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A non-stereogenic imidazolidinyl proline derivative as a pH-sensitive and functionalizable probe for tuning collagen triple helix stability

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Numerous compounds have been developed to study the conformational properties of proline-containing peptides and proteins as well as for peptide functionalization [1]. Most proline derivatives possess substituents at the C4-position, similar to the naturally occurring hydroxy group of proline [2,3]. Notably, 4S/4R aminoprolines produce a number of different conformational effects, but also provide sites for functionalization and pH response of peptides [4-7]. We recently developed a minimal 4-azaproline analogue based on a non-stereogenic pseudoproline-scaffold which can be prepared in a single synthetic step, named Imp. The conformational properties of this proline analogue were investigated through NMR spectroscopic studies of model compounds and DFT calculations, and confirm that both Imp and functionalized Imp effectively mimic proline and other proline-based residues. Incorporation of Imp into a collagen model peptide (CMP) produces pH responsive CMPs and CMPs that can be functionalized through the Imp side-chain with sterically bulky groups. Thermal denaturation studies of the CMPs corroborate computational insight and suggest that Imp possesses a dynamic nature in the context of a peptide where the lack of a stereogenic center at the 4-position of the ring can alleviate steric constraints of bulky substituents that would otherwise be created by a mismatched 4S/4R proline stereoisomer. We envision that Imp will provide a useful alternative for the synthesis of functionalized peptides, particularly in examples where a dynamic residue can benefit the overall stability.

[1] Mauger, A. B.; Witkop, B., *Chem. Rev.* **1966,** *66*, 47-86.
[2] Remuzon, P., *Tetrahedron* **1996,** *52*, 13803-13835.
[3] Dobitz, S.; Aronoff, M. R.; Wennemers, H., *Acc. Chem. Res.* **2017,** *50*, 2420-2428.
[4] Erdmann, R. S.; Wennemers, H., *Angew. Chem. Int. Ed* **2011,** *50*, 6835-6838.
[5] Erdmann, R. S.; Wennemers, H., *J. Am. Chem. Soc.* **2012,** *134*, 17117-17124.
[6] Siebler, C.; Erdmann, R. S.; Wennemers, H., *Angew. Chem. Int. Ed.* **2014,** *53*, 10340-10344.
[7] Egli, J.; Siebler, C.; Maryasin, B.; Erdmann, R. S.; Bergande, C.; Ochsenfeld, C.; Wennemers, H., *Chem. Eur. J.* **2017,** *23*, 7938-7944.

Single molecule TIRF microscopy unveils prolonged residency time of PRC2 on chromatin through DNA binding mediated by PHF1

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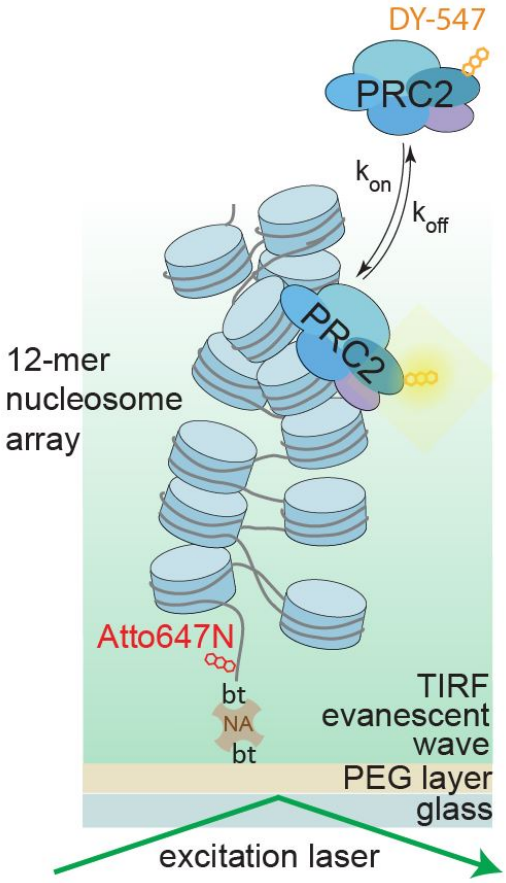
Polycomb repressive complex 2 (PRC2) trimethylates histone H3 at lysine 27 to mark genes for repression. We measured the dynamics of PRC2 binding on recombinant chromatin and free DNA at the single-molecule level using total internal reflection fluorescence (TIRF) microscopy. PRC2 preferentially binds free DNA with multisecond residence time and midnanomolar affinity. PHF1, a PRC2 accessory protein of the Polycomblike family, extends PRC2 residency time on DNA and chromatin. We further unveil that a winged-helix domain on PHF1 is responsible for the prolonged residency time of PHF1-PRC2, which renders it a more efficient H3K27 methyltransferase than PRC2 alone [1].



[1] Jeongyoon Choi, Andreas L. Bachmann, Nat Struct Mol Biol, 2017, 24, 1039-1047.

Click-code-seq: genome-wide nucleotide resolution mapping of 8-oxoG

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8-oxo-guanine (8-oxoG) is one of the most abundant DNA base lesions. It induces G to T transversions, which can be a resource of cancer mutagenesis. It is also linked to other oxidative stress-related diseases, such as atherosclerosis and diabetes, and to ageing. Moreover, there are indications that 8-oxoG can be a potential epigenetic mark regulating inflammation and immune responses. To better understand the links between 8-oxoG and its effects, a genome-wide scale mapping of 8-oxoG at single nucleotide resolution is highly demanded. Despite advances in high-throughput sequencing, this is not possible yet. However, by utilizing the specific recognition of 8-oxoG by the base-excision enzyme formamidopyrimidine-DNA glycosylase, we were able to target and excise oxidatively damaged guanine and replace it with a clickable nucleotide. Binding a coding sequence to this site via a click reaction forms an altered but biocompatible linkage with which it is possible to sequence the genome and map the sites of 8-oxoG. Preliminary results in yeast and human genomes revealed distinct patterns of 8-oxoG distribution, suggesting a good functionality of the method when compared to other techniques. Additionally, this method could be adapted to other DNA damages with corresponding glycosylases. In conclusion, we have developed a novel method allowing the mapping of 8-oxoG at single nucleotide resolution, which could aid in understanding the role of DNA lesions in biological and pathological processes.

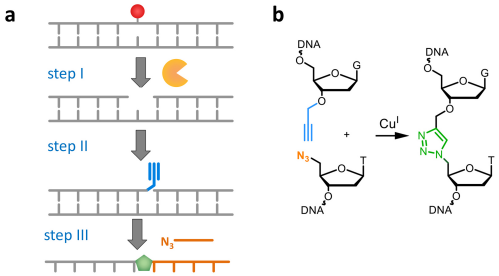

Figure. a) Three steps to label 8oxoG: base excision, propargyl-dGTP incorporation and ligation of the coding DNA. b) The click reaction to ligate the coding sequence to the DNA.

Single-molecule FRET studies on structural dynamics of chromatin regulated by a pioneer transcription factor

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Deciphering chromatin regulation at the molecular level is of fundamental importance for an understanding of cellular physiological and pathological processes. Chromatin is an extremely complex system due to its molecular organization, heterogeneous structure and multiscale dynamics induced by (1) post translational modifications on chromatin itself and (2) other regulatory effectors including transcription factors (TFs) [1]. One key class of chromatin interacting proteins are pioneer transcription factors. The main characteristic that distinguishes pioneer transcription factors from other TFs is their ability to specifically recognize their target DNA sequences in compacted chromatin and consequently to trigger chromatin opening, thus enabling the cellular machinery to locally access the DNA. In the context of cell fate reprogramming, this pioneer action is crucial, but its molecular mechanism is poorly understood [2]. Here, we study repressor-activator protein 1 (Rap1) as a model pioneer transcription factor [3]. Combining chemically defined synthetic chromatin segments and single-molecule FRET approaches [4], we explore how Rap1 invades and remodels compacted chromatin. These studies yield fundamental insights into the molecular mechanisms of gene regulation by molecular interactions.

[1] Olivier Cuvier, Beat Fierz, *Nature Reviews Genetics*, **2017**, 18, 457-472.
[2] Kenneth Zaret, Susan Mango, *Current opinion in Genetics & Development*, **2016**, 37, 76-81.
[3] Erik Feldmann, Roberto Galletto, *Biochemistry*, **2014**, 53(48), 7474-7483.
[4] Sinan Kilic, Beat Fierz, *Nature Communication*, **2018**, 9, 235.

Beyond Lipinski – an analysis of the PubChem chemical space

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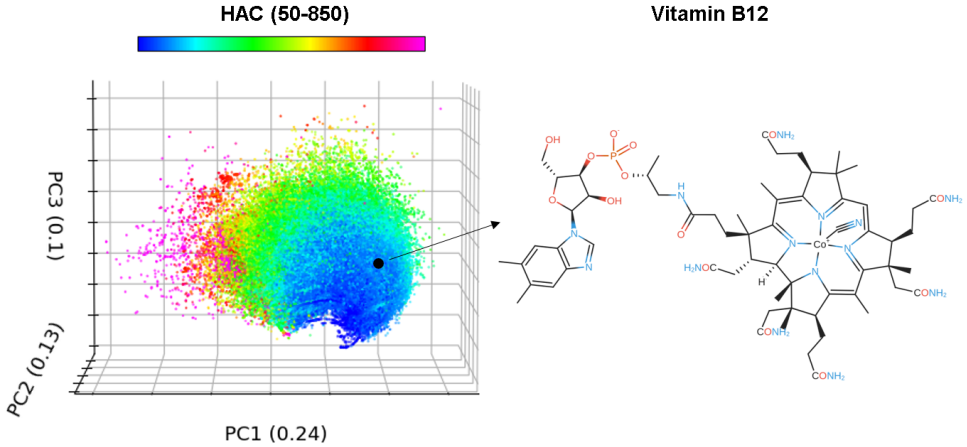
With 95.3 million compounds spanning from small organic fragments to large macromolecules, PubChem is one of the largest public databases of molecules.[1] To complement its website which allows for specific queries (https://pubchem.ncbi.nlm.nih.gov/), we previously reported color-coded interactive 2D-maps that provide a global overview of its contents, however these maps were limited to drug-like molecules as defined by the Lipinski "rule of 5".[2] Here we report an interactive visualization of the entire PubChem database including macromolecules beyond Lipinski based on our recently reported Faerun and SmilesDrawer applications, which are capable of handling very large datasets of molecules.[3] This analysis reveals the extent of chemical diversity available and potential for new explorations.



[1] S. Kim, P. A. Thiessen, E. E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B. A. Shoemaker, J. Wang, B. Yu, J. Zhang, S. H. Bryant, *Nucleic Acids Res.* **2016**, *44*, D1202-D1213.
[2] a) M. Awale, R. van Deursen, J. L. Reymond, *J. Chem. Inf. Model.* **2013**, *53*, 509-518; b) C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Delivery Reviews* **1997**, *23*, 3-25.
[3] a) D. Probst, J. L. Reymond, *Bioinformatics* **2017**, doi: 10.1093/bioinformatics/btx1760; b) D. Probst, J. L. Reymond, *J. Chem. Inf. Model.* **2018**, *58*, 1-7.

Crystallographic study of an antimicrobial bicyclic peptide from chemical space

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We recently reported a chemical space approach for the discovery of potent antimicrobial bicyclic peptides (AMBP) against multidrug resistant Gram-negative bacteria.[1] Here we report the X-ray crystal structure of three of these AMBPs, which reveals that the bicyclic peptide exists as an amphiphilic α-helix stabilized within the bicyclic structure, which probably explains their membrane disruptive mechanism of action.

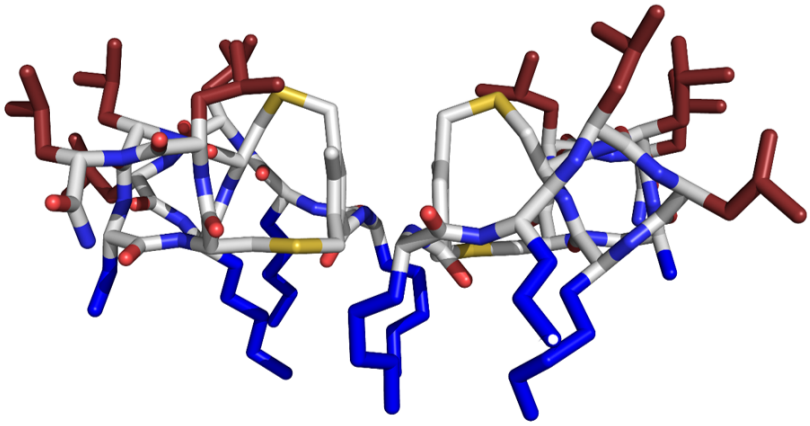

Figure 1: X-ray structure of an AMBP obtained by crystallizing the racemic product. Amino acids are color-coded, red: hydrophobic, blue: hydrophilic. The bicyclic structure is formed from a linear precursor peptide by double thioether ligation of an N-terminal 3,5-dichloromethyl-toluoyl group with a pair of cysteine residues in the chain.

[1] a) I. Di Bonaventura, X. Jin, R. Visini, D. Probst, S. Javor, B.-H. Gan, G. Michaud, A. Natalello, S. M. Doglia, T. Kohler, C. van Delden, A. Stocker, T. Darbre, J.-L. Reymond, *Chem. Sci.* **2017**, *8*, 6784-6798; b) I. Dibonaventura, S. Baeriswyl, A. Capecchi, B.-H. Gan, X. Jin, T. N. Siriwardena, R. He, T. Kohler, A. Pompilio, G. Di Bonaventura, C. van Delden, S. Javor, J.-L. Reymond, *ChemComm* **2018**, doi:10.1039/C1038CC02412J.

Synthesis of aminopyrazole analogues for the treatment of leishmaniasis: A University-DNDi Open Synthesis project.

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Leishmaniasis 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 leishmaniasis.In the frame of an Open Synthesis Network (OSN) between the University of Geneva and DNDi, we aimed at synthetizing new aminopyrazole analogues for early stage discovery into new treatments for leishmaniasis.

In order to explore the aminopyrazole chemotypes,we set up a 4-steps synthesis starting from beta-proline, 14 different aldehydes and 3 different aromatic cores to obtain 42 different products. The two key reactions are reductive amination, during which a first structural diversification occurs and the last coupling by amidation that leads to the final expected compounds. DNDi will test these analogues against leishmaniasis and the results added into a Master Data table, shared with all OSN participants.

[1] Sebastian R. Oehler, *et. al*, *MRes Drug Discovery & Development,* **2017**
[2] Daniel Fernández-Llaneza, *et. al*, *MRes Drug Discovery & Development,* **2017**
[3] Charles E. Mowbray, *et. al*, *J. Med.Chem,* **2015** 58 (24), 9615-9624

Accelerating Turnover Frequency in Nucleic Acid Templated Reactions

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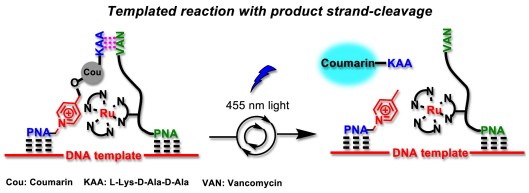
Abstract: Nucleic acid templated reactions have attracted attention as an important technology to sense oligonucleotides and to translate nucleic acid-based instructions into diverse outputs. Great progresses have been made in accelerating the reaction in order to improve signal amplification, reaching rates where substrate turn-over rather than chemical reaction is rate limiting. Herein we explore the utility of architectures inspired by three-way junction that yield a cleavage of a strand thus accelerating substrate turn-over. We demonstrate that such design can overcome product inhibition in templated reactions and operate close to the rate of hybridization.



[1] D. Chang, E. Lindberg, N. Winssinger, 'Critical Analysis of Rate Constants and Turnover Frequency in Nucleic Acid- Templated Reactions: Reaching Terminal Velocity',*J. Am.Chem. Soc.* **2017**, *139*, 1444 – 1447.

[2] D. Chang, K. T. Kim, E. Lindberg, N. Winssinger, 'Accelerating Turnover Frequency in Nucleic Acid Templated Reactions', *Bioconjug. Chem*. **2018**, *29,* 158 – 163.

Design, synthesis and photochemical properties of a new photoswitchable TRPV6 inhibitor

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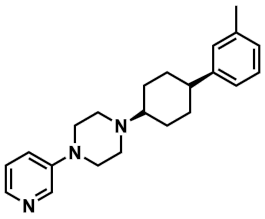
Since their discovery, the TRP family of ion channels became an interesting target due to its involvement in a diverse number of diseases [1]. One of its representatives, TRPV6 arouse as a key point in cancer progression, however, the physiopathological mechanisms remains unclear [2]. One effective strategy to access the spatiotemporal control of a chosen protein is to attach light responsive groups to known ligands [3]. To enable this approach, we incorporated the azobenzene moiety to *cis*-22a, a potent TRPV6 inhibitor previously discovered in our group [4]. Remarkably, we found a derivative that inhibited TRPV6 in micromolar range (IC_{50} = 2.6 ± 0.2 μM) and possess the required photoswitchable profile for its precise spatiotemporal control. This compound will now be used to deepen our understanding of TRPV6 mechanisms at the cellular level.



***cis*-22a**
IC_{50} = 0.3 ± 0.1 μM

[1] T. Rodrigues, F. Sieglitz, G. Bernardes, *Chem. Soc. Rev*, **2016,** *45*, 6130-6137.
[2] C. Fecher-Trost, U. Wissenbach, P. Weissgerber, *Cell calcium*, **2017,** *67*, 116-122.
[3] T. Fehrentz, M. Schönberger, D. M. Trauner, *Angew. Chem. Int. Ed.,* **2011,** *50*, 12156-12182.
[4] C. Simonin, M. Awale, M. Brand, R. van Deursen, J. Schwartz, M. Fine, G. Kovacs, P. Häfliger, G. Gyimesi, A. Sithampari, R.P. Charles, M. A. Hediger, J.L. Reymond, *Angew. Chem*, **2015,** *127*, 14961-14965.

Multifunctional harmonic nanoparticles targeting the microenvironment of lung cancer tumor for multimodal imaging and diagnosis

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

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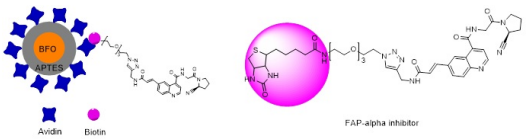
style="text-align: justify;">The recent and rapid progress in nanotechnologies paved the way for the investigation of nanomaterials in clinical settings for early detection, diagnosis and targeted treatment of cancer, which represents a major health burden in developed countries.[1] The ability to produce 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] While cancer cell biomarkers such as epithelial growth factor receptors have been widely investigated as therapeutic targets for the treatment of lung cancers, evidence has accumulated that the microenvironment of a given tumor has a crucial role in its progression, metastasis and response to chemotherapeutic intervention. In particular, carcinoma associated fibroblasts (CAFs), which are key components of the tumor stroma, appear as promising target for both diagnosis and treatment of several types of cancer.[4] A specific characteristic of CAFs is the expression of fibroblast activation protein α (FAP-α), a transmembrane protease which is not found in normal, healthy adult tissues, except in granulation tissue of healing wounds. This protease is a present in 90% of epithelial tumors and plays a role in their growth.[5]

In this context, we present the synthesis of a highly specific and selective inhibitor of FAP-α which was further functionalized to be evaluated as targeting ligand for lung tumor microenvironment. This ligand was evaluated for its selective association to FAP-α and was further conjugated to avidin coated bismuth ferrite ($BiFeO_3$, BFO) harmonic nanoparticles (NPs) through biotin-avidin interaction.



Taking advantage of the second harmonic generation properties of the BFO NPs, the resulting functionalized nanomaterials were evaluated for targeted lung cancer imaging by multiphoton microscopy.

