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A Covalent Strategy to Develop Highly Selective Chemical Probes Targeting PI3Kα

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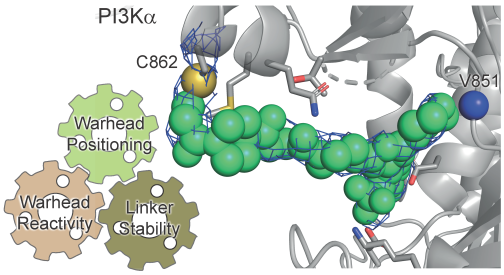
Inhibitors of the phosphatidylinositol 3-kinase (PI3K) – mechanistic target of rapamycin (mTOR) axis are considered as valuable assets in cancer therapy. A considerable effort has been dedicated to the development of drugs targeting the PI3K-mTOR pathway[1-4], and some inhibitors are currently evaluated in preclinical and clinical studies.

Herein we present a strategy to convert a phase II clinical candidate, a pan-PI3K inhibitor (PQR309, bimiralisib)[1,5], into highly selective PI3Kα-covalent inhibitors aiming to minimize the on-target metabolic side effects of PI3K inhibitor cancer therapy. We exploited a rational approach to increase target selectivity by covalently targeting a PI3Kα non-conserved nucleophilic amino acid side chain, namely Cys862. A reactive moiety, so called warhead, was introduced into a chemically modified bimiralisib.

A combination of warhead activity design, proximity and orientation allows a tight control of reversible inhibitor binding and isoform selective covalent binding. To avoid off-target reactions, we have set up a method to quantitatively evaluate warheads' reactivity and optimize for selective Cys862 modification. An extensive Structure Activity Relationship (SAR) study was performed and a wide range of linear and restricted rotation linkers introduced. A comprehensive understanding of the kinetics of irreversible inhibition allowed to interpret SAR and identify compounds with optimal kinact (maximum potential rate of inactivation). X-ray crystallography and mass spectrometry experiments validated the covalent modification of Cys862. Our pilot compounds exceed specificity and potency over an experimental dimethyl-substituted enone, CNX-1351[6]. Moreover, they are metabolically stable in rat liver microsomes and outperform the rapidly metabolized CNX-1351.

Our strategy to investigate and tune warheads' reactivity represents a major step forward in the rational design of covalent chemical tools. Moreover, we provide highly selective chemical tools to dissect PI3K isoform signaling in physiology and disease.



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Design of an internal salt bridge in zwitterionic SUCNR1-antagonists for improved oral exposure

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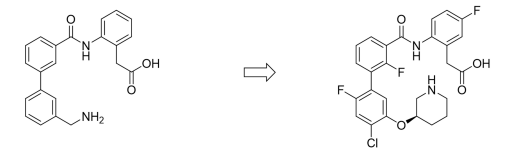
SUCNR1 (succinate receptor 1 or GPR91) is a G-protein coupled receptor sensing succinate, a signaling molecule when released into the extracellular space under certain pathological conditions.[1] SUCNR1 is implicated in various pathological conditions such as liver fibrosis, obesity or atherothrombosis and plays also an important role in inflammation.[2] Recently, a novel SUCNR1 antagonist scaffold has been discovered by our group by high-throughput screening.[3] The hit-to-lead optimization led to zwitterionic compounds which, however, suffered from poor absorption properties. In order to improve the absorption and oral exposure, formation of an internal salt bridge was envisioned for shielding of both opposite charges and as a consequence, the high polarity found in zwitterions with separated charges. Guided by a pair of rather close zwitterionic analogs with very different permeation and absorption properties new, potent, salt bridge-containing SUCNR1 inhibitors were designed. The new analogs demonstrated indeed much higher oral bioavailability and oral exposure. Importantly, such compounds were found to be of a chameleonic nature, i.e. they form the salt bridge allowing them for good permeation in their unbound form while the salt bridge can be disrupted in their bound form which allows for their binding into the receptor with both ions required to be separated.[3]



logPAMPA	<-5.3 cm/s
mouse BAV	10%
mouse Cl	22 mL/min/kg
mouse AUC po d.n.	206 nM.h/L

IC_{50} (hSUCNR1)	0.088 μM
logPAMPA	-5.1 cm/s
mouse BAV	74%
mouse Cl	0.6 mL/min/kg
mouse AUC po d.n.	37000 nM.h/L

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Discovery of a selective antitubercular small-molecule inhibitor targeting CoaBC

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Tuberculosis (TB) is one of the top ten causes of death worldwide, accounting for 1.8 million deaths per year.[1] The emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* (*Mtb*) has stimulated renewed efforts for the discovery of new anti-tubercular drugs.[1]

The biosynthesis pathway of coenzyme A (CoA) is essential in bacteria, and inhibiting the CoA pathway is considered a viable antibiotic strategy. In *Mtb*, the bifunctional enzyme CoaBC catalyses the formation of 4'-phosphopantothenoylcysteine from 4'-phosphopantothenate and *L*-cysteine through its CoaB domain, followed by decarboxylation of the cysteine moiety to 4'-phosphopantetheine via its CoaC domain.[2] Due to its considerable different to the human orthologue, CoaBC is a highly promising target for the development of novel selective anti-tubercular drugs.[3]

We have developed novel inhibitors of *Mtb* CoaBC using a fragment-based approach in conjunction with structure-guided design by X-ray crystallography. Our campaign started from an original fragment hit, and using fragment-growing strategies, we obtained a lead series of compounds inhibiting the *Mtb* CoaBC enzyme at nanomolar concentrations. This is the first series of drug-like small-molecule inhibitor of *Mtb* CoaBC which is active on whole cell *Mtb*, confirming the druggability of *Mtb* CoaBC and opening avenues to develop a new class of antitubercular drugs.

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Gamma-Secretase Modulators (GSM) for the potential treatment of Alzheimer’s Disease (AD); Novel phenyl bioisosteres

H. ratni[1]

[1]F. Hoffmann-La Roche

The prevalence of Alzheimer's Disease (AD) is growing and so will be the societal burden across the world due to the ageing of the population and the abscence of treatment. AD comprises a continuum of progressive neurodegenerative pathologies associated with cognitive, functional, and behavioral deficits. The earliest stages of AD include microscopic changes in the form of A-beta plaques arising from A-beta 42 aggregations.

Modulation of gamma-secretase appears as a safe approach to lower toxic A-beta 42. Here, we present our contribution to the field which has led to the identification of our clinical candidate RO7185876. Furthermore, in the frame of this project, we also discovered that bridge piperidine coul dbe use as a superior phenyl bioisoster.

Machine Learning Guides the Design of Non-Hemolytic Antimicrobial Peptides

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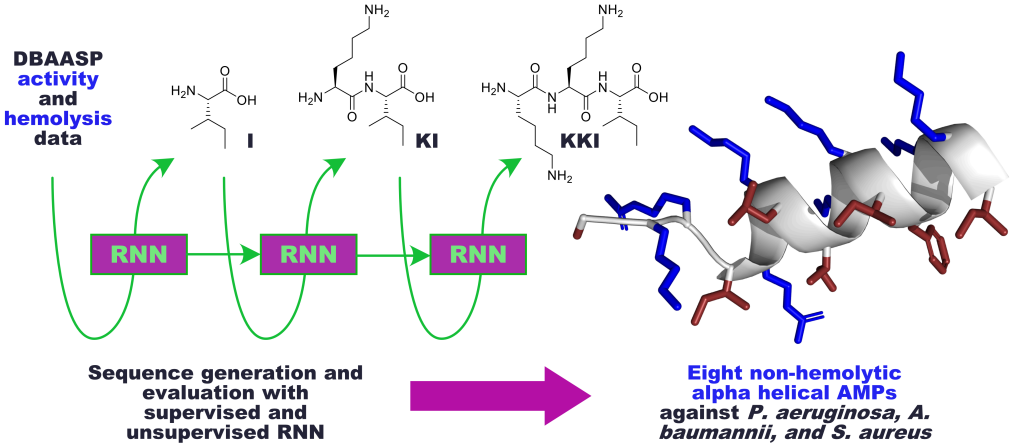
Antimicrobial peptides (AMPs) offer an opportunity to address antibiotic resistance, which represents one of the major global public health threats [1]. Most AMPs are membrane disruptive amphiphiles, and unfortunately this activity is often associated with toxicity to human red blood cells [2]. Here, we have trained a combination of supervised and unsupervised recurrent neural networks (RNN) using hemolysis and activity data from DBAASP (Database of Antimicrobial Activity and Structure of Peptides) [3] to design non-hemolytic AMPs. The synthesis and test of 28 generated peptides led to the identification of eight novel non-hemolytic AMPs against *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA).



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Discovery of an orally active and selective ALK2 kinase inhibitor for the treatment of a rare genetic bone disease

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Fibrodysplasia ossificans progressiva (FOP) is an ultra-rare disease leading to progressive soft tissue heterotopic ossification with no curative treatment available to date. It is caused by gain-of-function mutations in the BMP type I receptor kinase ALK2, triggering a search for efficacious small-molecule inhibitors. We optimized a chemical series of 2-aminopyrazine-3-carboxamides (Figure 1) for metabolic stability and off-target selectivity and identified a novel potent and selective ALK2 kinase inhibitor with excellent oral bioavailability in preclinical species, showing full suppression of heterotopic ossification in a pediatric mouse model of skeletal muscle injury-induced FOP.

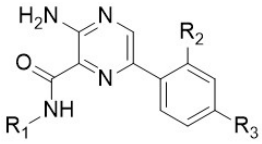

Figure 1

Cyclic Tetrapeptides as Next-Generation Remedies of Lead Poisoning

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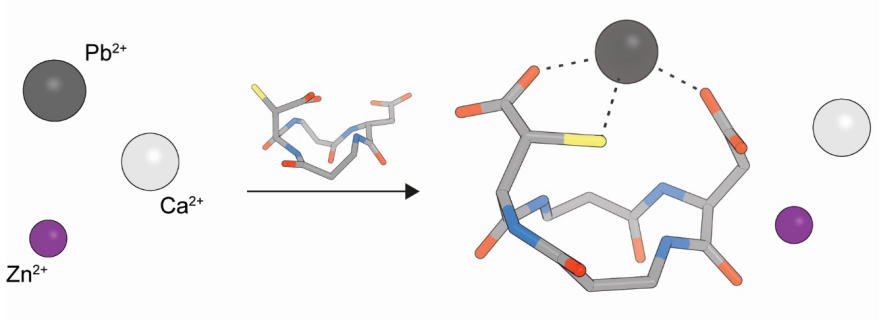
Lead (Pb) is the most abundant toxic metal that causes significant environmental and medicinal issues. Chelation therapy, in which a ligand called a chelating agent is administered to remove the poisonous metal, is the primary approach to handle Pb poisoning. To date, there is no ideal chelating agent for treating Pb toxicity, and the available chelating agents lack metal selectivity. Hence, they are highly toxic by themselves and subsequently prohibited from treating the most affected population segments, including children and pregnant women.[1]

Nature responds to metal poisoning through several creative solutions, most of them involve peptides and proteins as chelators.[2] Inspired by natural systems, we designed and synthesized a family of cyclic tetrapeptides. We investigated their ability to recover Pb-poisoned bacteria and human cells, where two candidates showed a distinguished potency, outcompeting the current clinical chelating agents. Characterizing the Pb-peptide complex of the leading ligand experimentally and computationally disclosed its surpassing metal affinity and selectivity, indicating its promising potential as a novel antidote for Pb poisoning.[3]



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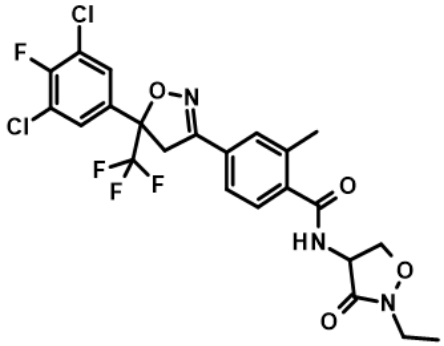
The Discovery of Isocycloseram: a Novel Isoxazoline Insecticide

M. El Qacemi[1]

[1]Syngenta Crop Protection

Isocycloseram is a novel insecticide discovered at Syngenta Crop Protection. It is a member of the isoxazoline class of insecticides, which acts as a non-competitive antagonist of the invertebrate GABA receptor at a site distinct to that of fiproles and cyclodienes, resulting in excellent efficacy against invertebrate pests.



