous media.



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Stabilizing inactive conformations of MALT1 as an effective strategy to inhibit its protease activity

J. Langlois[1], P. Erbel[1], N. Aubin[1], F. Bornancin[1], C. Wiesmann[1], F. Villard[1], B. Gerrits[1], J. Quancard[1], A. Schlapbach[1], M. Renatus[1]*

[1]Novartis Institutes for Biomedical Research

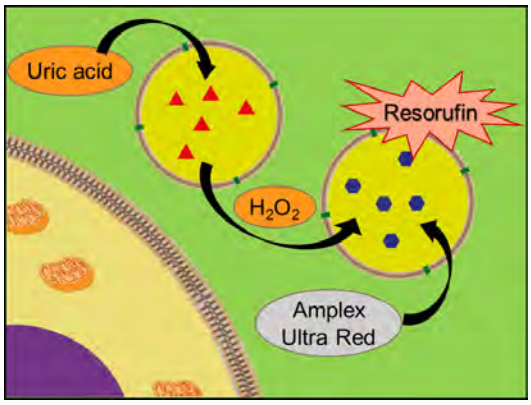
The paracaspase MALT1 (mucosa associated lymphoid tissue lymphoma translocation protein 1) plays an important role in various immune pathways and has been proposed as a therapeutic target for auto-immune disorders as well as cancers (i.e. DLBCL). We explored different mechanisms to inhibit the protease activity of MALT1 and discovered two unrelated chemical scaffolds. Biophysical and structural studies revealed that both scaffolds stabilize the protease in an inactive conformation. While one ligand binds to the allosteric site at the interface between the caspase and the IG3 domain, the other ligand binds to the active site in a so far undescribed mechanism. Iterative structure based drug discovery on one scaffold resulted in the identification of a potent, selective and bioavailable MALT1 inhibitor.

A nanotechnological approach to synthetic biology: cascade reactions in synthetic compartments.

A. Belluati[1], J. Liu[1], S. Thamboo[1], C. G. Palivan[1]*

[1]Department of Chemistry, University of Basel

Compartmentalization plays a fundamental role in biology: the spatiotemporal segregation of biochemical processes, both within the cell and between cells, allows a fine control of optimal reaction conditions, kinetics and overall protection from external environment. Much like phospholipids, amphiphilic block copolymers can self-assemble into spherical vesicles resembling cells, which can host a variety of enzymes and thus perform several specific reactions, with applications mainly in biomedicine and biosensing.[1]



Using more than a single enzyme in an interconnected, cascading system means that nanometre-sized objects start to behave as simple enzymatic networks, achieving complex responses depending on the polymer-enzyme assembly. In addition, the biocompatibility of the chosen poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA-PDMS-PMOXA) polymer means that cells can be phenotypically modified by assisting their native metabolism or outright expanding their environmental response.[2] The ever-growing complexity of nanometric catalytic assemblies leads them to behave dynamically and, in turn, to interact with living matter.

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From Setipiprant to ACT-774312: discovery of new potent CRTH2 antagonists

R. Siegrist[1], M. Holdnener[1], P. Bouis[1], C. Gnerre[1], J. Hazemann[1], S. Richard[1], C. Boss[1], H. Aissaoui[1]*

[1]Idorsia Pharmaceuticals Ltd.

Activation of the chemoattractant receptor-homologous molecule expressed on T helper-2 cells (CRTH2, also known as DP2) by prostaglandin D2 (PGD2) induces pro-inflammatory responses. Development of CRTH2 receptor antagonists could therefore be useful to treat allergic and type 2 inflammatory diseases such as allergic rhinitis, atopic dermatitis, asthma, or nasal polyposis [1].

We previously reported the discovery of setipiprant which was in advanced clinical development [2]. Late stage clinical studies with setipiprant in seasonal allergic rhinitis (Phase 3) [3] did not confirm efficacy findings made in earlier studies. Our efforts to identify a more potent backup compound started by modifying the core of the molecule and synthesizing tetrahydrocarbazole derivatives with drug-like properties. Further lead optimization guided also by *in-vitro* cytotoxicity profiling finally led to the discovery of the clinical candidate ACT-774312, which is currently in Phase 2 development in nasal polyposis [4].

In this presentation, we will describe our medicinal chemistry efforts that culminated with the identification of ACT-774312 [5].

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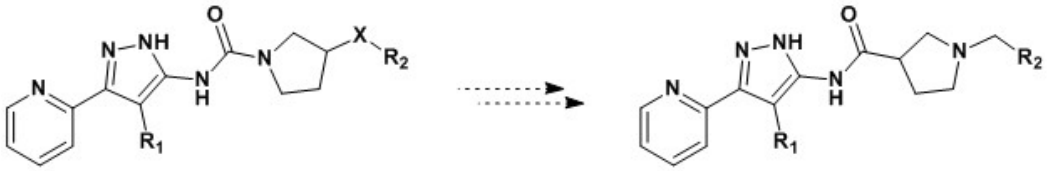
Synthesis of new aminopyrazole analogues with promising anti-leishmaniosis activity: A University-DNDi Open Synthesis Project

S. Tardy[1], S. Students from BUSP2[2], C. Cecchini[1], H. M. Ismail[1], N. Deschamps[1], L. Pellissier[1], A. Rutz[1], M. Fu[1], E. Varesio[1], L. Scapozza[1]*, B. Perry[3]*