[1] Tassa, C.; Shaw, S. Y.; Weissleder, R. *Acc. Chem. Res*. **2011**, *44*, 842-852; Yigit, M. V.; Moore, A.; Medarova, Z. *Pharm. Res*. **2012**, *29*, 1180-1188.
[2] Smith, L.; Kuncic, Z.; Ostrikov, K. K.; Kumar, S. *J. Nanomater*. **2012**, Article ID 891318, 7 pages.
[3] Malvezzi, M.; Bertuccio, P.; Levi, F.; La Vecchia, C.; Negri E. *Ann. Oncol*. **2014**, *25*, 1650-1656.
[4] Paulsson, J.; Micke, P. *Sem Cancer Biol* **2014**, *25*, 61-68.
[5] Zi, F.; He, J.; He, D.; Li, Y.; Yang, L.; Cai, Z. *Mol. Med. Rep.* **2015**, *11* (5), 3203–3211.

Structure-based Optimization of a selective TRPM4 inhibitor

C. Delalande[1,3], L. Ozhathil[2,3], D. Ross-Kaschitza [2,3], P. Arullampalam[2,3], M. Rubin[2,3], J. Gertsch[2,3], H. Abriel[2,3], S. Javor[1,3], J. L. Reymond[1,3]*

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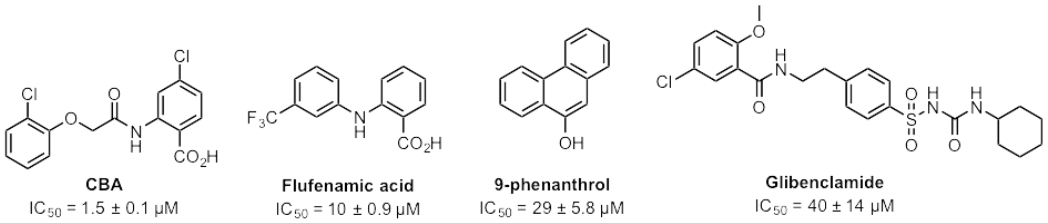
We recently reported the discovery of CBA (4-Chloro-2-(2-(2-chlorophenoxy)acetamido) benzoic acid) as the first potent and selective inhibitor of TRPM4, a monovalent cation channel and potential drug target in heart disease and cancer, using a targeted analog search starting from the known but weak and unselective TRPM4 inhibitors 9-phenanthrol, flufenamic acid and glibenclamide.[1] CBA inhibits TRPM4 in the micromolar range (IC_{50} = 1.5 ± 0.1 µM) without any detectable cross-reactivity with other TRP channels or any other off-target effects. Here, we used the recently reported X-ray crystal structure of TRPM4[2] to analyze the binding mode of our inhibitor. We obtained a consistent correlation between binding poses and experimentally observed IC_{50} values across a broad series of inhibitors, opening the way to structure-based optimization of CBA towards more potent analogs.



[1] L. C. Ozhathil, C. Delalande, B. Bianchi, G. Nemeth, S. Kappel, U. Thomet, D. Ross-Kaschitza, C. Simonin, M. Rubin, J. Gertsch, M. Lochner, C. Peinelt, J. L. Reymond, H. Abriel, *Br. J. Pharmacol.* **2018**, doi: 10.1111/bph.14220.
[2] J. Guo, J. She, W. Zeng, Q. Chen, X. C. Bai, Y. Jiang, *Nature*, **2017**, *552*, 205–209.

Coumarin-labeled Dinuclear Trithiolato-Bridged Ruthenium(II) Arene Complexes - Synthesis, Characterization and Spectral Properties

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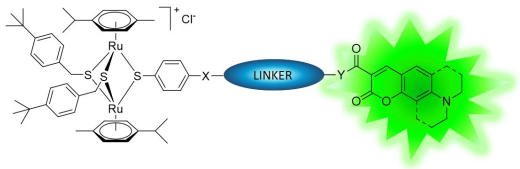
Following the identification of cationic trithiolato-briged dinuclear ruthenium(II) arene complexes of general formula $[(\eta^6\text{-arene})_2Ru_2(\mu_2\text{-SR})_3]^+$ as highly cytotoxic against human cancer cells[1] and potential anti-parasitic agents against *Toxoplasma gondii*[2] and *Trypanosoma brucei*, the investigation of the traffic of this type of compounds in cells and their possible mechanism of action has become a priority. To this end, the synthesis of fluorescent conjugates appear judicious, as they could bring information regarding the localization of this type of organometallic complexes in cells, and subcellular compartments. We chose coumarin derivatives as fluorophore labels, as they display broad pharmacological activities and conjugates combining metalorganic units with covalently linked coumarins have been shown to be versatile tools for imaging in the case of various fluorophore-tagged ruthenium, gold or iridium complexes.



Within this framework, we prepared a series of six new trithiolato-briged dinuclear ruthenium(II) arene complexes modified with coumarin fluorophores. Two carboxylic acid functionalized coumarin derivatives were used, namely 7-diethylaminocoumarin-3-carboxylic acid and coumarin 343 (the butterfly coumarin acid). The fluorophores were anchored *via* classical coupling reactions to three 'mixed' trithiolato precursors (general formula $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(\mu_2\text{-S-}CH_2\text{-}C_6H_4\text{-}p\text{-}Bu^t)_2(\mu_2\text{-S-}C_6H_4\text{-XH})]Cl$), bearing different functionalisable groups (-XH = -OH, $-NH_2$, $-CH_2CO_2H$). Different features were varied in the new conjugates as the type of connecting bond (ester *vs* amide), and the presence/absence of a spacer between the organometallic moiety and the fluorophore. The spectral properties of the new compounds were measured and compared to those of the unmodified fluorophores.

[1] Julien Furrer, Georg Süss-Fink, G. *Coord. Chem. Rev.* **2016**, 309, 36-50.
[2] Afonso P. Basto, Joachim Müller, Riccardo Rubbiani,b David Stibal, Federico Giannini, Georg Süss-Fink, Vreni Balmer, Andrew Hemphill, Gilles Gasser, Julien Furrer, *Antimicrob. Agents Chemother*. **2017**, 61, e01031-17.

Phenylcarbonates as reactive species in DNA templated reactions

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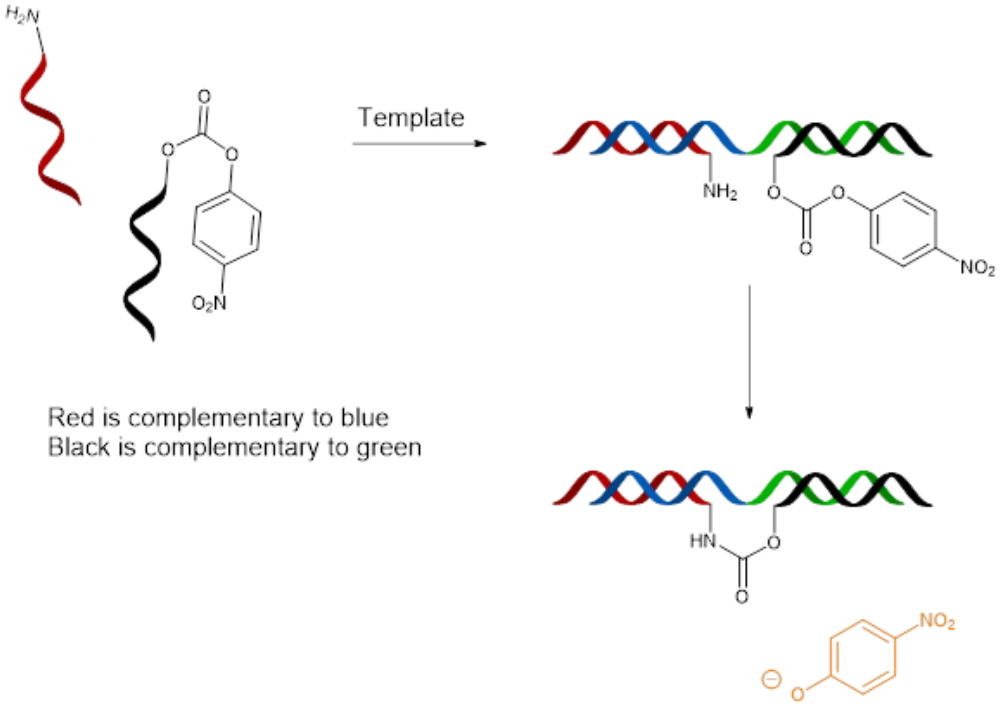
Nucleic acid templated reactions exploit the ability of nucleic acids to bind specifically to their complementary sequence, even at very low concentrations. The supramolecular interactions between two strands produce a highly pre-organized system that can be useful for synthetic chemistry or biosensing applications.[1]

Our research focuses on phenylcarbonates as reactive entities in DNA templated reactions. The carbonates are attached to DNA strands by “click” chemistry and used as electrophiles in amine/carbonyl reactions. Templation by DNA allows the reaction to take place at micro- or nanomolar concentrations.



When a reaction occurs, a reporter molecule such as a yellow coloured nitrophenolate or a fluorescent coumarin is released, allowing an easy reaction monitoring. For systems where a ligation occurs, a successful reaction can also be showcased by denaturing polyacrylamide gel electrophoresis.

[1] Katarzyna Gorska, Nicolas Winssinger, *Angew. Chem. Int. Ed.* **2013**, 52 (27), 6820–6843.

Functionalized Collagen Model Peptides

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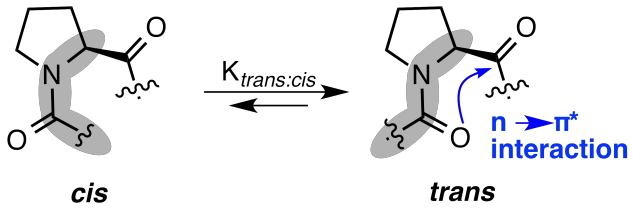
Collagen is the most prevalent protein in mammals.[1] The protein has a fibrous structure that arises from right-handed triple helices that are formed from three, parallel, left-handed all-*trans* polyproline (II)-like single strands. Studies with short-chain collagen model peptides (CMPs) showed that the *cis*/*trans* isomerization of the amide bonds is the rate-limiting step for triple helix formation.[2] Preorganized amide bonds within collagen single strands in all-*trans* conformations is therefore expected to accelerate triple helix folding and result in increased triple helix stability. In 2015, our group showed that an apolar environment favors the *trans* isomer of acetylated dimethylamide of proline over the *cis* isomer.[3] Based on this result, we reasoned that a hydrophobic environment pre-organizes the single strands into a *trans* conformation and should therefore result in stable, fast-folding triple helices. To create such a hydrophobic microenvironment, we covalently linked hydrophobic residues to CMPs, while maintaining the solubility in an aqueous environment. Thermal denaturation experiments and „temperature jump“ experiments showed that the CMPs with the hydrophobic moiety form remarkably more stable and faster-folding triple helices than CMPs without a hydrophobic moiety. NMR spectroscopic studies and native MS confirmed these observations. The results of these experiments as well as those obtained with other functionalized CMPs[4,5] will be presented.

cis/*trans* isomerization depends on polarity of environment



Hydrophobic solvent: $K_{trans:cis}$ ($CDCl_3$) = 8.8
Hydrophilic solvent: $K_{trans:cis}$ (D_2O) = 3.8

[1] Matthew D. Shoulders, Ronald T. Raines, *Annual Review of Biochemistry,* **2009,** 78, 929.
[2] Hans Peter Bächinger, Jürgen Engel., Peter Brucker, *European Journal of Biochemistry,* **1978**, 90, 605.
[3] Christiane Siebler, Boris Marysin, Michael Kümin, Roman S. Erdmann, Carla Rigling, Claudio Grünenfelder, Christian Ochsenfeld, Helma Wennemers, *Chemical Science,* **2015**, 6, 6725.
[4] Jasmine Egli, Christiane Siebler, Boris Maryasin, Roman S. Erdmann, Cedric Bergande, Christian Ochsenfeld, Helma Wennemers, *Chemistry - A European Journal,* **2017**, 23, 7938.
[5] Jasmine Egli, Roman S. Erdmann, Pascal Schmid, Helma Wennemers, *Organic & Biomolecular Chemistry,* **2017**, 53, 11036.

Tracking the Entry of Antimicrobial Peptide Dendrimer G3KL into Pseudomonas aeruginosa

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The peptide dendrimer **G3KL** (Fig. 1) exerts strong antimicrobial activity against multidrug resistant clinical isolates of various Gram-negative bacteria such as *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Escherichia coli.*[1,2] This dendrimer also shows pro-angiogenic effect in burn wound-healing processes[3]. In this work, we have modified **G3KL** at its C-terminus and obtained fluorescence labeled analogs that retain the antimicrobial activity of **G3KL**. We performed transmission electron microscopy, time lapse laser scanning confocal microscopy and stimulated emission depletion (STED) microscopy to image how these fluorescent **G3KL** analogs penetrate *P. aeruginosa* cells in real time.

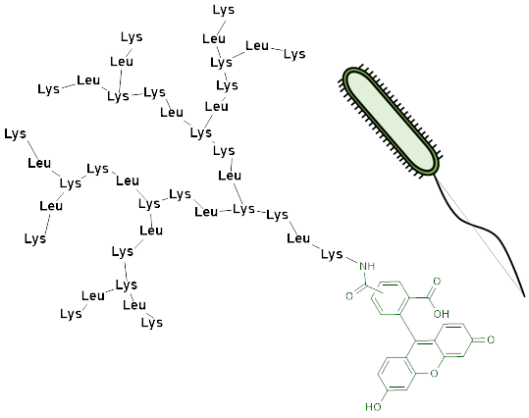

Figure 1: Structure of the fluorescein labeled peptide dendrimer **G3KL**.

[1] Stach M, Siriwardena TN, Köhler T, van Delden C, Darbre T, Reymond JL, *Angew. Chem. Int. Ed*. **2014***,* 53, 12827.
[2] Pires J, Siriwardena TN, Stach M, Tinguely R, Kasraian S, Luzzaro F, Leib SL, Darbre T, Reymond JL, Endimiani A*., Antimicrob Agents Chemother.* **2015**, 59, 7915.
[3] Abdel-Sayed P, Kaeppli A, Siriwardena T, Darbre T, Perron K, Jafari P, Reymond JL, Pioletti DP, Applegate LA, *Sci Rep.* **2016**, 6, 22020.

Protective Role of Polymeric Carriers in Chlorin Delivery Against Protein Binding

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Porphyrinic compounds are proven and prevalent photosensitizers in photodynamic therapy owing to their advantageous photophysical properties such as efficient light triggered phototoxicity, chemical stability, low dark toxicity, tumor accumulation and body excretion. However, the low solubility of porphyrins in biological media which is due to their high aggregation tendency is a limiting factor reducing the therapeutic efficiency. In addition, the intrinsic binding affinity of porphyrins towards human blood proteins affects the absorption, distribution, metabolism, and excretion (ADME) of a given photosensitizer thereby contributing to unfavourable pharmacokinetics. The application of polymer nanoparticles represents an elegant way to cope with the problems of undesired aggregation and protein-complex formation. [1] Photolon®, which is a chlorin e6 (Ce6) complex with polyvinylpyrrolidone (PVP) has been approved for medical application in PDT. [2] Previously, we have elaborated how aggregate structures, polymer encapsulation, and association dynamics depend on structural side chain modifications for a series of porphyrin and chlorin derivatives. [3, 4]

In the present study we examine the loading and disaggregation capability of two polymer matrices, PVP and Kolliphor P188 (KP188 - triblock copolymer) towards chlorin compounds (CEs). Moreover, chlorin ligand exchange between the polymer and various protein hosts is studied using NMR and fluorescence spectroscopy as main techniques. The analysis of chlorin ^{1}H-NMR resonances (chemical shift, linewidths) was used to follow the encapsulation and disaggregation by the polymer matrices as well as monitoring the ligand (chlorin) redistribution among the competitive host molecules, i.e. the polymer matrix and the protein. The results indicate that CE preferentially and strongly binds to PVP and the presence of HSA does not trigger CE-release but leads to the formation of tertiary (CE-PVP)-HSA complexes. On the contrary, if encapsulated in the KP188 matrix, ligand (chlorin) redistribution occurred between the polymer and the protein host. Flow cytometry measurements of HeLa cells incubated either with CE-PVP or CE-KP188 resulted in higher cell uptake of CE when encapsulated in PVP. The enhanced uptake most likely is related to the stronger binding to the PVP matrix and its protective role against serum proteins suggesting that PVP is the more efficient delivery system for CE as compared to KP188 micelles.

[1] Miceli, E., M. Kar, and M. Calderón. *J. Materials Chemistry B*, 2017. 5(23): p. 4393-4405.
[2] H.A. Isakau, M.V. Parkhats, V.N. Knyukshto, B.M. Dzhagarov, E.P. Petrov, P.T. Petrov, *J. Photochem. Photobiol. B*, 92, (2008) 165-174.
[3] Gjuroski I, Furrer J and M. Vermathen, *ChemPhysChem*, 2018, 19, 1089-1102.
[4] Hädener M, Gjuroski I, Furrer J, Vermathen M. 2015. *J Phys Chem B* 119:12117-28.

Chemical Surface Modification of Living Cells: Enzyme Immobilization and Light Guided Delivery

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Chemical surface functionalization offers many different possibilities of manipulating the cell surface with reactive groups presented on the cell. This strategy can be used to equip the cell surface in a controlled way with new abilities.[1] Our goal is to immobilize enzymes on the surface of a living cell and during this procedure preserve the catalytic activity of the enzyme. We use the eukaryote *Chlamydomonas reinhardtii* as a model organism, therefore leveraging its possibilities such as photosynthesis, photo taxis and CO_2 fixation.[2]

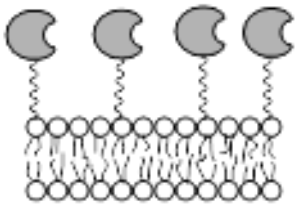

Figure 1: Schematic representation of the surface functionalization.

[1] Catarina A. Custódio, João F. Mano, *Chemistry of Nanomaterials for Energy, Biology and More*, **2016**, 2, 376-384.
[2] Elizabeth H. Harris, *Annual Review of Plant Physiology and Plant Molecular Biology*, **2001**, 52, 363-406.

Multivalent peptide probes for phase-separating chromatin states investigation

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Heterochromatin silencing relies on the binding of heterochromatin protein 1 (HP1) and other factors to post-translational modifications (PTMs) of histone tails such as trimethylation of lysine 9 (H3K9me3) that in turn trigger chromatin condensation and restrict access for the transcription machinery. Recent studies revealed that the formation of heterochromatin domains involves liquid-liquid phase separation, mediated by multivalent HP1 - chromatin interactions.[1, 2] The resulting membrane-less and distinct heterochromatin foci within the nucleus are characterized by rapid diffusion of proteins inside those domains. Motivated by these new discoveries, we wondered whether heterochromatin was the only chromatin state able to phase-separate, or if we can directly detect further histone PTM dependent sub-nuclear domains. We thus designed a modular strategy for the synthesis of multivalent peptide probes capable of detecting chromatin phase-separating states in living cells. Multivalent chromatin effectors, such as HP1, bind to the probe causing its accumulation at specific chromatin spots (Fig. 1). The sub-nuclear distribution of the probes is then imaged using confocal fluorescence microscopy. Our probe consists of a fluorescently labeled peptide scaffold, allowing the efficient coupling of histone fragments, containing combinatorial PTMs characterizing a specific chromatin state. We employed a combination of disulfide-directed modification (to couple a cell-penetrating peptide) and Huisgen azide-alkyne 1,3-dipolar cycloaddition (click chemistry) to rapidly derivatize the peptide scaffold with modified histone peptides. Designed for H3K9me3-dependent heterochromatin as well as S28 phosphorylated active regions, we expect that our novel reagents enable to visualize different chromatin compartments in single cells, providing a unique dynamic view of 3 dimensional chromatin state organization.