Innovative approaches for the delivery of modern agrochemicals such as multi-parameter optimization and faster cycles of Design-Synthesis-Test-Analysis (DSTA) were implemented during the research phase on the isoxazoline insecticidal class and led us to the discovery of Isocycloseram. The synthesis, optimization and biological efficacy aspects of this new chemical class will be presented.

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Disruption of mitochondrial redox homeostasis by enzymatic activation of a trialkylphosphine probe

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Intracellular redox balance is crucial for cell function and is primarily regulated by the relative concentrations of glutathione (GSH) and its oxidized dimer (GSSG). Multiple physiological processes, ranging from cell signaling to protein folding depend on redox homeostasis. In eukaryotes, this homeostasis is controlled at the level of subcellular compartments and each organelle possesses its own redox environment.

Mitochondria fulfill multiple essential tasks in the cell that depend on redox modulation. Disruption of this homeostasis leads to pathologies such as insulin resistance and type II diabetes. Whereas the effects of oxidative stress in mitochondria have been thoroughly investigated, reductive stress has remained significantly underexplored. We envisioned that the GSH/GSSG ratio could be manipulated by direct reduction of the disulfide bond in GSSG to GSH using trialkylphosphine derivatives. We could achieve mitochondria targeting by taking advantage of the local enzymatic activity of nitroreductases, triggering the release of tributylphosphine and a fluorescent reporter from a masked precursor.

A series of live cells experiments including fluorescence imaging and biological assays showed that reductive stress is eventually transformed into oxidative stress through the production of $O_2^{\cdot-}$. It activates the ATF4-ATF3-CHOP cascade, which upregulates the CHAC1 gene. This response is mediated by glutathione, suggesting a link between reductive and oxidative stress.

In this work, we have demonstrated the importance of trialkylphosphines as chemical probes to modulate redox biology by developing strategies to tune their reactivity and subcellular targeting. These probes have a potential impact in the development of new therapies and enriching our understanding of subcellular compartmentalization of redox signaling.

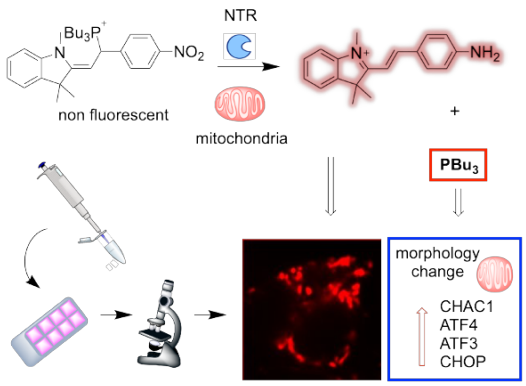

Figure. Mechanism of enzymatic activation of the probe by nitroreductases in mitochondria with simultaneous release of thributylphosphine and fluorescent reporter. Fluorescence microscopy shows localization in mitochondria. Transcriptomics analysis shows upregulation of genes from the integrated stress response.

J. Nguyen, A. Tirla, P. Rivera-Fuentes, *Org. Biomol. Chem.* **2021,** 2681-2687.

The Screening Compound Collection: A Key Asset for Drug Discovery

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[1]Idorsia Pharmaceuticals Ltd., Allschwil, Switzerland

In 2013 Actelion decided to establish a Compound Library Committee (CLC), a cross-functional team consisting of computational scientists, medicinal chemists, and biologists. The CLC was endowed with a significant annual budget for new screening compound purchases. Based on an initial library analysis performed in 2013, the CLC developed a New Library Strategy. The structural library quality was increased which was achieved by shifting the selection criteria from ‘druglike’ to ‘leadlike’ structures, enriching for non-flat structures, aiming for compound novelty, and increasing the ratio of higher cost ‘Premium Compounds’. Novel chemical space was gained by adding natural products, macrocycles, designed and focused libraries to the collection, and through mutual exchanges of proprietary compounds with agrochemical companies. A comparative analysis in 2016 provided evidence for the positive impact of all these measures on new lead discovery. Screening the improved library has provided several highly promising hits that are currently followed up in different Hit-to-Lead and Lead Optimization programs, including a compound in pre-clinical development. The goal of the CLC was not to achieve higher HTS hit rates, but to increase the chances of identified hits to serve as the basis for successful early drug discovery programs. Today, eight years later, lessons learned from successes and flaws will help the CLC shape the future Idorsia Library Strategy.

Exploring the function of microtubule post-translational modifications by semi-synthetic tubulin

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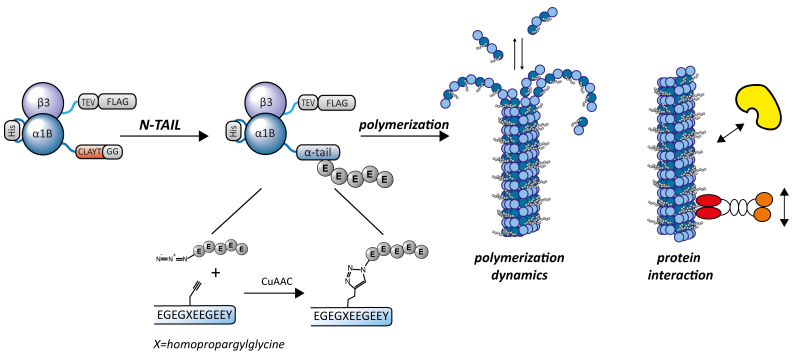
[1]École Polytechnique Fédérale de Lausanne (EPFL), [2]Université de Genève

Microtubule filaments, critical for cell structure, division and motility, are comprised of α- and β-tubulin dimers containing C-terminal tails with highly variable post-translational modifications (PTMs) such as detyronisation, polyglutamylation and polyglycylation. The tightly regulated spatial and temporal distribution of PTMs in the microtubule network underlines its importance for many cellular processes involving this major cytoskeleton component. Microtubules can form a local, specialized support as they are decorated by combinations of PTMs, which has been aptly described as the 'microtubule code'.[1] However, deciphering the microtubule code to unravel its role in cell biology or pathogenesis remains challenging. A major difficulty in studying PTMs lays in the unavailability of uniformly modified microtubules as we are limited to mostly natural resources of tubulin (i.e. cow or porcine brain cells).

Here we overcome this limitation by developing a semi-synthetic tubulin; a chemically defined C-terminal tail is fused to recombinant purified tubulin dimers. A recently developed ligation method, transpeptidase-assisted intein ligation (TAIL),[2] appeared extremely suitable for addition of the tubulin C-terminal tail as it requires a small recognition motif and only leaves a two amino-acid scar. We demonstrate the use of TAIL, where initially 1) a Sortase enzyme ligates an N-terminal intein onto the α-tubulin followed by 2) a protein trans-splicing reaction to fuse a pre-designed synthetic C-terminal tail bearing the intein counterpart. The two intein halfs can reassemble and propagate a thiolester-cysteine mediated ligation, excising itself and generating the semi-synthetic tubulin. With full chemical control of the α1B C-terminal tail composition (e.g. enabling the use of copper-catalyzed azide-alkyne cycloadditions) we produced a small set of α1B-tubulins containing either fluorescein or branches of one, five or ten glutamates. Dimers of α1B/β3 with the different PTMs were assessed for their microtubule polymerization dynamics and interaction with modifiers such as the newly discovered detyronisation enzyme vasohibin.

With this technology we will be able to test the individual effect of defined tubulin PTMs in a highly defined system using semi-synthetic tubulin proteins, carrying specific PTM patterns. These unique reagents will thus provide a key to decipher the tubulin code.



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Acceleration of New Medicines with “Discovery Process Research” at Janssen

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Janssen has established a “Discovery Process Research” group at the interface between Medicinal Chemistry and Process Chemistry. The group’s activities are concentrated in three areas: 1) development of new chemistry in the discovery lead-optimization space to enable and inspire structure-activity relationship profiling; 2) a traditional role of meeting material demands for tolerability studies in advance of development candidate declaration; and 3) acceleration of new candidates into Phase 1 clinical trials through early investment in route scouting to provide a line of sight for first multi-kg GMP batches. With a focus on recent advances in the field, application of High-Throughput Experimentation, and a mandate to deliver impactful chemical solutions, the group is producing exciting new methods with relevance to Pharma and beyond. The talk will highlight recent efforts and impact.

HaloTag9: an engineered protein tag for fluorescence lifetime multiplexing

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Self-labeling protein tags have become important tools in fluorescence microscopy. Their use in combination with fluorogenic fluorophores, which only become fluorescent when bound to their protein target, makes them particularly suitable for live-cell applications. The fluorogenic turn-on observed upon labeling as well as the photophysical properties of the fluorophore are mainly determined by the protein surface near the fluorophore binding site. However, up to now, most efforts have been invested in the development of new fluorophores and only little attention has been paid to the engineering of the self-labeling protein tag.

Here we report on the structure-guided engineering of HaloTag7 to modulate the brightness and fluorescence lifetime of bound rhodamines. Specifically, we developed HaloTag9, which showed up to 40% higher brightness *in cellulo* and 20% higher fluorescence lifetime than HaloTag7 upon labeling with rhodamines. This makes it an ideal tag for imaging techniques such as confocal microscopy or stimulated emission depletion microscopy. In addition, combining HaloTag7 and HaloTag9 enabled us to perform live-cell fluorescence lifetime multiplexing using a single fluorophore. The difference in fluorescence lifetime was further exploited to generate a chemigenetic fluorescence lifetime based biosensor to monitor cell cycle progression. Overall, our work highlights that the combination of protein engineering and chemical synthesis can generate imaging tools with outstanding properties. We expect HaloTag9 to be beneficial for a multitude of live-cell microscopy applications.[1]

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Cooperativity of nitrogenase in biological nitrogen fixation

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[1]Department of Inorganic and Analytical Chemistry, [2]Department of Organic Chemistry, [3] Department of Civil and Environmental Engineering

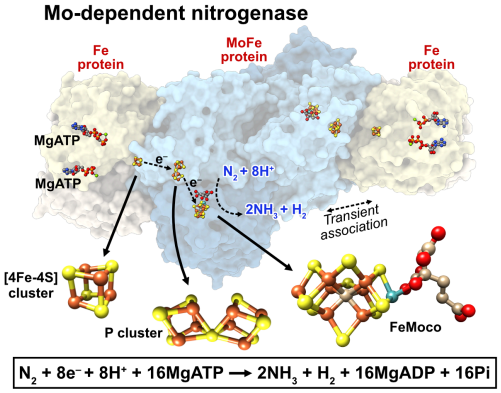
Nitrogenase is a metalloenzyme that catalyzes the reduction of kinetically inert N_2 to NH_3 as a bio-available source of nitrogen. Produced by select diazotrophic microorganisms, this enzyme catalyzes N_2 fixation at ambient temperature and pressure alongside the hydrolysis of adenosine triphosphate (ATP) by to the following equation:

$$N_2 + 8H^+ + 8e^- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

Nitrogenase is comprised of an electron-transferring ATP-hydrolyzing iron protein (Fe protein) and a catalytic molybdenum-containing protein (MoFe protein) where N_2 is reduced. The MoFe protein is a α2β2 heterotetramer and each symmetric αβ half undergoes repeated transient associations with the Fe protein for catalytic turnover. In 2016, Danyal and co-workers investigated pre-steady state cooperativity in nitrogenase by EPR and observed that electron transfer between the Fe protein and the MoFe protein on one-half inhibits electron transfer on the second half.[1]

Here, we report on cooperativity in nitrogenase in the steady state by disrupting association between the Fe and the MoFe proteins on one or both halves. A mutant MoFe protein exhibiting a surface cysteine close to the P cluster was reacted with maleimide-based linkers by thiol-Michael addition, including a Strep-tag peptide for affinity purification. We also report on a mutant MoFe protein where the "16th Fe" of nitrogenase (not known to be involved in the catalytic cycle) which bridges both αβ halves was disrupted.

