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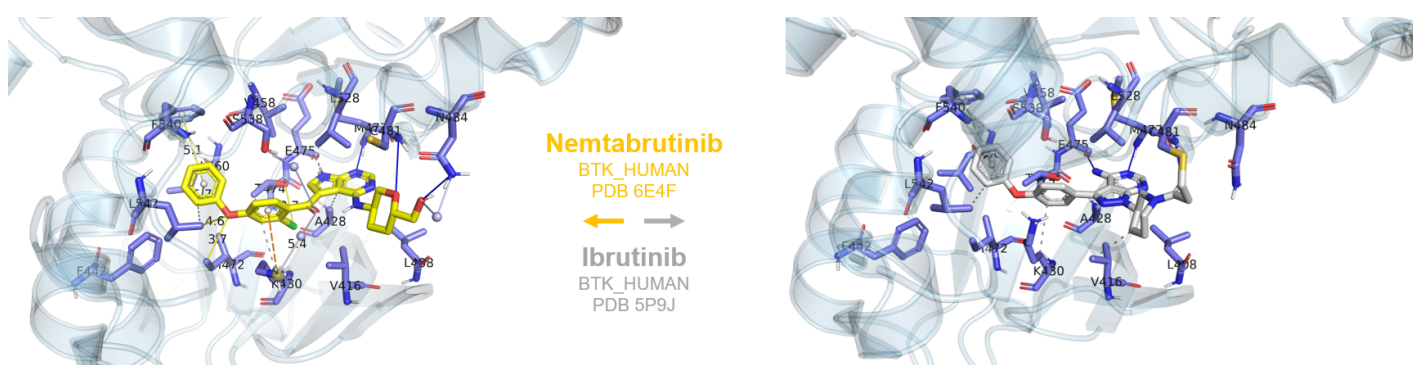
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Pharmacophore profiling highlights diverging binding modes of nemtabrutinib and ibrutinib across kinases

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Kinase inhibition is a major pillar of targeted anticancer therapy, but rational control of kinase selectivity remains challenging because their ATP-binding pockets are highly homologous and conformationally dynamic. This can lead to off-target inhibition, toxicity, and reduced clinical effectiveness. Here, we present a computational workflow to rationalize selective compound activity by capturing subtle structural differences across kinases. We applied it to nemtabrutinib and ibrutinib, two BTK-targeting inhibitors with divergent activity in cell lines derived from activated B-cell-like (ABC) and germinal-center B-cell-like (GCB) diffuse large B-cell lymphoma (DLBCL). In vitro activity was correlated with in silico selectivity scores computed across 20 kinases relevant in lymphomas.



To enable scalable analysis of ATP-binding pocket variants, we collected metastable kinase conformations defined by DFG-motif geometry and associated dihedrals using AlphaFold2 templating and publicly available structures. Structural alignment of this conformational dictionary, followed by structure-based pharmacophore analysis, generated rich interaction maps of kinase pocket features. Compound fitting against these maps enabled pharmacophore matching and relative selectivity scoring, using BTK as the baseline confirmed target.

The analysis showed that the divergent profiles of nemtabrutinib and ibrutinib can be rationalized by the combined contribution of ten discriminant positions in the extended ATP-binding pocket, structurally aligned to BTK residues Q412, V416, F442, M449, L460, I472, T474, C481, N484, and L542. These positions modulate covalent reactivity, hydrogen bonding, hydrophobic packing, sulfur–aromatic interactions, π -stacking, cation– π contacts, and halogen-bonding opportunities. Interaction fingerprints highlighted FYN, FRK, MAST1, and SIK1–3 as candidate kinases preferentially compatible with nemtabrutinib over ibrutinib, providing a mechanistic hypothesis for differential anti-proliferative effects in GCB DLBCL models.

Overall, this proof of concept establishes a structure-guided strategy for interpreting selective compound activity across conserved kinase families. The workflow can also be extended toward automated contrastive screening and explicit design of selective kinase inhibitors optimized for desired target engagement while minimizing off-target interaction potential across the kinome.

Discovery and Complex Dose-Dependent Pharmacology of a Potent SARM1 Base-Exchange Inhibitor in a Mouse Model of Neuropathy

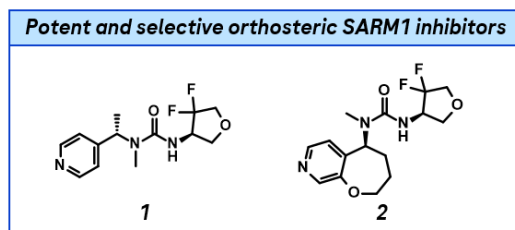
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SARM1 (Sterile alpha and TIR Motif Containing 1) is the central executioner of axonal decay and plays a pivotal role in the pathogenesis of various neuropathies and neurodegenerative diseases. Stress-induced activation of SARM1 triggers rapid NAD⁺ depletion, resulting in energetic failure and the physical breakdown of axons. Consequently, SARM1 has emerged as a high-priority target for small-molecule neuroprotective therapies.[1,2] Herein, we report the structure-based optimization of a known ligand class,[3] supported by quantum-mechanical (QM) calculations, which led to the discovery of two potent lead compounds, **1** and **2**.[4]

While both compounds demonstrated potent SARM1 inhibition in biochemical and cellular assays, high selectivity against related NADases, and robust protection of iPSC-derived motor neurons from chemically induced degeneration, compound **1** was prioritized for further development due to its superior ADMET profile.

In a spared nerve injury (SNI) mouse model of neuropathy, oral administration of compound **1** (50 mg/kg) significantly reduced plasma neurofilament light (NfL)—a clinical biomarker of axonal injury—confirming robust target engagement and in vivo efficacy. Interestingly, a lower dose (2 mg/kg) paradoxically increased NfL levels, suggesting a complex, dose-dependent pharmacology. These findings validate our rational design approach and provide critical insights into the development of base-exchange inhibitors for treating neurological disorders characterized by axonal degeneration.



[1] J. M. Osterloh, J. Yang, T. M. Rooney, A. N. Fox, R. Adalbert, E. H. Powell, A. E. Sheehan, M. A. Avery, R. Hackett, M. A. Logan, J. M. MacDonald, J. S. Ziegenfuss, S. Milde, Y.-J. Hou, C. Nathan, A. Ding, R. H. Brown, Jr., L. Conforti, M. Coleman, M. Tessier-Lavigne, S. Züchner, and M. R. Freeman, *Science*, 2012, 337, 481-484

[2] H. S. Loring, P. R. Thompson, *Cell Chemical Biology*, 2020, 27, 1-13

[3] M. Bratkowski, T. C. Burdett, J. Danao, X. Wang, P. Mathur, W. Gu, J. A. Beckstead, S. Talreja, Y.-S. Yang, G. Danko, J. H. Park, M. Walton, S. P. Brown, C. M. Tegley, P. R. B. Joseph, C. H. Reynolds, S. Sambashivan, *Neuron*, 2022, 110, 3711-3726

[4] M. Giroud, B. Kuhn, S. Steiner, P. Westwood, M. Mendel, A. Mani, E. Pinard, W. Haap, U. Grether, P. Caramenti, D. Rombach, C. Zambaldo, M. Ritter, P. Schmid, C. Gasser, N. Aregger, N. Séchet, A. Topp, M. Bilyard, A. Malnight-Alvarez, I. Plitzko, M. Hilbert, S. Kalayil, D. Burger, C. Bonardi, W. Saal, A. Haider, M. B. Wittwer, A. Brigo, J. Benz, James Keaney, *Journal of Medicinal Chemistry*, 2025, 68, 6558-6575

From structure-activity relationship study to solubility enhancement and development of photoswitchable TRPM4 inhibitors

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We conducted a focused and systematic structure-activity relationship study¹ on the 4-chloro-2-(2-phenoxyacetamido)benzoic acid scaffold, shared by known inhibitors of TRPM4, which is a Ca²⁺-activated, voltage-dependent monovalent cation channel associated with various diseases². Our series of synthetic compounds revealed valuable SAR trends and ultimately led to the discovery of PBA, which exhibited increased potency, ligand efficiency, aqueous solubility, and lipophilic ligand efficiency, as well as lower cytotoxicity than reference inhibitor NBA. In addition, PBA inhibited both mouse and human TRPM4, but had no effect on hTRPM5, a closely related Ca²⁺-activated, voltage-dependent monovalent cation channel. Building on these insights from the SAR study, our next goal is to develop chemical probes for the spatiotemporal control of TRPM4 using light. Azobenzene-functionalized compounds have so far demonstrated efficient photoswitchable behaviour, long thermal relaxation times and promising biological activity. Additionally, single-crystal X-ray diffraction structures of our 4-chloro-2-(2-phenoxyacetamido)benzoic acid-based compounds revealed strong bifurcated intramolecular hydrogen bonds stabilizing a “bent” and completely flat conformation. Through a synthetic structure-property relationship study combining X-ray, melting points, NMR, and solubility measurements, we discovered that these hydrogen bonds are retained in solution and probably decrease aqueous solubility due to π - π -stacking. We believe that the bent conformation is important for these inhibitors’ mode of action. Currently, we are working to increase the solubility of PBA by breaking the molecular planarity of the compounds.

[1] C. E. Gerber, B. S. Augustynek, P. Grossenbacher, B. Hauert, S. A. Singer, C. Peinelt, M. Lochner, *Journal of Medicinal Chemistry*, 2026, 69, 8763-8789

[2] B. Preti, J.-S. Rougier, I. Papapostolou, F. Bochen, C. E. Gerber, H. Abriel, M. Lochner, C. Peinelt, *CHIMIA*, 2022, 76, 1039–1044

Matched Molecular Pair Analysis and Chameleonic Effects in the Discovery of an Allosteric WRN Helicase Inhibitor

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The discovery of the allosteric WRN helicase inhibitor HRO761 illustrates how systematic analysis of structure–property relationships (SAR) can guide medicinal chemistry in challenging beyond–rule–of–five (bRo5) space. Targeting WRN as a synthetic lethal vulnerability required balancing high molecular weight and polarity with sufficient permeability, cellular activity, and *in vivo* efficacy.

Central to this approach was the prospective use of physics–based property prediction, systematic data capture, and collaborative idea triaging to define target property windows and to support rapid decision–making. Within this framework, matched molecular pair analysis (MMPA) played a key role in extracting robust medicinal chemistry insights from developing datasets. Single structural modifications were quantitatively linked to changes in potency, permeability, and cellular efficacy, enabling design hypotheses to be tested rapidly and effectively while establishing robust SAR.

MMPA revealed pronounced chameleonic effects, where subtle substitutions—such as a methyl group addition—led to non–intuitive improvements in permeability and cellular activity without increasing lipophilicity. These effects are consistent with conformation–dependent modulation of exposed polarity rather than simple logP–driven behavior.

Overall, this case study demonstrates how consistent application of MMPA enables the derivation of actionable design rules, supporting rational optimization of inhibitors for difficult targets operating in bRo5 space.

[1] S. Ferretti, J. Hamon, J. et al., *Nature*, 2024, 629, 443–449.