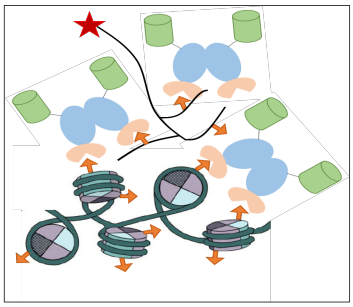

Fig. 1: Schematic representation of multivalent probe accumulation at phase-separating chromatin domains.

[1] Amy R. Strom, Alexander V. Emelyanov, Mustafa Mir, Dmitry V. Fyodorov, Xavier Darzacq and Gary H. Karpen, *Nature,* **2017**, 547, 241-245.
[2] Adam G. Larson, Daniel Elnatan, Madeline M. Keenen, Michael J. Trnka, Jonathan B. Johnston, Alma L. Burlingame, David A. Agard, Sy Redding and Geeta J. Narlikar, *Nature*, **2017**, 547, 236-240.

Pseudomonas metallothioneins - structure-function relationship

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Metallothioneins (MTs) are a superfamily of metalloproteins characterized by a high abundance of cysteine residues, low molecular weight and unique transition metal binding properties. They have the capability to participate in vital physiological functions e.g. metal homeostasis/detoxification and scavenging of reactive oxygen/nitrogen species. *Pseudomonas* MTs are a subfamily of bacterial metallothioneins (bacMTs) that were discovered as a consequence of sequencing efforts of numerous bacterial strains in the last decade. These MTs reveal unique divergences from cyanobacterial MTs[1], the most investigated bacMTs, on both the protein and the genome level. While they share a conserved Cys distribution pattern consisting of an N-terminal CxCxxCxC motif, a central YCC/SxxCAxxH stretch, as well as a C-terminal Cxxxx(x)CxC part, their primary structures also expose specific differences amongst *Pseudomonas* MTs (e.g. presence of a long C-terminal tail, varying number of histidine residues). Furthermore, while the cyanobacterial MT operon is well defined and understood[2], the *Pseudomonas* MT operon is not distinct and it is consisting of one MT gene and genes coding for hypothetical proteins, suggesting an alteration from functionalities previously assigned to bacMTs.

We are investigating not only how differences found in the primary structure of these novel bacMTs influence 3D structures, including protein fold and the metal clusters, but also how they define their biological function in order to ultimately deduce a structure-function relationship.

[1] Blindauer, C. A.; Harrison, M. D.; Parkinson, J. A.; Robinson, A. K.; Cavet, J. S.; Robinson, N. J.; Sadler, P. J. A Metallothionein Containing a Zinc Finger Within a Four-metal Cluster Protects a Bacterium from Zinc Toxicity. *Proc Natl Acad Sci U S A* **2001**, *98*, 9593–9598.
[2] Morby, A. P.; Turner, J. S.; Huckle, J. W.; Robinson, N. J. SmtB Is a Metal-dependent Repressor of the Cyanobacterial Metallothionein Gene smtA: Identification of a Zn Inhibited DNA-protein Complex. *Nucleic Acids Res* **1993**, *21*, 921–925.

Therapeutic approaches for erythropoeitic protoporphyria

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Erythropoeitic protoporphyria (EPP) is a genetic disorder affecting in average 1/100.000 individuals and causing light photosensitivity in patients who quickly develop skin irritation upon exposure blue light coming from natural or artificial sources [1]. EPP onset requires two independent genetic events on both alleles of the ferrochelatase (*FECH*) gene affecting the production of FECH protein [2]: in one allele, a non-sense or missense mutation prevents the synthesis of the functional enzyme; in the other, an intronic single nucleotide polymorphism (SNP) causes aberrant splicing of the pre-mRNA. Low levels of FECH lead to accumulation of its photoreactive substrate protoporphyrin IX (PPIX) in erythroid cells in the bone marrow and in the blood, as well as in the liver and the spleen.

We are investigating several strategies to restore FECH production. One strategy to treat EPP is to use splice-switching oligonucleotides (SSOs) - oligonucleotides specially designed to bind the pre-mRNA and sterically force the production of a functional *FECH* pre-mRNA. An active sequence was identified *in vitro* after screening and tested *in vivo* in an in-house disease model [3], where it was able to partially restore correct splicing in the liver and the spleen but failed to have an effect in the last disease-relevant tissue, bone marrow. Several peptide- and lipid- conjugates were therefore designed for an improved bone marrow vectorization and are being tested *in vivo*. In a parallel but independent manner, we are screening several approved small molecule drugs known to impact heme biosynthesis pathway, hoping to identify candidates able to increase FECH protein production.

Finally, we are working within this project with a technique developed for chemically modified oligonucleotides – the chemical-ligation qPCR (CL-qPCR) [4] – to quantify the amount of SSOs delivered within the cells *in vitro* or in tissues *in vivo*. The technique is currently being exploited as a platform to investigate the effect of oligonucleotide chemistry, length or conjugation on uptake, subcellular localization and biological activity.

[1] Lecha M. et al., *Orphanet J of Rare Diseases*, **2009**, 4:19
[2] Gouya L. et al., *Nat Gen*, **2002**, 30
[3] Barman-Aksözen, Ćwiek et al., *Dis Models Mech*, **2017**, 10(3), 225-233
[4] Boos J. et al., *Nucl Acid Res*, **2013**, 41(15)

$(\beta\text{-Ala})_2$ derivatives – Synthesis, Characterization & Self-Assembly (SCS)

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INTRODUCTION: Peptide (hydro-)gels are a promising class of soft biomaterials for cell culture, regenerative medicine, or drug delivery applications due to good biocompatibility, biodegradability and injectability[1,2]. Longer peptide systems (e.g. Max1, P_{11}-2[2]) as well as dipeptides like Fmoc-Phe-Phe[3] self-assemble mostly via hydrophobic π-π stacking, but other forces like H-bonding may also play a role[4,5]. In order to better understand the self-assembly process, we created a peptide library with one of the simplest amino acids, β-alanine, to study the packing and properties. **METHODS:** Eleven $(\beta\text{-Ala})_2$ derivatives (Fig. 1A) were synthesized by different standard liquid phase synthesises and characterized by ^{1}H- and ^{13}C-NMR, MS-ESI, TGA, DSC, FT-IR and PXRD. Crystallization and organogel formation trials were successfully performed with selected samples that were further analysed by SEM and AFM. **RESULTS:** Five crystal structures were obtained: Boc-$(\beta\text{-Ala})_2$-NH_2; Boc-$(\beta\text{-Ala})_2$-N_2H_3 (Fig. 1B), Fmoc-$(\beta\text{-Ala})_2$-OH, NH_3^+-$(\beta\text{-Ala})_2$-OH, and NH_3^+-$(\beta\text{-Ala})_2$-$N_2H_4^+$. In case of Boc$(\beta ala)_2N_2H_3$ organogel like structures were obtained in different solvents (Fig. 1C).

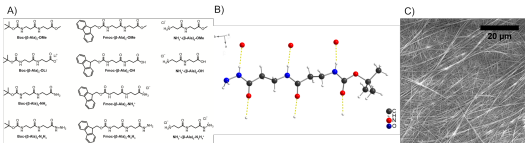

Figure 1: A) Structure and name of $(\beta\text{-Ala})_2$ derivatives which were synthesized; B) Crystal structure of Boc-$(\beta\text{-Ala})_2$-N_2H_3, H-bonds as dotted lines; C) SEM picture of an organogel-like structure

DISCUSSION & CONCLUSIONS: In general, the solid-state structure of the β-Ala-derivatives is governed by H-bonding motifs, which induce the formation of ribbons. The crystal structures vary however depending on the protecting group at the N-terminus (Boc, Fmoc or unprotected), as well as on the functional group at their C-terminus. Surprisingly, one of the samples, Boc-$(\beta\text{-Ala})_2$-N_2H_3, is capable of forming a gel-like structure in spite of the absence of π-π interactions and assembles into fibres as shown in Fig. 1C.

[1] S, Marchesan, L. Waddington, C.D. Easton, D.A. Winkler, L. Goodall, J. Forsythec and P.G. Hartleya, *Nanoscale*, **2012**, 4, 6752-6760
[2] W. Y. Seow and C.A.E. Hauser, *Materials Today*, **2014**, 17, 381-388
[3] T. Liebmann, S. Rydholm, V. Akpe and H. Brismar, *BMC Biotechnology*, **2007**, 7, 1472-6750
[4] M. Tena-Solsona, S. Alonso-de Castro, J.F. Miravet and B. Escuder, *J. Mater. Chem. B*, **2014**, 2, 6192-6197
[5] G. Fichman, E. Gazit, *Acta Biomaterialia*, **2014**, 10, 1671-1682

New chlorin-derivatives as potential dual-acting photodynamic agents

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[1]University of Zurich

Photodynamic therapy is an emerging method used for the treatment of various types of cancer. The principle of this method is based on activation of a chemical, so-called a photosensitizer, by a specific wavelength of light, the activated photosensitizer, in turn, produces different reactive oxygen species (ROS), which are highly toxic to cancer cells.[1]

Recently, highly phototoxic platinated porphyrins were developed in our group, which showed great phototoxicity together with low dark toxicity against various cancer cell lines.[2] However, porphyrins exhibit a quite low absorption in the red visible region, in which light penetrates deeper into body tissues.

In this study, we have developed an approach to synthesize two new chlorin derivatives of tetrapyridylchlorin and dipyridylchlorin, which have stronger absorption at wavelengths higher than 600 nm. Then, these chlorins were platinated with cisplatin and transplatin. The yielded products are subjects to further biological studies.

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

[1] Tegos, G.; Dai, T.; Fuchs, B.; Coleman, J.; Prates, R.; Astrakas, C.; St Denis, T.; Ribeiro, M.; Mylonakis, E.; Hamblin, M. *Frontiers in Microbiology* **2012**, *3,* 120.
[2] Naik, A.; Rubbiani, R.; Gasser, G.; Spingler, B. *Angewandte Chemie International Edition* **2014**, *53*, 6938-6941.

Development of multi-functional Cyclopentadienyl Complexes

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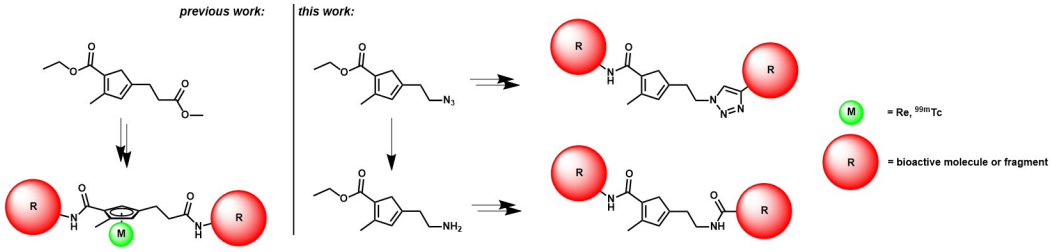
^{99m}Tc is widely used in nuclear medicine for diagnostics of various targets.[1] Using Cyclopentadiene (Cp) as chelator offers an interesting way of introducing ^{99m}Tc into a biologically active ligand system for the creation of radiopharmaceuticals as can be seen by the Re-analogues of ferrocifene.[2] Recently our group developed a multifunctional Cp bearing two ester functionalities which could be employed as a linker between up to two bioactive cancer targeting moieties as well as a chelator for Re and ^{99m}Tc.[3]



A new variant of this Cp bearing one ester and one azide functionality has been developed. This increases the orthogonality of the two binding sites and opens up new synthetic ways to introduce bioactive molecules to the Cp, such as “Click”-reactions that are already widely used in bio-, medicinal-, and radiochemistry.[4] Furthermore, the azide functionality can be reduced to an amine, resulting in a Cp bearing amine and carboxylic acid moieties and thereby mimicking an amino acid, which can be easily radiolabelled with Re or ^{99m}Tc.

[1] R. Dilworth, S. J. Parrott, *Chem. Soc. Rev.*, **1998**, *27*, 43-55.
[2] G. Jaouen, S. Top, A. Vessières, P. Pigeon, G. Leclercq, I. Laios, *Chem. Commun.*, **2001**, 383–384.
[3] A. Frei, B. Spingler, R. Alberto, Chem. Eur. J. 10.1002/chem.201801271 (Accepted Manuscript).
[4] K. Nwe, M. W. Brechbiel, *Cancer Biotherapy & Radiopharmaceuticals*, **2009**, *24*, 289-302.

The Neuronal Tau Protein Blocks in Vitro Fibrillation of the Amyloid-β (Aβ) Peptide at the Oligomeric Stage

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In Alzheimer's disease, amyloid-β (Aβ) plaques and tau neurofibrillary tangles are the two pathological hallmarks. The co-occurrence and combined reciprocal pathological effects of Aβ and tau protein aggregation have been observed in animal models of the disease. However, the molecular mechanism of their interaction remain unknown. Using a variety of biophysical measurements, we here show that the native full-length tau protein solubilizes the Aβ40 peptide and prevents its fibrillation. The tau protein delays the amyloid fibrillation of the Aβ40 peptide at substoichiometric ratios, showing different binding affinities toward the different stages of the aggregated Aβ40 peptides. The Aβ monomer structure remains random coil in the presence of tau, as observed by nuclear magnetic resonance (NMR), circular dichroism (CD) spectroscopy and photoinduced cross-linking methods. We propose a potential interaction mechanism for the influence of tau on Aβ fibrillation.

[1] Wallin C, Hiruma Y, Wärmländer SKTS, Huvent I, Jarvet J, Abrahams JP, Gräslund A, Lippens G, Luo J. The Neuronal Tau Protein Blocks in Vitro Fibrillation of the Amyloid-β (Aβ) Peptide at the Oligomeric Stage. J Am Chem Soc. 2018 May 22. doi: 10.1021/jacs.7b13623. (in press)

Thiol/Diselenolane-Mediated Cellular Uptake and Delivery of Biological Functions to Cells

R. Martinent[1], E. Bartolami[1], J. Saarbach[1], Y. Cheng[1], N. Chuard[1], L. Zong[1], Y. Okamoto[2], N. Sakai[1], T. Ward[2], N. Winssinger[1,1], S. Matile[1]*

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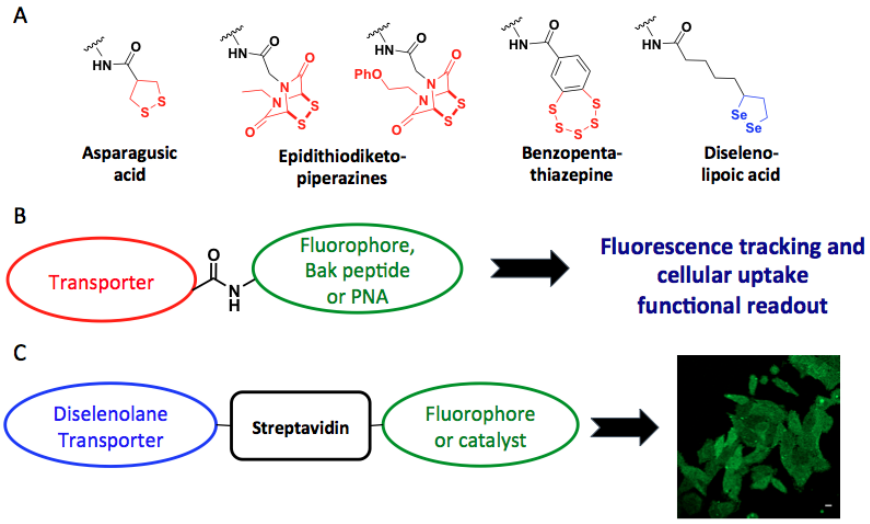
Intracellular delivery is an effortful but very attractive challenge in chemical biology research because of its numerous applications in medicine. In this way, the discovery of thiol-mediated and diselenolane-mediated uptake allowed the transport of cargos such as a fluorophore, linked to the different transporters (Fig. 1A), into living cells.[1,2] With this in hand, our ultimate goal is to deliver biologically relevant functions. Hence, a Bak-BH3 domain peptide (Fig. 1B) has been efficiently delivered to cells when tagged to an asparagusic transporter (Fig. 1A).[3] Most importantly, the adduct was able to induce a concentration dependent apoptosis, through inactivation of the BcL-2 pro-survival proteins, yielding a quantitative cellular uptake readout. Moreover, employing the same tagging method, the transport of PNAs (Peptide Nucleic Acids) to knockdown EGFR (Epidermal Growth Factor Receptor) expression in cells as a new quantitative readout is being developed (Fig. 1B). Finally, a new strategy combining diselenolane transporter with streptavidin-biotin biotechnology (Fig. 1C) enables both transport and catalysis to occur inside HeLa cells cytosol.



[1] Lili Zong, Eline Bartolami, Daniel Abegg, Alexander Adibekian, Naomi Sakai, Stefan Matile, *ACS Central Science*, **2017**, 3, 449-453.
[2] Nicolas Chuard, Amalia Isabel Poblador-Bahamonde, Lili Zong, Eline Bartolami, Jana Hildebrandt, Wolfgang Weigand, Naomi Sakai, Stefan Matile, *Chemical Science*, **2018**, 9, 1860-1866.
[3] Daniel Abegg, Giulio Gasparini, Dominic G. Hoch, Anton Shuster, Eline Bartolami, Stefan Matile, Alexander Adibekian, *Journal of the American Chemical Society*, **2017**, 139, 231-238.

Bicelles – Combining the Advantages of Micelles and Liposomes for Photosensitizer Delivery?

M. Meier[1], I. Gjuroski[1], J. Furrer[1]*, M. Vermathen[1]*

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To enhance the bioavailability of porphyrinic photosensitizers (PSs) in photodynamic therapy (PDT), encapsulation in nanoparticles has been shown to be a very promising approach, since it prevents self-association of the mostly hydrophobic PS in aqueous media and enhances their stability [1]. Previously, our group has shown that polymer-based nanoparticles including polyvinylpyrrolidone (PVP) [2] and block-copolymer micelles (BCMs) [3] can be suitable drug delivery vehicles (DDV) for amino acid derivatives of chlorin e6 (Ce6). NMR spectroscopy proved to be a powerful tool to describe the aggregate structures [4] and observe DDV encapsulation [2, 3]. However, for the more hydrophobic Ce6 derivatives Ce6-dimethyl- (Ce6 DME) and Ce6-trimethyl-ester (Ce6 TME) the polymer-based systems only exhibited limited encapsulation efficiency and other studies suggested the use of phospholipid-based nanoparticles as DDV [5].

The aim of the current study was to explore the suitability of phospholipid (PL)-based bicelles as DDV's and compare their DDV-relevant properties with those of the corresponding PL-micelles and PL-liposomes. Dihexanoyl-phosphatidylcholine (DHPC) micelles and dimyristoyl-phosphatidylcholine (DMPC)-DHPC bicelles (q=0.5) were characterized and compared as DDV for chlorin e4 (Ce4), Ce6 DME and Ce6 TME in PBS medium. Micelles showed loading of Ce4 which could be monitored by UV-VIS- as well as NMR-spectroscopy. Furthermore, encapsulation of Ce6 DME in micelles was observed by NMR measurements whereas Ce6 TME was not encapsulated and precipitated immediately. In the bicelles all three chlorins exhibited encapsulation as observed by NMR. Specifically, the interaction of the chlorin molecule with the DMPC moiety was visible through DMPC resonance broadening and intermolecular NOEs, indicating a high affinity for the long chain PL. The systems will be further investigated with respect to their photophysical properties and the results compared to DMPC-liposomes as a third carrier structure. To assess which DDV-chlorin system exhibits the most promising results, HeLa-cell studies will be included. While encapsulation of the chlorin is required in a first step, efficient cell uptake and drug release are equally important features. We hypothesize that the bicelles being capable of loading all porphyrinic compounds tested compared to micelles and having a less rigid structure than liposomes might be the "golden mean" of the three PL-carrier vehicles.