As researchers move towards the artificial reduction of the MoFe protein for ATP independent N_2 fixation, it will be important to understand how the organization of nitrogenase into two symmetrical halves assists N_2 reduction.



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The Antimicrobial Activity of Peptide Dendrimers and Polymyxin B Increases Sharply Above pH 7.4

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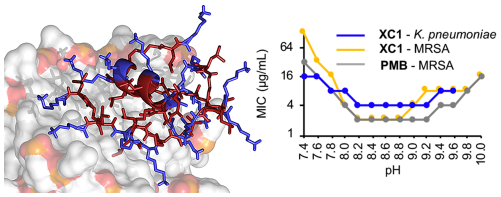
Recently we reported antimicrobial peptide dendrimer (AMPD) **G3KL** and **T7** with potent activities against *P. aeruginosa* and *A. baumannii*, two of the most problematic antibiotic-resistant nosocomial pathogens[1][2]. In our efforts to develop new AMPDs against Gram-negative bacteria, we investigated their activity at acidic and basic pH, which correspond to the conditions of the site of bacterial infections on skin or biofilms and chronic wounds respectively. Removing the eight low pKa amino termini by substituting the N-terminal lysine residues with aminohexanoic acid in our reference dendrimer **G3KL** provided the modified peptide dendrimer **XC1** with a broader pH-activity range. Furthermore, we discovered that raising the pH to 8.0 reveals strong activities against *Klebsiella pneumoniae* and methicillin at pH 7.4, an effect also observed with polymyxin B and tentatively assigned to stronger binding to the bacteria at higher pH as observed with a fluorescence labeled dendrimer analog. This work has been published in *Chemical Communication*.[3]



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Restoring HIF-2α Degradation in Clear Cell Renal Cell Carcinoma through PROTAC Technology

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Background: Dysregulation of the Ubiquitin-Proteasome System (UPS), such as the presence of non-functional E3 ubiquitin ligases, may result in oncogenic substrates overexpression. In particular, the lack of VHL E3 ubiquitin ligase in von Hippel-Lindau (VHL) disease has been shown to promote clear cell renal cell carcinoma (ccRCC) or renal cysts, which are correlated with the cell accumulation of HIF-1α and HIF-2α transcription factors.[1]

Aim: The growing evidence of HIF-2α oncogenic role fostered the development of HIF-2α antagonists.[2] In this work, we aim at inducing HIF-2α knockdown by taking advantage of the promising PROTAC Technology.[3] Proteolysis Targeting Chimeras (PROTACs) are heterobifunctional molecules that specifically bind and bring into proximity a target protein and a given E3 ubiquitin ligase. Contrary to protein inhibition, PROTACs act as degraders by using the cell's protein degradation pathway to remove specifically labeled proteins.

Methods: We designed and synthesized the warhead of our degraders, responsible for engaging HIF-2α, starting from the structure of HIF-2α antagonists. In parallel, as the HIF-2α binding site is completely buried, we conducted molecular modeling studies to identify linker attachment points allowing the binding affinity retention. We selected polyethylene glycol (PEG) linkers to connect the warhead to the corresponding E3 ligase binder, responsible for recruiting CRBN and MDM2 E3 ligases.

Results and conclusion: PROTACs *in vitro* activity has been accessed by multiple functional assays in RCC cells lacking VHL. In particular, our degraders showed activity in the nanomolar range after 4h treatment by western blot. In perspective, we plan proteomic analysis to quantitatively study protein expression in cells and we consider performing *in vivo* studies. So far, we demonstrated that our first class of degraders targeting HIF-2α are effective in restoring dysregulation of the Ubiquitin-Proteasome System (UPS) due to the lack of VHL.

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Optimization of Covalent Chemical Probes

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Phosphatidylinositol-3-kinase (PI3K) activity is aberrant in tumors, and PI3K inhibitors are investigated as cancer therapeutics.[1-5] The major obstacles to the successful implementation of PI3K-targeted cancer therapy are on-target adverse effects on insulin signaling. Isotype selective PI3K inhibitors have been exploited to answer fundamental questions regarding the role of PI3K isoforms in cell biology. However, the availability of claimed isoform-selective inhibitors is limited to BYL719 (Alpelisib)[6] and GDC0032 (Taselisib)[7], which do not maintain PI3Kα selectivity at a concentration required in cellular experimental settings and clinical applications. As the systemic inactivation of PI3Kα is embryonic lethal, genetic approaches are currently limited to organ-specific targeting, and a specific inactivation of PI3Kα in an adult organism has not been achieved up-to-date. Highly selective PI3Kα inhibitors are expected to represent ideal tools to elucidate the role of PI3Kα isoform in tumor development and insulin signaling.

Herein, we generate high-quality PI3Kα chemical probes to dissect the role of PI3Kα in cancer and metabolism. We exploit covalent inhibitors, permanently blocking target functions, as a strategy to enhance the ligand binding selectivity for proteins in the same family. The non-conserved nucleophilic amino acid Cys862 in PI3Kα represents a promising target for covalent modifiers. We converted the reversible scaffold of PQR514[5] into irreversible compounds. An extensive Structure Activity Relationship (SAR) study was performed using CNX-1351[8] reacting group and introducing different heteroaliphatic rings in the linker. X-ray crystallography and bottom-up LC-MS/MS based proteomics validated the covalent modification of Cys862. Our pilot chemical probes exceeded in vitro and cellular potency over CNX-1351. The generation of a novel class of covalent PI3Kα-specific inhibitors with improved selectivity and persistency of PI3Kα-inhibition will shed light on the role of PI3Kα in cancer and metabolism. Our results will pave the way for the dissociation of PI3Ki antitumor activity from adverse effects on insulin action.

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Novel Synthetic Strategies Enable the Efficient Development of Folate Conjugates for Radiotheragnostic Application

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The folate receptor (FR) is a promising target for radiotheragnostics due to its overexpression in numerous tumor types. The study aimed to establish a synthetic approach to facilitate and accelerate the preparation of a broad spectrum of new folate radioconjugates. Two solid support-based synthesis pathways were developed using orthogonal Fmoc-protection strategies for a modular buildup of oxidized (based on folic acid) and reduced (based on 5-methyltetrahydrofolate (5-MTHF) isomers) folate derivatives comprising an albumin-binding entity and the (radio-)metal chelator DOTA.[1]

Both strategies started with a resin-immobilized lysine linker but differed in the sequence of conjugating the single structural units to the linker. The approach that introduced folate as the last unit appeared particularly useful for the preparation of conjugates containing the less stable 5-MTHF as targeting entities. Each of the three folate conjugates (OxFol-1, 6*R*-RedFol-1 and 6*S*-RedFol-1) was synthesized in 12 steps within one week. In contrast to conventional liquid-phase chemistry methods, the solid-phase-supported strategy allowed the preparation of all three derivatives in high purity (>98%) and moderate yields (7–8%) after a single HPLC purification without the need to purify intermediate products. The conjugates were radiolabeled with lutetium-177 (50 MBq/nmol; >98% radiochemical purity) and the resulting radioconjugates were stable over at least 24 h. Preclinical biodistribution and SPECT/CT imaging studies in FR-positive KB tumor-bearing mice showed promising results for 6*R*-RedFol-1 with the highest tumor uptake (47 ± 4% IA/g, 24 h p.i.) and tumor-to-kidney ratio (2.0 ± 0.4, 24 h p.i.) achieved so far with a folate radioconjugate.[2]

The established synthesis pathway enabled a straightforward preparation of folic acid- and 5-MTHF-based FR-targeting agents and will serve for the synthesis of a broad spectrum of novel folate-based conjugates containing versatile functional entities to further optimize their tissue distribution characteristics.

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An immunomodulatory Peptide Dendrimer Inspired from Glatiramer Acetate

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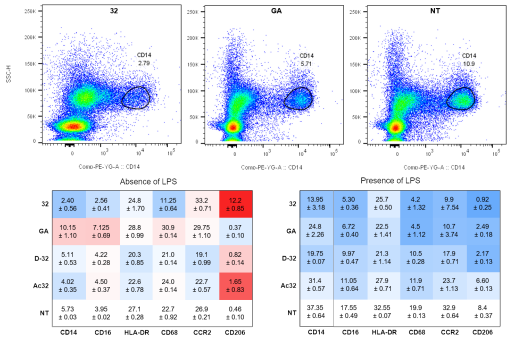
Glatiramer acetate (GA) is a random polypeptide drug used to treat multiple sclerosis (MS), a chronic autoimmune disease[1]. With the aim of identifying a precisely defined alternative to GA, we synthesized a library of peptide dendrimers with an amino acid composition similar to GA, also including stereorandomised sequences[2].



We challenged the dendrimers to induce the differentiation of monocytes towards an M2 (anti-inflammatory) state as GA does, however with a distinct immune marker profile. trigger the release of the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1Ra) from human monocytes, which is one of the effects of GA on immune cells[3,4]. Several of the largest dendrimers tested were as active as GA. Detailed profiling of the best hit showed that this Our peptide dendrimer provides the first example of an immunomodulatory molecule designed as GA analog. Using Flow Cytometry experiments we discovered action of peptide dendrimers on expression of selected surface markers on PBMC and compared it to action of GA.