[2] H. Moebitz, V. Bordas et al., ACS Spring, 2024, American Chemical Society

Discovery of de novo peptidic binders for undruggable proteins

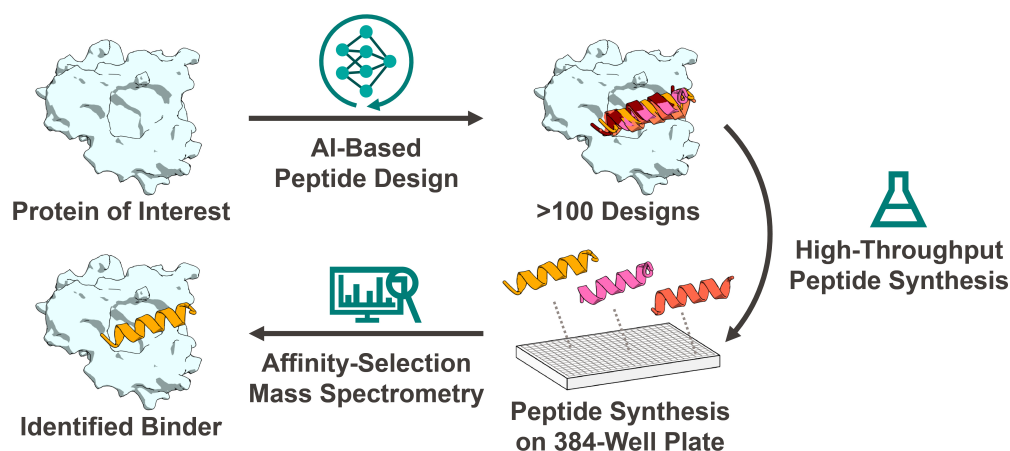
Y. Kamei^{1,2}, M. Pacesa³, G. Grammbitter¹, G. Menoud⁴, L. Nickel³, P. Franz¹, C. Heinis⁴, B. Correia³, J. Waser^{2*}, B. Fierz^{1*}

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Dysregulation of transcription often leads to carcinogenesis and cancer proliferation, making chemical modulation of chromatin factors a potential therapeutic strategy. However, protein-protein interactions (PPIs) are deemed undruggable by small molecules due to the lack of well-defined binding pockets. Peptides have emerged as a suitable modality for targeting such undruggable proteins by mimicking PPIs and engaging flat surfaces that are typically inaccessible to small molecules.

While artificial intelligence (AI) has enabled the generation of high-affinity protein binders¹, the design of peptidic binders by AI remains largely underexplored. To date, peptidic binders are typically identified by screening massive libraries (>10⁸ members) via display technologies² or high-throughput synthesis³.

Here, we describe a platform for the de novo design and rapid validation of peptidic binders that target typically undruggable proteins, including intracellular oncogenic chromatin factors. Our AI-designed peptides were synthesized by solid-phase peptide synthesis in a 384-well format and screened by affinity-selection mass spectrometry. We successfully identified binders for challenging targets. Our integrated platform may serve as a broadly applicable approach for targeting undruggable proteins.



[1] Martin Pacesa, Sergey Ovchinnikov, Bruno E. Correia *et al.*, *Nature* **2025**, 646, 483-492.

[2] Toby Passioura, Takayuki Katoh, Yuki Goto, Hiroaki Suga, *Annu. Rev. Biochem.* **2014**, 83, 727-752.

[3] Bradley L. Pentelute *et al.*, *Nat. Commun.* **2020**, 11, 3183.

Toward a Protein-Based Sensor for *S*-Nitrosylated Coenzyme A

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The *S*-nitrosylation of protein cysteine residues, resulting in the formation of *S*-nitrosothiols (SNOs), has emerged as an important signaling pathway of nitric oxide (NO).^[1] This well-regulated post-translational modification plays a role in protein activation or inhibition, as well as the modulation of protein-protein interactions and protein localization.^[2] Recently, *S*-nitrosylated coenzyme A (SNO-CoA) was identified as a key mediator of NO transfer.^[3,4]

To investigate SNO-CoA signaling, we aim to develop a genetically encoded SNO-CoA sensor based on the fusion of redox-sensitive green fluorescent protein (roGFP)^[5] with a CoA-binding protein (Figure 1). In this design, NO is transferred from the CoA-binding domain to the cysteine residues of roGFP, producing a ratiometric fluorescence change. To achieve efficient and selective NO transfer, we employ *de novo* protein design to engineer a structured linker that enforces the precise spatial orientation between the CoA-binding domain and roGFP.

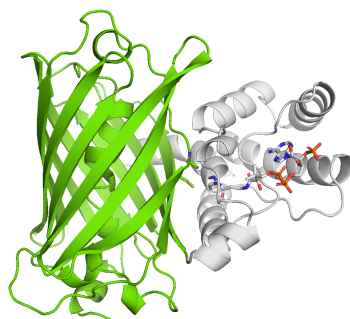


Figure 1. Fusion Protein Design. In green roGFP with cysteines represented as sticks, in grey CoA-binding protein with SNO-CoA bound.

This approach will enable measurements of SNO-CoA in live cells, providing a direct readout of the *S*-nitrosylation state of CoA and offering new insights into this emerging signaling pathway.

[1] Veani Fernando, Xunzhen Zheng, Yashna Walia, Vandana Sharma, Joshua Letson, Saori Furuta, *Antioxidants* **2019**, *8*, 404.

[2] Douglas T. Hess, Akio Matsumoto, Sung-Oog Kim, Harvey E. Marshall, Jonathan S. Stamler, *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 150–166.

[3] Puneet Anand, Alfred Hausladen, Ya-Juan Wang, Guo-Fang Zhang, Colin Stomberski, Henri Brunengraber, Douglas T. Hess, Jonathan S. Stamler, *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 18572–18577.

[4] Hua-Lin Zhou, Zachary W. Grimmett, Nicholas M. Venetos, Colin T. Stomberski, Zhaoxia Qian, Precious J. McLaughlin, Puneet K. Bansal, Rongli Zhang, James D. Reynolds, Richard T. Premont, Jonathan S. Stamler, *Cell* **2023**, *186*, 5812.

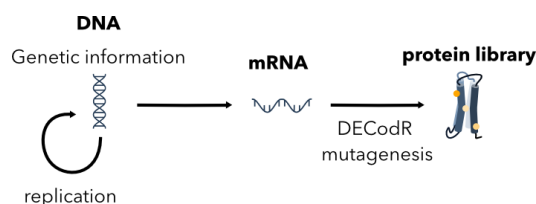
[5] George T. Hanson, Robert Aggeler, Devin Oglesbee, Mark Cannon, Roderick A. Capaldi, Roger Y. Tsien, S. James Remington, *J. Biol. Chem.* **2004**, *279*, 13044.

Arrayed Protein Libraries from DECodR Mutagenesis

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Directed evolution is a powerful technique to tailor a biomolecule for a desired application. Development cycles typically entail the generation of DNA libraries from a starting gene, the expression of different protein variants, and finally the identification of the best variants through screening. A commonly employed method for protein diversity generation is site saturation mutagenesis, the mutation of one or more residues to each of the 20 proteinogenic amino acids. We reimagine this diversity generation process through the lens of an “orthogonal central dogma”, and develop tools for the generation of protein variants by translation-level mutagenesis. We engineer a suite of genetic code expansion tools to efficiently refactor a set of stop and/or sense codons of a genomically recoded organism and demonstrate efficient encoding of the 20 proteinogenic amino acids at a single recoded codon or subsets of amino acids at multiple sites into model proteins. We apply the toolkit to different protein engineering challenges, including the optimization of fluorescent proteins, a self-labeling protein, and an oxidative enzyme. We anticipate that our approach will augment current protein engineering workflows and aid in the exploration of sequence-function relationships of proteins.



PAMs of GluN2B-containing NMDARs as enhancers of synaptic plasticity for the treatment of Alzheimer's disease

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Synaptic plasticity is the ability of synapses to strengthen or weaken over time, in response to increases or decreases in their activity. This phenomenon is proposed to be the basis for learning and memory of neuronal networks and has the potential to recover from synaptic loss. Substantial evidence exists correlating synaptic dysfunction/loss with degree of cognitive impairment and late stages of Alzheimer's Disease (AD) for which synaptic plasticity represents a potential therapeutic approach.

NMDA receptors are glutamatergic receptors that can be activated precisely during the coincidence of excitatory synaptic transmission with neuronal activity. This precise activation of the NMDA receptor is key for synaptic plasticity and remodeling neuronal circuits. Each NMDA receptor contains different subunits: two NR1 and two NR2 divided into four subtypes A-D. The NR2B subunit caught our attention. Its expression is developmentally regulated: a switch in NR2B-containing receptors by NR2A-containing receptors is seen after development which is associated with the closure of the “critical period of synaptic plasticity during brain development”. NR2B re-expression can restore critical period-like plasticity in adult sensory cortex, thus promoting learning and memory. Our aim is to identify PAMs of NR2B function to enhance synaptic plasticity and improve learning and memory in neuronal circuits with synaptic loss such as aged/AD brains.

Here we report the structure activity relationship of a series of NR2B PAMs pyridopyrazoles originated from a HTS campaign. Further characterization of advanced compounds revealed their potential as in vivo tools to understand the role of NR2B in synaptic plasticity and their potential application in neurodegenerative diseases.

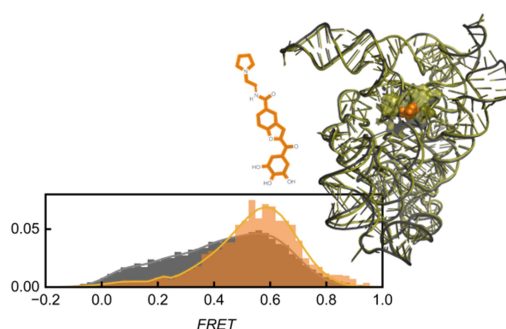
Locking RNA in motion: Dynamic Mechanism of Group II Intron Inhibition

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Group II introns are large self-splicing ribozymes. Since they exhibit considerable dynamic plasticity to attain catalytic activity, they serve as powerful model systems for studying RNA folding and splicing mechanisms. While being absent in the human genome, they occur in essential housekeeping genes of several human-pathogenic fungi and bacteria, making them promising antimicrobial targets with minimal host-cell toxicity.^[1] Intronistat B, a small-molecule inhibitor that binds the catalytic core, disrupts intron splicing.^[2] Although crystal structures provide static snapshots of inhibitor binding to the catalytic core, they offer valuable insights into the bound state. However, they do not fully capture how inhibition affects the structural rearrangements required for catalysis.^[3]

To address this limitation, we employ two-color single-molecule Förster Resonance Energy Transfer (smFRET) to follow structural rearrangement within the RNA. This approach allows us to follow conformational changes of individual RNA molecules during folding and catalysis, both in the presence and absence of Intronistat B. Our data reveal substantial alterations in domain-specific motions essential for catalytic activity, accompanied by pronounced changes in the dynamics of conformational rearrangements. These findings illuminate dynamic features of RNA when interacting with the small molecule that are otherwise inaccessible by static structural techniques, offering a foundation for the rational design of next-generation target-specific antimicrobials.



- [1] J. Nosek, M. Novotna, Z. Hlavatovicova, D. W. Ussery, J. Fajkus, L. Tomaska, *Molecular Genetics and Genomics* **2004**, 272, 173
- [2] O. Fedorova, G. E. Jagdmann, R. L. Adams, L. Yuan, M. C. Van Zandt, A. M. Pyle, *Nat. Chem. Biol.* **2018**, 14, 1073
- [3] I. Silvestri, J. Manigrasso, A. Andreani, N. Brindani, C. Mas, J.-B. Reiser, P. Vidossich, G. Martino, A. A. McCarthy, M. De Vivo, M. Marcia, *Nat. Commun.* **2024**, 15, 4980

Discovery and optimization of imidazole carboxamides, a new class of NLRP3 inflammasome inhibitorsT. Schuhmann¹¹Forum 1, Novartis Campus, CH-4056 Basel

The NLRP3 inflammasome is a key component of the innate immune system. It is activated by danger signals which leads to the production of pro-inflammatory cytokines IL-1 β and IL-18 as well as causes a type of pro-inflammatory cell death termed pyroptosis. Dysregulation of the NLRP3 inflammasome is associated with multiple inflammatory diseases including atherosclerosis¹ and Alzheimer's disease² making its inhibition an attractive therapeutic strategy. NLRP3 inhibitors that are currently subject of clinical investigation are largely limited to sulfonylurea-, and pyridazine-based scaffolds³. In a high-throughput screening campaign we identified novel naphthyl-substituted heteroaromatic amides which complement the toolbox of NLRP3 inhibitors. In our presentation, we will describe the binding mode of this new class of inhibitors, discuss its SAR and our multiparametric optimization strategy, which led to the identification of an advanced candidate for *in vivo* investigation.

