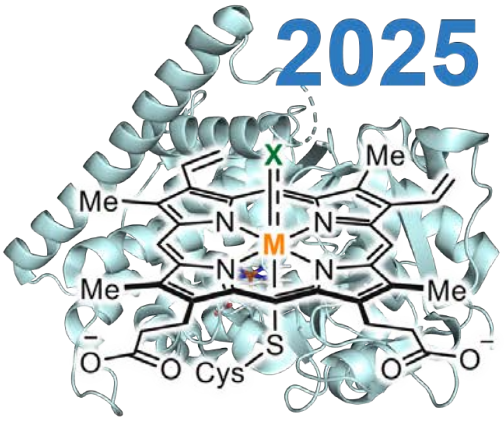

REPART ZYMES

Abstract Book

26-29 June 2025

Department of Chemistry, University of Basel, Switzerland

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Program

Thu, 26 June 2025

13:00 - 15:00	Registration	
15:00 - 15:15	Opening	
15:15 - 16:45		Chair: **Xiongyi Huang**
15:15	Laboratory Chemistry in a Protein…within a Cell…for Artificial Biosynthesis **John F. Hartwig**, *University of California, Berkeley, USA*	Plenary
15:55	*De Novo* Lanthanide Enzymes for Photoredox Catalysis **Cathleen Zeymer**, *Technical University of Munich, DE*	Invited
16:25	Artificial Enzymes Featuring Genetically Encoded Thiophenolate-Based Non-Canonical Amino Acids for Catalysis **Gerard Roelfes**, *University of Groningen, NL*	Selected
16:45 - 17:00	Coffee break	
17:00 - 18:30		Chair: **Marc Fontecave**
17:00	Design and Directed Evolution of Artificial Metalloenzymes for Selective Catalysis **Jared C. Lewis**, *Indiana University, USA*	Invited
17:30	Biocatalytic Transformations via Abiological Metal Hydrides **Dongping Chen**, *University of Basel, CH*	Selected
17:50	Designing Artificial Metalloenzymes **Yi Lu**, *University of Texas at Austin, USA*	Plenary
18:30	Apéro	Poster session 1

Fri, 27 June 2025

09:00 - 10:30		Chair: **Woon Ju Song**
09:00	Artificial CO_2 Reductases: Failures and Successes **Marc Fontecave**, *Collège de France, FR*	Plenary
09:40	Twisting the Reactivity of Cofactor-Dependent Enzymes for New-to-Nature Chemical Transformations **Rudi Fasan**, *University of Texas at Dallas, USA*	Invited
10:10	New Catalytic Reactions by Enzyme Engineering **Stephan Hammer**, *Bielefeld University, DE*	Selected
10:30 - 11:00	Coffee break	
11:00 - 12:30		Chair: **Thomas R. Ward**
11:00	Innovation by Evolution: Bringing New Chemistry to Life **Frances H. Arnold**, *California Institute of Technology, USA*	Plenary
11:40	Biocatalytic Atom Transfer Radical Polymerizations And Cyclizations **Nico Bruns**, *Technical University of Darmstadt, DE*	Invited
12:10	Accurate Structure Prediction with AlphaFold 3 **Zachary Wu**, *Google DeepMind, UK*	Selected
12:30 - 14:00	Lunch	Poster session 1
14:00 - 15:40		Chair: **Todd Hyster**
14:00	Designing a Model Metalloenzyme: From Active Site Geometry to Global Protein Dynamics **Hannah S. Shafaat**, *University of California, Los Angeles, USA*	Plenary
14:40	Photoenzymatic Hydroaminations and Hydroarylations via an Emergent Mechanistic Pathway **Felix Raps**, *Princeton University, USA*	Selected

15:00	Protein-Scaffold-Diverse Artificial Photoenzymes Powered by a Reversibly Binding BpAD Cofactor **Hui-Jie Pan**, *Nanjing University, CN*	Selected
15:20	Development of Biocatalytic Cobalt-Mediated Hydrogen Atom Transfer Chemistry **Andrew R. Buller**, *University of Wisconsin-Madison, USA*	Selected
15:40 - 16:10	Coffee break	
16:10 - 18:30		Chair: **Yi Lu**
16:10	Rational Design of Artificial Enzymes with Unnatural Entities **Yuzhou Wu**, *Huazhong University of Science and Technology, CN*	Plenary
16:50	Engineering Functional Metal Binding Sites into *De Novo*-Designed Protein Scaffolds **Angela Lombardi**, *University of Naples Federico II, IT*	Invited
17:20	Investigation of Flavin-Dependent Desaturases for Asymmetric Synthesis **Zhen Liu**, *National Institute of Biological Sciences, CN*	Selected
17:40	Break	
17:55	Merging Photocatalysis, Biocatalysis, and Organocatalysis for Stereoselective Radical Transformations **Paolo Melchiorre**, *University of Bologna, IT*	Plenary
18:35	Apéro	Poster session 2

Sat, 28 June 2025

09:00 - 10:30		Chair: **Hannah S. Shafaat**
09:00	Building Enzymes with New Function **Anthony Green**, *University of Manchester, UK*	Plenary
09:40	Iron-Dependent Enzymes for C–C Bond Formation, Cleavage, and Rotation **Alison Narayan**, *University of Michigan, USA*	Invited
10:10	Creating Photovoltaic Enzymes by Design and Evolution **Adrian Bunzel**, *ETH Zurich, CH*	Selected
10:30 - 11:00	Coffee break	
11:00 - 12:40		Chair: **Yuzhou Wu**
11:00	Reimagining Protein Scaffolds: How to Construct First Coordination Spheres for New Functions **Woon Ju Song**, *Seoul National University, KR*	Plenary
11:40	Artificial Non-Heme Copper Enzymes that Catalyze Stereoselective Inverse Electron-Demand Hetero-Diels-Alder Reactions **Nobutaka Fujieda**, *Osaka Metropolitan University, JP*	Selected
12:00	Repurposing Thiamine-Dependent Enzymes Using Radicals **Xiaoqiang Huang**, *Nanjing University, CN*	Selected
12:20	Unlocking New-to-Nature Enzymatic Transformations Through Precision Confinement **Marc Garcia-Borràs**, *University of Girona, ES*	Selected
12:40 - 14:10	Lunch	Poster session 2
14:10 - 15:40		Chair: **John F. Hartwig**
14:10	Unlocking New-to-Nature Transition Metal Catalysis in Nonheme Enzymes **Xiongyi Huang**, *Johns Hopkins University, USA*	Plenary
14:50	Mutation-Free Expansion of Substrate Scope in Cytochrome P450BM3 via Decoy Molecule Strategy **Osami Shoji**, *Nagoya University, JP*	Invited
15:20	Harnessing Glycyl Radical Enzymes for Asymmetric Catalysis **Mary C. Andorfer**, *Michigan State University, USA*	Selected
15:40 - 16:10	Coffee break	
16:10 - 18:15		Chair: **Paolo Melchiorre**
16:10	Repurposing Old Yellow Enzymes to Desaturases and Michaelases **Yuxuan Ye**, *Westlake University, CN*	Selected

16:30	Ultrahigh-Throughput Evolution of Enzymes Toward Abiotic Substrates Using Chemogenetic Platforms **Jeffrey D. Martell**, *University of Wisconsin–Madison, USA*	Selected
16:50	Harnessing Repurposed Enzymes with New-to-Nature Reactivity for Small Molecule Synthesis **Huimin Zhao**, *University of Illinois Urbana-Champaign, USA*	Invited
17:20	Break	
17:35	Photoenzymatic Catalysis — Using Light to Reveal New Enzyme Functions **Todd Hyster**, *Princeton University, USA*	Plenary
18:15 - 18:30	Closing remarks and poster prize	
19:30	Conference dinner *Restaurant Krafft, Rheingasse 12, 4058 Basel*	Optional: Only if booked separately!

Sun, 29 June 2025

11:00 - 13:30	Conference networking tour: “Brunch on the River Rhine” *Rhystärn, Schifflände 4, 4051 Basel*	Optional: Only if booked separately!

Sponsors

Gold partners

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Molecular Systems Engineering is a National Centre of Competence in Research (NCCR) funded by the Swiss National Science Foundation. The uniqueness of NCCR MSE relies on the combination of both chemical- and biological modules. In this approach, complex dynamic phenomena emerge as the result of the integration of molecular modules designed to create molecular factories and cellular systems whose properties are more than sum of the attributes of the individual modules.

The Swiss Nanoscience Institute (SNI) at the University of Basel is a center of excellence for nanosciences and nanotechnology. It was founded in 2006 by the University of Basel and the Swiss Canton Aargau in order to support research, education, and technology transfer in the nanosciences and in nanotechnology in Northwestern Switzerland. Since the SNI was founded, interdisciplinary teams of scientists of various research institutions in the SNI network have conducted basic and applied research and have supported actively the knowledge and technology transfer to industry.

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Metal-containing **ra**dical en**zymes** creates a PhD school across leading European universities that trains the next generation of scientists capable of tackling the design, development and implementation of novel enzymatic reactions in a holistic approach.

Poster Sessions

Session 1

Thu, 26 June 18:30; Fri, 27 June 12:30

1	Decoupling Cytochrome P450 Monooxygenase from Ferredoxin and Ferredoxin Reductase: A Fast Approach to Substrate Scope Screening **Fatemeh Aziziyan**, *University of Bern, CH*
2	The natural redox cofactor PQQ enables photocatalytic radical cyclizations **Srishti Ballabh Bahukhandi**, *Technical University of Munich, DE*
3	Identification and Engineering of Promiscuous Amidases for Multi-Purpose Applications **Thomas Bayer**, *University of Greifswald, DE*
4	Design of Artificial Catalytic Cobalt Enzymes using SpyTag/SpyCatcher Technology **Valentina Borghesani**, *University of Parma, IT*
5	Artificial metalloenzymes based on the Spy system exhibit versatile catalytic functions, from catecholase activity to hydrogen evolution **Chiara Bottoni**, *University of Parma, IT*
6	Installation of an organocatalyst into a protein scaffold creates an artificial Stetterase (ArtiSt) **Dominic Campopiano**, *University of Edinburgh, UK*
7	Artificial Metalloenzymes For Radical Biocatalysis **Bratislav Dačević**, *University of Groningen, NL*
8	Predicting Temperature induced Protein Unfolding via Structural Attribute trained Algorithms **Bastian Daniel**, *University of Graz, AT*
9	Reprogramming Enzymes: Engineering TOYE with Secondary Amine-Functionalized ncAAs **Ivana Drienovska**, *Vrije Universiteit Amsterdam, NL*
10	Photobiocatalysis: Expanding Reactivity Through Non-Biological Cofactors **Nicholas East**, *University of Melbourne, AU*
11	Computational Design of a Hyperstable Protein Platform for Multifunctional Biocatalytic Applications **Wael Elaily**, *University of Graz, AT*
12	Protein Nanomaterials for Solar Energy Conversion **Philipp Elbers**, *ETH Zurich, CH*
13	Effects of Superflavination on NOX Oligomerization and Activity **Elif Erdem**, *Technical University of Denmark, DK*
14	NHC Metal Peptide Conjugates for Abiotic Catalysis **Francisca Figueiredo**, University of Bern, CH
15	Focusing Metagenomic Screening on Stable and Functionally Divers Enzyme Representatives **Yuval Fishman**, *Weizmann Institute of Science, IL*
16	Small Molecule-Directed Metal-Catalyzed Prodrug Therapy (MCPT) **Damian Graf**, *University of Basel, CH*
17	Discovery and Development of Cytochrome P450BM3-Activating Agents Derived from Dicarboxylic Acids **Megumi Ishigami**, *Nagoya University, JP*
18	Engineered and Artificial Myoglobins: electrochemical and catalytic studies **Amanda Jarvis**, *University of Edinburgh, UK*
19	An Artificial Copper-carbene Transferase for the Asymmetric Synthesis of Chiral Organoboranes **Ru Jiang**, *University of Groningen, NL*
20	Photovoltaic enzymes by design and evolution **Eleftheria Kelefioti Stratidaki**, *ETH Zurich, CH*
21	Biocatalysis Under Pressure; driving reactions in a reduced atmosphere **Lisa Kennedy**, *University of Edinburgh, UK*
22	Harnessing Photoenzymatic Systems for Intermolecular C-H Fluorination **Dani Lawson**, *University of Texas at Austin, USA*
23	A Fiber Forming Artificial Metalloenzyme for Heterogeneous Biocatalysis **Vincent Lebrun**, *CNRS/University of Strasbourg, FR*