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Off-target profiling of Janus Kinase (JAK) inhibitors in rheumatoid arthritis: A computer-based approach for drug safety studies and repurposing

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Background: The Janus Kinase inhibitors (JAK) inhibitors tofacitinib and baricitinib are small molecule drugs used to treat inflammatory autoimmune disorders such as rheumatoid arthritis. Safety concerns associated with JAK inhibitors, e.g., the increased risk for thrombosis and viral infections, have emerged.[1-3] The underlying biological mechanisms remain unknown, suggesting potential off-target effects. Computer-assisted techniques, particularly machine learning and molecular docking, can be used to explore potential drug-target interactions and identify potential safety-related concerns. Moreover, off-target profiling opens doors for potential drug repurposing.
Objective: We applied machine-learning based target prediction to identify and experimentally-validate off-targets of baricitinib and tofacitinib, with a focus on targets related to thrombosis and viral infection. Methods: Potential macromolecular targets of tofacitinib and baricitinib were predicted using two machine learning tools [Target Inference GEneratoR (TIGER)[4] and SOM-based Prediction of Drug Equivalence Relationships (SPiDER)[5]]. Selected targets related to thrombosis or viral infection were experimentally evaluated at Eurofins Cerep (France-Celle L'Evescault, www.eurofins.com). Compounds were tested using biochemical (radioligand or enzymatic) or cell-based assays [calcium flux or inhibition of (cAMP)] assays. Dose-response curves were performed for IC_{50}/EC_{50} (half maximal inhibitory/effective concentration) determination and estimation of inhibition constant (K_i). Computational docking was performed to further investigate potential drug-target interactions.
Results: Overall, a total of 98 potential off-target binding effects were suggested (baricitinib n=40; tofacitinib n=58), of which 17 targets had a potential impact on thrombosis (baricitinib n=5; tofacitinib n=5) or viral infection (baricitinib n=4; tofacitinib n=3). Based on the availability of fee-based in vitro testing services, 11 targets were selected for characterization (Adenosine Receptor A2A [AA2AR], Epidermal growth factor receptor (EGFR), inducible NOS, PI3 Kinase (p110b/p85a), Phosphodiesterase 10A2 [PDE10A2], and Protein Kinase N2 [PKN2] for baricitinib; and Adenosine receptor A3, Arachidonate15-Lipoxygenase [15-ALOX], PKN2, Transient receptor potential cation channel subfamily M member 6 [TRPM6] and AA2AR for tofacitinib). The biological activity was confirmed on four targets. In particular, both tofacitinib and baricitinib showed inhibitory activity on PKN2 in the nanomolar range, and micromolar-range inhibition of PDE10A2 and TRPM6 for baricitinib and tofacitinib, respectively. Computational docking on the confirmed receptors suggested residues potentially relevant for the experimentally-observed interactions.
Conclusion: The results suggest the binding promiscuity of baricitinib and tofacitinib. However, the observed off-target inhibition is likely not related to the cardiovascular or viral infection risk. Drug interactions to targets playing an important role in blood coagulation and viral infection, such as AA2AR, 15-ALOX, and EGFR, were ruled out. Overall, the off-target profiling of baricitinib and tofacitinib from this study increases current knowledge on their mechanisms of action, and it opens opportunities for drug repurposing and to design safer JAK inhibitors. Some of the effects of the observed off-target interactions might include attenuation of pulmonary vascular remodeling, anti-fibrotic and anti-psychotic activities (PDE10A2), modulation of viral response (PKN2), and hypomagnesaemia (TRPM6), which is involved in cardiovascular diseases.

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Using chemical biology approaches to decipher chromatin ubiquitylation involving RNF168

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Recognition, integration and propagation of post-translational modifications (PTMs) on histone proteins play a crucial role in the DNA damage response. In particular, the ubiquitylation cascade mediated by the E3 ubiquitin ligase RNF168 is central in promoting either homologous recombination (HR) or nonhomologous end-joining (NHEJ) following DNA double-strand breaks. RNF168 is known to interact with ubiquitylation marks on H1[1] and ubiquitylates H2AK13-15[2], thereby acting as a reader and writer of transient chromatin PTMs. However, the exact mechanisms underlying RNF168 functionality remain elusive, as the limited availability of specifically poly-ubiquitylated H1 restricts mechanistic research on a molecular level.

Here, we develop a chemical approach to poly-ubiquitylate a target protein, H1, with site- and chain-length specificity. Combined with *in vitro* reconstitution approaches, we can tightly control and systematically vary the chromatin fibre modification state. Using such ubiquitylated ‘designer chromatin’, we are now dissecting the interaction and downstream activity of RNF168. This will reveal the chromatin-state-dependent activity of RNF168 in repair pathway choice on a molecular level.

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Functionalized harmonic nanoparticles for cancer theranostics and multimodal imaging.

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This project aims at developing multimodal theranostic tools based on harmonic nanoparticle (HNP) materials. The theranostic paradigm involves merging, in a single agent, specific tumor biomarker targeting, multimodal imaging techniques which allow to overcome the inherent limitations of classical methods, and the controlled delivery of anticancer compounds. This strategy thus aims at high diagnosis sensitivity for earlier tumor detection and the reduction of off-target effects, which are critical factors in patient survival rates. The HNP metal oxide nanomaterials used, characterized by a crystalline structure lacking inversion symmetry (e.g $LiNbO_3$, LNO), exhibit a non-linear optical response by generating second- and third- harmonic signals upon laser excitation. A silica-based coating layer allows for improved biocompatibility of the inorganic HNPs and for the introduction of surface azide moieties, which were exploited for subsequent functionalization through bioorthogonal copper-free click reaction with cyclooctyne-modified ligands, or standard copper-catalyzed click chemistry with alkyne-modified ligands. An erlotinib analogue (ELA) was chosen for therapeutic effect on neoplastic cells overexpressing the Epidermal Growth Factor Receptor (EGFR). In addition, the HNPs were covalently conjugated to a lanthanide (III) chelate, potentially acting as an MRI/X-ray contrast agent or a luminescent probe depending on the selected lanthanide atom, paving the way for multimodal imaging. Light-sensitive drug carriers were produced by the grafting of chemotherapeutics to the surface of HNPs through photosensitive tethers based on coumarinyl moieties. Irradiation of the HNPs with near-infrared (NIR) light allowed switching between imaging and treatment modalities by tuning of the excitation energy. Excitation at high wavelengths (> 1000 nm) is used for multi-harmonic detection, while lower wavelengths (~800 nm) results in the harmonic emission of ultraviolet light, inducing cleavage of the phototrigger and release of the therapeutic cargo. An erlotinib derivative caged to the surface of silica-coated LNO nanoparticles was released by NIR irradiation *in vitro* against EGFR+ DU-145 human androgen independent prostate cancer cells, and showed successful cell growth inhibition.

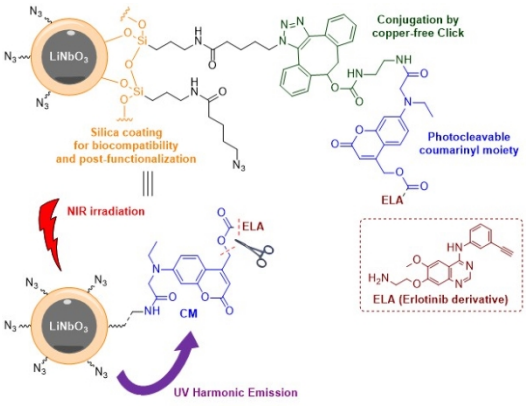

Figure 1: Controlled uncaging of therapeutic compound triggered by the harmonic emission of functionalized LNO HNPs.

Cys-Cys and Cys-Lys stapling of unprotected peptides enabled by hypervalent iodine reagents

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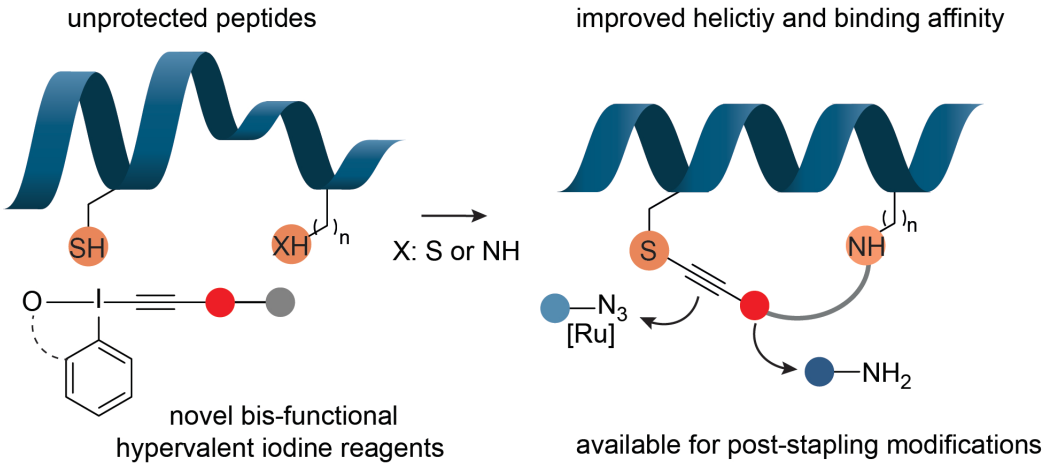
Protein-protein interactions (PPIs) are responsible for regulating many processes in our bodies.[1] Such interactions are often mediated by α-helical segments. These short helical segments can act as a starting point for an inhibitor design, but they lack stability and structure. Peptide stapling - covalent linkage of amino acid side chains, can be used to improve *in vivo* stability, cell-permeability and binding affinity of α-helical peptides.[1] Therefore, a method providing an easy access to a wide range of structurally varied stapled peptides is vital for the development of efficient inhibitors of PPIs.

Hypervalent iodine reagents for selective cysteine alkynylation have been previously introduced by our group.[2] This method has now been extended to cross-linking of peptides.[3] Herein, we will present the recent advances in development and application of novel structurally diverse hypervalent iodine reagents suited for Cys-Cys and Cys-Lys peptide stapling (Figure).This metal free peptide stapling utilises unprotected natural amino acid residues and yields unprecedented thioalkyne containing linkers. Post-stapling modifications can be achieved via amidation or via cycloaddition onto the formed thioalkyne. The ability for our method to provide good inhibitors for PPIs was demonstrated by stapling a peptide derived from p53 protein. Significant increase in helicity and 12 times increase of binding affinity to MDM2 protein was demonstrated.



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Chemical Approach to Stabilise the Structure of Mitochondrial Carrier AGC2 (SLC25A13)

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Aspartate-Glutamate Carrier 2 (AGC2, Citrin, SLC25A13) is a mitochondrial solute carrier that exchanges aspartate and glutamate across the inner mitochondrial membrane. The carrier activity is regulated by extramitochondrial calcium levels. Increased levels activate the exchange and vice versa. The transporter is highly expressed in liver, kidney, and heart tissue, but is also present in various other tissues throughout the body. (Begum, et al.[1]). The x-ray structure of the regulatory domain was solved in 2014 by the group of Edmund Kunji[2]. However, the structure of the carrier domain could not be solved yet. This is due to the highly dynamic nature of this domain and the resulting various conformational states. We pursue two approaches to stabilise particular conformations of the carrier for cryo-EM measurements (I) The first approach is based on introducing mutations that increase the stability of particular carrier states (II) The second approach relies on small molecules that also increase the stability of carrier states when bound. An increase in conformational stability was measured in a thermal shift assay by determining melting temperatures. We report the synthesis of glutamate and aspartate analogues that were incubated with AGC2 and characterised with respect to thermally stabilising AGC2 conformations. The most promising candidates were selected for structural studies by cryo-EM.

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Dual-Activatable Cell Tracker for Controlled and Prolonged Single Cell Labeling

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Dynamic cell behavior like cell migration, cell proliferation, and cell-cell interactions are key to many biological phenomena.[1,2] Fluorescent labeling of cells allows visualization of such processes over time. Fluorescent dyes applied in such experiments need to be non-toxic, compatible with experimental conditions, and should be specific for the cells of interest without leaking to the environment. We recently developed a dual-activatable fluorophore for long-term cell tracking experiments.[3] This molecule can be converted into a fluorescent dye through light irradiation after hydrolysis of the ester group on the fluorescent core by an esterase present in cells.[3] Additionally, upon photoactivation the dye forms a reactive ketene, through which the dye can bind covalently with nucleophilic macromolecules in its environment and therefore fixing the dye within the stained cell. [3] These features allow spatial and temporal control of cell staining. We demonstrated that the dye is not cytotoxic, and it is compatible with harsh fixation and permeabilization protocols. Furthermore, we applied the dye in a cell proliferation assay, *in vivo* homing experiments in mice and tracked a single splenocyte over 20 h on a lymphatic endothelial cell monolayer. Therefore, this dye is a valuable tool for future studies of cellular behavior in cell culture and *in vivo*.