[1] P. Duewell, H. Kono, K. J. Rayner, C. M. Sirois, G. Vladimer, F. G. Bauernfeind, G. S. Abela, L. Franchi, G. Nuñez, M. Schnurr, T. Espevik, E. Lien, K. A. Fitzgerald, K. L. Rock, K. J. Moore, S. D. Wright, V. Hornung, E. Latz, *Nature*, 2010, 464, 1357–1361.

[2] M. T. Heneka, M. P. Kummer, A. Stutz, A. Delekate, S. Schwartz, A. Vieira-Saecker, A. Griep, D. Axt, A. Remus, T. C. Tzeng, E. Gelpi, A. Halle, M. Korte, E. Latz, D. T. Golenbock, *Nature*, 2013, 493, 674–678.

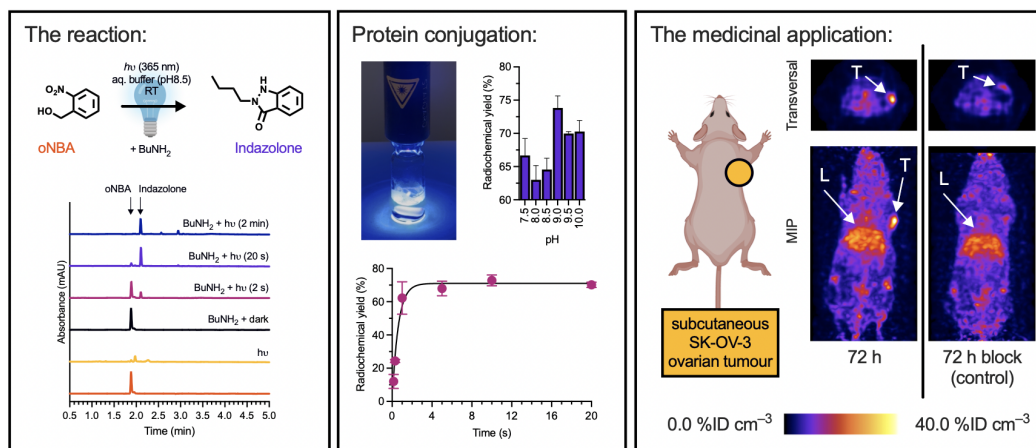
[3] A. Mackay, J. Velcicky, N. Gommermann, H. Mattes, P. Janser, M. Wright, C. Dubois, S. Brenneisen, S. Ilic, E. Vangrevelinghe, N. Stiefl, A. Boettcher, M. Schoenboerner, M. Vogelsanger, S. Muller-Bentz, M. Kamke, J. Rubert, M. Kauffmann, S. Desrayaud, M. Trunzer, H. Srinivas, A. Hinniger, N. von Burg, K. Beltz, C. Dekker, C. J. Farady, *J. Med. Chem.*, 2024, 67, 20780-20798.

Protein conjugates in a flash: light-activated *ortho*-nitrobenzyl alcohols for the production of antibodies against cancerJ. W. Southwell¹, C. Berton¹, S. Kichou¹, T. Chetot¹, J. Holland^{1*}¹University of Zürich, Zürich, Switzerland

In cases where their receptor is over-expressed in tumour tissue, the incredible specificity of monoclonal antibodies (mAbs) can be exploited for the targeted delivery of radionuclides for the imaging and treatment of cancer. Here, we report a versatile photochemical conjugation platform¹ using an *ortho*-nitrobenzyl alcohol (oNBA) motif² that forms a bioconjugate bond under mild, aqueous conditions and with excellent radiochemical conversions (RCC), 76.4±4.6% and isolated, decay-corrected radiochemical yields (RCY) 76.1±9.9% over a range of proteins and radionuclide combinations (*n*=6). Photoactivation of oNBA proceeds at 365 nm with high conversion on a millisecond timescale, to an indazolone conjugate bond through reaction with accessible lysine side-chains on the protein surface.

Whilst initial experiments showed that the photochemical reactivity of oNBA with a lysine mimic gave near quantitative conversion after ~20 s under purely aqueous conditions, the reaction was even faster on protein (~4 s). Next, a desferrioxamine-oNBA (DFO-oNBA) chelator was synthesised and quantitatively radiolabelled with the PET imaging radionuclide, zirconium-89. This complex was investigated for the radiosynthesis of [⁸⁹Zr]Zr-DFO conjugates with trastuzumab and onartuzumab for imaging ovarian and gastric cancer models, respectively. Both mAb conjugates retained excellent serum stability and immuno-competency *in vitro*. Subsequent imaging and biodistribution studies with [⁸⁹Zr]ZrDFO-indazolone-trastuzumab on immunocompromised athymic nude mice bearing sub-cutaneous SK-OV-3 xenografts showed excellent tumour uptake (23.9±2.8 %ID g⁻¹) and specificity (~72% tumour uptake reduction in blocking experiments). The onartuzumab conjugate also provided excellent imaging, where use of the *aza*-macrocyclic DOTA-oNBA chelator facilitated a therapy study using the beta-emitting radionuclide terbium-161.

This work establishes oNBA as a robust photoactive handle for developing diagnostic and therapeutic protein conjugates. We continue to explore use of the oNBA motif in the synthesis of other biomolecule conjugates to access novel pharmaceuticals and imaging agents.



1. Berton *et al. Chem. Commun.* **2025**, 61, 5234-5252
2. Guo *et al. Nat. Commun.* **2020**, 11 (1), 5472.

Can Molecular Dynamics Serve as a PostDocking Pose Filter?T. Steinbrecher¹¹Roche Pharma Research and Early Development, F. Hoffmann-La Roche Ltd, Switzerland

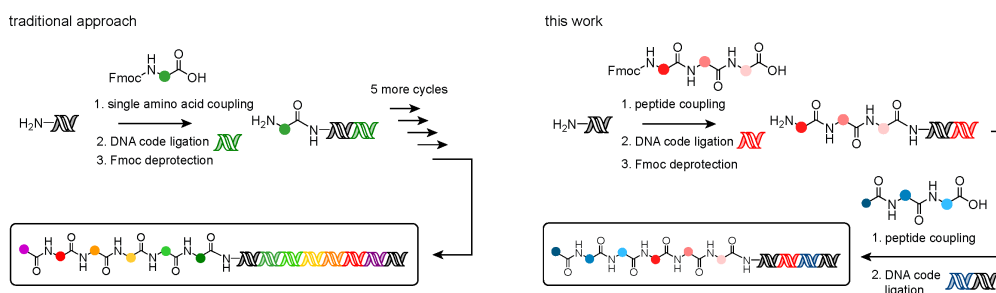
Molecular docking is a cornerstone of structure-based drug design but often fails to reliably identify the correct binding orientation of ligands, as commonly used scoring functions neglect protein flexibility and dynamic solvation effects. It is commonly assumed that a treatment of protein flexibility, e.g. by running molecular dynamics simulations, offers a more robust physics model of protein-ligand complexes. Here, we evaluate whether molecular dynamics (MD) simulations, run as a post-docking validation method, actually can confirm correct poses and flag incorrect ones. A curated benchmark of several hundred proteinligand complexes was assembled and automatically prepared for modelling. A semiautomated docking workflow generated both near-native and decoy poses to subject to explicit-solvent MD simulations. Pose stability and quality after MD were then evaluated. The findings confirm that short, GPU-accelerated MD simulations provide a simple and computationally feasible refinement step after docking.

Construction of DNA-Encoded Peptide Libraries with SPPS-Synthesized Building Blocks

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Many critical disease-related proteins, including cancer drivers like KRAS, β -catenin, and MYC, are difficult drug targets because they lack the binding pockets that traditional small molecule drugs require. Small peptides composed of mostly non-natural amino acids offer a promising solution, as these "beyond Rule of 5" compounds can bind flat protein surfaces while maintaining the ability to cross cell membranes.¹ However, successfully developing these molecules requires robust methods for generating and screening large combinatorial libraries.

DNA-encoded libraries (DELs) are a powerful platform for drug discovery, yet the success of their application to peptides has been limited. As many coupling steps are needed for the construction of peptides from amino acids, the quality of the final DELs is usually very low, distorting the genotype-phenotype linkage.² Here, we report the integration of solid phase peptide-synthesized (SPPS) building blocks into a DEL synthesis workflow to generate high-purity library in only two cycles. For this purpose, a minimal workup procedure for SPPS products without purification is employed, enabling the parallel synthesis of hundreds to thousands of building blocks in high quality. The coupling efficiencies of the synthesized tri- and tetramers were comparable to those of Fmoc-amino acids. A proof-of-concept 71 x 96 membered library featuring validated binders for MDM2 was synthesized. After affinity selection against MDM2, clear enrichment of the consensus sequence was observed, while the enrichment could be directly linked to the quality of the genotype-phenotype linkage.



[1] X. Ji, A. L. Nielsen, C. Heinis, *Angew. Chem. Int. Ed.*, **2024**, 136 (3), e202308251.

[2] M. Keller, D. Petrov, A. Gloger, B. Dietschi, K. Jobin, T. Gradinger, A. Martinelli, L. Plais, Y. Onda, D. Neri, J. Scheuermann, *Science*, **2024**, 384 (6701), 1259–1265.

STEREOCHEMICAL CONTROL OF SELF-ASSEMBLY IN EAK16 PEPTIDESY. AMER¹, J. REYMOND¹

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Self-assembling peptides such as EAK16 represent versatile biomaterials,¹ in which subtle variations in stereochemistry can profoundly influence self-assembly behavior.² EAK16 is well known to adopt β -sheet-rich conformations that readily assemble into nanofiber and hydrogel networks under physiological conditions.³ In this study, we employed circular dichroism spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, Thioflavin T fluorescence assays, and transmission electron microscopy (TEM) to characterize secondary structure formation, fibrillar morphology, and aggregation kinetics of various diastereomers of EAK16. The findings from this work are expected to contribute to the expanding body of knowledge on the structure–assembly relationships governing self-assembling peptide systems.

[1] Gelain, F., Luo, Z., Rioult, M. & Zhang, S. Self-assembling peptide scaffolds in the clinic. *npj Regen. Med.* 6, 9 (2021).

[2] Zheng, Y., Mao, K., Chen, S. & Zhu, H. Chirality effects in peptide assembly structures. *Front. Bioeng. Biotechnol.* 9, 703004 (2021).

[3] Zhang, S., Holmes, T., Lockshin, C. & Rich, A. Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc. Natl Acad. Sci. USA.* 90, 3334–3338 (1993).

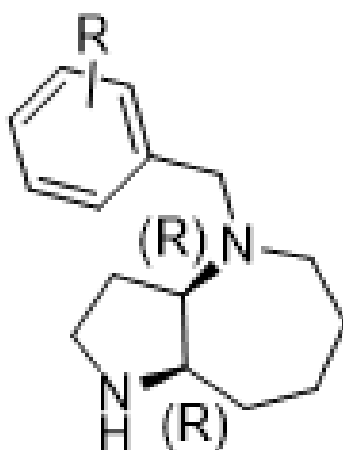
Synthesis of GDB Derived Bicyclic Diamines as Scaffolds for Medicinal Chemistry

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The Generated Data Base (GDB), curated by the Reymond group, offers stable, feasible, 3D-shaped chiral molecules, enabling exploration of a vast chemical space, providing promising scaffolds for Medicinal Chemistry. These molecules are innovative due to their polycyclic system, high sp^3 -hybridized carbon fraction and the presence of many quaternary carbons.