24 Designing fully computational and efficient de novo enzymes: insights into catalysis and foldability
Dina Listov, *Weizmann Institute of Science, IL*

25 Developing Myoglobin-Based Artificial Fluorinases
Mingyu Liu, *University of Edinburgh, UK*

26 Exploring Bacterial Hydrazine Biosynthetic Pathways Featuring Cupin/Methionyl tRNA Synthetase-like Enzymes
Kenichi Matsuda, *Hokkaido University, JP*

27 The use of SPY system for artificial photo-metalloenzymes
János Péter Mészáros, *University of Parma, IT*

28 Metalloenzyme engineering with non-canonical amino acid incorporation
Alexandria Deliz Liang, *University of Zurich, CH*

Session 2

Fri, 27 June 18:35; Sat, 28 June 12:40

1 Trying to catch the manganese: a SpyTag/SpyCatcher approach
Adriana Miller-Niewiarowska, *University of Parma, IT*

2 Discovery and evolution of enzymes for catalysis and chemical biology
Monica Neugebauer, *University of Wisconsin-Madison, USA*

3 Dual-Functional Designer Enzyme: Installing a Catalytically Active Multi-nuclear Metal Center while Preserving the Protein's Native Function
Yasunori Okamoto, *NINS Exploratory Research Center on Life and Living Systems, JP*

4 Enzyme Engineering to Access Biaryl Natural Products
Daniel Ong, *University of Michigan, USA*

5 Artificial Enzymes with Non-natural Active Centers Incorporated into Protein Matrices
Koji Oohora, *University of Osaka, JP*

6 Photoenzymatic Hydroaminations and Hydroarylations via an Emergent Mechanistic Pathway
Felix Raps, *Princeton University, USA*

7 Making metathesis essential
Elias Salvisberg, *University of Basel, CH*

8 Development of Artificial Metalloenzymes Based on Copper-Binding β-Helix Mimicking Peptides for C-C Bond Forming Reactions
Nele Schulte, *University of Strasbourg, FR*

9 Lighting Up Biocatalysis: New-to-Nature Photoenzymes Through Engineering of a Thioxanthonylalanine-Specific Aminoacyl-tRNA Synthetase
Marco Seifert, *University of Greifswald, DE*

10 In Vivo Continuous Evolution of an Artificial Metalloenzyme Toward Sustaining Cellular Metabolism
Michaela Slánská, *University of Basel, CH*

11 Design, synthesis and characterisation of artificial metallopeptides based on the Spy System
Andrea Lourdes Spinosa, *University of Parma, IT*

12 Guiding Generative Protein Language Models Towards High Fitness Regions with Reinforcement Learning
Filippo Stocco, *Pompeu Fabra University, ES*

13 Engineering Limonene Epoxide Hydrolases for the Transformations of Chiral Oxetanes
Zhoutong Sun, *Tianjin Institute of Industrial Biotechnology, CN*

14 Engineering a Light-responsive Synthase for Improved Optogenetic Control of Cyclic-di-GMP Dynamics
Aloysius Teng, *Nanyang Technological University, SG*

15 Optimisation of a Periplasmic Binding Protein (PBP) based Artificial Metalloenzyme (ArM) for Biocatalysis
Sean Adeoti Thompson, *University of York, UK*

16 Genetic Code Expansion with Novel Metal-Binding Unnatural Amino Acids for the Development of Artificial Metalloenzymes
Chris Thomson, *University of Edinburgh, UK*

17 A novel atom transfer radical polymerization initiation activity of galactose oxidase
Iuliia Ushakova, *Technical University of Darmstadt, DE*

18 Reactivity Studies of Artificial Metalloenzyme MspA-[Fe**III(TAML)]-**Using Nanopore Technology
Bart van Bree, *Wageningen University & Research, NL*

19	A Dual Cofactor Artificial Metalloenzyme for Synergistic Asymmetric Catalysis **Weijin Wang**, *EPFL, CH*
20	Computational Strategies for Engineering Non-Heme and Heme Iron-Dependent Enzymes **Yao Wei**, *University of Milan, IT*
21	Synergistic photobiocatalysis for enantioselective triple radical sorting **Zhongqiu Xing**, *Nanjing University, CN*
22	Repurposing ThDP-dependent Enzyme for Enantioselective Radical Acylation **Yuanyuan Xu**, *Nanjing University, CN*
23	Stereoselective Radical Reactions Enabled by Metalloenzyme Design and Evolution **Yang Yang**, *University of California Santa Barbara, USA*
24	Repurposing hemoproteins for metal-catalyzed H atom transfer in asymmetric radical biocatalysis **Xiang Zhang**, *University of Basel, CH*
25	Electricity-driven enzymatic dynamic kinetic oxidation **Beibei Zhao**, *Nanjing University, CN*
26	Unlocking Enamine Catalysis for Ketone Functionalization by Artificial Enzyme Design and Evolution **Zhi Zhou**, *Jiangnan University, CN*
27	Design and Evolution of Artificial Diels–Alderase by Covalent Incorporation of Artificial Secondary Amine Cofactor **Zhixi Zhu**, *Jiangnan University, CN*
28	*In vivo* catalysis by artificial metalloenzymes for the production of aldehydes **Ismaïl Benhamed**, *Université Grenoble Alpes / CEA, FR*

Plenary Talks

Innovation by Evolution: Bringing New Chemistry to Life

Frances H. Arnold, *California Institute of Technology, USA*

Chemistry encoded in DNA and optimized by evolution promises efficient, clean, sustainable routes to fuels, chemicals, materials, foods, medicines, and more. Evolution not only optimizes and tunes features such as activity or stereoselectivity--it also innovates. We are using evolution to create entirely new biocatalysts that catalyze reactions unknown in biology and sometimes unprecedented in human-invented chemistry. New-to-nature 'carbene transferase' and 'nitrene transferase' enzymes increase the scope of molecules and materials that can be made using biology's remarkable chemical machinery. Such enzymes unlock chemicial transformations that were inaccessible to small-molecule catalysts. And, with modern machine learning and AI tools to aid discovery and optimization, we are closer than ever to encoding a vast array of chemical transformations in DNA.

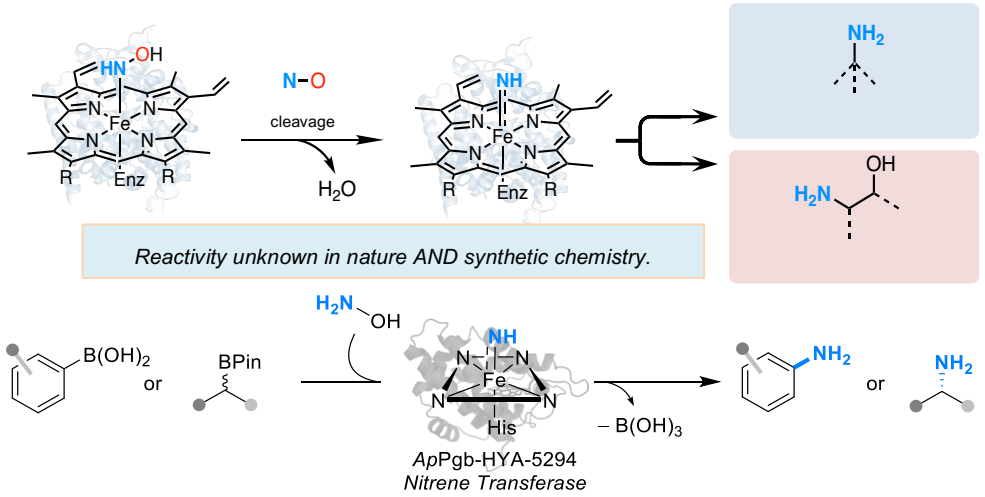


Figure *Example abiological reactions for which nitrene transferases have been developed. In contrast to previous work in chemical catalysis, these reactions can use simple hydroxylamine as the nitrene source.*

References

1. J. Yang et al., "Active Learning-Assisted Directed Evolution." *Nature Communications* **16**, 714 (2025). https://doi.org/10.1038/s41467-025-55987-8
2. E. Alfonzo et al., "Biocatalytic Synthesis of α-Amino Esters via Nitrene C-H Insertion." Journal of the American Chemical Society, 146(40), 27267-27273 (2024). https://doi.org/10.1021/jacs.4c09989
3. D. Hanley et al., "Stereospecific Enzymatic Conversion of Boronic Acid." *Journal of the American Chemical Society* **146**(28), 18781-19608 (2024). https://doi.org/10.1021/jacs.4c04190
4. R. Mao et al., "Biocatalytic, Enantioenriched Primary Amination of Tertiary C–H Bonds." *Nature Catalysis* **7**, 585-592 (2024). https://doi.org/10.1038/s41929-024-01149-w
5. S. Gao et al., "Enzymatic Nitrogen Incorporation Using Hydroxylamine." *Journal of the American Chemical Society* **145**(37), 20196-20201 (2023). doi: 10.1021/jacs.3c08053

Artificial CO_2 Reductases: Failures and Successes

Marc Fontecave, *Collège de France, FR*

The energy transition requires new sources of carbon, as alternatives to fossil sources, for the production of organic compounds for the chemical industry (fuels, polymers, etc..). Carbon dioxide transrformation to C1 or C2 compounds can be achieved by electroreduction or photoreduction using low-carbon electricity or sunlight directly, respectively. The reactions at work imply multiple electrons and protons and thus require catalysts for minimizing energy barriers and controlling selectivity. Whereas synthetic coordination complexes and solid metal materials are extensively studied, it is interesting to note that living organisms have evolved complex metalloenzymes for the reduction of CO_2 into formic acid and for the interconversion between CO_2 and CO, providing a source of inspiration for the design of biomimetic homogeneous and heterogeneous catalysts. Another class of bioinspired catalysts, namely artificial enzymes, deserves more attention. Such a strategy has been very little explored so far for CO_2 electro- or photo-reduction. This the theme of this presentation which will focus on our recent studies of artificial CO_2 reductases.[1,2]