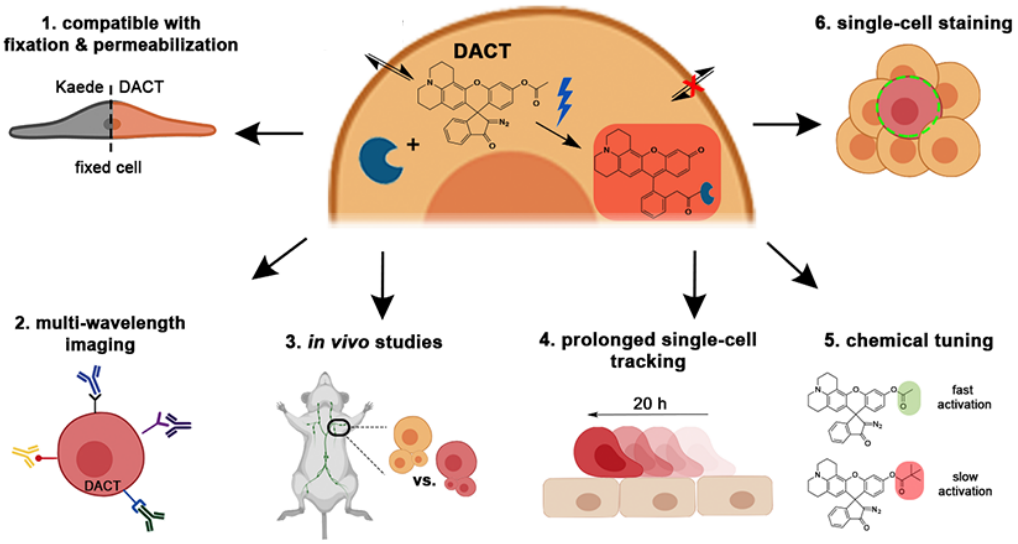

Figure 1. Overview of the working principle and applications of DACTs.

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Synthesis of potential protein-degrading drugs targeting the androgen-receptor in prostate cancer

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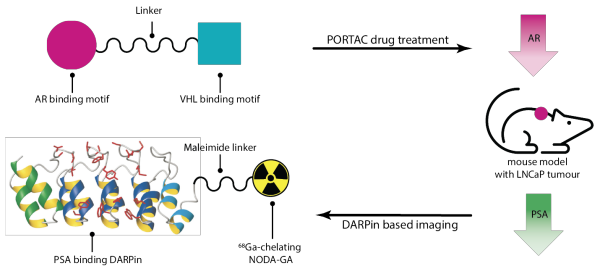
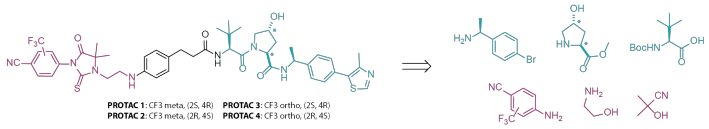
Prostate cancer (PCa) has one of the highest incidences in male cancer patients nationally and worldwide, with increasing mortality over the past 30 years.[1] PCa can progress to an aggressive, hormone refractory and often metastatic disease, castration-resistant prostate cancer (CRPC). One feature of CRPC is a persistently overexpressed androgen receptor (AR) signalling pathway and CRPC patients often develop resistance to first line antiandrogen therapy.[2,3] Our research focuses on addressing this problem in a multidisciplinary way to improve treatment with new protein-degrading drugs (PROTACs) that can also be measuring by using a companion diagnostic approach.



Here, we present the synthesis of a novel AR degrading PROTAC and related diastereomeric control compounds, that bind the androgen-receptor (AR)[4] (pink) and can potentially recruit the *von-Hippel Lindau* (VHL) E3 ligase (blue) pathway.[5] **PROTAC 1** – **4** were synthesised in 5.5%, 17.3%, 7.7%, and 4.5% yield over 7 steps for the longest linear sequence from the commercially available 4-bromophenylethylamine, (2*R*,4*S*)- or (2*S*,4*R*)-hydroxyproline-methyl esters, and *meta*- or *ortho*-CF_3-cyanoanilines. Cell proliferation assays show promising results for PROTAC 1. Experiments show that **PROTAC 1** can inhibit proliferation of LNCaP cells with an IC_{50} of ~8.4 x 10^{-8} M. Switching the position of the CF_3 group from a *meta* to an *ortho* position (**PROTAC 1** vs. **3**) inactivated the PROTAC. The control molecules (inactive epimeric VHL binder, **PROTAC 2** and **4**) were inactive confirming that the VHL recruiting unit is integral for the activity of **PROTAC 1**. Further cellular experiments are planned to probe changes AR signalling by monitoring expression levels of downstream proteins including prostate-specific antigen (PSA) by Western Blot and quantitative proteomics. In parallel, we are working on the development of novel positron emission tomography (PET) imaging agents based on site-specific functionalisation of designed ankyrin repeat proteins (DARPins) labelled with ^{68}Ga to detect changes in free-PSA expression levels as a means of non-invasively monitoring PROTAC treatment.



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A ratiometric sensor for live-cell imaging of dynamic glutathione concentration with subcellular precision

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Accurately measuring glutathione (GSH) levels is of great interest in understanding general redox biology and multiple pathophysiological mechanisms. Over the past decades, several significant advances have been made towards observing GSH dynamics in live cells using genetically encoded and small-molecule sensors.[1,2] Nevertheless, a general tool to assess dynamic changes in absolute GSH concentration featuring flexibility in subcellular targeting or spectral range is still lacking. To tackle this problem, we developed a hybrid sensor that unites some of the advantages of endogenous and small-molecule sensors. We combined a previously reported GSH-sensitive silicon rhodamine (SiR) core[2] with a covalently bound, targetable fluorescent protein reference (Figure 1b). The small-molecule sensor bears a chloroalkane linker that can react with an endogenously expressed HaloTag fluorescent fusion protein of spectrally distinct wavelength. Once linked together, the two parts of the sensor allow for a robust ratiometric read-out of GSH dynamics in live-cell imaging.

To test our design, we synthesized compound **1** and were able to show the response of the fluorescence signal from its HaloTag adduct to GSH. We further confirmed the accessibility of the sensor for GSH in the HaloTag-SiR conjugate by X-ray crystallography (Figure 1c). Improved compatibility with live-cell imaging was achieved with derivatives **2** and **3**, in which fluorescence is only turned on upon binding to the protein.[3] However, we were surprised to find that only compound **2** exhibits a measurable response to changes in GSH concentration. Validation of these results in live cells and additional X-ray crystallography will shed light on the mechanism behind these findings. We want to show that our sensor can detect changes in the cellular redox environment upon treatment and differences in GSH levels among subcellular compartments in further experiments.

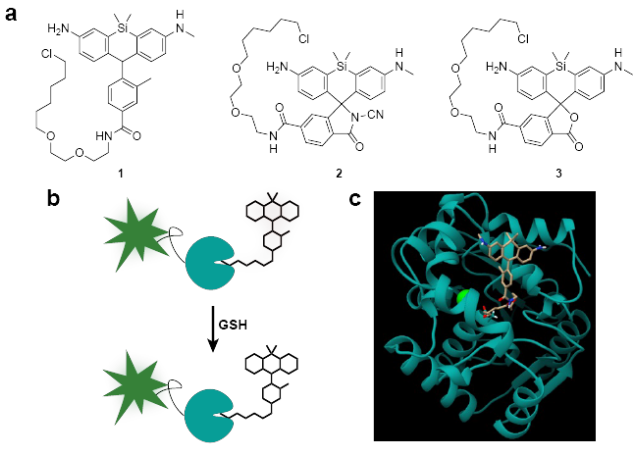

Figure 1: Structures of the tested GSH-sensitive SiRs (a), working principle of the ratiometric hybrid GSH sensor (b), and crystal structure of the HaloTag conjugate with **1** (c).

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Systematic Tuning of Rhodamine Spirocyclization for Super-Resolution Microscopy

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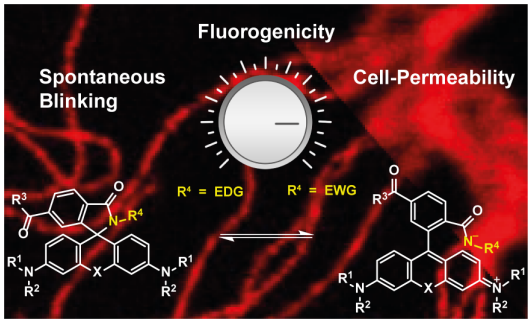
Rhodamines are the most important class of fluorophores for applications in live-cell fluorescence microscopy. This is mainly because rhodamines exist in a dynamic equilibrium between a fluorescent zwitterion and a non-fluorescent but cell-permeable spirocyclic form. Different imaging applications require different positions of this dynamic equilibrium, which poses a challenge for the design of suitable probes. We describe here how the conversion of the ortho-carboxy moiety of a given rhodamine into substituted acyl benzenesulfonamides and alkylamides permits the systematic tuning of the equilibrium of spirocyclization with unprecedented accuracy and over a large range. This allows to transform the same rhodamine into either a highly fluorogenic and cell-permeable probe for live-cell stimulated emission depletion (STED) microscopy, or into a spontaneously blinking dye for single molecule localization microscopy (SMLM). We used this approach to generate differently colored probes optimized for different labeling systems and imaging applications. [1]



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Rhenopaganib – an Organometallic Mimic of the Small Molecule Kinase Inhibitor Opaganib®

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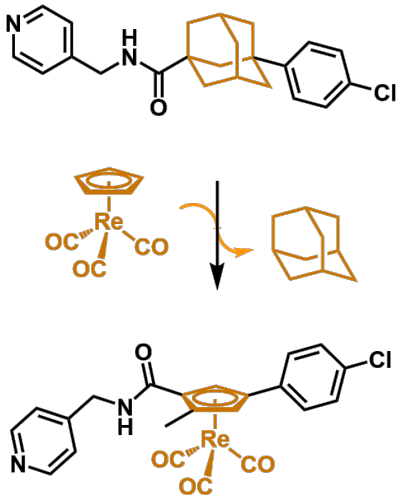
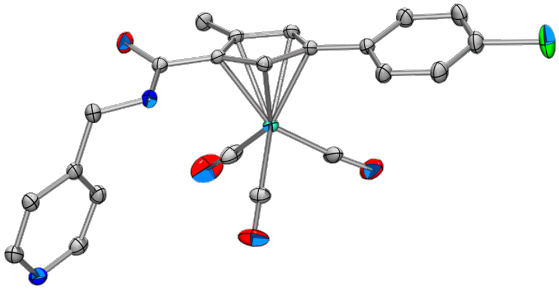
Incorporating transition metals into pharmaceutical lead structures is an area of great interest in medicinal chemistry. The metal might not only help in overcoming drug-resistance, but also introduces new properties such as 3D shape, redox behaviour, or even radioactivity to known pharmaceuticals.

We herein report the synthesis of a rhenium containing mimic of the small molecule kinase inhibitor (SMKI) Opaganib® where the purely organic adamantly function was replaced by the organometallic [CpRe(CO)$_3$] moiety. Since both functions are similar in their lipophilicity and have both a 3-dimensional structure, it is reasonable to assume that the biological activity should not be affected too strongly by the exchange. Biological assessment of the compound is currently undergoing.