[1] F. Moret, E. Reddi, *J. Porphyrins Phthalocyanines*, **2017**, 21, 239-256.
[2] M. Hädener, I. Gjuroski, J. Furrer, M. Vermathen, *J. Phys. Chem. B*, **2015**, 119, 12117-12128.
[3] I. Gjuroski, J. Furrer, M. Vermathen, *ChemPhysChem*, **2018**, 19, 1089-1102.
[4] M. Vermathen, M. Marzorati, P. Bigler, *J. Phys. Chem. B*, **2013**, 6990−7001.
[5] T. E. Zorina, I. V. Yankovsky, I. E. Kraychenko, T. V. Shman, M. V. Belevtsev, V. P. Zorin, *Biophysics*, **2015**, 60, 759-766.

Synthesis of new building blocks from the chemical universe database GDB

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The identification of new building blocks in medicinal chemistry is of high interest since these could not only lead to an enhancement of activity and selectivity for a biological target, but they also represent major opportunities in the field of intellectual property. Here we focused our attention on cyclic diamines, which represent privileged building blocks not only for medicinal chemistry but also for coordination chemistry or catalysis.[1] Comparing known diamines from public databases with those in the chemical universe database GDB-13, which lists all possible up to 13 atoms following simple rules of synthetic feasibility and chemical stability,[2-4] shows that the vast majority of possible bi- and tricyclic diamines are completely unknown either as exact molecule or as substructure. Here we present different synthetic strategies leading to simply yet novel bi- and tricyclic diamines, with focus on those containing quaternary centres embedded within chiral ring systems such as the trinorbornane ring system, which is a defining feature of GDB molecules particularly neglected among known molecules.[4-6]

Fig. 1. Examples of polycyclic ring systems from the GDB database.[4]

[1] O. O. Grygorenko, D. S. Radchenko, D. M. Volochnyuk, A. A. Tolmachev, I. V. Komarov, *Chem. Rev.* **2011**, *111*, 5506-5568
[2] L. C. Blum, J.-L. Reymond, *J. Am. Chem. Soc.* **2009**, *131*, 8732-8733
[3] M. Awale, R. Visini, D. Probst, J. Arús-Pous, J.-L. Reymond, *Chimia* **2017**, *71*, 661-666
[4] T. Fink, J.-L. Reymond, *J. Chem. Inf. Model.* **2007**, *47*, 342-353
[5] L. D. Bizzini, T. Müntener, D. Häussinger, M. Neuburger, M. Mayor, *Chem. Commun.* **2017**, *53*, 11399-11402
[6] R. Visini, J. Arús-Pous, M. Awale, J.-L. Reymond, *J. Chem. Inf. Model.* **2017**, *57*, 2707-2718

Second Generation Fidaxomicin Derivatives: Design, Synthesis and Biological Evaluation

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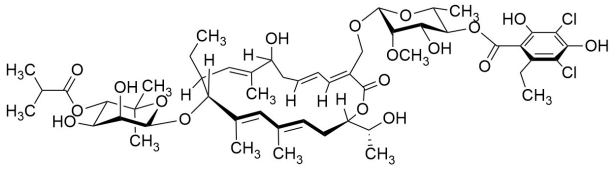
Fidaxomicin (**1**, tiacumicin B, lipiarmycin A3) is a marketed drug for the treatment of intestine infections caused by *Clostridium difficile*. It constitutes an aromatic glycosylated macrolide and was isolated from various strains of soil bacteria.[1] It shows good activity against many Gram-positive bacteria, including some resistant strains of *Mycobacterium tuberculosis*.[2] In 2015 our research group accomplished the first total synthesis[3] 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. Investigations of the mechanism of action revealed that Fidaxomicin binds to the RNA-polymerase (RNAP) and consequently inhibits the transcription.[4] Based on computational simulations on a homology-model of *Clostridium difficile* RNAP, we synthesized new semisynthetic Fidaxomicin analogs for SAR-studies, improved water-solubility and further mechanistic investigations.



Fidaxomicin (**1**, tiacumicin B, lipiarmycin A3)

[1] Review: William Erb, Jieping Zhu, *Natural Product Reports*, **2013**, 30, 161–174.
[2] Mekonnen Kurabachew, Stephen H. J. Lu, Philipp Krastel, Esther K. Schmitt, Bangalore L. Suresh, Anne Goh, John E. Knox, Ngai Ling Ma, Jan Jiricek, David Beer, et al., *Journal of Antimicrobial Chemotherapy*, **2008**, 62, 713–719.
[3] Elias Kaufmann, Hiromu Hattori, Hideki Miyatake-Ondozabal, Karl Gademann,*Organic Letters*, **2015**, 17, 3514–3517; Synthetic studies: Louis Jeanne-Julien, Guillaume Masson, Eloi Astier, Grégory Genta-Jouve, Vincent Servajean, Jean-Marie Beau, Stéphanie Norsikian, Emmanuel Roulland, *Journal of Organic Chemistry*, **2018**, 83, 921–929 and references therein.
[4] Hande Boyaci, James Chen, Mirjam Lilic, Margaret Palka, Rachel Anne Mooney, Robert Landick, Seth A. Darst, Elizabeth A. Campbell, *eLife*, **2018**, 7, 1–19; Wei Lin, Kalyan Das, David Degen, Abhishek Mazumder, Diego Duchi, Dongye Wang, Yon W. Ebright, Richard Y. Ebright, Elena Sineva, et al. *Molecular Cell*, **2018**, 70, 1–12 and references therein.

Impact of drug delivery systems on cell-uptake and photophysical properties of chlorin e6-based photosensitizers

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Porphyrin and chlorin based compounds are used as photosensitizers (PS) in photodynamic therapy (PDT) due to their ability to produce reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) upon light excitation (~400−700 nm). However, the efficacy of many hydrophobic PS is diminished in aqueous solutions because of self-aggregation, which reduces their fluorescence lifetimes and $^1O_2$ quantum yields. Therefore, the application of drug delivery systems has become an integral part in the development of more efficient PDT drugs. Ideally, the delivery system should be capable to monomerize the PS, enhance its cellular uptake and at the same time ensure favourable photophysical properties, i.e. high fluorescence and $^1O_2$ quantum yield. [1]

The aim of the current study is to systematically investigate how these photophysical properties and cellular uptake correlate with monomerization and encapsulation efficiency for selected systems. For this, a series of chlorin e6 (CE6) derivatives (xCE) with increasing hydrophobicity has been combined with different types of polymeric carriers including single molecule carriers such as polyvinylpyrrolidone (PVP), block-copolymer micelles (BCMs) [2] and phospholipid-based nanoparticles. The dark- and phototoxicity of xCE was determined with HeLa cells in vitro. Using CE6 as a reference xCE, e.g. serine-CE6 (SerCE), an amphiphilic chlorin, or chlorin e4 (CE4), a hydrophobic PS, showed high phototoxicity in the low micromolar range. Furthermore, flow cytometry (Image Stream) studies have been performed to determine the effect of PVP and BCM-based carriers on the cellular uptake of xCE. The more hydrophobic PS, CE4 and CE6-dimethylester (CEDME), benefitted greatly from encapsulation whereas the amphiphilic SerCE did not exhibit higher cellular uptake despite good monomerization. To determine whether the PS remains in monomerized form within the cell or if self-aggregation reoccurs, fluorescence lifetime microscopy will be performed. The monomerized form of PSs shows longer fluorescent lifetimes and typically results in higher singlet oxygen quantum yields. As a first step, time correlated single photon counting (TCSPC) is currently being used to measure the effect of encapsulation on the fluorescence lifetimes of xCE in solutions. In addition, the phosphorescence of singlet oxygen (emission 1270 nm) will be measured to further characterize the aggregation state of the previous mentioned PSs. Furthermore, the intracellular localisation and PS association are currently being elucidated using confocal microscopy to gain a better understanding of where within a cell the PS resides.

[1] Sasidharan Swarnalatha Lucky, Khee Chee Soo, Yong Zhang, *Chem. Rev*. **2015**, 115, 1990−2042.
[2] Ilche Gjuroski, Julien Furrer, Martina Vermathen, *ChemPhysChem*, **2018,** 19, 1089−1102.

Shaping the centromere – role of CCAN proteins in determining 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 in the form of long connected loops or superhelices.

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 labeled 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 CENP-A binding proteins contribute to defining the centromere chromatin structure. Overall, these studies are designed to reveal the unique organization of the centromeric chromatin.

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

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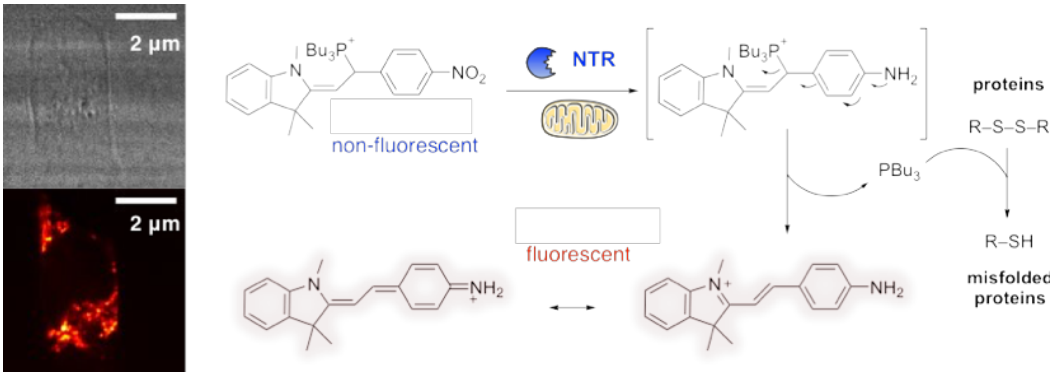
Mitochondria are responsible for the production of ATP through cellular respiration,[1] formation of reactive oxygen species (ROS), calcium signaling,[2] and apoptosis.[3] Disruption of mitochondrial redox state leads to pathologies such as insulin resistance, obesity and type II diabetes.[4] Mitochondria sense and respond to redox stress through activation of the mitochondrial unfolded protein response (^{mt}UPR).[5] In more severe cases, mitophagy,[6] a quality control mechanism that degrades impaired mitochondria through organelle-selective autophagy is triggered. Previously, we demonstrated that a photoactivatable trialkylphosphine mimics certain aspects of reductive stress, such as increased concentration of free thiols and protein misfolding and aggregation.[7] We developed a probe that could achieve mitochondria-specific reductive stress by taking advantage of the activity of mitochondrial enzymes to trigger the release of trialkylphosphine. Preliminary experiments demonstrated that trialkylphosphine activation induces mitochondrial reductive stress that lead to dramatic morphological changes, but do not trigger canonical mitophagy.[8]



[1] M.-C. Frantz, P. Wipf, *Environ. Mol. Mutagen.* **2010**, 51, 462–475.
[2] N. Kaludercic, S. Deshwal, F. Di Lisa, *Front. Physiol.* **2014**, 5, 285.
[3] H. Xiao, P. Li, X. Hu, X. Shi, W. Zhang, B. Tang, *Chem. Sci.* **2016**, 7, 6153–6159.
[4] J. Szendroedi, E. Phielix, M. Roden, *Nat. Rev. Endocrinol.* **2012**, 8, 92–103.
[5] C. Münch, J. W. Harper, *Nature* **2016**, 534, 710–713.
[6] R. J. Youle, D. P. Narendra, *Nat. Rev. Mol. Cell Biol.* **2011**, 12, 9–14.
[7] A. Tirla, P. Rivera-Fuentes, *Angew. Chem. Int. Ed.* **2016**, 55, 14709–14712.
[8] J. Nguyen, A. Tirla, P. Rivera-Fuentes, *manuscript in preparation*.

Activated Charcoal Promotes Surgical Wound Healing Effects of Musa Sapientum and Citrus Limon Peel Gel in Rattus Novergicus

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*Musa sapientum*known as banana is used as source of staple food for millions world over. Similarly, lemon, *Citrus limon(L)*is a specie of small evergreen tree in the family *Rutaceae*. The purpose of this study was to assess the effects of combining activated charcoal with banana and lemon peel gel on the healing of surgical wound s in rats . 36 wistar rats were divided into nine groups of 4 rats each. Wound control (Paraffin base) , Standard (Povidone iodine) , experimental groups(4% w/w *Citrus limon*, *Musa sapientum*gel ointment) and (activated charcoal mixed with *Citruslimon* peel and or *Musa sapientum*peel). Surgical wound of 40mm X 40mm was created dorsally on each rat, cleaned daily with 0.9% saline, treated with the formulated drugs. Measurement of wound contractions were done on 4, 8, and 12 days of the experiment. Wound contraction rates were found to be higher in wound treated with *Citrus limon* and *Musa sapientum* gel ointment formulated with activated charcoal . Order of increasing wound closure (unripe *M.sapientum* gel...> ripe *M.sapientum* gel ...> activated charcoal + ripe *M.sapientum* gel .....> activated charcoal + unripe *M. sapientum*gel .....> unripe *M.sapientum* gel + *C.limon* gel ....>activated charcoal + *C. limon* gel. Wound healing elicited by the drugs in this investigation following topical application clearly indicates that activated charcoal enhanced wound healing effects of *M. sapientum* and *C. limon* gel .

Key words: *Musa sapientum*, *Citrus limon*, activated charcoal , rats

Photocontrol of antimicrobial activity and cytotoxicity with green light and smart materials

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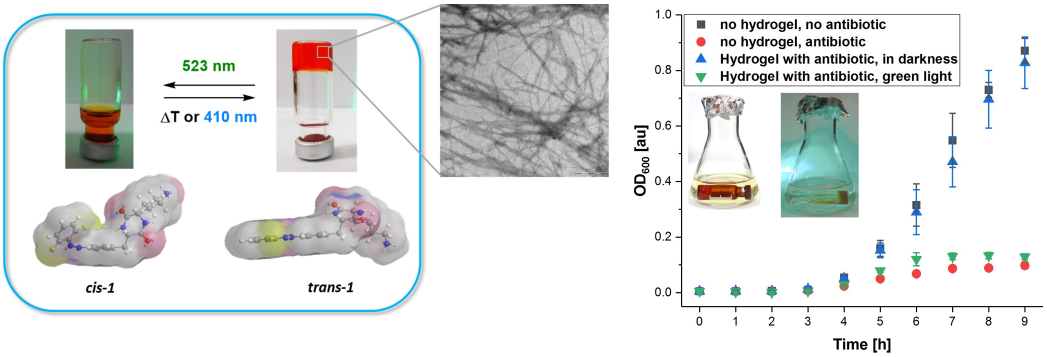
Here we report two photoresponsive drug-releasing supramolecular systems based on azobenzene molecules coupled with cyclic dipeptides. A prototypic self-healing hydrogel [1] reversibly turns into fluid (sol) upon UV light irradiation. After irradiation with 365 nm LED diode, the gel can selectively release previously encapsulated guest molecules, like an anticancer drug doxorubicin or long dsDNA. The use of UV light in biological systems is, however, limited by low tissue penetration and, to some extent, its cytotoxicity. By appending additional fluorine atoms on the photoswitch [2], visible light can be used for isomerization. We exploited this observation to create the second-generation hydrogel that can be activated with green light and is more suitable for *in vivo* applications. We demonstrated photocontrolled release of structurally unmodified antibiotic, anticancer, and anti-inflammatory drugs under physiological conditions. Using the antibiotic-loaded gel, we selectively inhibited bacterial growth with green light. Moreover, by exploring alternative gel loading strategies we discovered a photochromic compound that exhibits over 15-fold increase in cytotoxicity against mammalian cells upon irradiation with green light, and can be regarded as a prospective photochemotherapy agent [3,4].



Left: reversible transition of hydrogel into sol; **right**: green-light-induced antibiotic activity

[1] Z. Pianowski, J. Karcher, K. Schneider, *Chem. Commun.* **2016**, *52*, 3143-3146.
[2] D. Bleger, J. Schwarz, A. M. Brouver, S. Hecht *J. Am. Chem. Soc.* **2012**, *134*, 20597-20600.
[3] Z. Pianowski, J. Karcher, K. Schneider, *patent* DE 10 2015 014 834 A1
[4] J. Karcher, Z. Pianowski, "Photocontrol of Antimicrobial Activity and Cytotoxicity with Green Light", *full paper, submited*

Molecular Tools for the Cellular Study of Adenosine A_1 Receptors

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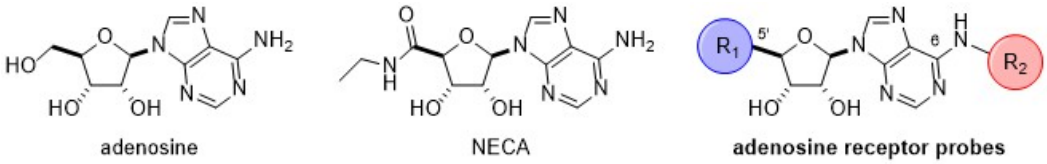
The purinergic nucleoside adenosine acts as an important signalling molecule by exerting its agonist activity at all subtypes of the adenosine receptors (ARs), which belong to the family of G protein coupled receptors (GPCRs). These subtypes A_1, A_{2A}, A_{2B} and A_3 have a wide and varying tissue distribution and are implicated in cardiovascular, respiratory, inflammatory and neurological disorders. In human cells, A_1R and A_3R mainly couple to the $G\alpha_i$ subunit inhibiting the production of cAMP, while A_2R subtypes couple to the $G\alpha_s$ elevating cAMP levels [1]. Because the same ligand is giving the opposite effect it is important to discover subtype selective ligands that could represent useful tools in the purinergic signalling research and warrant further assessment of their therapeutic potential.

We have previously discovered several potent and A_1R-selective agonists that are based on the adenosine and NECA structures [2]. These adenosine derivatives were modified at the N^6-position of the purine ring and at the C-5’ position of the ribose moiety. Here, we report the improved synthesis of a series of these A_1R-selective agonists. In particular, we have optimised the key S_NAr-reaction to introduce substituents at the purine C-6 position by using microwave chemistry among other things. Furthermore, we have synthesised diastereoisomers of some of our A_1R-selective agonists and assessed their biological activity. We also present some fluorescent adenosine and NECA derivatives that might be interesting tools to study the trafficking and activation of A_1R.



[1] Sachdeva, S.; Gupta, M. Adenosine and its receptors as therapeutic targets: an overview. *Saudi Pharm. J.* **2013**, 21, 245−253.
[2] Knight, A et al., *J. Med. Chem.* **2016**, 59, 947.

Fast approximate tree representations of high-dimensional chemical spaces using minimum spanning trees

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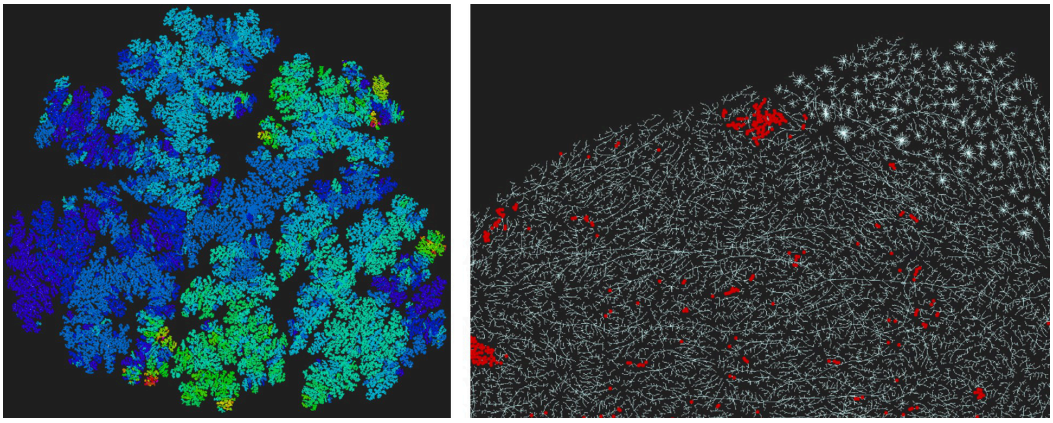
Embeddings of high-dimensional spaces into the plane are a challenging task due to inevitable distortions[1]. Dimensionality reduction methods such as PCA or t-SNE that leverage the effect of distortions are widely applied throughout a wide range of scientific fields. Here we introduce an algorithm for the embedding of chemical (metric) spaces into a tree metric using k-nearest-neighbour graphs and minimum spanning trees[2]. Compared to similar approaches based on the neighbour joining algorithm that is limited to thousands of data points, our solution can process databases such as ChEMBL (1.7 million distinct compounds) in their entirety and in full resolution, providing an alternative to sub-setting, clustering or binning. Moreover, the presented solution produces an unrooted tree where also non-leaf nodes convey meaningful information[3].