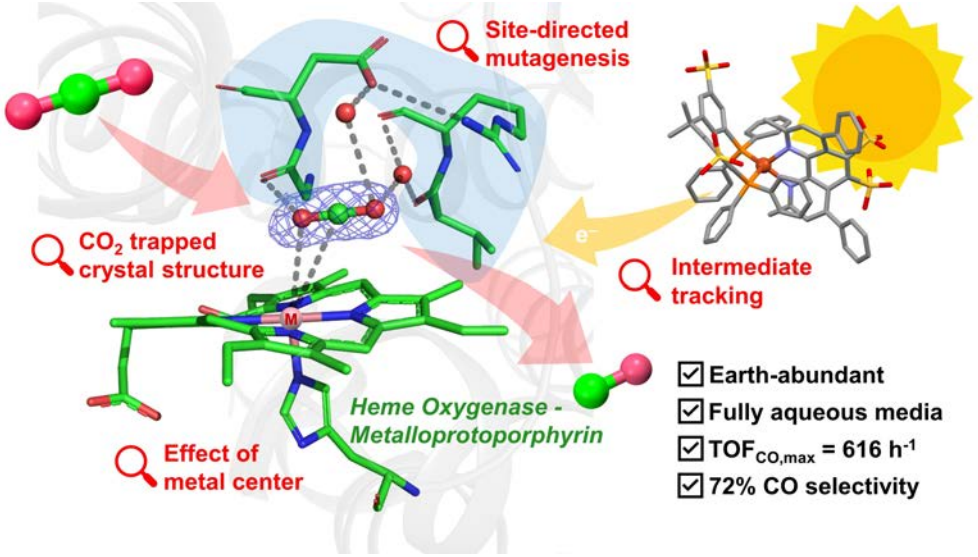


Figure *Structural and mechanistic strategies for the investigation of a CO_2 reductase, hybridiziing a Co-porphyrin and a heme oxygenase.*

References
1. R. J. Labidi, B. Faivre, P. Carpentier, G. Veronesi, A. Solé-Daura, R. Bjornsson, Y. Li, M. Atta, M. Fontecave, *J. Am. Chem. Soc.* **2023**, 145, 13640.
2. R.J. Labidi, B. Faivre, P. Carpentier, J. Perard, P. Gotico, Y. Li, M. Atta, M. Fontecave, *J. Am. Chem. Soc.,* **2024**, 146, 28296.

Building Enzymes with New Function

Anthony Green, *University of Manchester, UK*

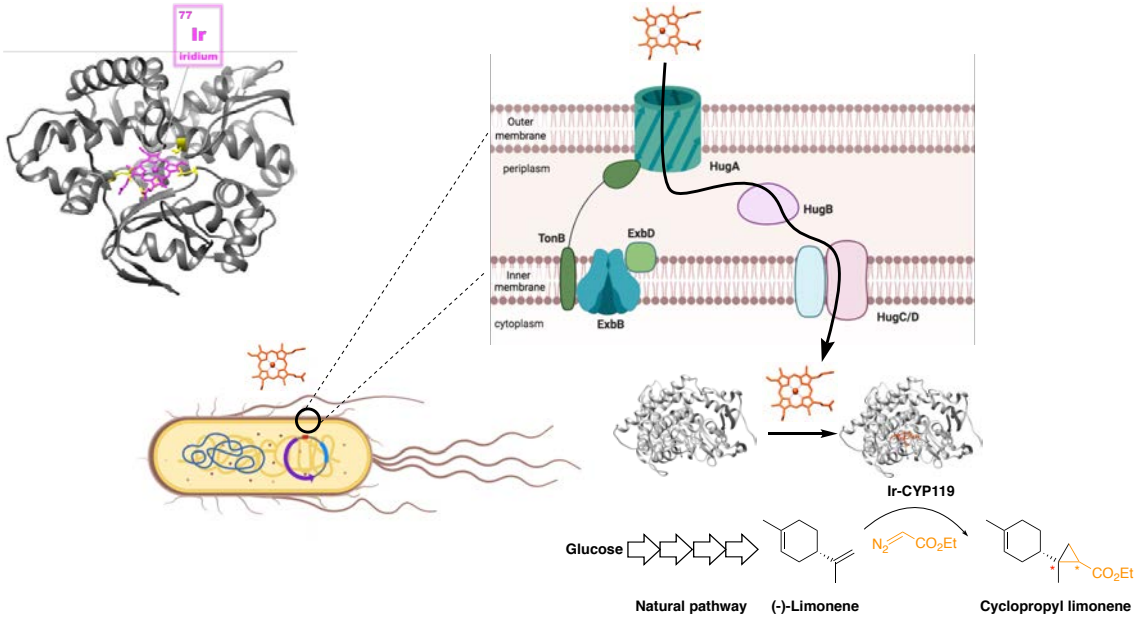
Protein cavities can offer highly versatile and engineerable environments for hosting new catalytic sites. However, only a narrow range of functional elements are available to enzyme designers when building new active sites, meaning that many important modes of reactivity are not accessible. Here I will discuss our efforts to overcome these limitations, by encoding new catalytic elements into proteins as non-canonical amino acid side chains. This approach has allowed us to build enzymes with new functions and reactivity modes that were previously inaccessible with protein catalysts. Significantly as our catalyts are genetically encoded, their activities and selectivities can be optimized using directed evolution workflows adapted to an expanded amino acid alphabet. We are optimistic that this integration of enzyme design, genetic code expansion and laboratory evolution can provide a versatile strategy for creating enzymes with catalytic functions not accessible to nature.

Laboratory Chemistry in a Protein…within a Cell…for Artificial Biosynthesis

John F. Hartwig, *University of California, Berkeley, USA*

The introduction of functional groups at the positions of typically unreactive C-H bonds site-selectively and the stereo- and regio-selective functionalization of unconjugated C=C bonds have been longstanding challenges in catalysis. To this end, our group has been motivated by the limits of small-molecule catalysts for such reactions to begin to investigate artificial metalloenzymes. These artificial metalloenzymes contain synthetic cofactors possessing abiotic metal centers that catalyze unnatural reactions with control over selectivity resulting from the protein environment. In the best-case scenario such reactions could occur within the cells of *E. coli* or other microorganisms and in the long-term to occur as part of an unnatural biosynthetic pathway to produce unnatural products by fermentation.

This talk will include results on new transformations, new mechanisms, new reactive intermediates, and new methods for *in vivo* assembly of artificial metalloenzymes. This combination of results has enabled us to combine an unnatural carbene-transfer reaction catalyzed by natural and artificial metalloenzymes with the biosynthesis of diazo compounds and natural reactions of a heterologous biosynthetic pathway to create engineered microorganisms that produce unnatural products by artificial biosynthesis encompassing organometallic chemistry.[1]



References

1. Key, H. M.; Dydio, P.; Liu, Z.; Rha, J. Y. E.; Nazarenko, A.; Seyedkazemi, V.; Clark, D. S.; Hartwig, J. F. Beyond Iron: Iridium-Containing P450 Enzymes for Selective Cyclopropanations of Structurally Diverse Alkenes. *ACS Cent. Sci.* **2017**, *3* (4), 302-308. DOI: 10.1021/acscentsci.6b00391. Huang, J.; Liu, Z.; Bloomer, B. J.; Clark, D. S.; Mukhopadhyay, A.; Keasling, J. D.; Hartwig, J. F. Unnatural biosynthesis by an engineered microorganism with heterologously expressed natural enzymes and an artificial metalloenzyme. *Nat. Chem.* **2021**, *13* (12), 1186-1191. DOI: 10.1038/s41557-021-00801-3

Unlocking New-to-Nature Transition Metal Catalysis in Nonheme Enzymes

Xiongyi Huang, *Johns Hopkins University, USA*

Repurposing natural enzymes to catalyze synthetic transformations absent in nature has emerged as a significant research field bridging chemistry and biology. A key challenge in this pursuit is the introduction of synthetic reaction mechanisms into natural protein scaffolds. Over the past decades, substantial breakthroughs have been achieved in this field, with many enzymatic systems developed to catalyze critical chemical transformations not previously observed in biology. However, much of this progress has focused on proteins or enzymes containing heme or organic cofactors. In this context, our group has drawn inspiration from mechanistic connections between synthetic and biocatalytic systems to explore the vast, untapped potential of nonheme enzymes for new-to-nature biocatalysis. This talk will highlight several enzymatic systems developed by our group over the past five years, which utilize diverse reaction mechanisms in transition metal catalysis for the formation of C–N, C–S, C–C, C–O, and C–halogen bonds. We hope these systems will further advance the integration of synthetic chemistry and biology to innovate chemical synthesis, as well as deepen our understanding of both biochemical and synthetic reaction mechanisms.

Photoenzymatic Catalysis — Using Light to Reveal New Enzyme Functions

Todd Hyster, *Princeton University, USA*

Enzymes are exquisite catalysts for chemical synthesis, capable of providing unparalleled levels of chemo-, regio-, diastereo- and enantioselectivity. Unfortunately, biocatalysts are often limited to the reactivity patterns found in nature. In this talk, I will share my groups efforts to use light to expand the reactivity profile of enzymes. In our studies, we have developed novel photoexcitation mechanisms involving common biological cofactors, such as nicotinamide, flavin, and pyridoxal, to facilitate electron transfer to substrates bound within enzyme active sites. Alternatively, proteins can be used to electronically activate substrates for reduction by exogenous photoredox catalysts enabling radical formation to be localized to the protein active site. The resulting radicals can engage in a variety of inter- and intramolecular reactions with high levels of enantioselectivity. These approaches enable biocatalysts to solve long-standing selectivity challenges in chemical synthesis.

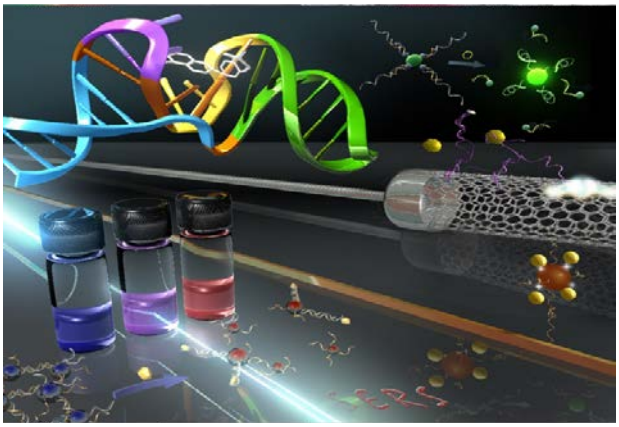
Designing Artificial Metalloenzymes

Yi Lu, *University of Texas at Austin, USA*

Metalloenzymes play important roles in many biological processes, yet the structural features underlying their remarkable reactivity and selectivity remain incompletely understood. To address this issue, we have designed artificial metalloenzymes (ArMs) using small, stable proteins as scaffolds. These scaffolds are designed to incorporate key residues essential for functions in native enzymes, including O_2 and N_2 reduction as well as nitrosylation and polysaccharide oxidative cleavage reactions.