A single-molecule multiplexed approach to study dCas9 nucleosomes invasion

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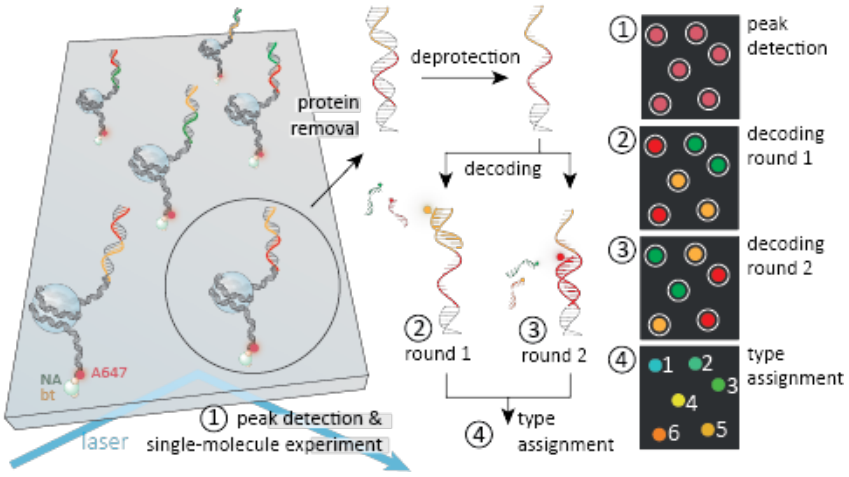
For genetic engineering of eukaryotic cells, the Cas9 nuclease must target chromatinized DNA. Similarly, the use of catalytically dead Cas9 (dCas9) for targeting genomic loci to modulate gene function relies on efficient chromatin binding. The position of Cas9 target sites within nucleosomes (in particular the PAM sequence), the presence of histone modifications as well as sgRNA specificity are important factors for Cas9 recognition and binding. However, we are lacking a systematic understanding of the mechanisms governing Cas9/dCas9 chromatin invasion. Single-molecule measurements provide detailed mechanistic insight into intricate molecular processes, and are thus suitable to reveal how Cas9 dynamically engages nucleosomes. However, single-molecule experiments, in particular of complex chromatin samples, are difficult to perform and the reproducible and quantitative determination of parameters can be challenging.

We addressed this challenge and developed a method to multiplex dynamic single-molecule observations, where the interactions of dCas9 to many different types of nucleosomes are observed simultaneously in one single-molecule experiment. We provide each nucleosome type with an identifying DNA sequence, called “barcode”, within its nucleosomal DNA. Parallel experiments are subsequently spatially decoded, via the detection of specific binding of dye-labeled DNA probes. Using the established multiplexing system, we reveal that the time required for stable dCas9 binding is greatly increased for target sequences located further within nucleosomes. Moreover, nucleosomes suppress non-specific binding by shortening the residence time of dCas9 for sgRNAs containing mis-matches. Together, these results provide mechanistic information on the role of chromatin in directing Cas9 activity in genome targeting and editing applications.



Amino Acid Influence on Ag^+ Binding Site in Model Peptides Inspired by the Silver Resistance Protein SilE

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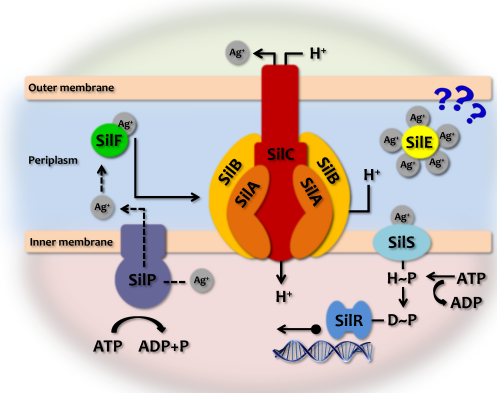
The silver cation Ag^+ and its compounds have been known for their antibacterial properties. [1] However, an increasing number of reports have highlighted the emergence of silver resistant bacterial strains isolated from burn care centers or silver contaminated media. The resistance is provided by the silver efflux pump, which contains eight proteins that act together to export silver ions. Among them, the SilE protein is the only one of which the role is still unknown, acting as a sponge for silver. [2]



Model peptides were studied to identify Ag^+-binding sites of the bacterial silver resistance protein SilE. [3] The silver ions are binding in a linear coordination mode on histidine (His, H) and methionine (Met, M) residues. Following this study, the amino acids (aa) present between these two residues could influence the coordination of the His and Met residues with Ag^+. Thus replacing one aa by an other one could increase or decrease the stability of the Ag^+-HXXM complexes (with X random aa). Furthermore, HXXH and MXXM peptides Ag^+ bindings were investigated to compare with their HXXM homologous. The goal of this study is to understand the contributions of different aa on the coordination of the His and Met residues with Ag^+.

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Development of a New Synthetic Route for the Convenient Synthesis of Benzoxazinoid Compounds

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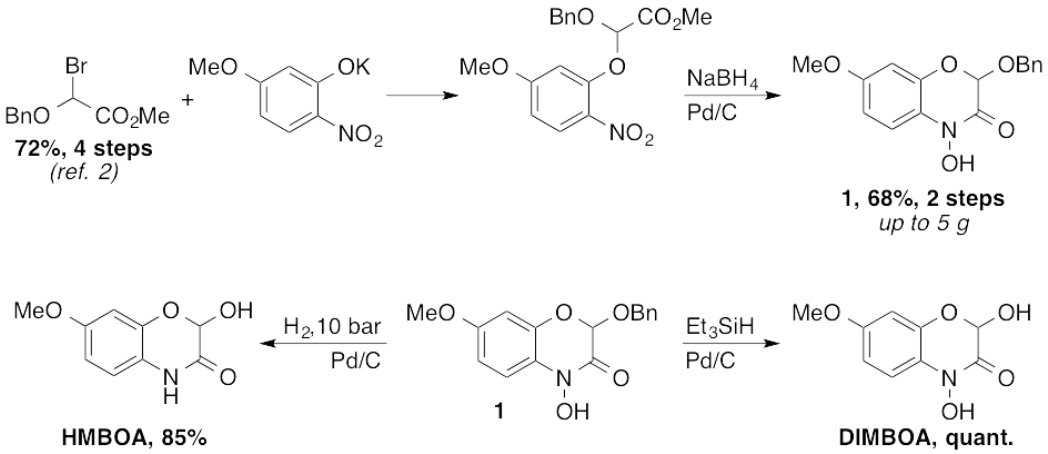
Benzoxazinoids belong to a large family of secondary metabolites that are present in crops such as wheat and maize. The study of these bioactive compounds is particularly relevant in chemical ecology due to their role as mediators of the interactions between plants and their environment. In this context, the IRC One Health investigates more specifically the benzoxazinoid-microbiome relationship along the food chain, at the interface between soil, cereals, cattle and mice (as a model for human beings). Nevertheless, the difficulties to obtain benzoxazinoids in a pure form and in sufficient quantities has been limiting their use for *in-vivo* experiments. Indeed, the different syntheses reported so far in the literature involve tedious reaction conditions[1] that lead to poor yield or purity in our hands. To solve this issue, we aimed at establishing a more convenient and robust access to the benzoxazinoid skeleton. We addressed this challenge by developing a reliable synthesis of the new common intermediate **1**, on a multi-gram scale. By simply changing the conditions for the benzyl deprotection, the two major benzoxazinoids DIMBOA and HMBOA could thus be obtained in excellent yield and purity from the same precursor. Noteworthy, a deuterated version of **1** could also be prepared via the same route.



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Development of stapled-peptide-based degraders of oncogenic chromatin factors

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Chromatin proteins, such as histones, are important factors that regulate gene expression in eukaryotic cells. Deregulation of this process leads to abnormal transcription or genetic instability with disastrous effect that lead to cancer formation. Recent studies on CENP-A, the centromeric variant of the canonical histone H3, revealed its overexpression and mislocation in cancer cells [1][2]. Recently, the protein DAXX was found to act as a chaperone for CENP-A, responsible for its mislocalization [3]. Moreover, DAXX itself is an oncogenic factor, as DAXX knockdown results in a decrease in tumor growth [4][5][6].

We thus hypothesize that DAXX could be an interesting target to the development of peptide-based degraders. Previous structural studies identified a single alpha-helix that maintains the interaction of DAXX with a transcriptional regulator, ATRX [7]. We thus base our peptide design on this interaction. We synthesized alpha-helical peptide, which were further stabilized with a newly developed staple based on Hypervalent iodine chemistry [8], in order to enhance its binding affinity, cell permeability and stability. The peptide is stapled between a Cys and a Lys residue separated by one turn (i+4) or two alpha-helix turns (1+7). Since our peptides design contains multiple lysine, we developed protocols to ensure stapling specificity. Finally, the staple moiety carries an alkyne group that can be further functionalized via click-chemistry in order to modify the peptide with a dye, allowing the characterization of its biophysical proprieties, or an E3 recruiting moiety for the development of a degrader.

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Shaping the centromere – The role of CENP-B in modifying centromere chromatin

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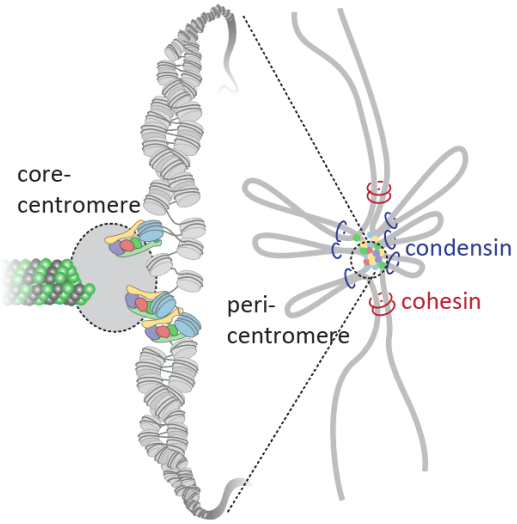
The centromere is an essential region in a chromosome responsible for its proper segregation to the daughter cells during mitosis. It acts as the point of recruitment of the kinetochore and attachment to the spindle microtubules. The centromere is marked epigenetically by the presence of a histone H3 variant know as CENP-A, which is selectively deposited on centromeric DNA repeats. A major structural consideration for CENP-A nucleosomes is that they need to be exposed to the surface of the chromosomes for efficient recruitment of the kinetochore. Thus, the CENP-A containing nucleosomes, together with CENP-B, CENP-C and CENP-N are hypothesized to form non-canonical and potentially highly dynamic chromatin structures.

The goal of our study is to determine these unique dynamics and structures of centromeric chromatin. To address this question, we combine recombinant and synthetic, fluorescently labelled DNA to form designer CENP-A containing chromatin fibers. We then employ single molecule Förster resonance energy transfer (smFRET) to directly detect the structure and dynamics of centromeric chromatin. We further probe how the centromere binding protein CENP-B contribute to defining the centromere chromatin structure. Overall, these studies are designed to reveal the unique organization of the centromeric chromatin.