[1]School of Pharmaceutical Sciences, University of Geneva, [2]Students from the second year of the bachelor in Pharmaceutical Sciences performing practicle exercice in pharmaceutical chemistry, [3] Drugs for Neglected Diseases initiative DNDi

Leishmaniosis is a neglected tropical disease, an illness that kills up to 30,000 people yearly. Existing drugs have serious drawbacks in terms of safety, resistance, stability, difficulty of administration and cost. Thus, there is a need for new treatments. The aminopyrazole class of compounds originally from Pfizer has shown promising early profiles for the treatment of both visceral and cutaneous leishmaniosis. [1] In the frame of an Open Synthesis Network (OSN) between the University of Geneva and the Drugs for Neglected Diseases initiative (DNDi), we aimed at synthetizing new aminopyrazole analogues for early stage discovery for new treatments for leishmaniosis.



In order to explore the aminopyrazole chemotypes, we set up a 5-steps synthesis to obtain theoretically 42 different products. The two key reactions are reductive amination and peptidic coupling during which structural diversification occurs. Biological activity has been assessed on *Leishmania infantum* and cytotoxicity on human and murine fibroblasts. In 2018, this multi-steps synthesis led to 14 new compounds that have been fully characterized by HRMS, ^{1}H-NMR, ^{13}C-NMR, IR and HPLC. All final compounds have been tested *in vitro* for both efficacy and toxicity. Two of them have shown high potency against *Leishmania infantum* (IC_{50} 0.3 and 1.5 microM) and a selectivity index (CC_{50}/IC_{50}) ranging from 27 to 213. The open source nature of this project aimed at deepening the learning of laboratory work in the context of students R&D practical work. The collaborative spirit of the students has led to the successful synthesis output and the development of a scientific rigor of work, which includes the preparation and the follow-up plans of experiment. The promising anti-leishmaniosis activity has clearly indicated an SAR and the possibility of further exploring the current chemotype to improve compounds efficacy and selectivity. The two most potent compounds have undergone in vitro ADME studies and have shown metabolism issue. In this context, a new set of 24 compounds was designed for praticle work in 2019…

[1] Mowbray C. E. *et al*. *J. Med. Chem.* **2015**; 58 (*24*): 9615-9624.

Discovery and optimisation of novel compounds for malaria vector control

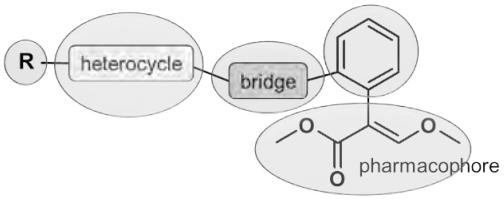
O. F. Hueter[1], M. Hoppé[1], P. Wege[2], R. Nourani[2], M. Daniels[2], P. Maienfisch[3]

[1]Syngenta Crop Protection, Schaffhauserstrasse, CH-4332 Stein, Switzerland, [2]Syngenta Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, UK, [3]Syngenta Crop Protection, Schwarzwaldallee 215, CH-4058 Basel, Switzerland

In excess of 30000 compounds, from over 150 chemical classes, have been screened at Syngenta against adult mosquitoes within a research and optimisation programme, undertaken as part of a Product Development Partnership sponsored by the IVCC (Innovative Vector Control Consortium).

In the initial screening effort, many classes with biological activity against mosquitoes were discovered.[1,2)] However, the class of synthetic analogues of the natural product strobilurin was selected for optimization as it represents a novel mode of action for control of the anopheline vectors of malaria, and displayed many of the characteristics required of vector control interventions.

The discovery, design, optimisation and associated structure activity relationships of strobilurin analogues will be described. As a result, promising lead candidates for further evaluation were identified.[3,4)]



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[3] Syngenta WO 2016/193267.
[4] Syngenta WO 2019/081575.

Advanced building blocks for medicinal chemistry from the chemical universe database GDB

K. Meier[1], J. Reymond[1]*

[1]University of Bern, Freiestrasse 3, CH-3012 Bern

With more than 30 drug candidates in clinical trials, fragment-based drug discovery (FBDD) has become a very successful strategy to identify bioactive molecules during the past 20 years.[1] However, the success of FBDD is often dependent on the quality of the fragment library. It is therefore critical to access new and advanced fragments or building blocks. Reymond *et. al.* extensively enumerated the chemical space of small molecules with up to 17 heavy atoms in the chemical space project. This project lead to different chemical universe databases, GDB-11 (26.4 million molecules), GDB-13 (970 million molecules), and GDB-17 (166 billion molecules). The vast majority of the molecules in these databases have not been described in synthetic literature and are therefore a source of inspiration for novel fragments or building blocks.[2-4] The use of GDB for drug discovery was exemplified with the successful identification of new Glutamate Transporter 1 (GLT-1) inhibitors. First, Aspartate and Glutamate were systematically diversified using GDB. High-throughput virtual screening followed by synthesis of high-ranking molecules then yielded a new Norborane-type inhibitor. These Aspartate analogues showed not only good micromolar activity but also high selectivity towards GLT-1.[5] Extensive analysis of molecules described in literature compared to molecules in GDB revealed that a large part of novelty resides in polycyclic compounds.[6] In this work we focussed on the synthesis of conformationally restricted structures bearing one quaternary carbon. So here we present a new approach to the design of the synthesis of novel polycyclic molecules from GDB, displaying pharmacophoric features of interest for medicinal chemistry.

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