This project exploits a subset of the GDB, listing 1139 mono- and bicyclic saturated diamine scaffolds containing 5, 6 or 7 membered rings, including 680 novel accessible scaffolds. Herein I discuss initial synthetic steps for preparing a target subset of these diamines based on Diels-Alder and ring expansion reactions. Of these compounds we present promising preliminary biological results. As a proof-of-concept molecule **1** was recently synthesised in the group, showing potent inhibition of monoamine transporter, with selectivity to norepinephrine and dopamine transporter. Moreover, the amine functional group within the cyclic scaffolds grants straightforward functionalisation with desired moieties.



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- [1] Buehler Y, Reymond JL. A View on Molecular Complexity from the GDB Chemical Space. *J Chem Inf Model*.
- [2] Ruddigkeit L, Van Deursen R, Blum LC, Reymond JL. Enumeration of 166 Billion Organic Small Molecules in the Chemical Universe Database GDB-17. *J Chem Inf Model*. 2012 Nov 26;52(11):2864–75.
- [3] Lovering F, Bikker J, Humblet C. Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success. *J Med Chem*. 2009 Nov 12;52(21):6752–6.
- [4] Carrel A, Yiannakas A, Roukens JJ, Reynoso-Moreno I, Orsi M, Thakkar A, et al. Exploring Simple Drug Scaffolds from the Generated Database Chemical Space Reveals a Chiral Bicyclic Azepane with Potent Neuropharmacology. *J Med Chem*. 2025;

What is a small self-cleaving ribozyme doing next to a DNA polymerase gene?

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Small self-cleaving ribozymes are catalytic RNAs that promote site-specific phosphodiester backbone scission through internal transesterification reactions and are encoded across all domains of life. However, the biological functions of most known families remain unresolved, including those of the HDV-like ribozyme class, one of the most widely distributed families, with candidates found even in the human genome.^[1, 2]

We recently showed that a subclass of minimal HDV-like *theta* ribozymes located adjacent to tRNA genes in bacteriophages participates in tRNA maturation, providing a rare functional assignment for this family.^[3] Beyond tRNA-associated *theta* ribozymes, a broad bioinformatical search of gut bacteriophage genomes uncovered hundreds of additional HDV-like ribozyme candidates. Notably, we identified a distinct and widespread group located in the 5' untranslated region of phage DNA polymerase genes. Refinement of the structural search motif revealed more than 1,600 candidate loci, consistently positioned in close proximity to the DNA polymerase start codon.

Sequence analysis shows strong conservation across these elements and metal ion dependent *in vitro* cleavage assays confirm robust catalytic activity. In addition, the genomic context suggests regulated ribozyme activity, pointing to a functional role in controlling gene expression. We propose that self-cleavage modulates DNA polymerase expression, potentially by interfering with the recruitment of factors involved in translation.

Together, these findings represent a new and widespread class of HDV-like ribozymes directly linked to regulation of the phage replication machinery.

[1] L. Sharmeen, M. Y. Kuo, G. Dinter-Gottlieb, J. Taylor, Journal of Virology, 1988, 62, 2674-2679

[2] K. Salehi-Ashtiani, A. Lupták, A. Litovchick, J. W. Szostak, Science, 2006, 313, 1788-1792

[3] K. Kienbeck, L. Malfertheiner, S. Zelger-Paulus, S. Johannsen, C. von Mehring, R. K. O. Sigel, Nature Communications, 15, 1559

General Platform for Divergent Construction of Phosphorus-Modified DNA/RNA Backbones

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Phosphorus-containing biomolecules, including nucleotides, cyclic dinucleotides, and related cofactors, play central roles in signaling, immunity, and enzyme regulation. However, the highly anionic nature of canonical phosphate linkages severely limits the therapeutic potential of nucleotide-based modalities by restricting membrane permeability, metabolic stability, and systemic delivery. While charge-neutral or non-canonical phosphorus linkages offer a promising solution, their development remains constrained by synthetic challenges, limited structural diversity, and poor compatibility with oligonucleotide assembly workflows.

Here, we present a unified and modular platform for the direct modification of phosphorus centers within DNA and RNA backbones. Building on recent advances in organophosphorus radical chemistry, this approach enables the selective engagement of diverse reaction partners under mild conditions, overcoming the reactivity and selectivity limitations typically associated with complex nucleotide substrates. The strategy provides access to a broad range of previously inaccessible phosphorus linkages without relying on unstable reagents or extensive protecting group manipulations.

By decoupling nucleic acid assembly from phosphorus diversification, this platform enables rapid exploration of chemically and functionally diverse backbone architectures. More broadly, it establishes phosphorus as a tunable element for modulating physicochemical and biological properties, opening new opportunities for the development of nucleotide-based probes and therapeutics.

Antibacterial Activity of Novel Oncocin-Peptoid HybridsJ. Bucchioni¹, R. Rauscher¹¹Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland

The escalating prevalence of multidrug-resistant Gram-negative bacterial infections necessitates the exploration of novel antimicrobial agents. Among synthetic analogues of antimicrobial peptides (AMPs), peptoids (N-alkylated oligoglycines) have been reported to act both by membrane disruption and on intracellular targets. Our research group has already demonstrated that substituting amino acids with their peptoid equivalents in analogues of polymyxin B [1] and the undecapeptide ln69 (KKLLKLLKLLL) [2] enhances antibacterial potency and reduces cell toxicities.

Based on this, we investigated the modification of the last nine C-terminal residues of Oncocin (VDKPPYLPRPRPPRIYNR-NH₂) [3] (Figure 1) by incorporating mixed peptide/peptoid chains, along with random chirality alterations. Selected amino acid residues were progressively substituted with their N-alkylated counterparts to influence pharmacokinetics, specifically cellular uptake. Preliminary biological assays reveal that new hybrids exhibit broader antimicrobial activity.

[1] Bonvin, E., et al. Antimicrobial Peptide–Peptoid Hybrids with and without Membrane Disruption. *ACS Infectious Diseases*. 2023, 12, 2593-2606.

[2] Bonvin, E., et al. Antimicrobial Peptide□Peptoid Macrocycles from the Polymyxin B2 Chemical Space. *Angew. Chem. Int.* 2025, 64, e202501299 doi.org/10.1002/anie.202501299.

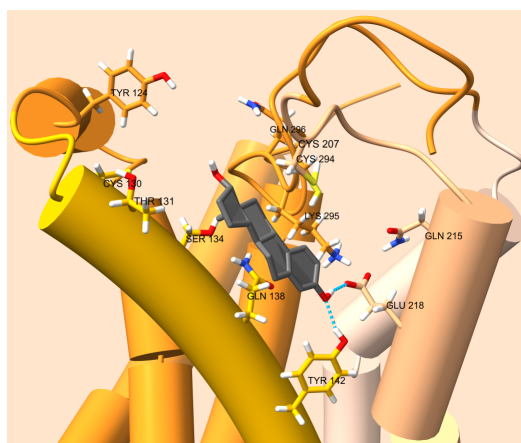
[3] Gan, B. H., et al. Stereorandomized Oncocins with Preserved Ribosome Binding and Antibacterial Activity. *J. Med. Chem.* 2024, 67, 19448-19459.

Molecular Mechanisms Underlying GPER Activation and Deactivation

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The G protein-coupled estrogen receptor (GPER) is a non-classical estrogen receptor that mediates rapid estrogen signaling and has emerged as an important therapeutic target in cancer, cardiovascular, and endocrine diseases [1-3]. Despite extensive experimental studies, the molecular mechanisms underlying GPER activation and inhibition remain incompletely understood [1-4]. In this work, we identified and characterized a binding pocket for 17 β -estradiol (EST) in GPER using molecular dynamics simulations. The thermodynamic stability of the proposed binding mode was evaluated through free energy calculations, providing quantitative support for EST binding. To elucidate ligand-dependent receptor regulation, we compared the structural and dynamic behavior of the apo receptor, the EST-bound complex, and complexes containing the selective GPER antagonists G15 and G36 [4, 5]. Our analyses revealed distinct conformational signatures associated with receptor activation and deactivation, including ligand-dependent rearrangements of transmembrane helices. In addition, machine learning-based analysis of simulation trajectories was employed to map the conformational landscape of GPER and identify dominant metastable states. The results demonstrate how agonist and antagonist binding differentially modulate GPER conformational dynamics and provide molecular insights into the mechanisms governing receptor activation. These findings contribute to the structural understanding of GPER pharmacology and may facilitate the rational design of selective modulators targeting estrogen-mediated signaling pathways.

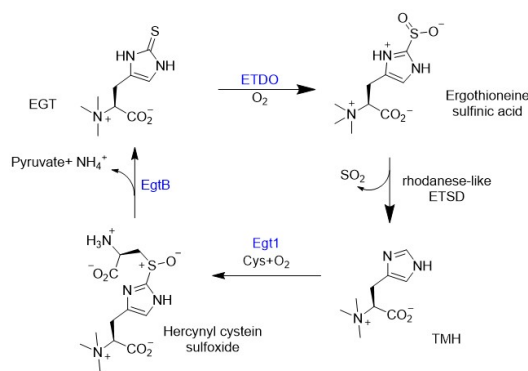


- [1] Eric R. Prossnitz, Matthias Barton, *Nature Reviews Endocrinology*, **2023**, 19, 407–424.
- [2] Jeffrey B. Arterburn, Eric R. Prossnitz. *review of pharmacology and toxicology*, **2023**, 63, 295-320.
- [3] Aisha Bushi et al., *Pharmacology & Therapeutics*, **2025**, 269, 108822.
- [4] Diana Méndez-Luna et al., *Journal of Biomolecular Structure and Dynamics*, **2015**, 33, 2161-2172.
- [5] Maryam Rouhimoghadam et al., *Frontiers in Endocrinology*, **2020**, 11, 591217.

Enzyme-Catalyzed Oxidative Degradation of Ergothioneine

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Ergothioneine (EGT) is a sulfur-containing antioxidant that protects cells against oxidative stress, yet pathways for the regeneration of oxidatively damaged EGT remain poorly understood in fungi. In many actinobacteria, EGT is synthesized from N α -trimethyl histidine (TMH) and subsequently degraded through O₂-dependent dioxygenation oxidation by an iron-dependent ergothioneine dioxygenase (ETDO), yielding ergothioneine sulfinic acid. Cleavage of this intermediate by ergothioneine sulfinic acid desulfonase (ETSD) regenerates TMH.^{1,2} Hence, many actinobacteria that contain enzymes to sulfurize and desulfurize TMH may be able to regulate the cellular EGT/TMH ratio via this oxidative cycle.³ Other organisms that contain no ETDO may utilize their ETSD to recycle EGT that was oxidized via uncatalyzed processes. In this presentation, we will discuss our latest effort to understand the mechanism of these enzymes and their involvement in EGT-based defense against oxidative stress.



[1] Nalivaiko, E.Y., Vasseur, C.M. and Seebeck, F.P., *Angewandte Chemie*, **2024**, 136(8), p.e202318445.

[2] E. Y.Nalivaiko, F. P.Seebeck, *ChemBioChem*, **2024**, 25, e202400131.

[3] Seebeck, F.P., *Journal of the American Chemical Society*, **2010**, 132(19), pp.6632-6633.