A robust ratiometric HaloTag reporter system for gene expression monitoring

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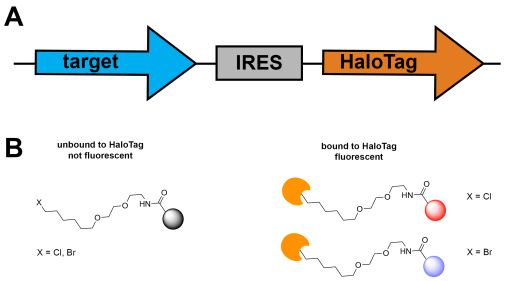
[1]EPFL, LOCBP, Lausanne, [2]ETHZ Zurich

Regulation of gene expression plays a key role in stress-response pathways. Common methods to study differential gene expression are western blots and reporter assays using luminescence or fluorescence.[1] However, western blotting is only suitable for bulk measurements and reporter assays often require exogenous substrates, cofactors or cell lysis. There is a need for simple techniques allowing dynamic measurement of gene expression in live cells that can be measured at the single cell level as well as in high-throughput screening. Here we report a robust and modular ratiometric fluorescence reporter system for gene expression monitoring in live mammalian cells. The expression of the self-labeling HaloTag protein[2] is correlated to that of the target gene through co-expression of both genes. The IRES RNA element is employed to avoid the use of fusion proteins that can impair the protein's function. Two fluorogenic HaloTag ligands with different spectral properties and binding kinetics are used to label the HaloTag protein and result in a ratiometric fluorescence output. We envisioned that through adjustment of the concentrations of the two fluorogenic ligands, the system can be tuned to reflect differential expression with a large dynamic range. As proof-of-principle, we chose to monitor the expression of the chaperone BiP upon induction of the unfolded protein response (UPR) of the endoplasmic reticulum (ER).[3] CRISPR-Cas9 was used to edit mammalian cells for co-expression of BiP and HaloTag with the CRIS-PITCh system for cassette knock-in based on microhomologies.[4] This system is highly modular with easy modification of microhomology regions to change the target gene. Moreover, the fluorogenic HaloTag ligands spectral properties can be tuned to suit specific multicolor imaging requirements.



Scheme. A. Expression of HaloTag is linked to that of target gene through the IRES RNA element. **B.** HaloTag fluorogenic ligands are non-fluorescent unless they are bound to the HaloTag.

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Towards PROTAC-mediated degradation of CBP/EP300

L. Palaferri[1], I. Cheng-Sánchez[1], K. Rollins[1,2], M. Kirillova[1], E. Laul[1], A. Müller[2], L. Wiedmer[2], A. Caflisch[2]*, C. Nevado[1]*

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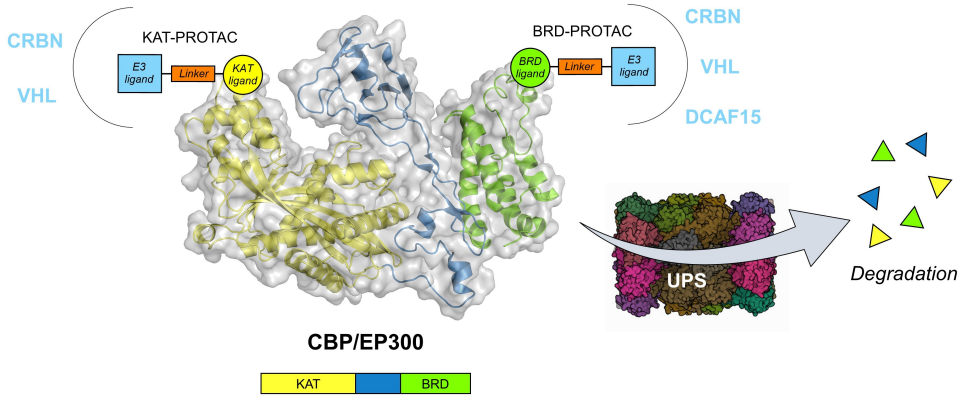
CREB Binding Protein (CBP) and EP300 are two proteins with a high sequence homology that participate in chromatin remodelling and act as co-activators of several transcription factors.[1] Their lysine acetyl transferase domain (KAT) and bromodomain (BRD), which respectively acetylate and bind acetylated lysine residues on histone tails and other proteins, are crucial for these functions.[1] CBP/EP300 are often overexpressed, mutated or dysregulated in cancer, making them extremely interesting targets for medicinal chemistry.[1]

After developing CBP/EP300 bromodomain-binding molecules with sub-micromolar potency and promising bioavailability,[2] we now aim to elucidate the contribution of CBP/EP300 to gene regulation by inducing their degradation through PROteolysis TArgeting Chimeras (PROTACs). PROTACs consist of a binder for a protein of interest (POI) connected to an E3 ligase ligand through a linker. With a catalytic mechanism, PROTACs promote the formation of a ternary complex with the POI and the E3 ligase leading to polyubiquitination of the POI and consequent proteasome-mediated degradation. In such way, PROTACs enable loss of function of the whole protein independently from the bound domain.[3]

Starting from the analysis of X-ray crystal structures of our in-house[2] and other published probes[4] in complex with CBP/EP300, we have designed and synthesised a series of bifunctional compounds with the ability to simultaneously bind either the BRD or KAT domain and a E3 ligase. Empirical exploration of several linkers and E3 ligands allowed optimisation of the probes' potency, cell permeability and target engagement. Binding affinity and ternary complex formation have been measured with biochemical assays while cellular target engagement, degradation and phenotypical effects were evaluated in multiple cell systems. In conclusion, we developed a library of compounds with some valid candidates to enable CBP/EP300 degradation as a powerful therapeutic alternative to classical protein inhibition.



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X-ray crystal structures of short mixed chirality α-helical antimicrobial peptides

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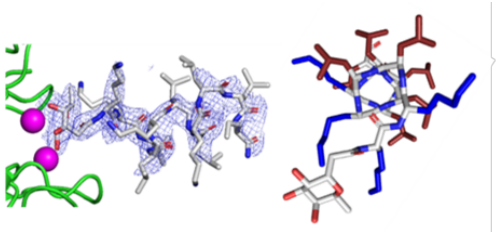
Peptide α-helicity mostly depends on its amino acid sequence and is right or left-handed depending on amino acids chirality (respectively L- or D-). However, mixed-chirality sequences are usually unfolded. In case of antimicrobial peptides (AMPs), an amphiphilic α-helix is generally required to be active and research on mixed chirality AMPs is poorly documented. We recently reported the first X-ray crystal structures of mixed chirality short bicyclic and linear AMPs as complexes of fucosylated analogs with the bacterial lectin LecB.[1] Our data suggest that this secondary structure is conserved both in a membrane environment and as helix bundle determined by X-ray crystallography in water. Furthermore, mixed chirality linear peptide displays lower hemolytic activity and better stability in human serum while keeping its antimicrobial behavior compare to its homochiral parent.



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Structure-activity relationship study to find novel potent and selective adenosine A_1 receptor agonists

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Adenosine receptors (ARs) belong to the GPCRs family and play an important role in both the central nervous system (CNS) and in the peripheral nervous system (PNS). They are linked to cancer, cardiovascular, neurological, respiratory and inflammatory disorders. ARs consists of four subtypes, namely A_1, A_{2A}, A_{2B}, and A_3, which couple to different Gα subunits and therefore activate different downstream signaling pathways. Depending on the activated signaling cascade, unwanted side effects can occur, making these receptors difficult drug targets. With GPCRs remaining one of the most important target class in medicinal chemistry, it is crucial to find molecules that activate only particular intracellular signaling pathways.

Last year we have shown that A_1R agonist BnOCPA has analgesic properties but no adverse effects such as bradycardia, hypotension and respiratory depression[1]. This long sought agonist behavior was achieved because BnOCPA is able to stabilize a A_1R conformation that couples to one particular Gα subunit in a highly efficient and selective manner. We decided to embark on a new structure-activity relationship study based on the BnOCPA scaffold (figure 1) in order to better understand how agonist structure is linked to Gα-signaling bias.

Here we show the synthesis and the characterization of novel A_1 receptor agonists. In order to achieve the desired target compounds, we had to optimize some steps of our original synthetic route. In particular, efforts were made on two critical reactions: a difficult *O*-alkylation and an unconventional Mitsunobu reaction to introduce phenoxy substituents. Due to the robustness and high reproducibility of the chemistry we succeeded in synthesizing a new library of novel A_1R agonists. Pharmacological studies revealed that some of the newly obtained compounds have a higher potency than BnOCPA without losing their selectivity for A_1R. These novel tool compounds will be used to understand GPCR-mediated signaling pathways both *in vitro* and in *in vivo*.

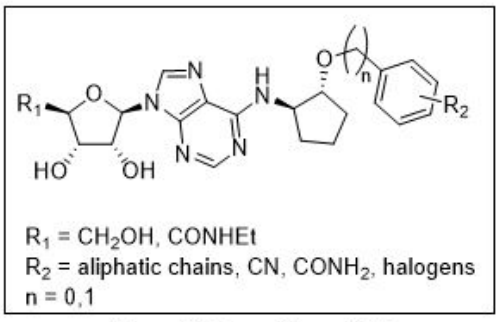

***Figure 1** A_1R agonists scaffold*

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Targeting RNA conformational ensemble of *LMNA* gene via Small Molecules against Hutchinson-Gilford Progeria Syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is a very rare, sporadic, autosomal dominant disorder resulting in early death. HGPS is caused by a point mutation in exon-11 of the *LMNA* gene [1]. This mutation promotes recognition of a cryptic alternative splicing site in exon-11 and reduces the recognition of the authentic splicing site resulting in the production of progerin, a truncated toxic protein leading to an aberrant nuclear morphology, genetic instability, and premature senescence [2, 3]. Progerin accumulates in the cell nuclei of HGPS patients and its accumulation is the biomarker of the disease. Symptomatic treatments of HGPS are available but no disease modifying treatment is existing [2, 4].

In this work, to address the unmet medical need of HGPS, we aim at understanding the relationship between the conformational plasticity of pre-mRNA structure of exon-11 carrying the mutation around its cryptic splice site and the splicing behavior to validate this structural element as a target for small molecules that would abolish the production of progerin.

Different point mutations in exon-11 of *LMNA* gene and 2-Aminopurine tagged RNA hairpins have been designed to assess hairpin conformational plasticity. Various small molecules have been filtered and experimentally tested to appraise their binding and splicing efficiency to *LMNA* mRNA. Two different binding assays were utilized to determine small molecules as suitable hits. Hits were tested according to their RNA-binding ability and their splicing modifying characteristics in patient-derived cells. Results give first insights into the relationship between hairpin conformational plasticity and splicing modulation and suggest ideas for the development of small molecules reducing progerin pre-mRNA, which may open new avenue for other splicing-linked diseases after the first successful example of modifying TSL2 conformation to modulate the splicing of *SMN2* in SMA [5].

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Total Synthesis of FR252921 and Analogues: New Era in Immunosuppression?

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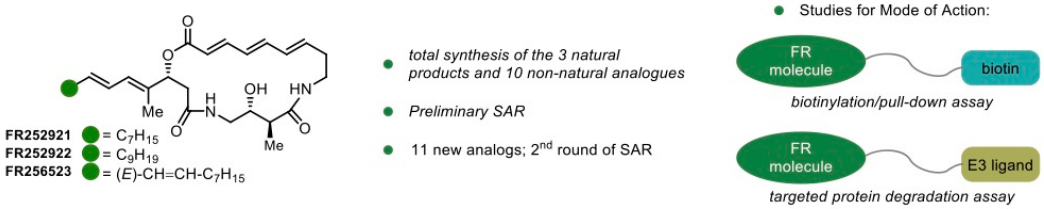
Current immunosuppressive therapy relies on state-of-the-art medications that are fraught with side effects for patients. We propose herein the development of potentially novel immunosuppressive agents, inspired by the natural products FR252921, FR252922 and FR256523.[1-3] The isolation report for these secondary metabolites already suggested in 2003 that they derive their immunosuppressive activity from a *novel Mode of Action*. We present the total synthesis of these macrocycles as well as a series of 10 non-natural analogues which has allowed for a preliminary structure-activity relationship (SAR) and quick design of a nanomolar-potency derivative.[4]



Herein, multiple further modifications are proposed that shall expand the SAR, allowing thorough structural evaluation towards achieving highest potency. Furthermore, intensive studies are undertaken to shed light in the Mode of Action of the FR252921 family. We expect the development of highly active compounds that shall usher in a new era in immunosuppressive therapy, replacing the current state-of-the-art medications and the high risks they present to patients.