Our findings reveal that replicating the primary coordination sphere may suffice for creating structural models of metalloenzymes. However, achieving functional ArMs with high activity and turnover rates—on par with native enzymes—requires precise engineering of non-covalent secondary coordination sphere interactions, such as hydrophobic effects and hydrogen bonding, including those mediated by water molecules.

This presentation will highlight recent advances in ArM design, the insights gained from these studies, and the expansion of ArM activities beyond the capabilities of natural enzymes.

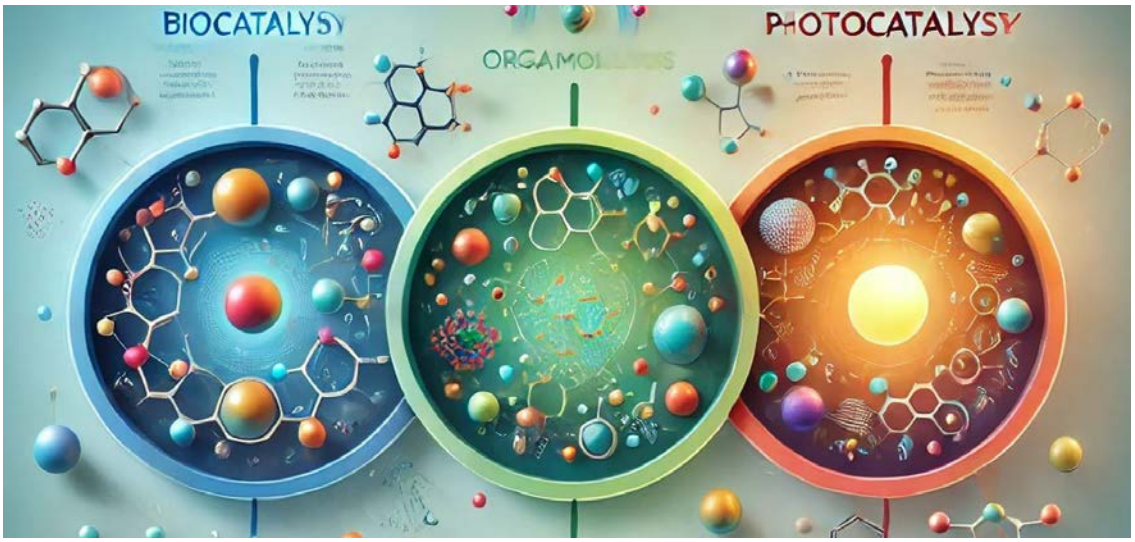


Merging Photocatalysis, Biocatalysis, and Organocatalysis for Stereoselective Radical Transformations

Paolo Melchiorre, *University of Bologna, IT*

The combination of photocatalysis, biocatalysis, and organocatalysis provides a powerful yet underexplored strategy for addressing major challenges in asymmetric synthesis.[1] By combining these distinct catalytic disciplines, we aim to develop novel enantioselective radical processes that are otherwise difficult to achieve using conventional approaches. Central to this concept is the ability to harness enzyme-bound organocatalytic intermediates as photoactive intermediates, unlocking new mechanistic pathways for radical generation and control.

Recently, we developed a new approach to light-driven biocatalysis, where engineered enzymes utilize iminium ion intermediates—formed transiently within their active sites—as single-electron oxidants upon visible-light excitation.[2] This strategy enables the activation of chiral carboxylic acids, triggering radical decarboxylation and subsequent stereospecific cross-coupling to construct complex chiral architectures with multiple stereocenters and complete enantiocontrol. Notably, the enzyme's active site prevents racemization of chiral radicals via a rare "memory of chirality" mechanism, ensuring high stereochemical fidelity. By leveraging the unique synergy between light, biocatalysts, and organocatalytic intermediates, this work expands the scope of radical chemistry and sets the stage for new, sustainable methods in asymmetric synthesis.[3]



References
1. Hyster, T. K. *et al*. Photobiocatalytic strategies for organic synthesis. *Chem. Rev.* **2023**, *123*, 5459–5520.
2. Tseliou, V.; Kqiku, L.; Berger, M.; Schiel, F.; Zhou, H.; Poelarends, G. J.; Melchiorre, P. *Nature* **2024**, *634*, 848–854.
3. This research is supported by the European Research Council (ERC-2023-AdG 101141690 –PHOTOZYME).

Designing a Model Metalloenzyme: From Active Site Geometry to Global Protein Dynamics

Hannah S. Shafaat, *University of California, Los Angeles, USA*

Metalloenzymes catalyze the challenging chemical reactions that lie at the core of vital life processes, from carbon and nitrogen fixation to photosynthesis and respiration. Nickel-containing enzymes, specifically, are essential for global hydrogen and carbon cycling and the metabolisms of diverse microbes, with implications in human health, clean energy conversion, and sustainable fuel generation. In this presentation, I will discuss our recent efforts to recapitulate key structural and functional elements of microbial nickel enzymes such as hydrogenase, carbon monoxide dehydrogenase (CODH), and acetyl coenzyme A synthase using protein-based scaffolds. By designing model metalloenzymes from the “inside out”, each contribution can be clearly delineated. Functional studies of our model proteins are combined with diverse spectroscopic techniques and computational investigations, allowing us to obtain a comprehensive understanding of how the entire protein matrix contributes to reactivity. These fundamental structure-function-dynamics relationships will be discussed in the context of understanding native metalloenzymes and providing design guidelines for new biological and anthropogenic catalyst development.

Reimagining Protein Scaffolds: How to Construct First Coordination Spheres for New Functions

Woon Ju Song, *Seoul National University, KR*

Protein scaffolds offer an expansive platform to construct novel structures and functions of metallocofactors. While numerous methods have been developed to optimize protein environments near or distant from active sites, the design of the first coordination sphere still relies largely on structure-based approaches derived from coordination chemistry. Here, we present two strategies to build first coordination spheres for new functions: (i) pinpointing specific positions for mutations to create divalent transition metal-binding sites by developing an *in silico* program, *Metal-Installer*, and (ii) genetically incorporating noncanonical amino acids followed by symmetry-based ligand mulitplication. By integrating geometric parameters derived from both natural metalloproteins and synthetic inorganic complexes, we created tailor-made metal –binding sites that mimic the structure and chemical properties of mono- and dinuclear metalloproteins. These designs closely matched our structural predictions and fulfilled the minimal requirements for metal-dependent catalysis or photochemical properties. This work significantly broadens the accessible chemical space of metalloproteins, enabling the repurposing of natural protein/enzyme scaffolds for various applications such as metalloenzyme mimics, biocatalysts, and protein-based photochemical materials.

References
1. T. Vornholt, F. Leiß-Maier, W. J. Jeong, C. Zeymer, W. J. Song, G. Roelfes, T. R. Ward, *Nat. Rev. Method. Prim.*, **2024**, *4*, 78.
2. W. J. Jeong, W. J. Song, *Nat. Commun.*, **2022**, *13*, 6844.
3. W. J. Jeong, S. Ha, W. J. Song, *in revision*, **2025**.
4. J. Lee, S. Lee, W. Kim, W. J. Song, et al. *manuscript in preparation*, **2025**.

Rational Design of Artificial Enzymes with Unnatural Entities

Yuzhou Wu, *Huazhong University of Science and Technology, CN*

Biomanufacturing is a transformative technology with the potential to revolutionize diverse industrial sectors. An underlying limitation in this field is the lack of enzymes capable of driving unnatural transformations necessary for the synthesis of a broad range of chemical products. However, enzyme design is inherently restricted by the availability of structural and functional units provided by nature. This presentation will detail our ongoing efforts to develop new enzymes that incorporate unnatural entities into their scaffolds, with a particular emphasis on photoenzymes that utilize synthetic photosensitizers. I will discuss strategies for incorporating these entities, including genetic encoding approaches and chemical modification techniques, and studying their compatibility with high-throughput directed evolution in living cells. Additionally, I will highlight the unique catalytic properties observed in these artificial enzymes and their potential to be integrated with natural cellular metabolic pathways. We envision that artificial enzymes, enriched with unnatural entities, will serve as versatile tools for constructing "cell factories" adaptable to a variety of synthetic applications.

Invited Talks

Biocatalytic Atom Transfer Radical Polymerizations And Cyclizations

Nico Bruns, *Technical University of Darmstadt, DE*

Atom transfer radical reactions are chemical transformations that do not occur naturally in biological systems. Yet, metalloenzymes can catalyze such new-to-nature reactions, thereby paving the way to biocatalytic routes to atom transfer radical polymerizations (bioATRP) and atom transfer radical cyclizations (bioATRC). However, native heme proteins such as myoglobin and horseradish peroxidase display a limited degree of control or activity in these reactions. By rational design, myoglobin mutants that show enhanced performance over their wild-type counterparts in radical polymerizations and greatly enhanced catalytic turnover in radical cyclization reactions were created. Moreover, bioATRP is not only an enzymatic route to polymers but allows the synthesis of polymers *in situ* in biological systems. This opens up the possibility to engineer living cells on their surface and within their cytosol by biocatalytic radical polymerizations and to create artificial cells that can express their own proteins. Thus, repurposed metalloenzymes play a crucial role in developing novel communicating life-like biomaterials, semi-synthetic engineered living materials, and synthetic biology systems.

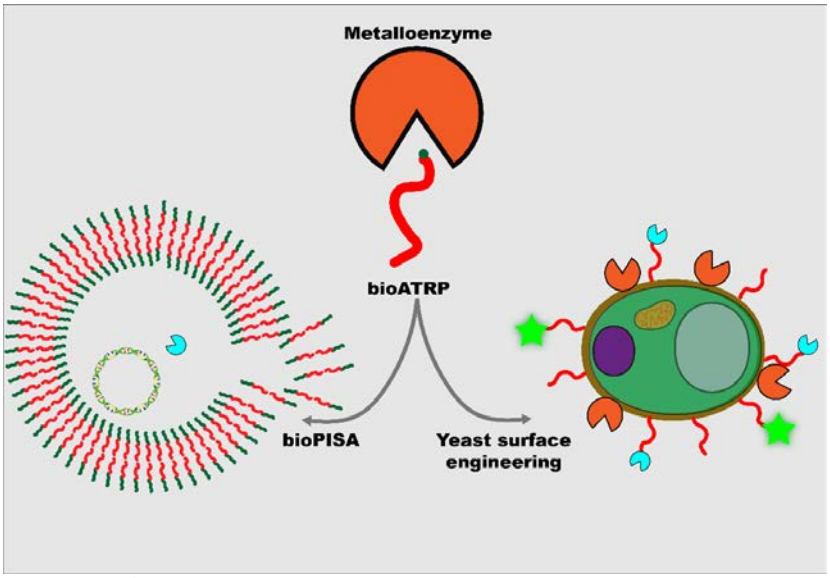


Figure *Applications of biocatalytic ATRP: Repurposed metalloenzymes catalyze the synthesis of artificial cells via biocatalytic polymerization-induced self-assembly (bioPISA) and allow the engineering of yeast cell surfaces with synthetic polymers.*

References

1. A. Lubskyy, C. Guo, R. J. Chadwick, A. Petri-Fink, N. Bruns, M. M. Pellizzoni, *Chem. Commun.* **2022**, *58*, 10989-10992.
2. A. Belluati, D. Happel, M. Erbe, N. Kirchner, A. Szelwicka, A. Bloch, V. Berner, A. Christmann, B. Hertel, R. Pardehkhorram, A. Reyhani, H. Kolmar, N. Bruns, *Nanoscale* **2023**, *15*, 19486-19492.
3. A. Belluati, S. Jimaja, R. J. Chadwick, C. Glynn, M. Chami, D. Happel, C. Guo, H. Kolmar, N. Bruns, *Nat. Chem.* **2024**, *16*, 564-574.
4. E. Ornati, J. Perrard, T. A. Hoffmann, R. Bonon, N. Bruns, *J. Am. Chem. Soc.* **2025**, DOI:10.1021/jacs.4c17257

Twisting the Reactivity of Cofactor-Dependent Enzymes for New-to-Nature Chemical Transformations

Rudi Fasan, *University of Texas at Dallas, USA*

Expanding the reaction scope of biological catalysts beyond the realm of enzymatic transformations occurring in nature can create new opportunities for the exploitation of biocatalysis for asymmetric synthesis and other applications. In this seminar, I will present recent progress made by our group toward the design and application of engineered cofactor-dependent enzymes for catalyzing 'new-to-nature' transformations, with a focus on selective C(sp^3)–H functionalization reactions achieved through abiological reactive intermediates and the synergistic integration of enzyme catalysis with chemical hydrogen atom transfer. These systems make available new approaches for the asymmetric construction of carbon-carbon and carbon-heteroatom bonds beyond the scope of currently available biocatalytic or chemocatalytic methods.