Development of novel orthosteric-allosteric bitopic ligands to study adenosine A₁ receptors

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Adenosine receptor (AR) subtypes (A₁, A_{2a}, A_{2b}, A₃) are implicated in a range of physiological and pathological functions, thus rendering them interesting drug targets.^{1,2} The downstream signalling is mediated by interactions with different G proteins, so not only subtype but also G protein selectivity (biased agonism) is important.³ BnOCPA is the first adenosine A₁ receptor (AA₁R) biased agonist (signalling only through G_{ob}).⁴ Nevertheless, increasing potency, subtype and G protein selectivity with monovalent orthosteric ligands is challenging.⁵ Simultaneously targeting the orthosteric and allosteric binding sites of GPCRs has the potential to overcome these limitations.^{5,6} Several studies have demonstrated that adenosine-based bitopic ligands result in biased AA₁R agonism. However, they are also active at the AA_{2b}R.^{5,7}

The aim of this project is to develop novel orthosteric-allosteric bitopic AA₁R ligands. Phenylloxycyclopentyladenosine derivatives (PhOCPA), which are highly potent and AA₁R selective, tolerate various substituents in *meta*-position of the phenoxy ring.⁸ Based on these findings, the PhOCPA scaffold was functionalised with different linker-PAM conjugates to identify an optimal bitopic ligand. The binding affinities of these compounds for the A₁ and A₃ receptor subtypes were evaluated using a NanoBRET competition assay.

- [1] J. F. Chen, H. K. Eltzschig, B. B. Fredholm, *Nature Reviews Drug Discovery*, 2013, 12, 265–286.
- [2] P. A. Borea *et al.*, *Physiological Reviews*, 2018, 98, 1591–1625.
- [3] K. A. Jacobson *et al.*, *Frontiers in Cellular Neuroscience*, 2019, 13, 124.
- [4] M. J. Wall *et al.*, *Nature Communication*, 2022, 13, 4150.
- [5] L. Aurelio *et al.*, *Journal of Medicinal Chemistry*, 2018, 61, 2087–2103.
- [6] J. R. Lane, P. M. Sexton, A. Christopoulos, *Trends in Pharmacological Sciences*, 2013, 34, 59–66.
- [7] J. K. Awalt *et al.*, *Journal of Medicinal Chemistry*, 2022, 65, 9076–9095.
- [8] B. Preti *et al.*, *Journal of Medicinal Chemistry*, 2022, 65, 14864–14890.

Synthesis of fluorescent ligands to study the adenosine A₁ receptors

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The adenosine receptor is a GPCR with four receptor subtypes, named A₁R, A_{2A}R, A_{2B}R and A₃R, that are involved in different pathological conditions like cancer, respiratory, cardiovascular and neurological diseases.[1] Therefore, the adenosine receptors are interesting targets for the development of new treatments with different pharmacological mechanisms.[1] To study these therapeutic targets a biological tool is required. A fluorescent ligand seems to be an ideal instrument to visualize unmodified GPCRs and monitor their protein-protein interactions, mobility or localized expression level.[2] There are examples of fluorescently labelled ligands for different ARs, however, a fluorescent agonist with high selectivity for the adenosine A₁ receptor (AA₁R) is lacking.[3][4]

The aim of this project is to develop a AA₁R selective fluorescent probe to study signalling processes. Phenyloxycyclopentyladenosine derivatives (PhOCPA) were discovered as highly selective and potent agonist for the AA₁R in our group.[5] This agonist is used as orthosteric ligand that is connected to a fluorophore using different linkers. The agonists *meta*-position of the phenoxy group tolerates various substituents and is therefore chosen for linker functionalisation. It is crucial that the receptor tolerates the linker and fluorescent dye without disturbing binding affinity of the agonist. Thus, a library of different linkers is chosen, to identify a fluorescent ligand that maintained high selectivity and potency. Here the multistep synthesis of these fluorescent adenosine A₁R agonists is described.

[1] F.J. Chen, H.K. Eltzschig, B.B. Fredholm Nature Reviews Drug Discovery 2013 12, 265–286.

[2] A.J. Vernall *et al.* Journal of Medicinal Chemistry 2012 55, 1771–1782.

[3] R.J. Middleton *et al.* Journal of Medicinal Chemistry 2007 50, 782–793.

[4] E. Kozma *et al.* Biochemical Pharmacology 2013 85:1171-81.

[5] B. Preti *et al.* Journal of Medicinal Chemistry 2022 65, 14864-14890.

Synthesis of PEG decorated amine functionalized Zn-CP and investigation of its capability for doxorubicin loading and controlled release

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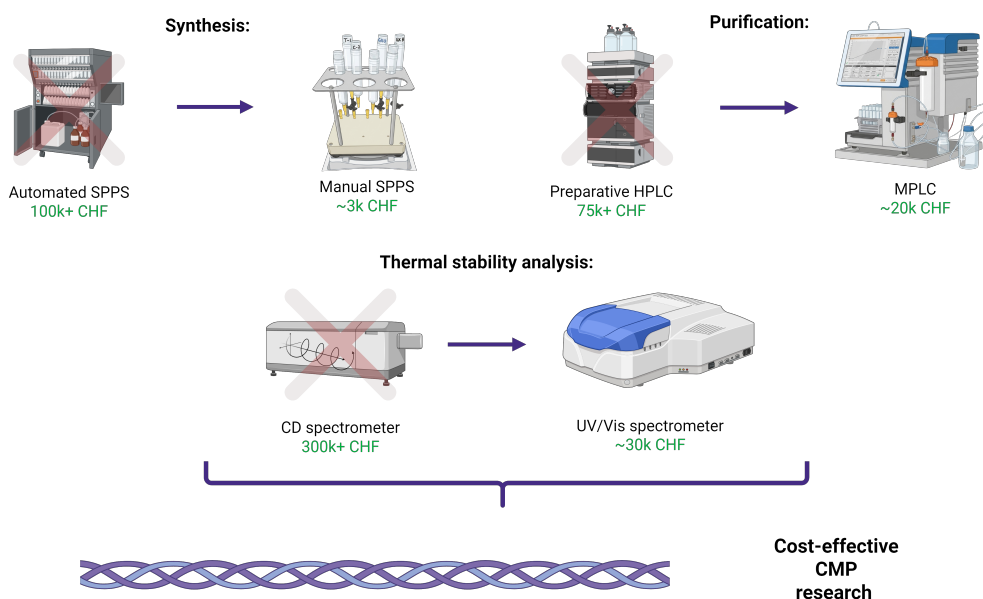
Metal-organic frameworks (MOFs) have received much attention as promising drug delivery systems due to their unique properties. In our previous investigation, a new zinc coordination polymer of 5-aminoisophthalic acid called Zn-CP was prepared which showed promising drug carrier properties. In the present study, the surface of previously-prepared Zn-CP was modified with -NH_2 groups, loaded with doxorubicin (Dox) as the model drug, PEGylated with polyethylene glycol (PEG), and its cytotoxic efficiency was investigated on breast cancer cells. Moreover, various properties of the carrier such as drug loading and loading efficiency, hydrodynamic stability, and colloidal stability were studied and compared to the results of Zn-CP and Zn-CP-SO₃H carriers. According to the results, the synthesized Zn-CP-NH₂ has a cubic structure with an average diameter of 195 nm and a zeta potential of around -35 mV. The Dox loading and loading efficiency of Zn-CP-NH₂@PEG were 41, and 70.64%; respectively. The in-vitro cytotoxicity of the drug delivery system was evaluated on MCF7 and MCF10 cells by MTT assay. The results revealed that it could significantly inhibit the proliferation of cancerous cells. NH₂-functionalized Zn-CP improved cellular uptake and cytotoxicity in MCF7 breast cancer cells compared to Zn-CP-SO₃H and Zn-CP due to the enhanced Dox loading, intracellular stability, and delayed drug-release capability. Hence, functionalized Zn-CP could be considered as a promising candidate for drug delivery.

Democratizing collagen research: a cost-effective concept for the synthesis and biophysical studies of collagen model peptidesM. Jaros¹, T. Fiala^{1*}¹Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, Brno 625 00, Czech Republic

Collagen model peptides (CMPs) represent valuable tools for studying the structure and stability of collagen – one of the most abundant protein families in the animal kingdom. However, their broader utilization is often limited by the high cost of peptide synthesis, purification, and biophysical characterization. Here, we present a cost-effective workflow for the preparation and analysis of CMPs, aimed at making collagen peptide research more accessible. The methodology combines:

- 1) efficient solid-phase peptide synthesis carried out under simplified laboratory conditions without the use of an automated peptide synthesizer,
- 2) purification on a common medium-pressure liquid chromatography instrument, without the need for preparative HPLC,
- 3) triple helix stability analysis by thermal denaturation with UV/Vis absorbance monitoring, without the need for a circular dichroism (CD) spectrometer.

The presented approach provides an affordable platform for collagen peptide research and biophysical studies in resource-limited environments.



Using Repurposed Metalloenzymes to Address Challenges in Organic Synthesis

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Metalloenzymes, as nature’s catalysts, offer unmatched advantages over small-molecule organic catalysts in selectivity, sustainability, cost, and evolvability. By repurposing native metalloenzymes for new-to-nature transformations, we aim to address long-standing challenges in organic synthesis through biocatalytic strategies. Here, we report the engineering of cytochrome P450s to enable metal hydride hydrogen atom transfer (MHAT) toward unactivated alkenyl substrates. This transformation generates highly reactive carbon-centered radical intermediates under mild conditions, while the chiral environment of enzyme pocket enables precise controls of subsequent reactivity. Leveraging this strategy, we achieved stereoselective intermolecular C–C and C–O bond formations that difficult to achieve with organosynthetic methods. This biocatalytic platform provides a powerful approach for selective molecular diversification, with potential applications in late-stage functionalization and pharmaceutical synthesis.

Towards a Modular Platform for Tissue/ Cell-specific Transfection: mRNA Transfection using Branched Peptides

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The COVID-19 pandemic has brought big advances in the field of mRNA delivery. The success of the mRNA vaccines has underscored the potential of therapeutic mRNA and has revitalized the research for delivery systems. To treat chronic or rare diseases research must go beyond immunization which presents additional challenges for the development of delivery systems. For example, to restore the function of a protein, expression levels a 1000 times higher than what is typically necessary for immunization are needed.¹ Therefore, the development of viral vectors, lipid nanoparticles (LNPs), polymers, and branched peptides has been pushed forward extensively. Drawbacks as immunogenicity, undisclosed structures, or nonstandard synthetic procedures has motivated this research group to explore branched peptides as a nucleic acid delivery system.²

In this work, we aim to establish a platform for specific mRNA delivery. Using cysteines in specific positions on the branched peptide allows for overnight modification with targeting moieties in a mix and match manner. As a proof of concept, the conjugates with a Cardiomyocyte-Targeting-Peptide (CTP) are tested on the immortalized cardiomyocyte cell line AC16.³

[1] E. Rohner, R. Yang, K. S. Foo, A. Goedel, K. R. Chien, *Nat. Biotechnol.* 2022, 40 (11), 1586-1600

[2] M. Heitz, S. Javor, T. Darbre, J. L. Reymond, *Bioconjugate Chem.*, 2019, 30, 2165-2182

[3] D. Sahagun, M. Zahid, *Biomolecules*, 2023, 13, 1690.