Based on our previous work on large data sets, the resulting tree embeddings can be interactively visualized on the web[4,5].

[1] Fakcharoenphol, J., Rao, S. and Talwar, K., *Journal of Computer and System Sciences*, **2004**, 69(3), 485-497
[2] Kruskal, J.B *Proceedings of the American Mathematical society*, **1956**, 7(1), 48-50
[3] Lu, J. and Carlson, H.A., *Bioinformatics*, **2016**, 32(23), 3584-3592
[4] Probst, D. and Reymond, J.L., *Journal of chemical information and modeling*, **2017**, 58(1),1-7
[5] Probst, D. and Reymond, J.L., *Bioinformatics*, **2018**, 34(8), 1433–1435

Strategies to crystallize the 1st human ribozyme: the CPEB3 ribozyme and its mutants

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The *Hepatitis delta virus* (HDV) ribozyme is one of the best studied ribozymes. The native fold of the ribozyme is complicated, having **two pseudoknots** involving five helical regions (P1.1, P1, P2, P3, and P4) [1]. The HDV ribozyme has a **remarkably stable structure**, demonstrating self-scission activity in up to 18 M formamide condition [2]. Until 2006 the HDV ribozyme was considered to be the only representative of a small ribozyme with such a complex fold. However a genome-wide search identified the human **cytoplasmic polyadenylation element-binding protein 3 (hCPEB3) ribozyme** as the first of the HDV-like ribozymes [3]. Since this day, the HDV-like ribozyme family has largely expanded. Indeed, the HDV-like ribozymes are found in nearly all branches of life [4]. These ribozymes display the secondary structure in a nested double-pseudoknot with only six conserved nucleotides fulfilling functional or structural roles.

In order to better understand the complex folding of HDV-like ribozymes and to locate the metal ions involved in that particular fold, we would like to solve the **three-dimension structure** of the human CPEB3 ribozyme by **X-ray crystallography.** In order to stabilize the structure of the hCPBE3 and to improve its crystallization, we started with two different strategies: (i) introduction of one supplementary base pair in P2 helical region of the hCPEB3 ribozyme and (ii) introduction of spliceosomal U1A protein binding site as a crystallization module [1]. Our first results pinpoint several outlines towards the crystallization of the hCPEB3 ribozyme: (i) the short leader sequence above the cleavage site in required for selection of catalytically active ribozymes, (ii) construct with the U1A binding site within the P4 region is still catalytically active thus indicating that internal folding of the hCPEB3 ribozyme is not disturbed, (iii) the binding of the U1A protein to the hCPEB3 ribozyme was successful and the complex remain stable, (iv) the first crystals of the complex were obtained.

[1] Ferré-D'Amaré A. R. & Doudna J.A., *Nature*, **1998**, 395, 567-574
[2] Sarah P. Rosenstein and Michael D. Been, *Biochemistry,* **1990**, 29 (35), 8011-8016.
[3] Salehi-Ashtiani, K. A., *Science*, **2006**, 313, 1788–1792.
[4] Webb, C.H. et al., *Science*, **2009**, 326, 953

Manufacturing of Novel Ultra-High Molecular Weight Branched Biopolymers for use with Antibody Based Bioconjugate Therapies

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Purpose: Development of an aqueous, optically clear biocompatible polymer platform that can pair with therapeutic antibodies to generate next generation therapies with enhanced durability and stability as compared to current agents.

Methods: We designed a multi-branched polymerization initiator and studied polymerizations with methacrylate-based phosphorylcholine containing monomers with the goal of producing very large (>2,000 monomers) water-soluble polymers with controlled molecular weight, polydispersity, composition, chain architecture, and site-specific functionality. The desired antibodies of choice can be attached to the biopolymer via a stable covalent linkage. Key success factors were the parallel optimization of initiator architecture, monomer features and the fine-tuning of controlled/living polymerization chemical process parameters.

Results: A manufacturing process was developed and successfully applied in the production of a well characterized custom biopolymer under current good manufacturing practices on batch scales approaching 10 kilograms. This is sufficient manufacturing scale to support clinical development through early commercialization of Kodiak’s ophthalmic biologics pipeline.

Conclusions: A novel biopolymer platform was developed that features a high molecular weight phosphorylcholine based biopolymer that is manufactured with high-fidelity and allows for the development of well-characterized, optically clear antibody based bioconjugate therapies.

Anti-cancer activity of metallodrug RAPTA-C in combination with repurposed medication

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RAPTA-C is a ruthenium-based experimental compound presenting anti-cancer, anti-metastatic and anti-angiogenic activities with a good safety profile. Therefore, it seems to be a promising alternative to the platin-based anti-cancer drugs for cancer treatment.

In our screen for possible combinations of RAPTA-C with clinically approved drugs used for non-oncological indications, we used a simple *in vitro* screen for cell viability performed in human colorectal and renal carcinoma cell lines, as well as in epithelial non-cancerous cells. The effects on cell viability, cell cycle analysis and expression of apoptotic markers for the drug combinations chosen for each cell line were measured.

RAPTA-C itself administered at low doses did affect cell viability or cell cycle. However, in combination with one or two repurposed drugs, the synergistic tumor cell viability inhibition was observed together with the cell cycle regulation leading to interesting cell death fate.

Sesquiterpene lactones from Artemisia argyi - determination of absolute configurations and immunosuppressive activity

J. K. Reinhardt[1], A. M. Klemd[2], M. De Mieri[1], M. Smieško[1], R. Huber[2], T. Bürgi[3], C. Gründemann[2]*, M. Hamburger[1]*

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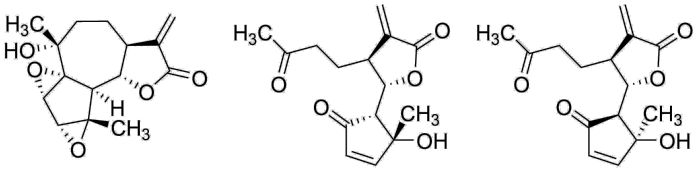
Autoimmune diseases like rheumatoid arthritis or multiple sclerosis are characterized by an increased T cell proliferation.[1] Since no causal therapies are currently available, autoimmune diseases are typically treated with immune suppressive drugs possessing severe side effects.[2] Therefore, there is a need for new and better tolerated treatment options, which could be found among natural products.[3] A library of 435 extracts from plants used in Traditional Chinese Medicine (TCM) was screened. Cell division progress was determined by staining of T lymphocytes with carboxyfluorescein diacetate succinimidyl ester and flow cytometric analysis. The ethyl acetate extract of *Artemisia argyi* Levl. et Vant. significantly inhibited T lymphocyte proliferation *in vitro*. HPLC based activity profiling lead to the targeted isolation of three stereoisomers of canin and two *seco*-tanapartholides that significantly inhibited T lymphocyte proliferation (IC_{50} 0.30-0.96 µg/ml). Using Electronic Circular Dichroism (ECD), the ACs of the three stereoisomers of canin were determined as 1*R*-canin (**1**), 1*S*-10-*epi*-artecanin and 1*S*-artecanin. While the structures of canin and artecanin were known, the structure of 10-*epi*-artecanin was confirmed for the first time in this work. For the *seco*-tanapartholides, ECD gave ambiguous results. Thus, the ACs were determined via Vibrational Circular Dichroism (VCD) as [4R,5R,6S,7S]-seco-tanapartholide A (**2**) and [4S,5S,6S,7S]-seco-tanapartholide B (**3**).ECD and VCD proved to be a powerful combination for establishing the absolute configurations of flexible compounds such as *seco*-tanapartholides A and B. Both compounds showed promising activity *in vitro* and possess drug like properties showing no rule-of-five violations



1 **2** **3**

[1] D. D. Chaplin, *J. Allergy Clin. Immunol,* **2010**, 125, 3-23.
[2] M. Her, *et. al., J. Allergy Clin. Immunol,* **2016**, 137 (1), 19-27.
[3] G. Appendino *et. al.*, *Comprehensive Natural Products II*. Elsevier, Oxford, **2010**, 205-236.

A novel bioorthogonal ligation method and its application for in vivo glycan labelling

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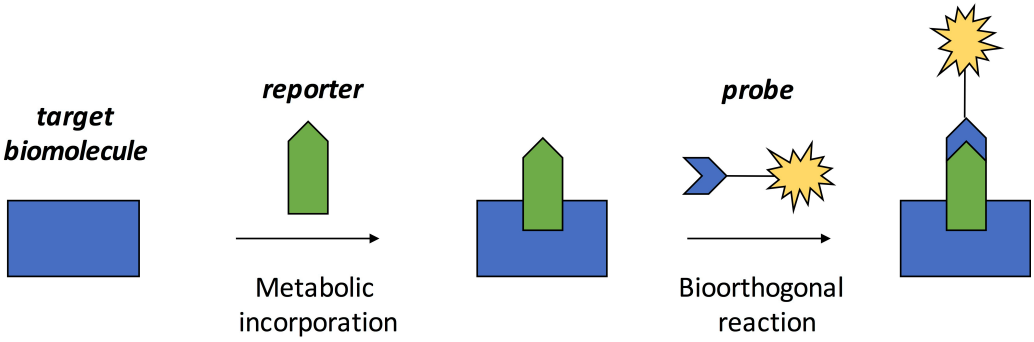
[1]ETH Zurich

The development of chemical reactions suitable for application in biological environments is important for the study of biomolecules in living systems and thus the elucidation of complex biological processes.[1] The "chemical reporter strategy" represents one of the most powerful methods for the labeling of biomolecules in their native environment.[2] This strategy utilizes the cell's own biosynthetic machinery to incorporate non-natural metabolites with unique functional groups into specific cellular components. In the second step these metabolites, 'reporters', are selectively targeted with a complementary functional group that is bound to a detectable probe. Thus, this method allows for the visualization and isolation of specifically targeted biomolecules such as glycans,[3] lipids[4] and proteins.[5] Despite recent improvements in reactivity and selectivity of bioorthogonal reactions, limitations remain. Here we present a new type of bioorthogonal ligation with high reactivity and selectivity. This new ligation method works under physiological conditions and is orthogonal to most commonly used bioorthogonal ligation methods including strain promoted azide alkyne cycloaddition (SPAAC),[6] copper catalyzed azide alkyne cycloaddition (CuAAC)[7] and the Staudinger ligation.[8] The selectivity and applicability of this new ligation method was demonstrated both in *in vitro* and *in vivo* by imaging metabolically labelled glycans on cell membranes.



[1] David M. Patterson, Lidia A. Nazarova, Jennifer A. Prescher, *ACS Chem. Biol.*, **2014**, 9, 592-605.
[2] Jennifer A. Prescher, Carolyn R. Bertozzi, *Nat. Chem. Biol.*, **2005**, 1, 13-21.
[3] Lara K. Mahal, Kevin J. Yarema, Carolyn R. Bertozzi, *Science,* **1997**, 276, 1125-1128.
[4] Yoonjung Kho, Sung C. Kim, Chen Jiang, Deb Barma, Sung W. Kwon, Jinke Cheng, Janis Jaunbergs, Carolyn Weinbaum, Fuyuhiko Tamanoi, John Falck, Yingming Zhao, *Proc. Natl. Acad. Sci. U. S. A.,* **2004**, 101, 12479-12484.
[5] B. Albert Griffin, Stephen R. Adams, Roger Y. Tsien, *Science,* **1998**, 281, 269-272.
[6] Nicholas J. Agard, Jennifer A. Prescher, Carolyn R. Bertozzi, *J. Am. Chem. Soc.,* **2004**, 126, 15046-15047.
[7] Qian Wang, Timothy R. Chan, Robert Hilgraf, Valery V. Fokin, K. Barry Sharpless, M. G. Finn, *J. Am. Chem. Soc.,* **2003**, 125, 3192-3193.
[8] Eliana Saxon, Carolyn R. Bertozzi, *Science,* **2000**, 287, 2007-2010.

Study of 2-aminoethoxydiphenyl borate (2-APB) analogues in MDA-MB-231 and MCF-7 cells towards Orai isoform-specific modulation of store-operated calcium entry (SOCE)

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Calcium signalling is crucial for various physiological functions such as cell differentiation, proliferation, muscle contraction, neurotransmission and fertilization. The endoplasmic reticulum (ER) is the main intracellular calcium store in eukaryotic cells. The ER calcium store is refilled by the interplay of two types of membrane proteins: the calcium sensing stromal interaction molecule (STIM1/2), located in the ER membrane and the plasma membrane resident Orai1/2/3. [1]

The isoforms of Orai proteins, Orai1, 2 and 3, exhibit different expression levels in a variety of cells. Specific Orai isoforms were reported to be upregulated in various cancer types and thus they are interesting targets for pharmacological studies. Consequently, Orai isoform specific modulators would prove to be beneficial tools for such studies.

In our effort to find Orai isoform specific modulators we screened our previously published library of 2-aminoethoxydiphenyl borate (2-APB) derivatives [2]. For this screening we used the breast cancer cell lines MDA-MB-231 and MCF-7 which were reported to accomplish calcium entry after store-depletion mainly through Orai1/STIM1 and Orai3/STIM1, respectively [3]. Based on the results of the screening, we designed, synthesized and tested several new 2-APB analogues.

[1] M. Prakriya, R.S. Lewis, *Phys. Rev.* **2015**, *95*, 1383-1436.
[2] A. Hofer, G. Kovacs, A. Zappatini, M. Leuenberger, M.A. Hediger, M. Lochner, *Bioorg. Med. Chem.* **2013**, *21*, 3202–3213.
[3] R. K. Motiani et al. *J. Biol. Chem.* **2010**, *285*, 19173-19183.

Long-lasting and fast-acting in vivo efficacious antiplasmodial azepanylcarbazole amino alcohol.

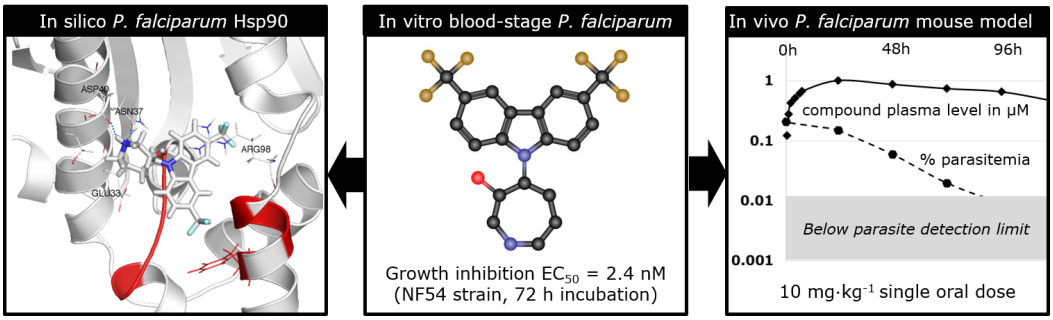
T. Spangenberg[1]

[1]Coinsins, VD, Switzerland

Nada Abla,[1,3] Sridevi Bashyam,[4] Susan A. Charman,[8] Béatrice Greco,[1] Philip Hewitt,[2] Maria Belén Jiménez-Díaz,[6†] Kasiram Katneni,[8] Holger Kubas,[2] Didier Picard,[9] Yuvaraj Sambandan,[4] Laura Sanz,[6] Dennis Smith,[7] Tai Wang,[9††] Paul Willis,[3] Sergio Wittlin,[5] Thomas Spangenberg[1]*

[1]Merck Global Health Institute, Ares Trading S.A., a subsidiary of Merck KGaA (Darmstadt, Germany), Coinsins, Switzerland, [2]Merck, KGaA, Darmstadt, Germany, [3]Medicines for Malaria Venture, Geneva, Switzerland, [4]Syngene International, Bangalore, India, [5]Swiss TPH, Basel, Switzerland, [6]GSK,Tres Cantos, Spain, [7]MMV Expert Scientific Advisory Committee; [8]Centre for Drug Candidate Optimisation, Monash University, Parkville, VIC, Australia ; [9]Département de Biologie Cellulaire, Université de Genève, Sciences III, 30 quai Ernest-Ansermet, CH-1211, Genève 4, Switzerland.

With ~429,000 deaths in 2016, malaria remains a major infectious disease where the need to treat the fever symptoms, but also to provide relevant post-treatment prophylaxis, is of major importance. An azepanylcarbazole amino alcohol is presented with a long- and fast-acting in vivo antiplasmodial efficacy and which meets numerous attributes of a desired post-treatment chemoprophylactic antimalarial agent. The synthesis, the parasitological characterization, the animal pharmacokinetics and pharmacodynamics of this compound are discussed along with a proposed target.



A Crystal Structure of EgtB, from Ergothioneine Biosynthesis, reveals an Alternative Catalytic Architecture to Catalyze an Identical Reaction

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Oxygen activation and C-H bond cleavage are chemically challenging reactions. Numerous enzymes, primarily iron-dependent, catalyze such reactions. Yet our understanding of the mechanism by which these enzymes achieve such transformations is poorly understood. EgtB is an iron dependent mono-oxygenase that catalyses the central step in the biosynthesis of Ergothioneine (Fig 1.), a thiourea derivative of histidine with antioxidant properties. The discovery of EgtB represents the creation of a new class of iron oxygenases, the sulfoxide synthases, with a novel structure and distinct mechanism. Structural and kinetic characterization of a new cysteine utilizing EgtB has revealed a novel catalytic architecture, unique to that of other known EgtBs, to catalyze an identical reaction. This alternative active site unveils a novel mechanistic strategy for O_2 activation, augmenting Nature's arsenal for such transformations. This active site plasticity provides us with a powerful and unique approach to tease out mechanistic features and to isolate the core catalytic principles of these catalysts *via* comparative enzymology.

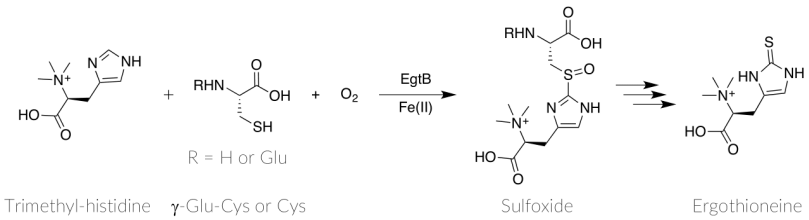

Figure 1: EgtB is an iron-dependent oxygenase that catalyses sulfoxidation as the central step in the biosynthesis of Ergothioneine.