Design and Directed Evolution of Artificial Metalloenzymes for Selective Catalysis

Jared C. Lewis, *Indiana University, USA*

Metalloenzymes perform some of the most remarkable transformations in nature under ambient conditions in complex cellular milieu. The possibility of leveraging molecular recognition and evolution for non-biological metal catalysts has driven efforts to engineer artificial metalloenzymes (ArMs), hybrid catalysts comprised of synthetic metal cofactors linked to protein scaffolds. In this talk, I will discuss recent efforts from my group to design and evolve ArMs containing dirhodium and metal polypyridine cofactors for selective catalysis.

Engineering Functional Metal Binding Sites into *De Novo*-Designed Protein Scaffolds

Angela Lombardi, *University of Naples Federico II, IT*

Metalloenzymes are capable of catalyzing a variety of reactions, and a given metal ion can be used in a number of oxidative, reductive, and hydrolytic transformations in different enzymes. This functional diversity arises from a strong partnership between the metal cofactor and protein matrix: the metal ion provides the protein with an array of chemical properties, while the protein stabilizes it in solution and directs its reactivity toward a unique and distinct path.[1]

Bioinorganic chemists tackled the challenge to unravel the mechanisms that allow the protein matrix to modulate the catalytic activity of metal-containing cofactors, through the development of artificial systems.[2-4] In this respect, *de novo* protein design, involving the construction of proteins "from scratch", has contributed to tremendous advances in manufacturing metalloenzymes with unique structures and functionalities.[5]

This lecture will give an overview of our results on the design of artificial helical bundles, housing different metal cofactors, which catalyze a variety of oxidative reactions. Starting from the Due Ferri (DF) family of artificial diiron-oxo-proteins,[6] our design has integrated rational and computational strategies to engineer mononuclear and dinuclear copper sites, mimicking natural lytic polysaccharide monooxygenases (LPMOs) and polyphenol oxidases.[7]

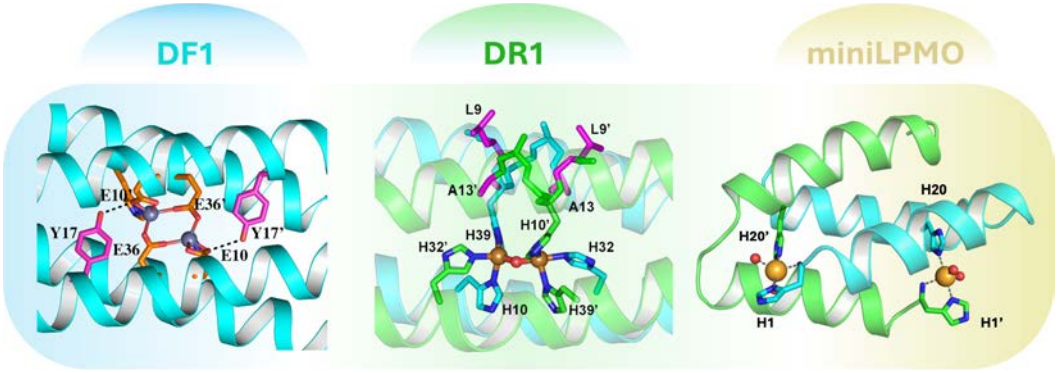


Figure *Metal site structure in de novo designed metalloenzymes: (a) diiron site in DF1; (b) T3 copper site in DR1; (c) Histidine-Brace motif in miniLPMO.*

References

1. L. Leone, M. De Fenza, A. Esposito, F. Nastri, A. Lombardi, *J. Pep. Sci.* **2024**, *30*, e3606.
2. S. La Gatta, V. L. Pecoraro, *Curr. Op. Chem. Biol.* **2025**, *86*, 102586.
3. F. Nastri, D. D'Alonzo, L. Leone, G. Zambrano, V. Pavone, A. Lombardi, *Trends Biochem. Sci.* **2019**, *44*, 1022.
4. T. Vornholt, F. Leiss-Maier, W. J. Jeong, C. Zeymer, W. J. Song, G. Roelfes, T. R. Ward, *Nat. Rev. Methods Primers* **2024**, *4*, 78.
5. M. J. Chalkley, S. I. Mann, W. F. DeGrado, *Nat. Rev. Chem.* **2022**, *6*, 31.
6. A. Lombardi, F. Pirro, O. Maglio, M. Chino, W.F. DeGrado, *Acc. Chem. Res.* **2019**, *52*, 1148.
7. F. Pirro, S. La Gatta, F. Arrigoni, A. Famulari, O. Maglio, P. Del Vecchio, M. Chiesa, L. De Gioia, L. Bertini, M. Chino, F. Nastri, A. Lombardi, *Angew. Chem.* **2023**, *135*, e202211552.

Iron-Dependent Enzymes for C–C Bond Formation, Cleavage, and Rotation

Alison Narayan, *University of Michigan, USA*

Chemical methods that facilitate a desired transformation with precise chemo-, site- or stereoselectivity can allow for more efficient synthetic routes, thus expanding the practical-to-target molecules. Biocatalytic methods present the opportunity to develop exquisite catalyst-controlled selectivity, enabling highly streamlined synthetic routes. This is exemplified by nature's ability to make intricate secondary metabolites with potent biological activity such as taxol and vancomycin. Although biocatalysis has been embraced by industrial chemists for the commercial production of pharmaceutical agents, more work is needed to promote the implementation of biocatalysis in target-oriented synthesis more broadly, including limitations in the breadth of well-developed reactions, the unknown substrate scope of functionally characterized enzymes, and the perceived incompatibility with multistep, preparative-scale sequences. In this talk, the use of iron-dependent enzymes for C–C bond formation, C–C bond cleavage, and C–C bond rotation will be discussed from mechanism to application in the synthesis of complex molecule synthesis.

Mutation-Free Expansion of Substrate Scope in Cytochrome P450BM3 via Decoy Molecule Strategy

Osami Shoji, *Nagoya University, JP*

Cytochrome P450BM3 is a highly efficient heme enzyme, but its native activity is largely restricted to long-chain fatty acids. To overcome this limitation without relying on mutagenesis, we developed a decoy molecule strategy that employs inert compounds mimicking native substrates to trigger oxygen activation. This approach enabled wild-type P450BM3 to hydroxylate abiological substrates such as benzene, propane, and methane. Optimized decoy molecules, particularly those derived from N-acyl amino acids and amino acid dimers, enhanced substrate turnover and, in some cases, promoted crystallization of the enzyme, which facilitated structural analysis. This strategy has also been successfully applied to other P450 enzymes, demonstrating its broader applicability. In addition, by combining the decoy approach with directed evolution, we developed variants capable of utilizing microbial signaling molecules such as N-acyl homoserine lactones as functional decoys. Our decoy molecule strategy offers new opportunities for sustainable and programmable oxidation chemistry.

De Novo Lanthanide Enzymes for Photoredox Catalysis

Cathleen Zeymer, *Technical University of Munich, DE*

Cerium photoredox catalysis is a powerful method to activate organic molecules under mild conditions. However, it remains a major challenge to achieve stereocontrol in these light-driven radical reactions. We thus developed a cerium-dependent photoenzyme enabling this chemistry in the chiral environment of a de novo protein. Our work is based on a de novo TIM barrel scaffold designed previously in a physics-based approach.[1] We equipped the protein with a high-affinity metal binding site for lanthanide ions and demonstrated its photocatalytic potential. Upon visible-light irradiation, the cerium-bound enzyme enables the radical C–C bond cleavage of 1,2-diols in aqueous solution.[2] To optimize the initially low activity and enantioselectivity, we redesigned the scaffold computationally. We decreased the cavity size between the two independently folded domains and used *ProteinMPNN* to redesign the sequences.[3] Selected redesigns were characterized experimentally and showed significantly improved k_{cat}/K_M and enantiomeric excess in kinetic resolutions of diols.

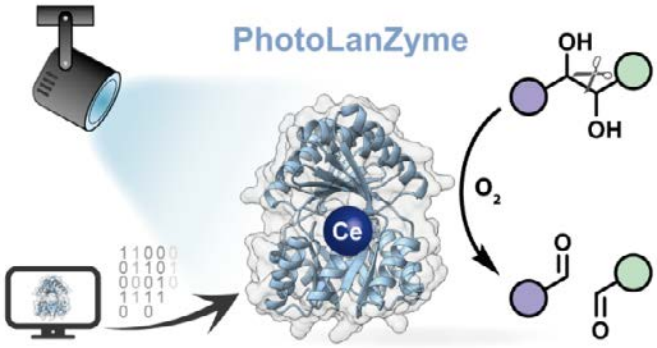


Figure *Lanthanide-dependent artificial photoenzymes (PhotoLanZymes) have been engineered to catalyze radical C–C bond cleavages upon visible-light irradiation. This approach brings cerium photoredox chemistry into the world of biocatalysis.*

References
1. Caldwell, Haydon, Piperidou, Huang, Bick, Sjöström, Hilvert, Baker, Zeymer, *Proc. Natl. Acad. Sci. U.S.A.* **2020**, *117*, 30362
2. Klein, Leiss-Maier, Mühlhofer, Boesen, Mustafa, Kugler, Zeymer, *J. Am. Chem. Soc.* **2024**, *146*, 25976
3. Dauparas, (…), Baker, *Science* **2022**, *378*, 49

Harnessing Repurposed Enzymes with New-to-Nature Reactivity for Small Molecule Synthesis

Huimin Zhao, *University of Illinois Urbana-Champaign, USA*

Enzymes have been increasingly used for practical synthesis of chemicals, fuels, and materials thanks to recent advances in enzyme engineering, synthetic biology, artificial intelligence (AI)/machine learning (ML), and laboratory automation. In this talk, I will discuss our recent effort in designing repurposed enzymes with new-to-nature reactivity for asymmetric synthesis by exploring the synergy between enzymatic catalysis and photocatalysis. The representative new-to-nature photoenzymatic reactions that we have demonstrated so far include but are not limited to intermolecular radical hydroalkylation, intermolecular radical conjugate addition, and intermolecular radical hydroamination. In addition, I will introduce a new strategy to address the scalability issue of these new-to-nature photoenzymatic reactions by directly integrating them into microbial metabolism. Finally, I will highlight the development of machine learning and laboratory automation tools for enzyme discovery and engineering. Taken together, these strategies and tools should greatly accelerate the development of biocatalysts for applications related to human health, energy, and sustainability.