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Glutathione-Inspired Octapeptides as Chelating Agents for Lead

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[1]University of Zurich

Lead (Pb) is an abundant toxic metal, posing a public health problem worldwide. More than a million deaths annually are attributed to Pb poisoning,[1] and every third child is considered to be poisoned,[2] which is a troublesome circumstance since they are the most vulnerable for suffering long-term damages from Pb exposure. The current treatment modality consists of chelation therapy. Clinically used chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and dimercaptosuccinic acid (DMSA), suffer various drawbacks, such as lacking selectivity and inherent toxicity, which limit their efficacy.[3] In this project, we aimed to develop a rationally designed, cyclic peptide library for selective metal detoxification. To achieve this, an octameric scaffold inspired by glutathione (GSH) was explored by selectively varying individual structural parameters. Subsequently, members of this library were assessed for their capability of detoxifying Pb in an *in vitro* assay using human cells. The design principles of this library were proved effective, as the peptides show greater efficacy for detoxifying Pb than the standard of care. In addition, specific criteria were identified that increase potency of peptides.

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Discovery of new inhibitors of human divalent metal transporter hDMT and hZIP8 by fragment-based lead discovery from GDB17 database

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The iron transporter hDMT and the zinc transporter hZIP8 are divalent metal transporters with few or no known modulators precluding their deeper investigation. A variety of diseases have been associated with both transporters and their disfunction (e.g. hemochromatosis, neurodegenerative disorders, obesity, Crohn's disease)[1-2]. Despite the scarce availability of information, these proteins are known to possess suitable pockets for small molecule inhibition[3]. To identify inhibitors of these two targets, we selected a diverse set of fragment-like molecules form the generated database GDB-17, which consist of all molecules up to a certain size following simple rules of chemical stability and feasibility[4-6]. The cell-based assays of this library resulted in the identification of two weak inhibitors of hDMT and the first described inhibitor of hZIP8.

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Modelling the active site of New Delhi Metallo-β-lactamase-1

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[1]Department of Chemistry, University of Fribourg

The bacterial metallo-β-lactamases induce antibiotic resistance by participating in the hydrolysis of a whole class of antibiotics, the β-lactams (except monobactams), which are currently used worldwide.[1] As illustrated below, an example of such an active site of these enzymes can comprise two zinc ions which are mainly coordinated by histidine, cysteine and aspartate, with a water molecule bridging the two zinc ions.

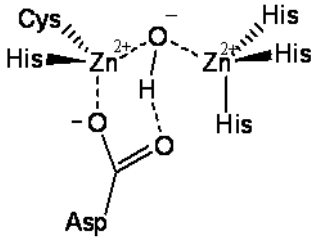

Figure 1: *The active site of the metallo-β-lactamase NDM-1 [2].*

One possible way to inactivate these metallo-proteins could be the use of chelating drugs to remove the zinc ions and to subsequently substitute the zinc ions by other metal ions such as bismuth and gold.[2]

In order to study the active site in more details and to be able to predict metal ion replacement, we decided to model the active site of NDM-1 by synthesizing a peptide which should allow to imitate the zinc binding situation in the original enzyme and to clarify the affinity of the active site for different metal ions. Several techniques were then used to investigate step by step the binding affinities for zinc and other metal ions.

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Development of Positron-Emission Tomography and Fluorescent Tracers for the Imaging of Calcium Sensing Receptors (CaSR) in Parathyroid Glands

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Primary hyperparathyroidism (HPT) is caused by the pathological growth of parathyroid glands [1] which affects kidneys, digestive system, bones, muscles and brain [2]. Surgical removal of the affected glands is the treatment of choice and a pre-surgical localization of the affected gland is necessary [3]. The development of a selective tracer for the parathyroid gland would reduce the likelihood of false negative results compared to existing localization-tools [4]. The calcium sensing receptor (CaSR) is highly expressed on the surface of the parathyroid glands and could be used as a target for ^{18}F-positron emission tomography (^{18}F-PET). Previously, highly potent CaSR inhibitors have been developed for the treatment of osteoporosis [6]. We have used these structures to develop PET-tracers. The challenge is to preserve the high CaSR activity of these compounds when synthetically altering their structures. We synthesized several non-radioactive ^{19}F-fluorinated CaSR inhibitor analogues and have determined activities in the low nanomolar range. We have also synthesized CaSR inhibitors with appended small fluorescent dyes. However, all compounds showed strong unspecific binding. Therefore, the synthesis of more polar structures was initiated to decrease the logP value. The most promising compounds will be 18F-labeled and evaluated as PET tracers in live animals.

Development of a single-molecule approach to observe ubiquitination dynamics in defined chromatin states

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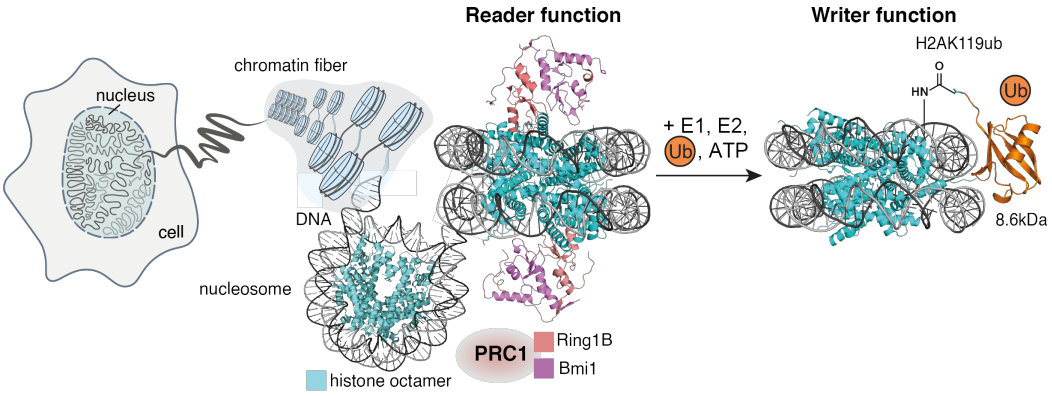
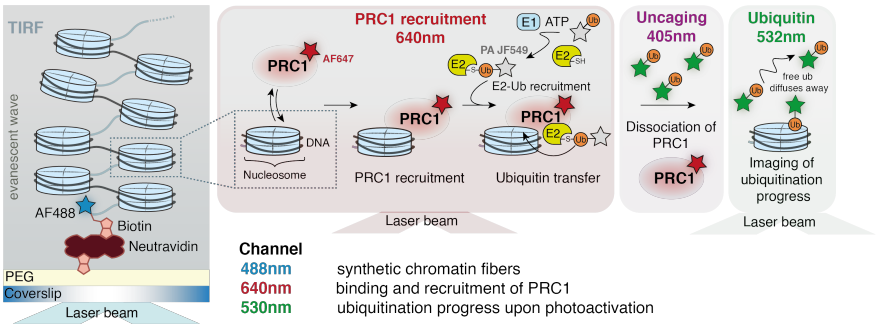
Dynamic regulation of transcription is orchestrated by a large cohort of enzymes, among which chromatin modifiers or 'writers' install histone post-translational modifications (PTMs) controlling the recruitment of chromatin 'readers'. A specific subset of chromatin modifiers includes Polycomb group (PcG) proteins, which drive the inheritance of a repressed chromatin state during development and cell differentiation by preventing abnormal oncogenic transformations.

PcG members Polycomb Repressive Complex (PRC) 1 in its canonical (cPRC1) or variant (vPRC1) form function as H2A-specific E3 ligases and drive gene repression. vPRC1 is involved in the majority of ubiquitin deposition in the context of repressed chromatin, without the requirement of primary installation of additional PTMs.



We hypothesize that chromatin modification, i.e. ubiquitination, by vPRC1 is controlled by its recruitment dynamics on underlying chromatin and furthermore its subunit composition. Here, we present a single-molecule approach, which allows to directly measure vPRC1 binding dynamics at defined chromatin states. In the following, this will allow us to identify protein-protein interactions (PPIs) that are key for enzymatic activity and reveal chromatin states that are specifically targeted. Moreover, we are extending this approach to directly observe vPRC1 dependent H2A ubiquitination in real time on the single-molecule scale. This will allow us to gain a mechanistic view of 'reading' and 'writing' by vPRC1 in real time and to elucidate its contribution to gene regulation.



Designing of new anticancer peptides using a Genetic Algorithm and Machine Learning

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[1]University of Bern, Department of Chemistry, Biochemistry and Pharmaceutical Sciences, Freiestrasse 3, Bern

Anticancer peptides (ACPs) represent small peptides possessing selective and toxic properties toward cancer cells. Designed ACPs can improve affinity, selectivity and stability for enhancing anticancer properties. Therefore, development of simple and efficient predictor for designing new ACPs with promising clinical applications is of great interest. Recently in our group was reported peptide design genetic algorithm (PDGA) – a computational tool capable of producing peptide sequences in proximity of any molecule of interest in a chemical space.[1] Based on one compound of interest such as Lasioglossin III,[2] a linear peptide with potent antimicrobial and anticancer activities, PDGA was used to generate new set of peptides with specified parameters, to which the Bioactivity and Hemolysis recurrent neural network (RNN) classifiers were then applied in order to predict active compounds.[3] After further filtration and clustering a final library contained 14 compounds was extracted. Selected peptides were synthesized and tested for their activity on HeLa, MCF-7, MDA-MB-231 cancer cell lines and hemolyticity against human blood red cells. Three of these peptides were active against cancer cells. And one «Hit» compound out of the library showed similar activity as Lasioglossin III against chosen cell lines without affecting human erythrocytes with at least tenfold selectivity. Preliminary mechanistic studies shed some light on apoptotic over necrotic mode of action of «Hit» compound.

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Synthesis of new building blocks from the chemical universe database GDB

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Drug discovery is in constant need of new molecules to develop drugs addressing unmet medical needs. To assess the chemical space available for drug design our group developed tools to enumerate, visualize and search chemical space. The Generated DataBases (GDBs) list billions of possible organic small molecules following simple rules of chemical stability and synthetic accessibility. [1a-1d] These databases are treasure troves of new molecules because the vast majority of GDB molecules (>99%) is unknown. Much of the originality and novelty of GDB molecules lies in new combinations of rings including 3D-shaped saturated ring systems, often containing chiral and quarternary centers. Molecules showcasing these properties have recently been recognized as desirable building blocks for drug candidates. [2] This has been demonstrated in our group by the synthesis of Triquinazine, a selective inhibitor of Janus Kinase I related to the marketed drug Tofacitinib. [3]

To identify interesting ring systems for drug design we analyzed the GDB4c database containing 916’130 possible ring systems. We focused our attention on novel aliphatic bicyclic diamines which represent interesting building blocks for medicinal chemistry. Here we present versatile synthetic strategies to access libraries of fused and spirocyclic diamines starting from simple commercially available building blocks.

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