Mechanosensitive membrane probes: Let's twist again

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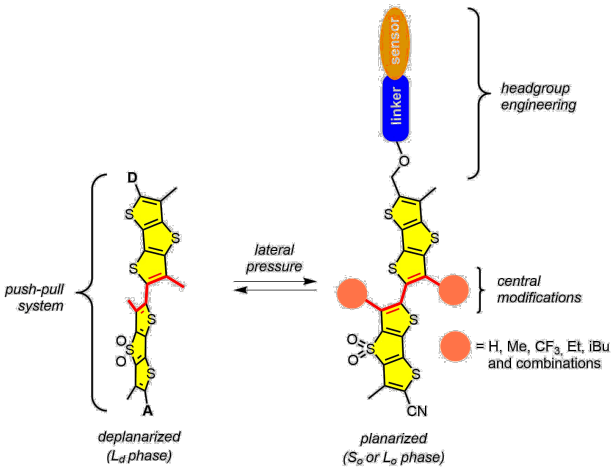
[1]Department of Organic Chemistry, University of Geneva, [2]Biochemistry Department, University of Geneva

Considering the biological relevance of understanding the behaviour of cellular membranes, conceptually innovative mechanosensitive "flipper" probes have been developed. These fluorescent push-pull dithienothiophene dimers can sense forces and differences in lateral organization of membranes by a combined effect of ground state planarization and polarization.[1]

To understand better the relationship between structure and function in terms of mechanosensitivity and spectroscopic properties, our flipper probes have been tailored almost atom by atom. Our attention is now focused on modifying the central region of the probe to tune the twist of the molecule,[2] and varying the push-pull system of the chromophore.[3] Furthermore, a broad headgroup engineering with the aim to target our probes to specific locations or create a turn-on probe is ongoing.[4,5]



[1] Marta Dal Molin, Quentin Verolet, Adai Colom, Romain Letrun, Emmanuel Derivery, Marcos Gonzalez-Gaitan, Eric Vauthey, Aurélien Roux, Naomi Sakai, Stefan Matile, *Journal of the American Chemical Society*, **2015**, 137, 568–571.
[2] Mariano Macchione, Nicolas Chuard, Naomi Sakai, Stefan Matile, *ChemPlusChem*, **2017**, 82, 1062–1066.
[3] Quentin Verolet, Marta Dal Molin, Adai Colom, Aurélien Roux, Laure Guénée, Naomi Sakai, Stefan Matile, *Helvetica Chimica Acta*, **2017**, 100, e1600328.
[4] Saeideh Soleimanpour, Adai Colom, Emmanuel Derivery, Marcos Gonzalez-Gaitan, Aurélien Roux, Naomi Sakai, Stefan Matile, *Chemical Communications*, **2016**, 52, 14450–14453.
[5] Karolina Strakova, Saeideh Soleimanpour, Marta Diez-Castellnou, Naomi Sakai, Stefan Matile, *Helvetica Chimica Acta*, **2018**, in press.

Metabolic Labeling and Selective Cross-linking of Duplex DNA

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A wide variety of bioorthogonal chemical reactions can be used for post-(bio)synthetic modification of nucleic acids in vitro and in vivo.[1] Among these, azide-containing nucleosides offer attractive reactivities because they can be detected using strain-promoted azide-alkyne cycloaddition (SPAAC) reactions that do not require a toxic Cu(I) catalyst. To date, metabolically incorporated DNA analogs have not been labelled in the native environment of duplex DNA due to steric hindrance. To overcome the limited reactivity of nucleoside analogs in duplex DNA, we focused on 5-(azidomethyl)-2'-deoxyuridine (AmdU) and dibenzocycloocta-1,5-diyne (CODY), known as Sondheimer diyne.[4] The application of Sondheimer diyne to azide-modified biomolecules has been limited to cell-surface and cell-free systems due to its poor water solubility.[5] Here we designed and synthesized a water-soluble Sondheimer diyne derivative ("DiMOC") by introducing cationic, hydrophilic side chains. DiMOC showed good solubility in buffers (> 10 mM) and was able to intercalate into duplex DNA and thereby gain access to azide groups for two, highly efficient SPAAC reactions. Furthermore, DiMOC facilitated the formation of DNA-DNA interstrand cross links via double SPAAC reaction in both cell-free and cell-based system.

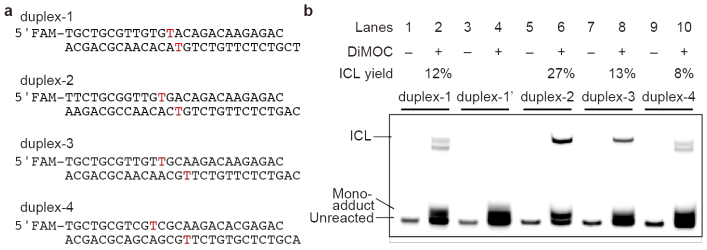

Figure. Inter-strand cross-linking of the azide-modified duplex DNA by DiMOC. (a) Duplex sequences containing AmdU-modification (in red letters). (b) Inter-strand cross-linking detection by PAGE analysis. Duplex-1' has only single azide-modification on the FAM-labeled sequence.

[1] J. T. George, S. G. Srivatsan, *Methods* **2017**, *120*, 28.
[2] A. B. Neef, N. W. Luedtke, *ChemBioChem* **2014**, *15*, 789.
[3] X. Ren, A. H. El-Sagheer, T. Brown, *Analyst* **2015**, *140*, 2671.
[4] H. N. C. Wong, P. J. Garratt, F. Sondheimer, *J. Am. Chem. Soc.* **1974**, *96*, 5604.
[5] I. Kii, A. Shiraishi, T. Hiramatsu, T. Matsushita, H. Uekusa, S. Yoshida, M. Yamamoto, A. Kudo, M. Hagiwara, T. Hosoya, *Org. Biomol. Chem.* **2010**, *8*, 4051.

Hypervalent iodine reagents for fast and efficient peptide and protein labelling

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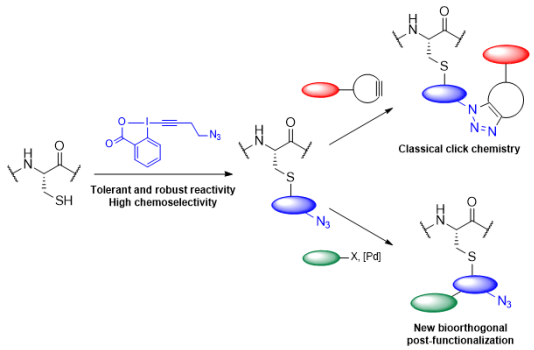
[1]Laboratory of Catalysis and Organic Synthesis, EPFL SB ISIC LCSO, BCH 4306, CH-1015 Lausanne, Switzerland, [2]Laboratory of Biophysical Chemistry of Macromolecules, EPFL SB ISIC LCBM, CH-1015 Lausanne, Switzerland.

Efficient and flexible functionalization for one amino acids among many unprotected other amino acid residues is a key challenge for selective protein modifications. Furthermore, the requisite for fast kinetics and mild conditions makes the development of practical labelling methods highly challenging. Because of their critical role in structural stability and catalytic activity of proteins, as well as their high nucleophilicity, cysteines are well-established targets for protein labelling. Previously, our group developed an efficient thio-alkynylation of small organic molecules, using the exceptional properties of hypervalent iodine reagents.[1]

This reaction proceeded under mild conditions, without any metal and did not require thiol pre-functionalization. Later, this technique was applied on living cells for intracellular labelling of cysteines. Nonetheless, this functionnalization was limited to the case of hyperreactive cysteines.[2]



Herein, we will present a new approach for a general cysteine labelling in aqueous buffer at room temperature, promoted by bench stable hypervalent iodine reagents. This method leads to the quantitative functionalization of any cysteine-containing peptides within few minutes. During this transformation, the hypervalent iodine bond was transferred to the peptide, allowing selective functionalization via either cross-coupling or cycloaddition.[3]

[1] a) R. Frei, J. Waser, *J. Am. Chem. Soc.* 2013, *135*, 9620. b) R. Frei, M. Wodrich, D. Hari, P.-A. Borin, C. Chauvier, J. Waser, *J. Am. Chem. Soc.* 2014, *136*, 16563.
[2] D. Abegg, R. Frei, L. Cerato, D. Hari, C. Wang, J. Waser, A. Adibekian, *Angew. Chem., Int. Ed.* 2015, *54*, 10852.
[3] R. Tessier, J. de Ceballos, N. Guidotti, R. Simonet-Davin, B. Fierz, J. Waser, *Manuscript in preparation.*

A Photoactivatable Probe for Super-Resolution Imaging of Nitroreductase Activity in Live Cells

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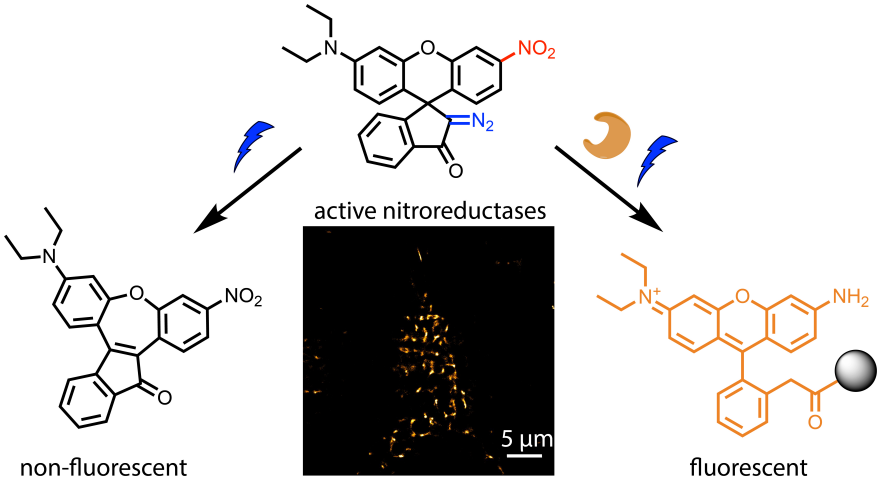
[1]Laboratory of Organic Chemistry, ETH Zurich, Zurich

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 rearrangement yields different products. The nitro group withdraws electron-density from the xanthene-system, which favors a non-emissive ring-expanded photoproduct upon irradiation. If nitroreductases reduce the nitro group to an electron-donating amine, the tricyclic core becomes electron-rich and the photoinduced Wolff-rearrangement produces a highly emissive rhodamine derivative. Thus, signal is only obtained at locations where active nitroreductases are present. Experiments in solution and in live cells confirm high selectivity towards nitroreductases. Furthermore, the photoactivated probe does not diffuse away from the original location of activation, due to covalent bonds that form between a ketene-intermediate and surrounding macromolecules. This feature can be used to track labeled enzymes and determine their diffusion coefficients within the cells. Photoactivated localization microscopy was used to reconstruct super-resolved images of the intracellular distribution of nitroreductase activity. This study proves that the sensing mechanism that we developed previously for esterases can be applied to other biologically relevant targets for single-molecule detection.[2]



[1] E. A. Halabi, Z. Thiel, N. Trapp, D. Pinotsi, P. Rivera-Fuentes, *J. Am. Chem. Soc.* **2017**,*139*, 13200–13207.

[2] Z. Thiel, P. Rivera-Fuentes, *manuscript in preparation*.

Organelle targeting with short peptides that contain asparagusic acid

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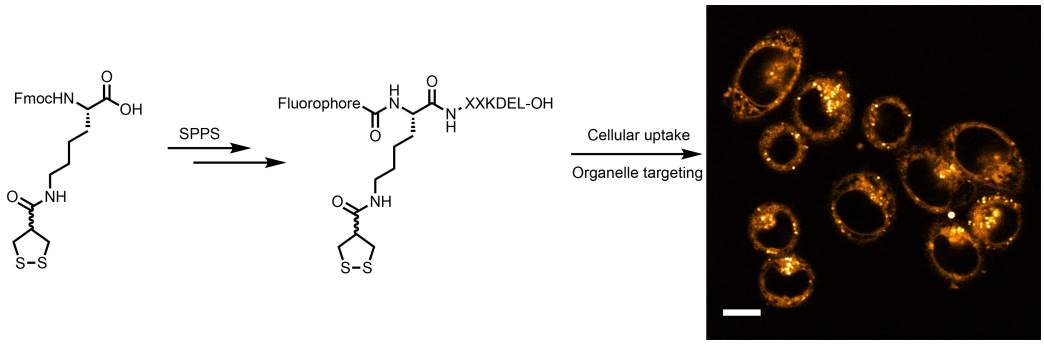
Large or highly polar molecules, such as peptides, are less able to cross the cellular membrane due to a highly regulated mechanism that controls the uptake of compounds.[1] To overcome this barrier, several delivery methods have been developed such as liposomes[2], viral vectors[3], polymers[4], cell-penetrating peptides [5] and cyclic disulfides.[6]

Cyclic disulfides, such as asparagusic acid, have been shown to increase the uptake of different cargoes inside the cell. We synthesized an asparagusic acid-modified lysine[7] and used this amino acid in the solid phase synthesis of peptides that can not only enter the cell, but target specific subcellular locations such as the endoplasmic reticulum, Golgi apparatus, or lysosomes.



[1] A. G. Torres, M. J. Gait, *Trends Biotechnol.* **2012**, *30*, 185–189.
[2] T. M. Allen, P. R. Cullis, *Adv. Drug Deliv. Rev.* **2013**, *65*, 36–48.
[3] Y. Ma, R. J. M. Nolte, J. J. L. M. Cornelissen, *Adv. Drug Deliv. Rev.* **2012**, *64*, 811–825.
[4] R. Haag, F. Kratz, *Angew. Chem. Int. Ed.* **2006**, *45*, 1198–1215.
[5] C. Bechara, S. Sagan, *FEBS Lett.* **2013**, *587*, 1693–1702.
[6] D. Abegg, G. Gasparini, D. G. Hoch, A. Shuster, E. Bartolami, S. Matile, A. Adibekian, *J. Am. Chem. Soc.* **2017**, *139*, 231–238.
[7] A. Tirla, M. Hansen, P. Rivera-Fuentes, *Synlett* **2018**, DOI: 10.1055/s-0036-1591847.

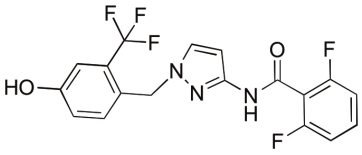
Synthesis of functionalized probes based on GSK7975A to study SOCE

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

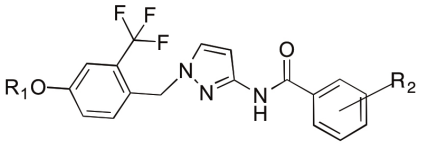
[1]Department of Chemistry and Biochemistry, [2]Institute of Biochemistry and Molecular Medicine

A wide array of physiological functions are regulated by calcium 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.

GSK7975A



Novel probes featuring additional functionalities



With this in mind, we synthesized novel probes based on the known SOCE-inhibitor GSK7975A. This class of probes feature two functionalities: a photo-crosslinking moiety for covalent target modification as well as a handle for bioorthogonal chemical modifications. Herein, we present the synthesis of these novel probes and their preliminary biological assessment.

Prakriya, M. & Lewis, R. S. *Physiological Reviews* **2015** 95(4)**,** 1383-436.

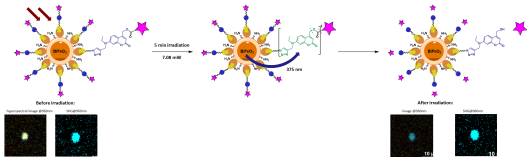
Light triggered release of encaged molecular cargos from the surface of functionalized harmonic nanoparticles

J. Vuilleumier[1], R. de Matos[1], V. Kilin[2], L. Bonacina[2], S. Gerber-Lemaire[3]

[1]Ecole Polytechnique Fédérale de Lausanne, [2]University of Geneva, [3]Institute of Chemical Sciences and Engineering, Gr

Nowadays, cancer is the leading cause of death in developed countries. The emergence of new multimodal nanodevices for in vivo imaging offers the perspective of cancer detection at a very early stage.[1] The recent progress in nanotechnologies has generated high expectation that nanomaterials could provide unprecedented contrast agents in imaging set-ups and multifunctional platforms for drug delivery.[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 recently disclosed efficient protocols for the biocompatible coating [4] and post-functionalization of bismuth ferrite (BiFeO3, BFO) HNPs as well as their favorable properties for targeted imaging of human cancer cells and tissue [5]. Herein, we report the conjugation of BFO HNPs to caged molecular cargos through a photocleavable linker based on coumarinyl derivatives. Excitation of these functionalized HNPs in the near IR region generated second harmonic UV emission [6] and subsequent selective release of a conjugated fluorescent dye, used as model for surface functionalization. In addition, we studied the different mechanisms involved in the uncaging of molecular cargos from the HNPs surface.



The suitability of this methodology for the decoupled imaging of cancer cells and exposure to uncaged molecular cargos was investigated in lung cancer neoplastic cells by tuning the excitation wavelength in multiphoton imaging setup.

[1] Smith, L.; Kuncic, Z.; Ostrikov, K. K.; Kumar, S. J. Nanomater. 2012, Article ID 891318, 7 pages.
[2] Bogart, L. K.; Pourroy, G.; Murphy, C. J.; Puntes, V.; Pellegrino, T.; Rosenblum, D.; Peer, D.; Lévy, R. ACS Nano 2014, 8, 3107-3122. He, C.; Liu, D.; Lin, W. Chem. Rev. 2015, 115, 11079-11108. Nguyen, K. T.; Zhao, Y. Acc. Chem. Res. 2015, 48, 3016-3025.
[3] Bonacina, L. Mol. Pharm. 2012, 10, 783-792.
[4] Staedler, D.; Passemard, S.; Magouroux, T.; Rogov, A.; Maguire, C. M.; Mohamed, B. M.; Schwung, S.; Rytz, D.; Juestel, T.; Hwu, S.; Mugnier, Y.; Le Dantec, R.; Volkov, Y.; Gerber-Lemaire, S.; Prina-Mello, A.; Bonacina, L.; Wolf, J.-P. Nanomedicine: NBM 2015, 11, 815-824.
[5] Passemard, S.; Staedler, D.; Sonego, G.; Magouroux, T.; Schneiter, G. S.; Juillerat-Jeanneret, L.; Bonacina, L.; Gerber-Lemaire, S. J. Nanopart. Res. 2015, 17:414; Rogov, A.; Irondelle, M.; Ramos-Gomes, F.; Bode, J.; Staedler, D.; Passemard, S.; Courvoisier, S.; Yamamoto, Y.;Waharte, F.; Ciepielewski, D.; Rideau, P.; Gerber-Lemaire, S.; Alves, F.; Salamero, J.; Bonacina, L.; Wolf, J.-P. ACS Photonics 2015, 2, 1416-1422.
[6] Staedler, D.; Magouroux, T.; Passemard, S.; Schwung, S.; Dubled, M.; Schneiter, G. S.; Rytz, D.; Gerber-Lemaire, S.; Bonacina, L.; Wolf, J.-P. Nanoscale 2014, 6, 2929-2936.

Development of Personalized Cancer Nanovaccines against Neoantigens

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Cancer immunotherapy has made unprecedented progress in the last few decades. In particular, checkpoint blockade and adoptive T cell therapy have shown remarkable clinical results. However, the clinical efficacy of therapeutic cancer vaccines remain modest. Recent studies provided solid evidence that cancer vaccines against tumor neoantigens—immunogenic peptides resulting from somatic mutations—can be immunologically and therapeutically effective in human patients. Here we developed a novel nanovaccine platform for synthetic long peptides (SLPs) to target neoantigens in order to enhance the efficacy and safety of cancer vaccines. The SLP nanovaccines were prepared through reversible chemical crosslinking of antigen and adjuvant components to form a gel like nanoparticle-nanogel (NG) with controlled size and responsiveness for release. The NG with optimized size targeted lymph nodes (LNs) and dendritic cells (DCs) effectively in vivo. The responsiveness of chemical linkers was designed to facilitate the intracellular release and subsequent cytosol delivery of SLPs for enhanced cross-priming of cytotoxic T cells. The SLP-NG vaccines elicited 3.5-fold higher frequencies of antigen-specific cytotoxic T cell than Montanide, one of the most potent vaccine adjuvants used in clinical trials. In conclusion, the SLP-NG provided a promising platform for the development of personalized neoantigen-based cancer vaccines.