Selected Talks

Harnessing Glycyl Radical Enzymes for Asymmetric Catalysis

Mary C. Andorfer, *Michigan State University, USA*

Glycyl radical enzyme (GRE) hydroalkylases use amino acid residue-based radicals to directly convert C(sp³)–H bonds into stereodefined C–C bonds. Despite their potential for biocatalysis, GRE hydroalkylases are underutilized due to the challenge of installing these amino acid-based radicals in vitro.[1] We recently developed a platform that overcomes this limitation, enabling radical generation within purified enzyme.[2] Using this approach, we demonstrated that a wild-type GRE efficiently catalyzes its native reaction—stereoselective toluene addition to fumarate—with high total turnover (>17,000 TTN) and robust catalysis (kcat = 18 s^{-1}). The glycyl radical species persists in an anaerobic environment without losing activity for up to 11 days. Moreover, we have found that this enzyme accommodates a range of simple hydrocarbons and heterocycles as substrates and that scope and selectivity are tunable through mutagenesis. Beyond native reactions, we have also discovered non-native transformations that are only catalyzed by enzyme variants. This work lays the foundation for developing GRE hydroalkylases as versatile biocatalysts for selective coupling reactions.

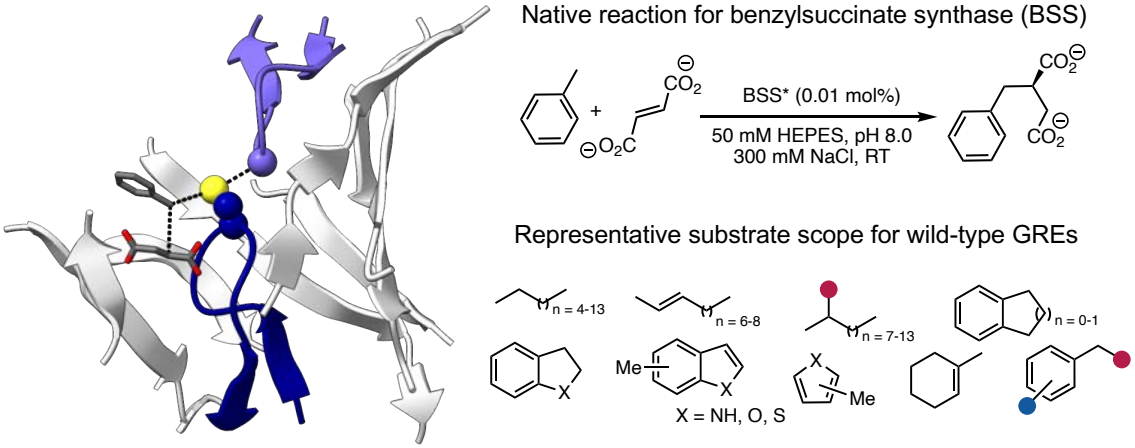


Figure *In GRE active sites, a β-barrel houses a Cys loop (blue) and a Gly loop (purple), both essential for catalysis. Upon substrate binding, a stable glycyl radical (purple sphere) can form a transient thiyl radical on a nearby Cys residue. The structure shown above is benzylsuccinate synthase (BSS), a GRE that catalyzes selective addition of toluene to fumarate (shown as grey sticks). The native reaction catalyzed by BSS is also shown, along with the initial substrate scope for the C–H substrate explored in our lab for BSS and an alkane-activating GRE.*

References

1. L. R. F. Backman, M. A. Funk, C. D. Dawson, C. L. Drennan, *Crit. Rev. Biochem. Mol. Biol*, **2017**, *52*, 674.
2. M. C. Andorfer, D. T. King-Roberts, C. N. Imirch, B. G. Brotheridge, C. L. Drennan, *iScience* **2023**, *26*, 106902.

Development of Biocatalytic Cobalt-Mediated Hydrogen Atom Transfer Chemistry

Andrew R. Buller, *University of Wisconsin-Madison, USA*

We report the in vivo synthesis of a new cofactor where cobalt instead of iron is inserted into protoporphyrin. The resulting cofactor can access a meta-stable metal-hydride that can be directed to perform metallo-hydrogen atom transfer chemistry. We report the molecular mechanisms of how this new cofactor is made in *E. coli*.[1,2] When this cofactor is inserted into P450 scaffolds, reaction with phenylsilanes access a unique cobalt-hydride intermediate. Synthetic limitations of using phenylsilane as a hydride source are discussed, as well as potential solutions. Evolution for reductive deallylation led to the serendipitous discovery of a novel mode of reductive dearomatization, which was characterized through detailed enzymological study.

References

1. Perkins, L. J.; Weaver, B. R.; Buller, A. R.; Burstyn, J. N. De Novo Biosynthesis of a Nonnatural Cobalt Porphyrin Cofactor in E. Coli and Incorporation into Hemoproteins. Proc Natl Acad Sci U S A 2021, 118 (16), 2017625118. https://doi.org/10.1073/PNAS.2017625118.
2. Weaver, B. R.; Perkins, L. J.; Fernandez Candelaria, F. O.; Burstyn, J. N.; Buller, A. R. Molecular Determinants of Efficient Cobalt-Substituted Hemoprotein Production in E. Coli. ACS Synth Biol 2023, 12 (12), 3669–3679. https://doi.org/10.1021/ACSSYNBIO.3C00481.

Creating Photovoltaic Enzymes by Design and Evolution

Adrian Bunzel, *ETH Zurich, CH*

The global energy crisis challenges us to develop more sustainable strategies to produce electricity. Given the excellent efficiency of natural photosynthetic complexes, biohybrid photovoltaic devices present an attractive solution for solar energy conversion. However, the low stability and high complexity of natural photoactive systems limit their application in photovoltaics.

Previously, our group has combined computational design and directed evolution to engineer *de novo* enzymes.[1-3] Here, we adopted this strategy to create photoenzymes that overcome the limitations of natural photosystems.[4] Photobiocatalysts were designed by introducing photosensitizer binding sites into heme-containing helical bundle proteins. The designed binding sites were highly specific for the target photosensitizer and reached nanomolar ligand affinity. Photosensitizer binding to the protein scaffold improved photostability by at least an order of magnitude, substantially extending the lifetime in a model biohybrid solar cell. By screening directly for photocurrents, photovoltaic activity could be improved 4-fold within just two initial rounds of directed evolution. This promising result suggests that enzyme engineering can yield photocatalysts with activities approaching those of state-of-the-art solar cells.

Our work provides a robust methodological framework for creating photoenzymes addressing critical sustainability challenges, such as solar energy conversion, nitrogen fixation, carbon capture, and hydrogen production.

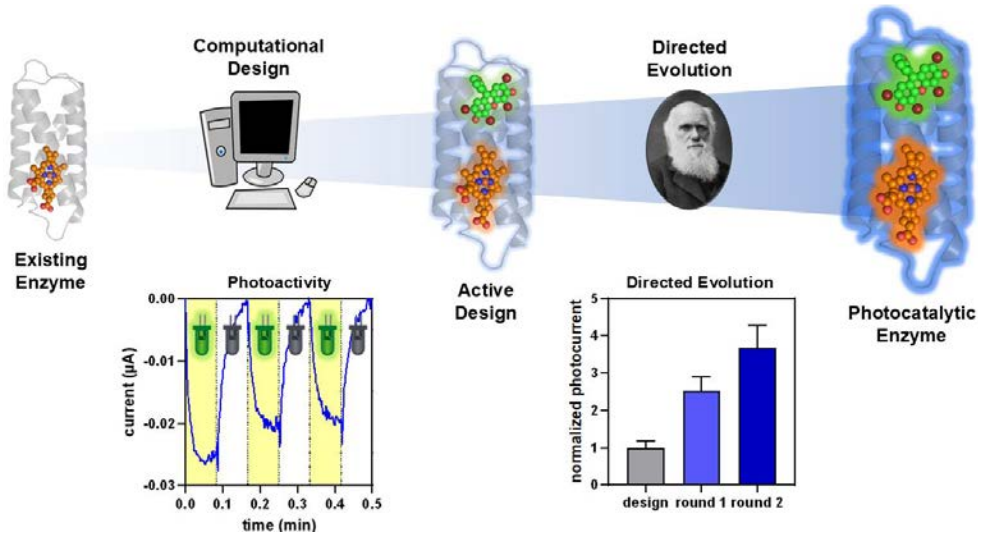


Figure *Creating Photoenzymes by computational desing and directed evolution.*

References

1. Bunzel, H. A. *et al. Nature Chemistry* **13**, 1017–1022 (2021).
2. Bunzel, H. A. *et al. Current Opinion in Structural Biology* **67**, 212–218 (2021).
3. Otten, R. *et al. Science* **370**, 1442–1446 (2020).
4. Bunzel, H. A. *et al. bioRxiv* 2022.12.20.521207 (2022).

Biocatalytic Transformations via Abiological Metal Hydrides

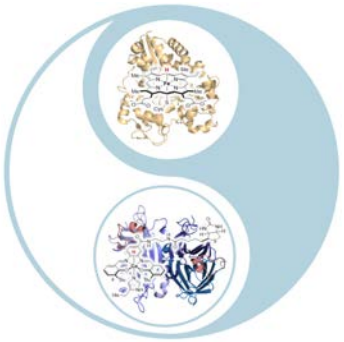
Dongping Chen, *University of Basel, CH*

Metal-hydride species are pivotal in organometallic chemistry, enabling a wide range of transformations of unsaturated C=X (X = CR_2, O, NR, etc.) bonds. While metal-catalyzed hydrogen atom transfer (HAT) offers an appealing alternative through an outer-sphere, radical- based pathway, achieving stereoselectivity in homogeneous catalysis remains a formidable challenge. Enzymes provide a blueprint for stereocontrol—thanks to their chiral microenvironments—and have been shown to facilitate radical reactions with remarkable selectivity. However, natural metal-hydride containing enzymes are rare and often exhibit limited catalytic activities compared to synthetic catalysts. To bridge this gap, we explored the integration of abiotic metal-hydrides into biological contexts to achieve enantioselective radical transformations using RepArtZymes (Repurposed and Artificial metalloenzymes).