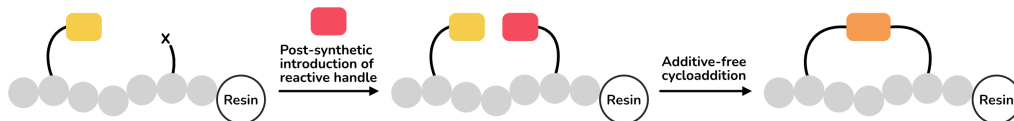
Solid-Phase Peptide Macrocyclization based on Cycloaddition Reactions

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Macrocyclic peptides are emerging as promising scaffolds for therapeutic development, as their restricted conformation grants them pharmacological advantages over linear analogs.^[1] To access these structures, a broad array of macrocyclization methods has been developed over the years, using either native side-chain functionalities or non-native bioorthogonal chemistry. While established strategies such as olefin ring-closing metathesis or [3+2] cycloaddition reactions are highly effective, they often rely on transition-metal catalysis. This requires intensive post-synthetic purification to ensure biocompatibility, driving the need for robust metal-free stapling methodologies.^[2]

Here, we report a new macrocyclization strategy based on additive-free cycloaddition reactions performed on solid support. Linear precursors were synthesized using microwave-assisted automated Fmoc-SPPS, followed by chemoselective peptide labelling to introduce reactive handles for cycloaddition. We optimized the subsequent on-resin cyclization through solvent, time, and temperature screenings to maximize conversion, and carried out kinetic studies to evaluate the impact of handle length and structural properties. Our approach proceeds with high conversion, and the resulting macrocyclic peptides can be isolated on a preparative scale. Furthermore, we assessed the compatibility of various amino acid sequences and *i+n* connectivities with our methodology, demonstrating its robustness across a diverse chemical space. To streamline scope generation, we synthesized peptide libraries using isokinetic amino acid mixtures.^[3,4] After simultaneous labelling of the resulting peptide pool, we were able to monitor progress of the subsequent cyclization by HPLC-MS and track individual components of the mixture. Finally, the scope was extended further to target biologically-relevant peptides.



1. Alexander A. Vinogradov, Yizhen Yin, Hiroaki Suga, *J. Am. Chem. Soc.* **2019**, 141, 4167-418.
2. Wanglin Zhan, Hongliang Duan, Chengxi Li, *Chem. Bio. Eng.* **2024**, 1, 593-605.
3. John M. Ostresh, James H. Winkle, Vince T. Hamashin, Richard A. Hough, *Biopolymers* **1994**, 34, 1681-1689.
4. John S. Albin, Gha Y. Lee, Corey Johnson, Dimuthu A. Vithanage, Wayne Vuong, Bradley L. Pentelute, *Chem* **2025**, 11, 102501.

Stabilization or Refolding? - Identifying the role of an RNA-binding protein in ribozyme activity

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RNA-binding proteins play a central role in shaping the folding landscapes of catalytic RNAs. RNA helicases in particular, are thought to promote folding of the catalytically active structure through local interactions. The mechanistic role of these helicases, however, remains unresolved: do they stabilize the active fold, or do they unwind misfolded structures in an ATP-dependent manner to enable productive refolding?

Here, we combine photoisomerization-related fluorescence enhancement (PIFE) with Förster resonance energy transfer (FRET), two complementary single-molecule techniques, to investigate the interaction mechanism of a helicase with a group II intron. Our preliminary findings challenge the current understanding of a purely local interaction, as the helicase can translocate along single stranded RNA. This allows for potential strand unwinding in a global, processive manner.

This work combines the insights of single molecule PIFE and FRET measurements as powerful tools for probing RNA-protein interactions in large ribozymes. Furthermore, we clarify the RNA remodeling capabilities of a member of a conserved RNA helicase family and provide new insights into the chemical and mechanistic basis of helicase assisted ribozyme folding.

[1] S. Mohr, M. Matsuura, P. S. Perlman, and A. M. Lambowitz, Proceedings of the National Academy of Sciences of the United States of America, 2006, 103, 3569–3574

[2] A. M. Pyle, Annual review of biophysics, 2016, 45, 183–205

[3] E. Ploetz et al., Sci Rep, 2016, 6, 33257

[4] H. Hwang and S. Myong, Chemical Society reviews, 2014, 43, 1221–1229

Combine and shine: Modular enzymatic and chemical approaches to generate site-specific labeled RNA for smFRET

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Site-specific labeling is essential for investigating RNA structure and dynamics, particularly in single-molecule FRET (smFRET) studies, where positioning of fluorescent dyes enables accurate distance measurements and conformational analysis. However, achieving site-specific labeling of RNA remains challenging due to its limited chemical diversity compared to proteins.

Here, we overcome these limitations by integrating complementary enzymatic and chemical strategies into a unified workflow. By combining ligase-mediated assembly of RNA fragments, engineered polymerases [1] and hydrazone-based dye conjugation [2], we use the modularity of RNA to enable adaptable site-specific labeling. In this work, we applied this multi-method strategy to generate diverse variants of labeled riboswitch constructs that would be difficult to access using exclusively a single technique.

These synergistic approaches establish a versatile platform for site-specific RNA labeling, expanding the toolbox for robust and reliable smFRET studies.

[1] R. Haslecker, V.V. Pham, D. Glänzer, *et al.*, Nat Commun, 2023, 14, 8422.

[2] E. Ahunbay, F.D. Steffen, S. Zelger-Paulus, R.K.O. Sigel, Methods in Molecular Biology, 2022, 2439.

Application of Peptide-Encoded Libraries of Small Molecules for the Discovery of Chemical Inducers of ProximityM. Masota¹, N. Grob^{1*}¹Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Bioscience, ETH Zurich

Targeted protein degradation (TPD) is an innovative therapeutic approach that relies on chemically induced proximity to recruit an E3 ligase to a specific target protein, ultimately triggering its degradation. While the early chemical inducers of proximity (CIPs) were discovered mostly by serendipity, modern discovery techniques are often low-throughput and highly labor-intensive due to the challenging nature of these neo-protein–protein interactions.¹ Consequently, there is a need for efficient, high-throughput screening methodologies to discover novel CIPs tailored for applications in TPD.

To address the challenges of modern CIP discovery, we employ peptide-encoded libraries (PELs) featuring a modular chemical design to efficiently discover novel CIPs targeting therapeutically relevant proteins. PELs are uniquely suited for this approach because the chemical stability of the peptide tag ensures the synthesis of high-quality libraries with full synthetic control.² Our preliminary data confirm productive ternary complex formation in a model system using a peptide-encoded derivative of a known CIP and its relevant protein partners. This poster presentation will discuss our progress and the challenges encountered in the development of a robust screening workflow that integrates in-solution affinity selection–mass spectrometry (AS-MS). Ultimately, this project aims to establish a versatile, high-throughput platform for discovering novel CIPs across a diverse range of protein targets.

1. Stuart Schreiber, *Cell Chemical Biology*, **2024**, 31, 1050-1063.
2. Simon Rössler, Nathalie Grob, Stephen Buchwald and Bradley Pentelute Science, **2023**, 379, 939-945.

Harnessing PROTAC-mediated degradation to trigger cytosolic uncoating of an engineered nucleocapsid

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A major bottleneck in mRNA delivery is the lack of mechanisms that actively trigger cargo release after cytosolic entry. While engineered protein nanocarriers can package RNA with high precision, strategies to program their intracellular disassembly remain largely unexplored. Here we investigate whether targeted proteasomal degradation can be harnessed as a molecular trigger for nucleocapsid uncoating inside mammalian cells.

Our system is based on NC4, a 240-subunit $T=4$ capsid evolved from *Aquifex aeolicus* lumazine synthase that selectively packages its own encoding mRNA¹. To enable conditional degradation, we engineered HaloTag domains on the capsid exterior, allowing recruitment of E3 ubiquitin ligases through HaloPROTAC molecules. Cytosolic delivery of capsids into HeLa cells was monitored using a split-luciferase reporter system in which capsids display HiBiT peptides that complement cytosolic LgBiT.

Electroporation of capsids produced a strong luminescence signal, confirming cytosolic access, whereas endocytic uptake generated minimal signal. Upon addition of HaloPROTAC, luminescence decreased rapidly over time, consistent with targeted degradation of capsid subunits through the ubiquitin–proteasome pathway. In contrast, capsids remained stable in the absence of the degrader.

These results provide the first evidence that engineered nucleocapsids can be selectively targeted for proteasomal degradation in the cytosol using small molecules. Ongoing work aims to determine whether this process enables controlled release of encapsidated mRNA. More broadly, this strategy introduces a programmable intracellular disassembly mechanism for protein nanocarriers, enabling the design of delivery systems in which cargo release can be externally controlled.

[1] Tetter, S. *et al.* Evolution of a virus-like architecture and packaging mechanism in a repurposed bacterial protein. *Science* **372**, 1220–1224 (2021).

Chemically Programmable Bacterial Probes for the Recognition of Cell Surface Proteins

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In living cells, information is processed and transferred through a series of recognition and signaling events, typically initiated by the binding of cell-surface receptors to extracellular signals like small molecules or proteins. There has been significant interest in engineering cells with artificial receptors to impart new functionalities. We have recently developed a method for decorating bacterial surfaces with self-assembled synthetic receptors using modified DNA duplexes.¹⁻² Integrating this approach with DNA nanotechnology enables the modification of bacteria with well-defined nanostructures, which can be constructed in a programmable and predictable manner via simple self-assembly processes. This structural programmability has allowed us to create bacterial probes (B-probes) capable of labeling distinct types of cancer cells with different colors. We have also engineered highly fluorescent B-probes with enhanced sensitivity by spatially separating multiple dyes along the DNA scaffold to prevent self-quenching. Furthermore, we have demonstrated that a similar approach can be used to confer new properties to bacteria, such as the ability to emit different colors, adhere to surfaces, and interact with proteins or cells. These capabilities hold promise for the development of cell-imaging techniques, living materials, devices, as well as therapeutic and diagnostic systems based on live cells.

1. N. Lahav-Mankovski, P. Kishore Prasad, N. Oppenheimer-Low, G. Raviv, T. Dadosh, T. Unger, T. Meir Salame, L. Motiei, D. Margulies, *Nat. Commun.* **2020**, 11, 1299.

2. P. Kishore Prasad, N. Eizenshtadt, I. Goliand, L. Liat Fellus-Alyagor, R. Oren, O. Golani, L. Motiei, D. Margulies, *Mater. Today Bio.* **2023**, 20, 100669.

Rhodamine based biosensor for the investigation of labile ironA. M. Nicoli¹, M. S. Frei^{1*}¹Laboratory of Organic Chemistry, ETH Zürich, Vladimir-Prelog-Weg 3, 8093 Zürich, Switzerland

Iron, one of the most abundant transition metals found in the human body, is involved in numerous biological processes. High cellular concentrations of free-dissolved iron(II) ions, often referred to as the labile iron pool (LIP), can induce a particular type of cell death known as ferroptosis. Moreover, imbalances in LIP levels are associated with various diseases. Despite the importance of the ferroptotic process, the specific mechanism that initiates this cell death pathway is still unknown. Using synthetic chemistry and biomolecular approaches we developed an iron(II)-specific sensor for monitoring cellular and subcellular iron(II) levels by fluorescence microscopy in live cells. In particular, we leverage the paramagnetic quenching effect of iron(II) on the fluorescence lifetime of silicon rhodamine upon binding to a conjugated chelator. Targeting via a self-labeling protein such as HaloTag will additionally allow us to read out subcellular concentrations, providing deeper insight into the complex cellular signaling pathways that involve this metal in human cells.