On-support synthesis of alkylamino linked oligonucleotides and their bypass by DNA polymerases

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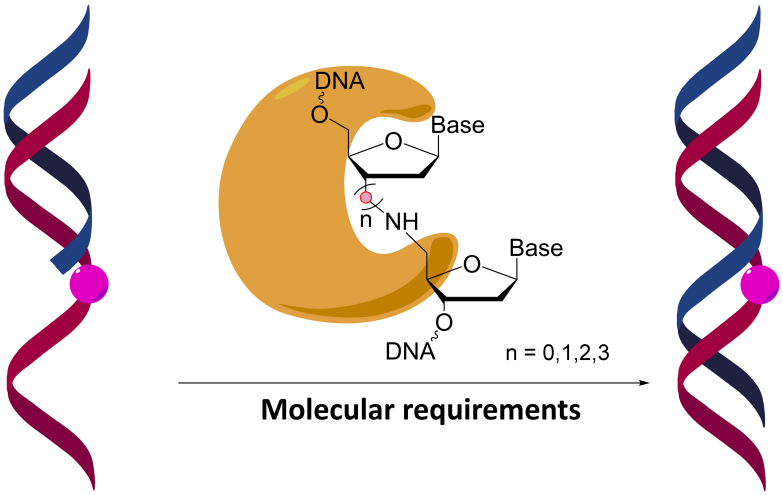
Phosphodiester bonds are essential to the basic cellular functions of all known life, as they make up the backbone of the strands of nucleic acid. However, phosphodiester bond could be replaced by other carbon-, nitrogen-, or oxygen-based backbone variants. These backbone modified oligodeoxynucleotides (ODNs) are endowed with novel properties than natural DNA, which are useful tools for molecular biology, biotechnology and medicinal chemistry. The synthesis of backbone modified ODNs are usually achieved with nucleoside dimer phosphoramidite blocks. Here, we report a convenient and inexpensive method to synthesis alkylamino linked ODNs. The fully protected 5'-OH ODN on a solid support is mildly oxidized by Moffat reaction. The resulting 5'-aldehyde ODN is then reacted with 3'-amino modified nucleoside analogues. Full length ODN is further synthesized with standard phosphoramidite chemistry. After deprotection and purification, the fidelity of replication through these alkylamino linked ODNs are studied by primer extension, PCR, qPCR and Sanger sequencing, revealing the essential backbone structure for error free read through by DNA polymerases. The results are expected to initiate the synthesis of alkylamino linked functional ODN or gene for biochemical or therapeutic use.



Peptide dendrimers as delivery systems for nucleic acids

S. J. Zamolo[1], M. Heitz[1], J. L. Reymond[1]*, T. Darbre[1]*

[1]University of Bern

Gene therapy is a powerful technique that allows the treatment of many diseases through introduction of genetic materials into the cells to compensate abnormal gene's behaviour. The introduction of a plasmid DNA allows the overexpression of the desired protein while the internalization of siRNA permits the knockdown of the corresponding mRNA. Due to the lack of efficient delivery systems, the transfection procedure is often very challenging. Kwok and co-workers demonstrated in 2013 that peptide dendrimers/lipid hybrid systems are efficient DNA transfection reagents[1] and in 2016 that the same systems can also efficiently deliver siRNA into HeLa cells[2,3].

We are now exploring a new library of third generation peptide dendrimers. The optimization of hydrophilicity, hydrophobicity and the introduction of stereoisomeric centres in the structure, allowed us to obtain systems displaying both high siRNA and promising DNA transfection efficiency in absence of helper lipid. In the first step, biological experiments were performed treating HeLa cells by the new transfection reagents and siRNA targeting GAPDH (siGAPDH) or scrambled (siNC), with L2000 as positive control, and the knockdown efficiency was measured by monitoring GAPDH enzyme activity. In the second step, biological assays were performed by transfecting HeLa cells with plasmid DNA (pCI-Neo-FL-wt) and monitoring the expression of the luciferase protein by measuring the luminescence in relative light units (RLU).

Furthermore, the addition of a fluorophore on the dendrimer gave us the opportunity to study dendrimer/siRNA complexes properties by Förster Resonance Energy Transfer (FRET) experiments.

[1] Albert Kwok, Gabriella Eggimann, Jean-Loius Reymond, Tamis Darbre, Florian Hollfelder, *ACS Nano,* **2013**, 7, 4668-4682.
[2] Albert Kwok, Gabriella Eggimann, Mar Heitz, Jean-Loius Reymond, Florian Hollfelder, Tamis Darbre, *ChemBioChem.* **2016**, 17, 1-8.
[3] Marc Heitz, Albert Kwok, Gabriela A. Eggimann, Florian Hollfelder, Tamis Darbre, and Jean-Louis Reymond, *Chimia,* **2017**, 71(4), 220-225.

Photophysical investigation on the interaction between Pt(II) complexes and an RNA G-quadruplex

W. Zenghui[1], F. D. Steffen[1], R. Börner[1], R. K. Sigel[1]*

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As non-canonical nucleic acid structures, G-quadruplexes (G4s) are believed to play crucial roles in gene regulation[1]. Small molecules are being designed and developed to specifically interact with G4s and interfere with their biological function in order to alter gene expression. Metal complexes are highly suitable for this purpose due to their predefined coordination geometry and positive charge. A detailed understanding on how these small molecules interact with G4s is crucial for further binding optimization and selectivity but very rarely available[2,3]. Here, we applied various photophysical methods based on fluorescent spectroscopy to elucidate the interaction between a BCL-2 (B-cell lymphoma 2) RNA G4 and two fluorescent Pt(II) complexes. Performing time-correlated fluorescence lifetime and dynamic fluorescence anisotropy studies we successfully determined the binding stoichiometry and affinity between the complexes and RNA G4. Furthermore, we directly observed a structural change of both complexes upon binding to the RNA G4 based on measuring the fundamental dynamic fluorescence anisotropy. Such a finding is unprecedented in the field. Hence, our results yield a highly detailed picture of the this particular interaction between a RNA G4 with two Pt(II) complexes and provide the opportunity for a future rational design of metal complexes as RNA G4 binders.

[1] Shankar Balasubramanian, Laurence H. Hurley, Stephen Neidle, *Nature Reviews Drug Discovery* **, 2011**, 10, 261–275.
[2] Pierre Murat, Yashveer Singhb, Eric Defrancq, *Chem. Soc. Rev.*, **2011**, 40, 5293–5307.
[3] Qian Cao, Yi Li, Eva Freisinger, Peter Z. Qin, Roland K. O. Sigel, Zong-Wan Mao, *Inorg. Chem. Front.*, **2017**, 4, 10-32.

Near-Infrared Confocal Imaging of Single-Walled Carbon Nanotube Uptake in Nanobionic Bacterial Cells

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The distinctive properties of single-walled carbon nanotubes (SWCNTs) have inspired the development of innovative applications in the field of cell nanobiotechnology. Most studies to date have focused on eukaryotic cells capable of internalizing SWCNTs functionalized with a variety of non-covalent wrappings. However, the effect of SWCNT functionalization on transport across the thick cell wall of prokaryotes remains unexplored. In this study, we explore the uptake of SWCNTs in Gram-negative cyanobacteria and demonstrate selective internalization of SWCNTs decorated with charged protein wrappings. The functionalized SWCNTs are shown to traverse the outer cell wall of both filamentous and unicellular strains of cyanobacteria, independent of whether the strain is naturally competent for DNA uptake, with adsorption and internalization rate constants of k_{ads}= (9.08 ± 0.16) x $10^{-8}s^{-1}$and k_{in}= (1.466 ± 0.011) x $10^{-4}s^{-1}$, respectively. A custom-built, spinning disc confocal microscope was used for the first time to directly image near-infrared (NIR) SWCNT fluorescence within cells, revealing a highly inhomogeneous distribution of SWCNTs that is otherwise overlooked using conventional NIR widefield imaging. The nanobionic cells show sustained photosynthetic activity and growth, offering a powerful avenue for engineering photosynthetic organisms with augmented and even inherited nanobionic capabilities.

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Cannabinoid receptor 2 antagonists - challenges on the way towards clinics

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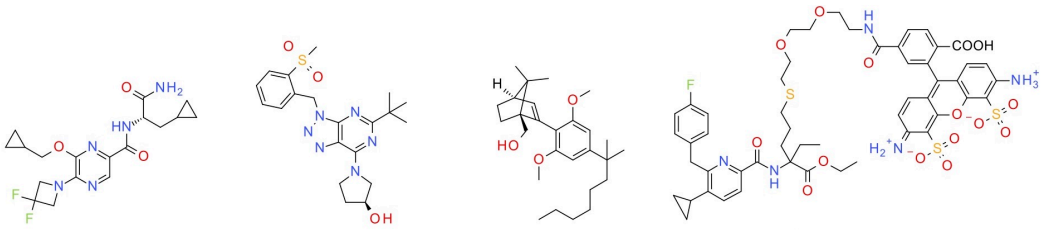
The type 2 cannabinoid receptor (CB_2R) plays an important role in cell migration and immunosuppression and is therefore a promising GPCR drug target for the treatment of tissue injury and inflammatory diseases. Highly selective CB_2R agonists show robust efficacy in various animal models of central and peripheral diseases. The successful development of new drugs strongly depends on the understanding of their underlying molecular and cellular mechanism of action as well as on knowledge about their *in vivo*target engagement. The lack of specific anti CB_2R antibodies and of suitable biomarkers for target occupancy hampers the clinical development of CB_2R agonists. 2,5,6-Trisubstituted pyridine/pyrazine[1] (**I**), triazolopyrimidine[2] (**II**) and novel cannabinoid derived ligands[3] (**III**) were found to be highly potent and selective CB_2 drug candidates.



These compound classes furthermore offer excellent starting points for generating improved chemical probes to help answering biological questions and have been exploited for generating enhanced CB_2 specific radioisotope and fluorescence labels, Raman probes as well as bifunctional probes which will be the subject of this communication. We will report results on CB_2 and CB_1 binding and functional activity which guided the probe optimization. Early absorption, distribution, metabolism and excretion properties of advanced probes including e.g. solubility, permeation, lipophilicity and selectivity data will be disclosed. First applications of novel probes such as the CB_2fluorescence label **IV** will be shown.

[1] R. Slavik et al., *J. Med. Chem.***2015**, *58*, 4266.
[2] M. Nettekoven et al., *ChemMedChem***2016**, *11*, 179.
[3] M. Soethoudt et al., *Nat Commun.* **2017**, *8*, 13958.

Peptide-Stabilized Platinum Nanoparticles Selectively Attack Liver Cancer Cells

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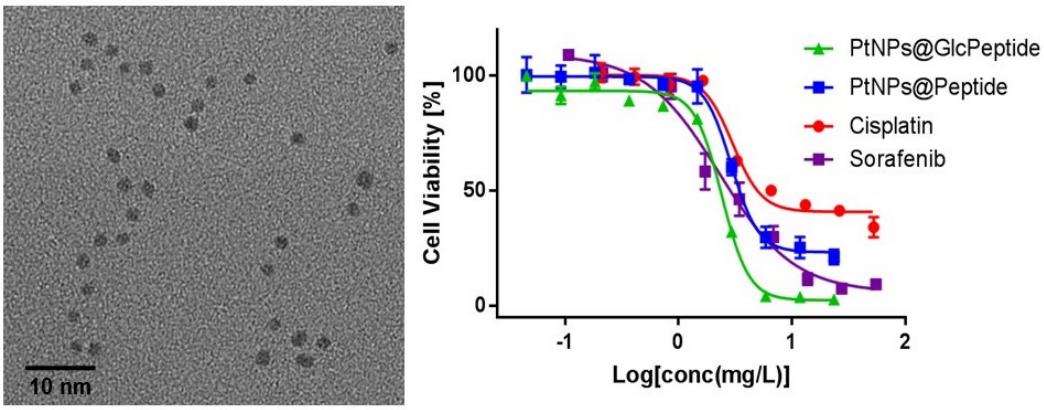
[1]Postdoctoral fellow, [2]Professor of Organic Chemistry

Hepatocellular carcinoma (HCC) is the sixth most common but has the second highest mortality rate amongst cancer disease world-wide. The only chemotherapeutic agent in use for HCC, Sorafenib, suffers from narrow activity range and severe side effects. Thus, there is a clear need for the development of new liver specific chemotherapeutics.

Platinum nanoparticles (PtNPs) have recently been considered as a source for cytotoxic Pt(II) ions, with the intention to reduce the viability of cancer cells. However, this approach to improve the efficacy of platinum-based chemotherapeutics has not yet achieved good cytotoxicity or tumor specificity. The success of this strategy mainly depends on three fundamental features: (1) formation of stabilized, small, and water soluble PtNPs; (2) selectivity of the PtNPs towards carcinogenic tissue and (3) oxidation of the nontoxic PtNPs to the cytotoxic Pt(II) ions upon cell penetration.

To address these challenges, we have developed peptide-coated PtNPs that are stable for more than 12 months. The peptide was identified from a combinatorial screening of more than 3000 different sequences and further optimized to provide high water solubility and long-time stability. The PtNPs form in a single-step without any ligand exchange process and are small and monodisperse (2.5 ± 0.7 nm). They were covalently functionalized with moieties that allow for monitoring and improving their cellular uptake. This resulted in PtNPs that exhibit high cytotoxicity and specificity for HCC cells as compared to nine other examined cell lines.



Discovery of SPL-707: A Potent, Selective and Orally Bioavailable SPPL2a Inhibitor

J. Velcicky[1], T. Brandl[1], P. Rigollier[1], R. Epple[2], U. Bodendorf[1], D. Guerini[1], P. Smith[1], D. Beisner[2], B. Wen[2]

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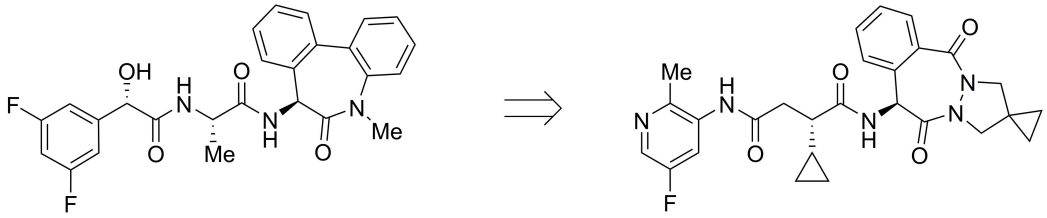
SPPL2a, Signal peptide peptidase-like 2a, a recently discovered[1] aspartyl protease has been shown to play an important role in the development and function of antigen presenting cells such as B lymphocytes and dendritic cells.[2] Due to similarity to presenilin, the catalytic subunit of the γ-secretase complex, γ-secretase inhibitor **LY-411,575** was identified as a potent while unselective SPPL2a inhibitor with poor oral exposure in rodents. Since inhibition of γ-secretase leads also to inhibition of Notch-1 causing serious side effects such as intestinal toxicity or development of skin cancer, the main goal of our chemistry efforts was to gain selectivity against γ-secretase/Notch processing. Subsequent optimization of the pharmacokinetic parameters led to discovery of the first, potent, selective and orally bioavailable SPPL2a inhibitor **SPL-707**.[3]



LY-411,575

IC_{50} (SPPL2a):	0.01 μM
IC_{50} (γ-secretase):	0.0003 μM
IC_{50} (SPP):	0.05 μM
rat AUC po d.n.:	63 nM.h

SPL-707

IC_{50} (SPPL2a):	0.08 μM
IC_{50} (γ-secretase):	6.1 μM
IC_{50} (SPP):	3.7 μM
rat AUC po d.n.:	8787 nM.h

SPL-707 significantly inhibits processing of the endogenous SPPL2a substrate (CD74/p8 fragment) in rodents at ≤10 mg/kg bid po. In addition, the phenotype seen in Sppl2a-deficient mice (reduced number of specific B cells and myeloid dendritic cells) could be recapitulated by **SPL-707**. Thus, we believe that SPPL2a represents a novel and druggable pharmacological target with a potential for treatment of antigen driven autoimmune diseases.

[1] Elena Friedmann, Ehud Hauben, Kerstin Maylandt, Simone Schleeger, Sarah Vreugde, Stefan F. Lichtenthaler, Peer-Hendrik Kuhn, Daniela Stauffer, Giorgio Rovelli, Bruno Martoglio,*Nat. Cell Biol.*, **2006**, 8, 843−848.
[2] Torben Mentrup, Regina Fluhrer, Bernd Schröder, *Eur. J. Cell Biol.*, **2017**, 96, 372−382.
[3] Juraj Velcicky, Ursula Bodendorf, Pascal Rigollier, Robert Epple, Daniel R. Beisner, Danilo Guerini, Philip Smith, Bo Liu, Roland Feifel, Peter Wipfli, Reiner Aichholz, Philippe Couttet, Ina Dix, Toni Widmer, Ben Wen, and Trixi Brandl, *J. Med. Chem.* **2018**, 61, 865−880.

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

E. Ebberink[1], N. Agashe[1], C. Janke[2], P. Gonczy[3], B. Fierz[1]*

[1]Laboratory of Biophysical Chemistry of Macromolecules, EPFL, Switzerland, [2]Institute Curie, PSL Research University, France, [3]UPGON, EPFL, Switzerland

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 difference in type and extent of PTMs over various microtubule structures (e.g. centriole versus cilia microtubules) indicates an important role of PTMs on microtubule structure and function previously described as the 'tubulin code' [1]. However, the effect of PTMs on microtubules remain elusive. Difficulty in studying PTMs lays in the unavailability of uniformly modified tubulins.

Here we fuse synthetic, uniform C-terminal tails to recombinant tubulin by use of an intein based, protein trans-splicing approach. Thereby, two intein halves can reassemble and propagate a thiolester-cysteine mediated ligation of the flanking peptide segments, excising itself. As proof of principle, α-tubulin containing an N-terminal intein half (Ava^N) was co-expressed with native β-tubulin in insect cells. Subsequently, we synthesized a C-terminal peptide tail bearing the intein counterpart (Npu^C) and an alkyne group for click chemistry of various tags. Following copper-catalyzed azide-alkyne cycloaddition of fluorescein we were able to monitor fusion of our fluorescent C-terminal tail to α-tubulin in cleared cell lysate.

In summary, this technology will enable us 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.

[1] Sudarshan Gadadhar, Satish Bodakuntla, Kathiresan Natarajan, Carsten Janke, *Journal of Cell Science*, **2017**, 130, 1347-1353.

Discovery of ADEPIDYN™ a new broad spectrum foliar fungicide

D. Stierli[1], H. U. Haas[1], R. Rajan[2], H. Walter[3], J. Cassayre[1]*

[1]1Syngenta Crop Protection AG, Schaffhauserstrasse, CH-4332 Stein, [2]2Syngenta Biosciences Private Limited, Santa Monica Works, Corlim Ilhas GOA, [3]-

Succinate dehydrogenase (SDH) has been demonstrated as a promising target for fungicide discovery.

N-methoxy-(phenyl-ethyl)-pyrazole-carboxamide is a new chemical group within the FRAC code 7 that will deliver differentiated and new sustainable solutions to the growing world population and will meet customer needs in key markets.

ADEPIDYN™, Pydiflumetofen (ISO common name), 3-(difluoromethyl)-*N*-methoxy-1-methyl-*N*-((*RS*)-1-methyl-2-(2,4,6-trichlorophenyl)ethyl)pyrazole-4-carboxamide is a novel, potent broad-spectrum fungicide molecule with a SDHI mode of action that was developed by Syngenta. It sets a new performance standard against many fungal diseases in various crops.