Artificial metalloenzymes (ArMs)[1] present a fascinating means of introducing non-natural metallic complexes into an evolvable protein scaffold, providing a unique approach to tackle some of great challenges in homogeneous catalysis. A Schiff-base cobalt complex was anchored into a chimeric streptavidin scaffold relying on the biotin-streptavidin supramolecular interaction as well as an axial histidine coordination to the cobalt-ion. The resulting Artificial Radical Cyclase was engineered to catalyze the formation of enantioenriched bicyclic terpenoid scaffolds via a Co–H mediated hydrogen atom transfer radical cyclization.[2]

Repurposed metalloenzymes offer an alternative strategy to achieve enantioselective radical reactions. Building on our finding with Artificial Radical Cyclase and relying on enzyme engineering, we repurposed hemoproteins to catalyze asymmetric abiological radical reactions, proposing a transient Fe–H species as key intermediate. Tailored variants of cytochrome P450s catalyzed enantioselective radical cyclizations through hydrofunctionalization of unactivated alkenes, proceeding via a homolytic metal-hydride HAT mechanism.[3]

These findings highlight the fusion of abiological metal-hydride catalysis with enzymatic stereocontrol, offering new avenues for enantioselective synthesis in biocatalysis and expanding the catalytic repertoire of enzymes.



References

1. Schwizer, F.; Okamoto, Y.; Heinisch, T.; Gu, Y.; Pellizzoni, M. M.; Lebrun, V.; Reuter, R.; Köhler, V.; Lewis, J. C.; Ward, T. R. *Chem. Rev*. **2018**, *118*, 142-231.
2. Chen, D.; Zhang, X.; Vorobieva, A. A.; Tachibana, R.; Stein, A.; Jakob, R. P.; Zou, Z.; Graf, D. A.; Li, A.; Maier, T.; Correia, B. E.; Ward, T. R. *Nat. Chem* **2024**, *16*, 1656-1664.
3. Zhang, X.; Chen, D.; Ward, T. R. *ChemRxiv*, **2024**, 10.26434/chemrxiv-2024-dhxpx.

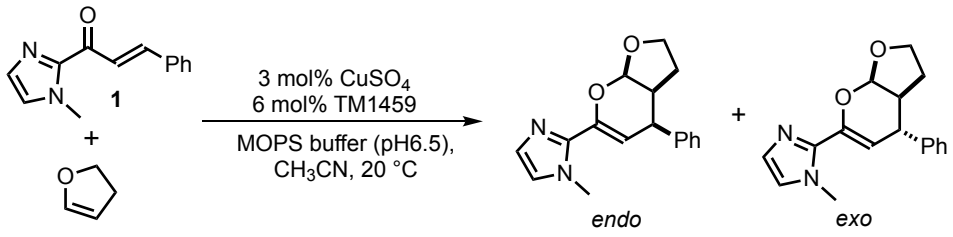
Artificial Non-Heme Copper Enzymes that Catalyze Stereoselective Inverse Electron-Demand Hetero-Diels-Alder Reactions

Nobutaka Fujieda, *Osaka Metropolitan University, JP*

In recent years, it has become clear that the Diels-Alder reaction occurs in biological system, where the Diels-Alderases catalyze this reaction as a part of metabolic pathway in living organisms. As the enzymes could be applied to the synthesis of desired cycloadducts, they would be very promising as an environmentally-friendly catalyst because of their industrial usefulness. However, such application has not been reached yet due to its high substrate specificity. On the other hand, a variety of approach toward developing artificial metalloenzymes (ArMs) have recently emerged all over the world. ArMs are defined as highly regio- and/or enantioselective catalysts consisting of a protein matrix and a synthetic metal complex. Therefore, ArMs can harness excellent reactivity derived from the metal complexes as well as enzymatic ability such as exquisite chemical environment to accelerate even difficult and desirable chemical reactions.

Our group developed artificial metalloenzymes with a cupin-type protein (TM1459) obtained from hyperthermophile, Thermotoga maritima, where well-defined amino acid residues are disposed around the metal center. This metal binding motif consists of 4-histidine tetrad in a almost identical geometry to that of the tris(2-pyridylmethyl)amine (TPA) ligand system. Using this protein as metal-ligands, we have recently developed the artificial non-heme metalloenzymes with high stereoselectivity by mutating 4 histidines at the metal binding site.[1-4]

In this study, we screened thus obtained mini-library of mutants for the inverse electron- demand hetero-Diels-Alder reaction (Scheme). As a result, H52A mutants which has 3-his triad, showed high endo-selectively, but low enantioselectivity and yield as well. Therefore, the pose of substrate docked into cavity by in silico simulation suggested that there are some steric repulsion between the substrate and surrounded amino acids. Based on this hypothesis, we constructed the H52G/I108A mutant which showed excellent selectivity and yield. Finally based on this notion, we constructed the Cu-H52G/I108D mutant which showed enhanced selectivity (94 % ee) and yield (92 %). The X-ray crystallographic analysis of this mutant exhibited that copper center would migrate during the catalytic cycle. In addition, further substrate scope was investigated and the series of substrate also showed good stereoselectivity.



Scheme *Asymmetric Diels-Alder reaction catalyzed by Cu-TM1459.*

References
1. N. Fujieda, S. Itoh et al., *J. Am. Chem. Soc.*, **2017**, 139, 5149.
2. N. Fujieda, S. Itoh et al., *Angew. Chem. Int. Ed.*, **2020**, 59, 7717.
3. R. Matsumoto.; N. Fujieda. et al., *Chem. Sci.*, **2023**, 14, 3932.
4. N. Fujieda, S. Itoh, et al., *Chem. Euro. J.*, **2024**, e202402803

Unlocking New-to-Nature Enzymatic Transformations Through Precision Confinement

Marc Garcia-Borràs, *University of Girona, ES*

Enzymes catalyze complex chemical reactions with high specificity, efficiency, and selectivity. Many enzymes require cofactors that participate in biocatalytic cycles involving the formation of organometallic, ionic, or radical reactive intermediates. These reactive intermediates can follow divergent reaction pathways, leading to enzymatic promiscuity and providing opportunities for designing new challenging enzyme-catalyzed transformations. Experimental characterization of these “fleeting” reactive intermediates is challenging due to limitations in structural and spectroscopic techniques.[1] However, computational methods offer a powerful alternative for describing these intermediates with atomic-level and real-time precision.

Our research program aims to develop and apply new multiscale computational protocols to study, characterize, and rationally improve the formation and stabilization of highly reactive intermediates in enzyme active sites, directing them towards desired reaction pathways. This can be achieved through designing precision confinement and specific polar environment within the protein’s active site. The ultimate goal is to implement new synthetically useful biocatalytic reactions. Herein, we present recent successful cases in computationally guided enzyme engineering, showcasing how precision confinement and tailored active-site electrostatics can be leveraged to direct enzymatic transformations toward synthetically valuable outcomes. These strategies have led to the successful implementation of metalloenzyme-catalyzed non-natural oxidative processes, carbene and nitrene transfers, and radical-mediated C–H functionalization.[3-5] Our findings provide atomistic descriptions and fundamental insights into enzymatic catalysis and offer a blueprint for designing enzymes capable of performing novel transformations with potential applications in green chemistry, pharmaceutical synthesis, and sustainable catalysis.

References

1. M. Reetz, M. Garcia-Borràs, *J. Am. Chem. Soc.*, **2021**, 143, 14939.
2. A. Romero-Rivera, M. Garcia-Borràs, S. Osuna, *Chem. Commun.*, **2017**, 53, 284.
3. (a) M. Garcia-Borràs, S. B. J. Kan, R. D. Lewis, A. Tang, G. Jimenez-Osés, F. H. Arnold, K. N. Houk, *J. Am. Chem. Soc.*, **2021**, 143, 7114.; (b) C. Calvó-Tusell, Z. Liu, K. Chen, F. H. Arnold, M. Garcia-Borràs, *Angew Chem Int Ed,* **2023***, 62, 35, e202303879.*
4. (a) Soler, J.; Gergel, S.; Hammer, S.; Garcia-Borràs, M. *J. Am. Chem. Soc.*, **2022**, 144, 35, 15954. DOI: 10.1021/jacs.2c02567; (b) Gergel, S.; Soler, J.; Klein, A.; Schülke, K.; Hauer, B.; Garcia-Borràs, M.; Hammer, S., *Nat Catal,* **2023,** *6, 7, 606-617.*
5. Rui, J.; Zhao, Q.; Huls, A. J.; Soler, J.; Paris, J. C.; Chen, Z.; Reshetnikov, V.; Yang, Y.; Guo, Y.; Garcia-Borràs, M.; Huang, X. *Science*, **2022**, 376, 6595, p.869-874.

New Catalytic Reactions by Enzyme Engineering

Stephan Hammer, *Bielefeld University, DE*

Enzyme catalysis is frequently highly efficient and selective, as enzymes precisely control the entire environment in which a reaction takes place. Beyond primary catalytic interactions, enzymes provide a multitude of secondary interactions that can pre-organize substrates (reagents), control conformations of highly reactive intermediates (e.g., radicals and carbocations) in each step of a catalytic cycle and distinguish between competing reaction pathways and transitions states. To me, one of the most exciting questions in catalysis is: What reactions can proteins catalyze that are not readily achieved with other types of catalysts?[1,2]

Our research group combines approaches from organic chemistry, enzymology and directed evolution to design, evolve, understand, and apply new enzyme function. We develop biocatalysts for sought-after chemical transformations that currently lack efficient catalytic solutions. This includes enzymes for Wacker-type alkene oxidation, regioselective N-alkylation of azoles with “off the shelf” reagents, as well as asymmetric hydration to synthesize chiral alcohols simply from alkenes and water (see figure).[3-9] During this talk, I will discuss unpublished examples of directed evolution, mechanistic studies and applications of new biocatalysts developed in our research group.

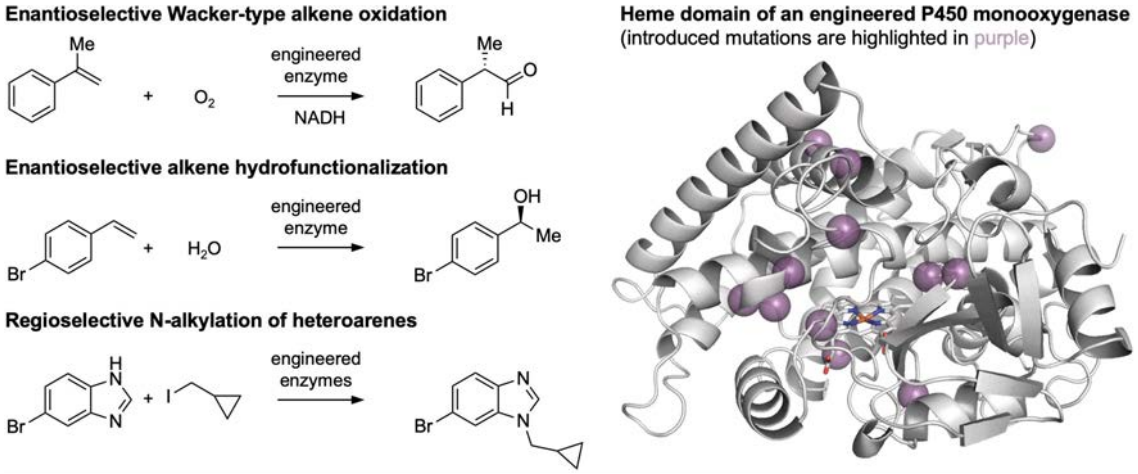


Figure *Examples of sought-after chemical reactions recently enabled using enzyme engineering.*

References
1. C. Klaus, *et al.*, *Trends Chem.* **2022**, *4*, 363.
2. S. Jain, *et al.*, *JACS Au* **2024**, *4*, 2068.
3. S.C. Hammer *et al.*, *Science* **2017**, *358*, 215.
4. J. Soler *et al.*, *J. Am. Chem. Soc.* **2022**, *144*, 15954.
5. S. Gergel *et al.*, *Nat. Catal.* **2023**, *6*, 606.
6. L.L. Bengel *et al.*, *Angew. Chem. Int. Ed.* **2021**, *60*, 5554.
7. F. Ospina *et al.*, *Angew. Chem. Int. Ed.* **2022**, *61*, e202213056.
8. K.H. Schülke *et al.*, *ChemBioChem* **2024**, *25*, e202400079.
9. M. Gajdoš *et al.*, *Angew. Chem. Int. Ed.* **2023**, *62*, e202215093.