High-Efficiency Photochemical Bioconjugation: Optimising Benzophenone and Introducing Novel Furan Architectures for Stable Radioimmunoconjugates

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Introduction

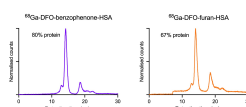
Photochemistry is a highly versatile approach that can be used in radiochemistry, offering significant bioconjugation advantages such as rapid reaction kinetics and mild operational conditions.^[1] Benzophenone serves as an important foundational photoreactive residue, generating a highly reactive ketyl diradical upon ultraviolet irradiation to facilitate covalent cross-linking. Building on this established chemistry, compact alpha-keto amides have emerged as excellent alternatives that can be easily functionalised to capture specific biological interactions.^[2,3] This study explores the optimisation of these fundamental systems to streamline the production of stable radioimmunoconjugates.

Results and Discussion

A desferrioxamine(DFO)-benzophenone probe was synthesised and characterised via ¹H-NMR, ¹³C-NMR and mass spectrometry. The construct demonstrated excellent radiolabelling compatibility with both ⁸⁹Zr and ⁶⁸Ga, as confirmed by radio-iTLC and radio-HPLC, maintaining the integrity of DFO coordination sphere. Optimisation of the bioconjugation parameters under 365 nm irradiation resulted in a significant breakthrough covalent attachment to the model protein, human serum albumin (HSA) reached 80%, more than doubling the standard literature yields (~30%)^[1]. Furthermore, a novel DFO-furan derivative was synthesised, characterised and evaluated, achieving a robust efficiency of 67±3 % (n=3). Mechanistic mapping against a panel of amino acids revealed that while both motifs preferentially target methionine residue, the furan derivative exhibits an expanded reactivity profile that warrants further investigation.

Conclusion

These findings established a high-efficiency framework for light-driven bioconjugation in radiopharmaceutical science. By significantly surpassing traditional yield benchmarks and introducing a versatile furan-based photophores this work provides a robust toolkit for the rapid generation of stable radioimmunoconjugates. The broader reactivity observed in the furan motif suggests potential for more diverse protein-targeting applications, currently under active study.



[1] D. F. Earley, A. Guillou, S. Klingler, R. Fay, M. Gut, F. d'Orchymont, S. Behmaneshfar, L. Reichert, J. P. Holland, “, *JACS Au* **2022**, 2, 646–664.

[2] E. Ota, K. Usui, K. Oonuma, H. Koshino, S. Nishiyama, G. Hirai, M. Sodeoka, *ACS Chem. Biol.* **2018**, 13, 876–880.

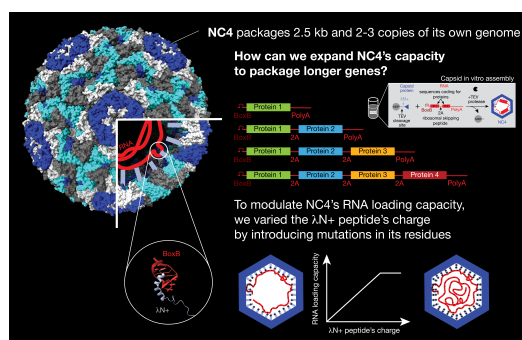
[3] T. Moriyama, D. Mizukami, M. Yoritake, K. Usui, D. Takahashi, E. Ota, M. Sodeoka, T. Ueda, S. Karasawa, G. Hirai, *Chemistry A European J* **2022**, 28, e202103925.

Charge-driven expansion of the RNA packaging of an engineered protein cage

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RNA-based therapeutics are a promising area of research, yet their clinical application is limited by challenges in RNA packaging and delivery. A bacterial enzyme was engineered to form a 240-subunit icosahedral capsid that packages and protects its own mRNA, providing a model for studying capsid assembly dynamics and designing nanocarriers. Capsid formation in bacteria is driven by the specific interaction between RNA packaging signals and the λN^+ peptide, analogous to the arginine-rich motifs (ARMs) found in natural ssRNA viruses. These viruses typically package 1.5–2 nucleotides per unit of ARM charge, and our capsid achieves a similar ratio of 1.7, encapsulating approximately 2.5 kb of RNA. The aim of this study is to expand the RNA loading capacity of the capsid and enable packaging of longer RNAs. By mutating the λN^+ peptide to increase its net positive charge, we achieved enhanced RNA loading in vivo, extending the packaging capacity to ~4 kb. Furthermore, we employed a previously developed in vitro assembly (IVA) method to assemble the capsid around a purified mRNA of interest. Analytical methods including native agarose gels, denaturing PAGE, and UV/Vis spectroscopy were used to assess the effect of capsid charge on RNA packaging efficiency and capacity. This in vitro assembly platform enables systematic evaluation of RNA encapsulation by capsids with varying charges and allows the engineering of RNA carriers capable of packaging RNAs of different sizes.



[1] Stephan Tetter, Naohiro Terasaka, Angela Steinauer, Bingham J. Richard, Sam Clark, Andrew J. P. Scott, Nikesh Patel, Marc Leibundgut, Emma Wroblewski, Nenad Ban, Peter G. Stockley, Reidun Twarock, Donald Hilvert, *Science*, **2021**, 372 (6547), 1220–1224.

[2] Mao Hori, Angela Steinauer, Stephan Tetter, Jamiro Hälgl, Eva-Maria Manz, Donald Hilvert, *Nature communication*, **2024**, 15, 3576.

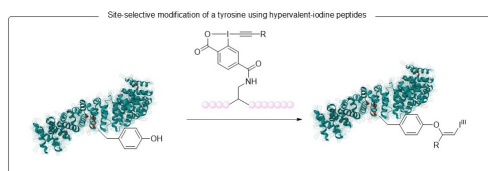
Site-Selective β -Catenin Modification Using Peptides Bearing a Hypervalent Iodine Group as a Covalent Warhead

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Achieving site-selective protein modification remains a central challenge in chemical biology and drug discovery, particularly for targets that lack well-defined small-molecule binding pockets. Peptides occupy a unique chemical space between small molecules and biologics, combining favorable features of both classes. Compared to small molecules, peptides offer enhanced specificity and efficacy due to their larger surface area and ability to engage in multiple, directional interactions with protein targets [1]. Relative to biologics, peptides are generally more cost-effective to produce and can exhibit improved membrane permeability, reducing the need for parenteral administration.

Despite these advantages, peptide modulators that act exclusively through competitive binding are not suitable for all targets. The incorporation of covalent fragments, or “warheads,” into peptide scaffolds provides an effective strategy to increase binding affinity and expand the range of tractable protein targets. In this work, we investigate hypervalent iodine-bearing peptides as a platform for site-selective covalent protein modification. By integrating a hypervalent iodine moiety into rationally designed peptide sequences, we harness the distinctive electrophilic reactivity of hypervalent iodine species to enable selective covalent modification of nucleophilic amino acid residues [2], a tyrosine, under mild, biologically relevant conditions. The peptide component confers sequence-driven molecular recognition, while the hypervalent iodine warhead enables controlled covalent bond formation, thereby minimizing off-target reactivity. This dual-function design allows precise modulation of protein activity and improved selectivity compared to non-targeted covalent reagents. Collectively, these findings establish peptide-conjugated hypervalent iodine warheads as a versatile and modular platform for site-selective protein modification, with potential applications in the development of next-generation covalent chemical probes and therapeutic modalities.

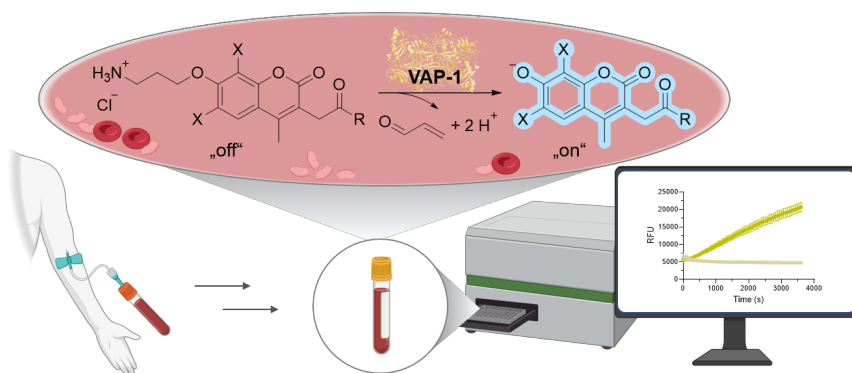


[1] A. Yeste-Vázquez, F. M. Paulussen, M. Wendt, R. Klintrot, C. Schulte, K. Wallraven, L. van Gijzel, B. Simeonov, M. van der Gaag, A. Gerber, et al., *Angew. Chem. Int. Ed.* **2024**, 63, e202411749.

[2] C. Marty, X. Ji, S. Nicolai, C. Heinis, J. Waser, *J. Am. Chem. Soc.* **2025**, 147, 42524–42531.

Coumarin-Based Fluorogenic Assay for Evaluating VAP-1 Activity *In Vitro* and *Ex Vivo*L. M. Poller¹, H. Wennemers^{1*}¹Laboratory of Organic Chemistry, ETH Zürich, Vladimir-Prelog-Weg 3, 8093 Zürich, Switzerland

Inflammatory, hepatic, and cardiovascular disorders are often characterized by acute or chronic inflammation and vascular changes. Central to inflammatory responses is the recruitment of leukocytes, a process in which vascular adhesion protein-1 (VAP-1/AOC3) plays an essential role.[1] Located in the vasculature, VAP-1 functions as both a cell adhesion molecule and a semicarbazide-sensitive amine oxidase (SSAO).[1] The enzyme catalyzes the oxidative deamination of small primary amines, generating aldehydes and the pro-inflammatory byproducts H₂O₂ and ammonia.[1] Soluble VAP-1 (sVAP-1) is currently being investigated as a biomarker, facilitated by the enzyme's accessibility in serum.[2] Elevated sVAP-1 levels are present in diseases such as systemic sclerosis (SSc), liver diseases, and stroke.[3] Nevertheless, a comprehensive investigation of VAP-1 activity, rather than mere protein concentration, is vital for elucidating its catalytic role in pathology. Current strategies for quantifying VAP-1 activity are, however, susceptible to endogenous interference or require specialized facilities.



In this work, we developed a straightforward and sensitive fluorogenic reagent with high robustness and reliability, enabling the direct monitoring of endogenous sVAP-1 activity in human serum. The translational utility of the fluorogenic probe was demonstrated by evaluating sVAP-1 activity in clinical serum samples from patients with systemic sclerosis (SSc), sourced from the USZ biobank. We anticipate that this tool will be valuable for the screening of VAP-1-targeted drug candidates and for further deciphering the role of VAP-1 in inflammatory pathologies.

[1] a) M. Salmi, S. Jalkanen, *Antioxidants & Redox Signaling*, **2019**, 30, 314–332.

[2] a) P. Bhattacharjee, M. R. Iyer, *Medicinal Research Reviews*, **2025**, 45, 1564–1596.

[3] a) K. Yanaba, A. Yoshizaki, E. Muroi, F. Ogawa, K. Shimizu, S. Sato, *International Journal of Rheumatic Diseases*, **2013**, 16, 442–447; b) R. Kurkijärvi, G. G. Yegutkin, B. K. Gunson, S. Jalkanen, M. Salmi, D. H. Adams, *Gastroenterology*, **2000**, 119, 1096–1103; c) L. Airas, P. J. Lindsberg, M.-L. Karjalainen-Lindsberg, I. Mononen, K. Kotisaari, D. J. Smith, S. Jalkanen, *Neuropathology and Applied Neurobiology*, **2008**, 34, 394–402.

[4] B. Zhao, F. A. Summers, R. P. Mason, *Free Radical Biology and Medicine*, **2012**, 53, 1080–1087.