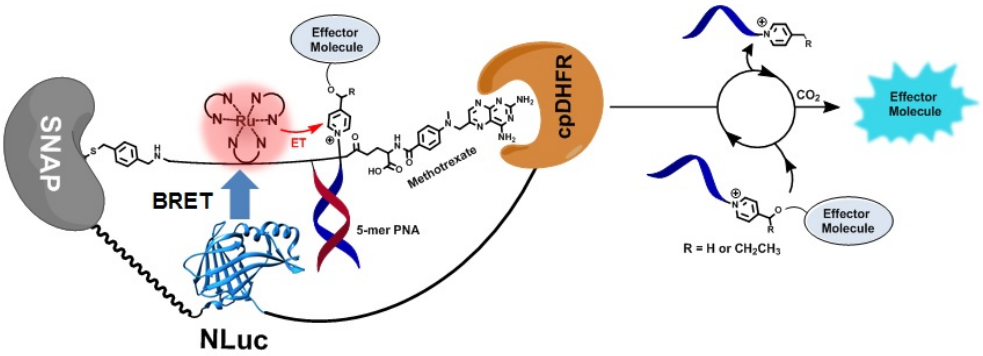
Herein, a brief history of SDHI's, the discovery and optimisation of novel N-methoxy-pyrazole-carboxamide SDH inhibitor's as well as biological aspects will be discussed.

Luciferase-induced photoreductive uncaging of small-molecule effectors

S. Angerani[1], E. Linberg[1], M. Anzola[1], N. Winssinger[1]*

[1]Department of Organic Chemistry, University of Geneva

Bioluminescence resonance energy transfer (BRET) is extensively used to study dynamic systems and has been utilized in sensors for studying protein proximity, metabolites, and drug concentrations. [1,2,3] We demonstrate that BRET can activate a ruthenium-based photocatalyst which performs bioorthogonal reactions. BRET from luciferase to the ruthenium photocatalyst was used to uncage effector molecules with up to 64 turnovers of the catalyst, achieving concentrations > 0.6 mM effector with 10 nM luciferase construct. Using a BRET sensor, we further demonstrate that the catalysis can be modulated in response to an analyte, analogous to allosterically controlled enzymes. The BRET-induced reaction was used to uncage small-molecule drugs (ibrutinib and duocarmycin) at biologically effective concentrations *in cellulo*.



[1] Stijn J. A. Aper, Pieterjan Dierickx, Maarten Merkx, *ACS Chem. Biol*. **2016**, 11, 2854-2864.
[2] Anca Dragulescu-Andrasi, Carmel T. Chan, Abhijit De, Tarik F. Massoud, Sanjiv S. Gambhir, *Proc. Nat. Acad. Sci*. **2011**, 108, 12060-12065.
[3] Franz X. Schaub, Md Shamim Reza, Colin A. Flaveny, Weimin Li, Adele M. Musicant, Sany Hoxha, Min Guo, John L. Cleveland, Antonio L. Amelio, *Cancer Res*. **2015**, 75, 5023-5033.

Lessons learned from the Neuropeptide S project at Idorsia

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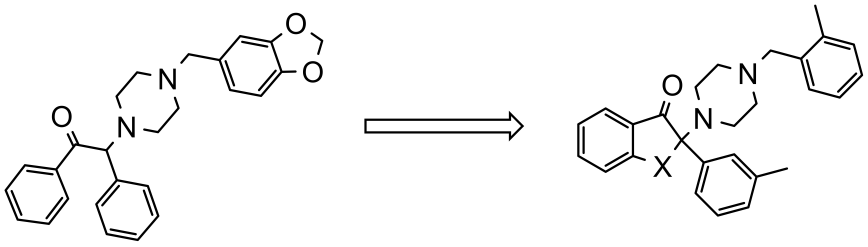
Neuropeptide S (NPS) is a small peptide (20 amino acids) whose sequence and possible physiological function were first described in 2004 [1]. It binds to a G-protein-coupled receptor (NPSR) expressed especially in the brain, in the hypothalamus, thalamus, amygdala and certain cortical regions. NPSR -/- mice display reduced arousal in different paradigms, suggesting a physiological role for this receptor. Small molecule antagonists of NPSR could therefore offer novel therapeutic approaches to insomnia and stress-related disorders [2].

High-throughput screening of our in-house compound collection for hNPS antagonists in a FLIPR assay revealed aminoketone 1 as a hit, antagonizing the hNPS receptor with an IC50 of 170 nM.



1

2a (X=CO)
2b(X=CH_2)

A medicinal chemistry effort to increase the chemical stability, control the integrity of the chiral center and obtain patentable analogues of 1, delivered indanones 2a and indan-1,3-diones 2b together with two other series of yet undisclosed potent NPSR antagonists. Eventually, detailed and consequent structure activity and structure property relationships could be established and 4 proof of concept compounds exhibiting suitable ADME and brain penetrating properties for in vivo experiments were discovered. These compounds were crucial for investigating the pharmacological potential of NPSR antagonists in animal models.

[1] Lu et al., Neuron 2004, 43, 487-497; Reinscheid, Handbook of Biologically active peptides, 2013, 869-874.
[2] Duangdao et al., Behav. Brain Res. 2009, 205(1), 1-9.

Development of radiotracers for non-invasive imaging of the co-stimulatory molecule CD80 by PET

M. F. Taddio[1], T. Bollmann[1], D. Schmid[1], A. Müller Herde[1], C. Keller[1], L. Mu[2], S. M. Ametamey[1], R. Schibli[1]*, S. D. Krämer[1]*

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The co-stimulatory protein CD80 is expressed on activated monocytes and granulocytes. It is a marker of inflammation. The goal of this project is to develop positron emission tomography (PET) radiotracers for non-invasive imaging of immunogenic processes. Several small molecules with specific affinity to human CD80 (hCD80) in the low nanomolar range have been described, among them AM7 (**Figure 1**).[1,2] Our own *in vivo* experiments with the carbon-11 labelled lead structure ^{11}C-AM7 revealed poor pharmacokinetic behavior.[3] Therefore, the structure was modified to improve the pharmacokinetic profile and conserve strong affinity to hCD80, which resulted in the novel structure MT107 (**Figure 1**) with similar affinity to hCD80 (K_D = 4.9 nM), as determined by surface-plasmon resonance (SPR), and a higher experimental log *D* (pH 7.4) of 2.1 compared to 0.1 for AM7. Despite the increased lipophilicity, liposomal bilayer permeability[4] did not improve. *In vivo* experiments with SCID mice bearing hCD80 positive xenografts showed an improved pharmacokinetic profile of [^{11}C]-MT107.[5] This led to higher blood activity levels and improved but heterogeneous uptake into hCD80 positive xenografts (**Figure 2**). Further structural modifications, focusing on the pharmacokinetic optimization, resulted in additional ligands with binding affinity in the low nanomolar range and promising characteristics for further development as PET tracers.

The project is funded by the Swiss National Science Foundation (SNSF #153352).

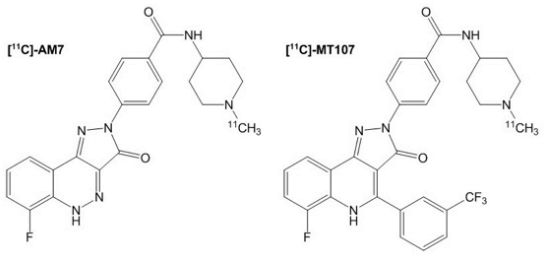

Figure 1: [^{11}C]-labelled AM7 and MT107 for PET imaging

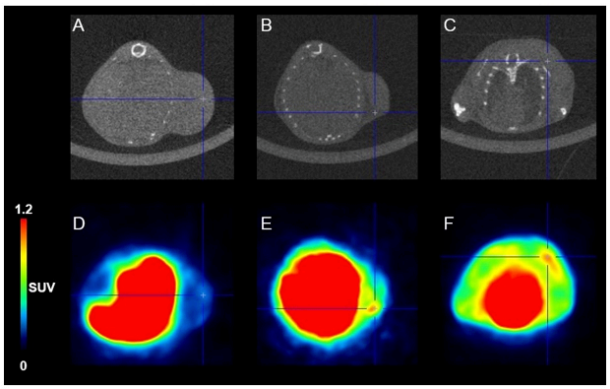

Figure 2: Coronal CT (A-C) and PET (D-F) images of Raji (hCD80+) xenograft bearing C.B.17 SCID mice after tracer intravenous injection. PET images averaged from 1-61 min. A,D) 8.2MBq (3.3 nmol/kg) [^{11}C]-AM7; B,E) 5.5MBq (14.5 nmol/kg) [^{11}C]-MT107; C,F) 11.5MBq (13.0 nmol/kg) [^{11}C]-MT107 with cyclosporine to block biliary excretion (50mg/kg p.i. 30min before tracer injection). SUV, standardized uptake value. Cross hairs indicate maximal uptake in the xenografts.

[1] Neil J. Green et al., *Bioorganic and Medicinal Chemistry*, **2003**, 11, 2991-3013.
[2] Philip Huxley et al., *Chemistry & Biology*, **2004**, 11, 1651–1658.
[3] Adrienne Müller et al., *International Journal of Cardiology*, **2014**, 174, 503-515.
[4] Katharina Hermann et al., *European Journal of Pharmaceutical Sciences*, **2017**, 104, 150-161.
[5] Marco F. Taddio et al., *Contrast Media & Molecular Imaging*, in press.

Development of a Natural Product-Like DNA-Encoded Macrocycle Library for Screening against Biologically Relevant Protein Targets

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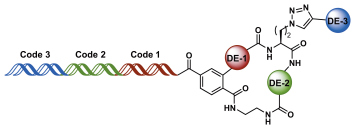
DNA-encoded chemical libraries (DECLs) were first proposed by Brenner and Lerner in 1992.[1] This technology uses the advantages of combining genetic sequencing and combinatorial chemical synthesis. Due to this, large collections of molecules (up to billions) can quickly be synthesized and tested all at once. Potential protein binders are identified by the attached unique DNA code. Therefore, screening assays become much faster, more cost effective and less storage space for the library is needed.
Macrocycles, however, still are an underdeveloped class of compounds, which show unique properties and have a big potential for medicinal chemistry research. There are a couple of very effective macrocycles on the drug market as well as many very important natural products contain a macrocyclic scaffold.[2]



We therefore synthesized a DECL comprising of natural product-like macrocycles (approx. 1.5 million members) with very diverse macrocyclic scaffolds (>2000). The diversity was introduced using three building blocks that were incorporated by amide bond formation and click reaction. The individual building blocks were subsequently encoded by enzymatic DNA elongation reactions. The first building blocks (DE-1) were designed to bear typical natural product elements such as polyenes, alkyl chains, substituted aromatic rings or heteroaromatic moieties. Diversity element 2 (DE-2) consists of a set of natural and non-natural amino acids. The third building block (DE-3) gives the major diversity using copper-catalyzed click reactions. We tried to cover as much chemical space as possible by the selection of a diverse set of chemical functionalities and sterics. The obtained library was then tested against biologically relevant proteins to find potential binders. PCR amplification, followed by next generation sequencing revealed the binding structures. Chemical resynthesis and protein binding affinity measurements of the elucidated hits gave an idea of the efficiency of our macrocycles in binding to our protein targets.

[1] S. Brenner, R. A. Lerner, *Proc. Natl. Acad. Sci. USA*. **1992**, 89, 5381-5383.
[2] A. Whitty *et al*. *Drug Discovery Today*, **2016**, 21, 712-717.

Lanthanide-Loaded Dendrons as Antibody Labels for Mass Cytometry Applications

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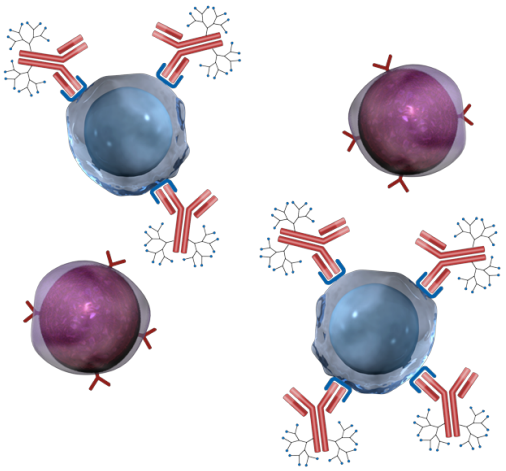
Time-of-flight mass cytometry (CyTOF) is a technique that enables the simultaneous quantification of over 40 cellular parameters at the single cell level [1]. Metal isotope-labeled antibodies are employed to stain cellular epitopes. As the sensitivity of CyTOF linearly increases with the metal isotope-to-antibody ratio, polymeric probes are needed to label antibodies with multiple copies of a metal isotope. Dendrimers are branched macromolecules with well-defined structures and sizes [2]. The possibility to conjugate multiple chemical entities on the surface of dendrimers makes them attractive scaffolds for biomedical applications. [3]. Despite that, dendrimers are suboptimal candidates for CyTOF applications as the probes must react with a single antibody to avoid undesirable cross-linking and maintain homogeneity. The controlled conjugation of a dendritic scaffold with a single antibody is therefore best achieved with a dendron. Herein the use of antibody-dendron conjugates in CyTOF is for the first time described. Two generations of well-defined dendrons were designed and synthesized. Multiple metal isotopes were inserted on the periphery of the dendrons while their focal point was used for conjugation to an antibody. The conjugates were demonstrated to specifically bind to their receptor using human peripheral blood mononuclear cells.



[1] Matthew H. Spitzer, Garry P. Nolan, *Cell*, **2016**, 165, 780-791.
[2] Cameron C. Lee, John A. MacKay, Jean M. J. Frechet, Francis C. Szoka, *Nat.* Biotechnol., **2005**, 23, 1517-1526.
[3] Sonke Svenson, *Chem. Soc.* Rev., **2015**, 44, 4131-4144.

Site-specific two-color labeling of long RNAs for single-molecule FRET

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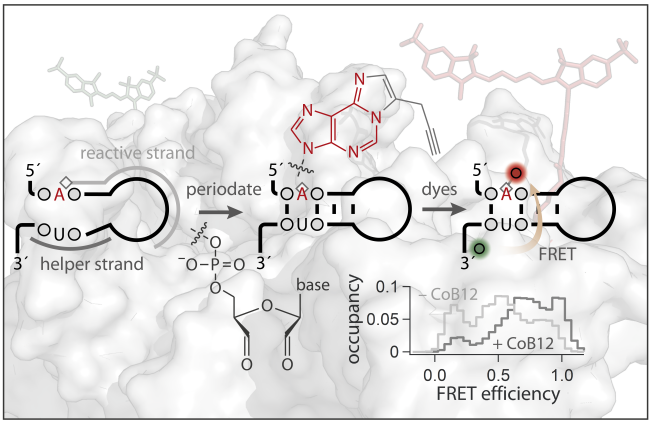
Fluorescent labeling of long RNA molecules in a site-specific yet generally applicable manner is integral to many spectroscopic applications. By introducing a pair of organic dyes at strategic sites within the RNA, one can monitor conformational dynamics via FRET. Here, we propose a novel, covalent labeling method that is position-selective and scalable to long, intricately folded RNAs. [1] In this modular approach, we first locally and temporarily disrupt the secondary structure of the target RNA and then let a custom-designed DNA probe bring an *in situ* activated functional group close to a preselected adenine or cytosine residue. [1,2] The resulting etheno adduct carries an alkyne handle which can be coupled to an azide functionalized fluorophore. Concomitantly to this RNA-templated transfer, the 3'-terminal ribose of the RNA is oxidized, offering room for a second dye to be incorporated bio-orthogonally.



We validate our labeling protocol using a B12-riboswitch of 240 nucleotides in length. The integrity of the fluorophores and their motional flexibility within the RNA environment is evaluated by fluorescence lifetime and dynamic anisotropy decays. [1,3] Native folding and function of the riboswitch in response to its metabolite is confirmed on the single-molecule level. We use FRET as a sensor to probe the conformational equilibrium of the riboswitch that is tuned through binding of coenzyme B12. [1,4] Overall, our labeling strategy overcomes size and site constraints that have hampered routine production of labeled RNA beyond 200 nucleotides in length.

[1] Meng Zhao, Fabio D. Steffen, Richard Börner, Michelle F. Schaffer, Roland K.O. Sigel, Eva Freisinger, *Nucleic Acids Res.*, **2018**, 46, e13.
[2] David Egloff, Eva Freisinger *et al., ACS Chem. Biol.*, **2016**, 11, 2558-2567.
[3] Fabio D. Steffen, Roland K.O. Sigel, Richard Börner, *Phys. Chem. Chem. Phys.*, **2016**, 18, 29045-29055.
[4] Georg A. Perdrizet, Tao Pan *et al.*, *Proc. Natl. Acad. Sci.*, **2012**, 109, 3323-3328.

RNA Therapeutics: a medicinal chemists perspective

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RNA therapeutics have come a long way from the first FDA approval of Vitravene back in 1998 to the most recent and much awaited launch of Spinraza. Focusing on Locked Nucleic Acids (LNA) based RNA therapeutics, this lecture will cover the latest developments in the field and discuss the impact and opportunities of medicinal chemistry to further develop this therapeutic modality.

Long-Term Nanoscopic Imaging of Vesicle Dynamics in Living Cells with a Pump-and-Blink Probe[1]

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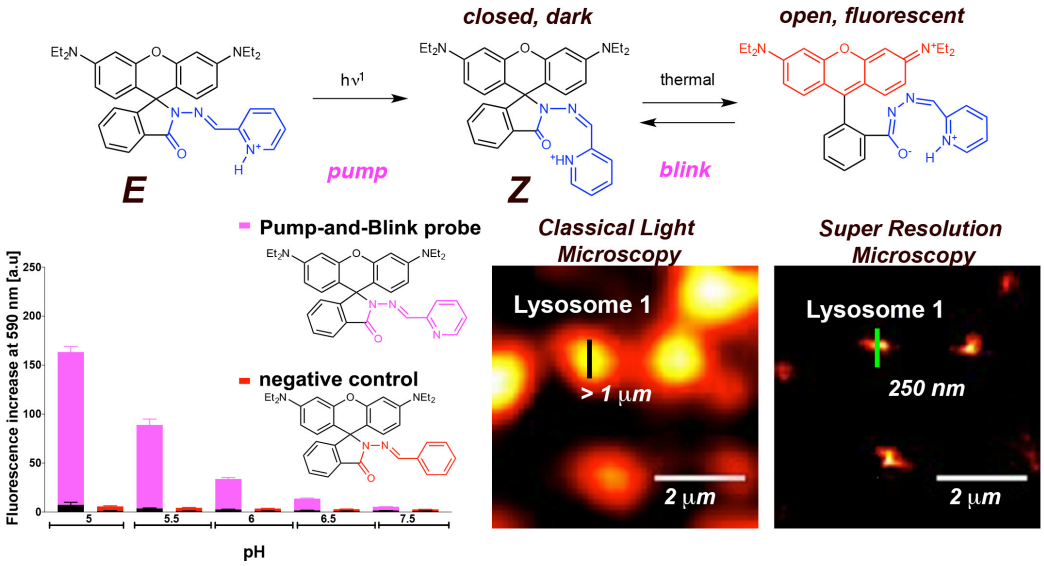
Spontaneously blinking dyes interconvert between emissive and dark species in the ground state, facilitating time-lapse, single-molecule localization microscopy (SMLM) by decreasing photobleaching and photoxicity. Previously reported fluorophores fail to blink at low pH, which prevents imaging of acidic organelles. We solved this problem by developing a probe that can be isomerized after a single photoactivation (pump) pulse and subsequently undergoes proton-promoted, spontaneous blinking specifically in lysosomes and synaptic vesicles (pH < 6.5, Pearson's correlation coefficient P = 0.8). Whereas time-lapse SMLM is normally limited to a few seconds, this pump-and-blink mechanism allowed us to record super-resolved movies for more than 30 minutes with a temporal resolution of 1 s. Individual acidic vesicles could be tracked using this pump-and-blink probe, in two and three spatial dimensions, with nanometric precision. Finally, these studies revealed details, previously undetected, of the three-dimensional organization and trafficking of synaptic vesicles in mammalian neurons.



[1] E. A. Halabi, D. Pinotsi, P. Rivera-Fuentes, *manuscript in preparation.*