SYNTHESIS OF NOVEL POLYCYCLIC DIAMINE SCAFFOLDS DERIVED FROM TROPINONE

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Small molecule drugs often consist of rigid scaffolds equipped with reactive handles, typically amino groups functionalized with pharmacophores. To explore previously unknown chemical space, we can use generated databases (GDBs), which are a large collection of generated chemical scaffolds adhering to principles of synthetic feasibility and chemical stability. Comparing the generated databases (GDB) with biologically active small molecules in ChEMBL reveals that many scaffolds, even structurally simple ones, have never been synthesized (1). From the GDB we have found interesting families of tricyclic and bicyclic chemical scaffolds which can be accessed synthetically from tropinone. In this poster we describe the simple synthesis to obtain these novel poly-cyclic diamine scaffolds from tropinone which can be easily functionalized to explore differentiated medicinal chemistry space.

[1] Ye Buehler and Jean-Louis Reymond, *J. Chem. Inf. Model.* **2023**, 63, 20, 6239–6248.

[2] Leon Rebhan, Ye Buehler and Jean-Louis Reymond, *Helvetica.* **2024**, 108, 1522-2675.

[3] Aline Carrel, Adonis Yiannakas, Jaap-Jan RoukensInes, Reynoso-Moreno, Markus Orsi, Amol Thakkar, Josep Arus-Pous, Daniele Pellegata, Jürg Gertsch and Jean-Louis Reymond. *J. Med. Chem.* **2025**, 68, 9, 9176–9201

Investigation on Nitrogenase Cooperativity Mechanism

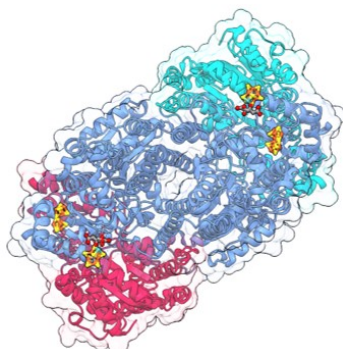
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Cooperativity during nitrogenase turnover is a focal topic of research. The heterotetrameric C_2 -symmetrical architecture of the MoFe protein has now been shown to extend beyond the idea of simply improving the stability of the enzyme, and has been shown to (i) impact substrate reduction, (ii) result in the formation of fibril-like structures during O_2 -mediated protection in obligate aerobes, and (iii) be involved in enzyme regulation in archaea.

Our group recently published a method for the post-purification modification of the MoFe protein with a steric-inhibitor on one MoFe:Fe protein interface, observing negative cooperativity during catalysis and confirming that N_2 fixation occurs even when only one half of the MoFe protein is enzymatically active. Asymmetry in catalysis was achieved by exploiting the cysteine-maleimide Michael addition reaction, limiting studies of cooperativity to MoFe proteins that are initially symmetrical prior to post-purification modification in vitro. [1]

Here, we present early work in the development of a method that permits truly asymmetric MoFe proteins to be isolated from *Azotobacter vinelandii*, thereby bypassing the need for inefficient heterologous or in vitro maturation. The introduction of an additional *nifD* gene to the genomic DNA and the use of tandem affinity tags permits well-defined asymmetric MoFe proteins to be purified, and subsequently enables the mechanical coupling of cooperativity in nitrogenase to be characterized and deciphered.



[1] C. Cadoux, D. Ratcliff, N. Maslac, W. Gu, I. Tsakoumagkos, T. Wagner, R. D. Milton, JACS AU, 2023, 3, 5, 1521-1533.

Design of a high-throughput screening system for the evolution of an artificial metathaseE. Salvisberg¹, Z. Zou¹, T. R. Ward^{1*}¹Department of Chemistry, University of Basel

Overcoming the current limitations of directed evolution for artificial metalloenzymes is a key challenge for future biosynthetic applications. Herein we present an approach based on a high-throughput screen of an artificial metathase. This artificial metalloenzyme consists of a modified Hoveyda-Grubbs 2nd generation catalyst ([Ru1]) incorporated into a *de novo* designed Tandem Repeat Protein (dnTRP). Previous directed evolution efforts revealed the variant dnTRP R5 maintaining ring-closing metathesis activity even at neutral pH and in living *E.coli* cells^[1]. This work aims to combine this exciting finding with a fluorescent read-out of metathesis activity. The ring-closing metathesis triggered release of a fluorophore would allow the application of a fluorescence-activated cell sorting (FACS) system to screen for further improved variants in a high-throughput fashion.

[1] Zou, Z., Kalvet, I., Lozhkin, B., Morris, E., Zhang, K., Chen, D., Ernst, M.L., Zhang, X., Baker, D., Ward, T.R., *Nat Catal* **8**, 1208–1219 (2025)

Monitoring Tissue Remodeling via a Lysyl Oxidase-activatable Green Fluorescent Probe

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Fibrosis is a progressive scarring process arising from tissue damage or inflammation that can lead to organ damage and failure.¹ It is characterized by excessive accumulation of extracellular matrix (ECM) components, such as collagen, and is associated with dysregulated lysyl oxidase (LOX) activity. LOX is an amine oxidase that initiates collagen cross-linking by oxidizing lysine residues to their aldehyde counterpart, allysine.² This process is essential for tissue stability but is often altered in fibrotic and malignant diseases. Precise detection of abnormal LOX activity and collagen cross-linking is therefore important for improving diagnosis and treatment of these disorders.

Building on our previously established LOX probe,³ we present here a TokyoGreen-based fluorescent sensor with an emission profile in the green region of the visible spectrum. Enzymatic assays demonstrated successful unmasking of the sensor upon incubation with LOX, resulting in fluorescence recovery. Conjugation of the sensor to linkers with aminooxy targeting moiety enabled covalent anchoring of the probe to endogenous allysine residues within the ECM and provided high selectivity for LOX over other amine oxidases. Application of the probe in a mouse embryonic fibroblast assay enabled direct monitoring of LOX-mediated ECM remodeling by widefield fluorescence microscopy. Collectively, these results demonstrate the potential of this probe as a tool for studying LOX activity and pathological ECM remodeling in fibrotic disease models.



[1] N. C. Henderson, F. Rieder, T. A. Wynn, *Nature*, **2020**, 587, 555-566.

[2] a) H. M. Kagan, W. Li, *J. Cell. Biochem.*, **2003**, 88, 660-672. b) S. D. Vallet, S. Ricard-Blum, *Essays Biochem*, **2019**, 63, 349-364.

[3] M. R. Aronoff, P. Hiebert, N. B. Hentzen, S. Werner, H. Wennemers, *Nat. Chem. Biol.*, **2021**, 17, 865-871.

ESIPT Nano-Emitter to Probe Lysosome Biogenesis in Live CellsM. Virmani¹, M. Jayakannan^{1*}¹Department of Chemistry, IISER Pune, Pune, India

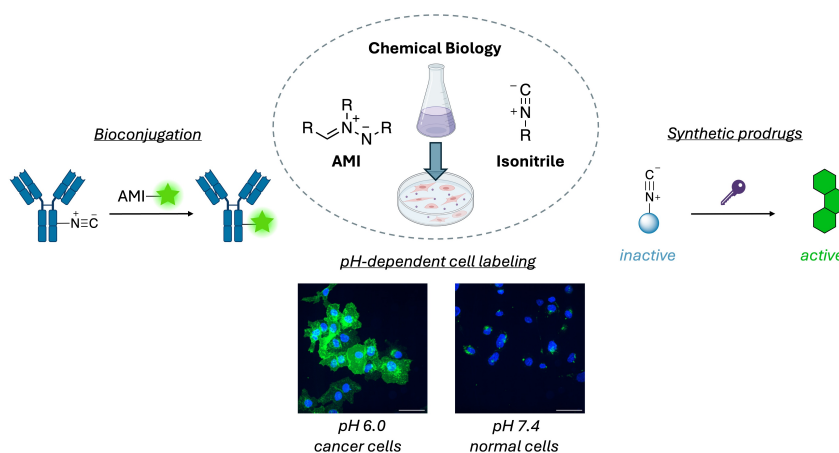
Fusion and fission of endosomes and lysosomes are central to diverse cellular processes, ranging from endocytosis to cellular homeostasis. Further, endosome–lysosome fusion and endo-lysosome fission-mediated lysosome biogenesis are crucial in regulating cellular health, and their dysregulation signifies disease. However, real-time tracking of these dynamic events with minimal perturbation remains challenging due to the lack of single-component probes capable of simultaneously distinguishing both organelles. In this work, we designed an amphiphilic π -conjugated imine probe that self-assembled into micellar nano-emitters in aqueous media. The probe enabled simultaneous and distinct labeling of endosomes and lysosomes under monochromatic excitation (405 nm). The ESIPT-active nano-emitter exhibited red fluorescence at endosomal pH, while its hydrolysis at lysosomal pH generated a fluorescent amine (PEG-Naph) emitting green fluorescence, allowing dual-color visualization. Using live-cell time-lapse imaging, we tracked endosome–lysosome fusion and fission-mediated lysosome biogenesis in real time. Furthermore, multiplexed imaging with doxorubicin revealed that, in addition to lysosomes, endosomes and endo-lysosomes also participated in drug sequestration in MCF-7 cells. Notably, doxorubicin-loaded endo-lysosomes underwent fission to generate additional drug-sequestering lysosomes, thereby limiting nuclear localization of the drug. These findings demonstrate that such single-component, dual-responsive probes provide a powerful platform for visualizing organelle dynamics and offer new insights into lysosome-mediated drug sequestration, with potential implications for improving therapeutic strategies.

Azomethine Imines and Isonitriles in Chemical Biology – Bioorthogonal Chemistry and the Controlled Synthesis of Heterocycles

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Bioorthogonal chemistry enables the interrogation and manipulation of biological processes and is therefore a valuable tool in chemical biology.^[1] To proceed in biological environments, these reactions need to be highly chemoselective and fast – requirements that only a few reactions fulfill. The discovery of new bioorthogonal reactions is thus highly sought-after. Azomethine imines (AMIs), as well as isonitriles, are well-known molecules in traditional organic synthesis, but their chemical reactivity is underexplored in biological settings.



Here, we show that *N*-acyl AMIs are stable in cellular environment and react with isonitriles with exquisite chemoselectivity and high reaction rate.^[2] A unique feature is the pH dependence. The ligation proceeds faster at acidic (pH 6) than at neutral pH, which is valuable for targeting acidic tumor microenvironments.^[3] Beyond *in vivo* chemistry, the AMI-isonitrile ligation is useful for the synthesis of bioconjugates, such as ADCs.^[4] Furthermore, the bioorthogonal reactivity of isonitriles can be exploited for the on-demand generation of heterocycles under physiological conditions via a prodrug approach, for example, for the controlled synthesis of tubulin polymerization inhibitors in living cells.^[5] Together, our work showcases the value of AMIs and isonitriles, traditionally only used in organic synthesis, for applications in chemical biology.

[1] Review: S.L. Scinto, D.A. Bilodeau, R. Hincapie, W. Lee, S.S. Nguyen, M. Xu, C.W. am Ende, M.G. Finn, K. Lang, Q. Lin, J.P. Pezacki, J.A. Prescher, M.S. Robillard, J.M. Fox, *Nat. Rev. Methods Primer.* **2021**, *1*, 30.

[2] A. Markos¹, M.P. Biedermann¹, J. Heimgärtner, A. Schmitt, K. Lang, H. Wennemers, *J. Am. Chem. Soc.* **2023**, *145*, 19513-19517.

[3] M.P. Biedermann¹, A. Markos¹, I. Warm, J. Heimgärtner, A. Schmitt, K. Lang, H. Wennemers, *Chem* **2026**, *12*, 102752.

[4] I. Warm, M. Trabesinger, A. Markos, H. Wennemers, *manuscript in preparation*.

[5] M.P. Biedermann¹, I. Warm¹, H. Wennemers, *manuscript in preparation*.