Repurposing Thiamine-Dependent Enzymes Using Radicals

Xiaoqiang Huang, *Nanjing University, CN*

Thiamine diphosphate(ThDP)-dependent enzymes are versatile biocatalysts that naturally facilitate asymmetric C−C forming/breaking through the enzymatic Breslow intermediate via a 2-electron umpolung polar mechanism.[1] Recently, our group has repurposed ThDP-dependent enzymes for non-natural asymmetric radical transformations triggered by single-electron radical pathways.[2] Mechanistically, an active site ketyl radical is generated by the oxidation of the resting enzymatic Breslow intermediate through photoredox catalysis. Concurrently, a prochiral benzylic radical is formed via single-electron trasnfers from an appropriate precursor. These radicals can undergo enantioselective cross-coupling within the active site, leading to the synthesis of chiral ketones. Specifically, we successfully employed N-(acyloxy)phthalimides, which are one-step synthesized from carboxylic acids, as radical precursors.[3] More recently, we designed in situ generation of prochiral radicals through the addition of electron-deficient carbon-centred radicals to olefins, followed by enzymatic acyl radical transfer, achieving a three-component photobiocatalytic transformation.[4] In this talk, I will also present our latest progress, including a triple activation strategy to utilize C-H bonds as radical precursors, as well as a radical repositioning strategy.

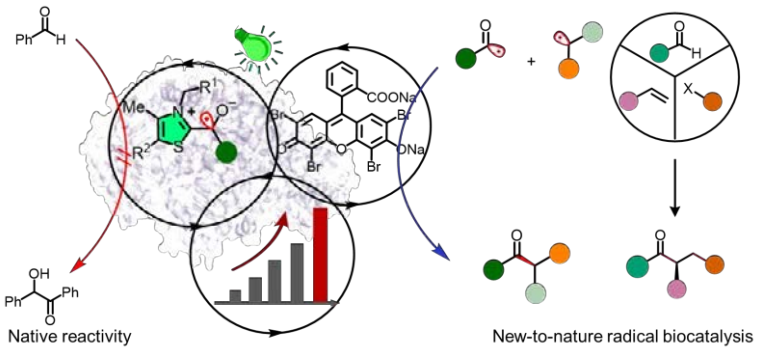


Figure *Repurposing thiamine-dependent enzymes with radicals.*

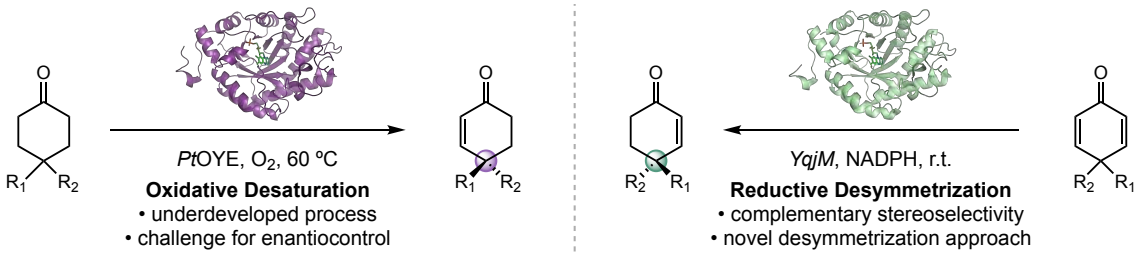
References

1. Hailes, H. C. *et al.* Engineering stereoselectivity of ThDP-dependent enzymes. *FEBS J.* **2013**, *280*, 6374-6394.
2. M. Yan, J. C. Lo, J. T. Edwards, P. S. Baran, *J. Am. Chem. Soc.* **2016**, *138*, 12692-12714.
3. Y. Xu, H. Chen, L. Yu, X. Peng, J. Zhang, Z. Xing, Y. Bao, A. Liu, Y. Zhao, C. Tian, Y. Liang, X. Huang, *Nature* **2024**, *625*, 74-78.
4. Z. Xing, F. Liu, J. Feng, L. Yu, Z. Wu, B. Zhao, B. Chen, H. Ping, Y. Xu, A. Liu, Y. Zhao, C. Wang, B. Wang, X. Huang, *Nature* **2025**, *637*, 1118-1123.

Investigation of Flavin-Dependent Desaturases for Asymmetric Synthesis

Zhen Liu, *National Institute of Biological Sciences, CN*

Efficient methods for achieving desaturation of *α,β*-unsaturated carbonyl compounds are highly sought after in organic chemistry. In contrast to traditional synthetic approaches, enzymatic desaturation systems offer the potential for enhanced sustainability and selectivity but have remained elusive. In this talk, I will introduce a versatile and general enzymatic desaturation system based on flavin-dependent ene-reductases for desymmetrizing cyclohexanones.[1] This innovative platform facilitates the synthesis of a wide array of chiral cyclohexenones bearing quaternary stereocenters—structural motifs commonly present in bioactive molecules—with remarkable yields and enantioselectivities. Mechanistic insights into this novel enzymatic desaturation process are provided through a combination of experimental investigations and computational studies. Furthermore, leveraging these insights gained, we have devised an additional biocatalytic strategy for the synthesis of *α,β*-unsaturated carbonyl compounds by reductively desymmetrizing cyclohexadienones. This method yields the opposite enantiomer compared to our desaturation system, underscoring the complementary nature and broad applicability of our flavin-based desymmetrization approaches.



References

1. Zeng, Q.-Q. *et al.* Biocatalytic desymmetrization for synthesis of chiral enones using flavoenzymes. *Nat. Synth.* **3**, 1340–1348 (2024).

Ultrahigh-Throughput Evolution of Enzymes Toward Abiotic Substrates Using Chemogenetic Platforms

Jeffrey D. Martell, *University of Wisconsin–Madison, USA*

We have developed new platforms for directed evolution that merge cellular protein expression systems with chemical synthesis, enabling ultrahigh-throughput selection of enzyme mutants (from libraries of millions) with high activity toward abiotic substrates. In one project area, we are evolving enzymes for enhanced activity in plastic recycling. Existing methods to evolve plastic-degrading enzymes require low-throughput testing, creating a bottleneck in discovery of high-activity mutants. To overcome this limitation, we developed an ultrahigh-throughput platform to evolve polymer-degrading enzymes, combining yeast display with the synthesis of a probe resembling the target polymer chain.[1] We discovered mutants of polyethylene-terephthalate (PET)-degrading enzymes with enhanced activity in degrading bulk plastics. We are applying the platform to diverse enzymes, synthetic polymer chains, and reaction conditions. In another project area, we are using high-throughput evolution with rational protein engineering and mechanistic analysis to discover catalysts with improved activity for generating reactive intermediates, which can be employed for "proximity labeling" inside living cells to map the locations of specific biomolecules.[2,3] The biocatalysts evolved using our approach also have applications for green synthetic methodology.

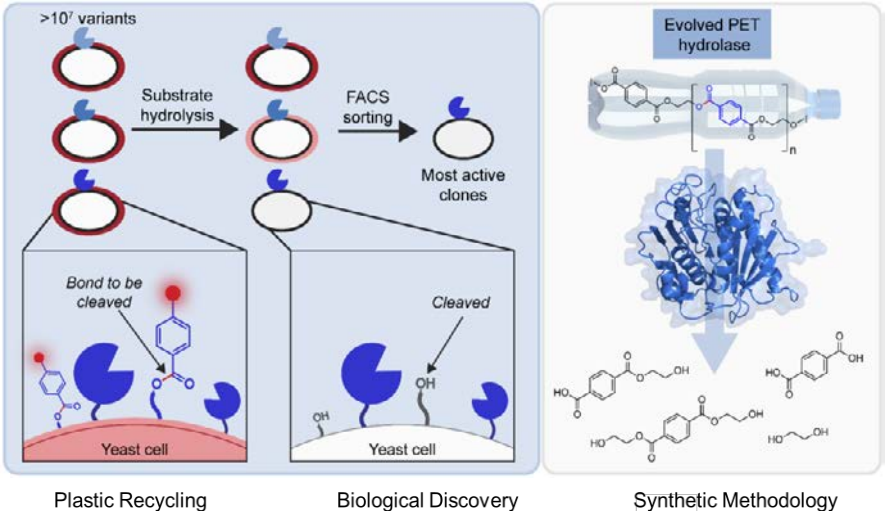


Figure *Chemogenetic directed evolution platform for polymer-degrading enzymes. A yeast library containing >107 enzyme mutants is rapidly evaluated for activity in cleaving a synthetic polymer probe.*

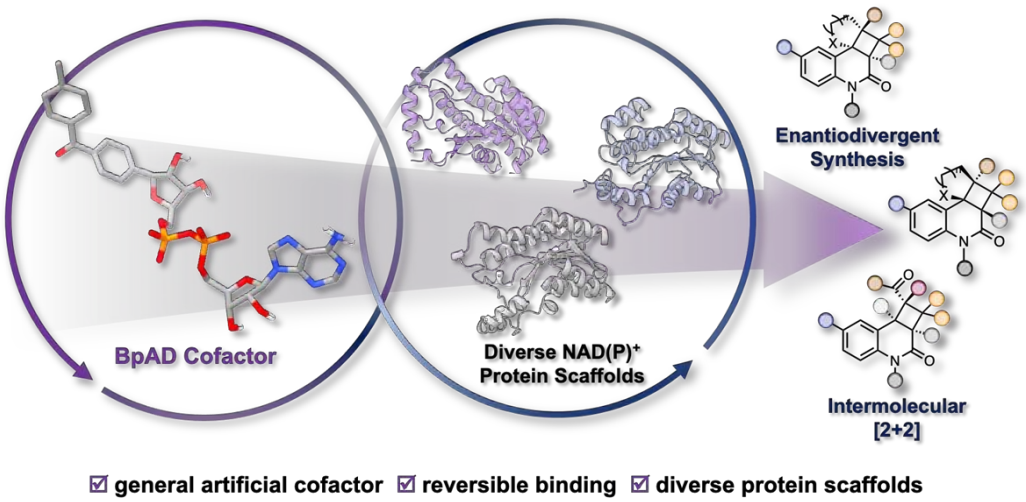
References

1. Cribari, M.A.; Unger, M.J.; Unarta, I.C.; Ogorek, A.N.; Huang, X.; Martell, J.D. Ultrahigh-Throughput Directed Evolution of Polymer-Degrading Enzymes Using Yeast Display. *J. Am. Chem. Soc.*, **2023**, *145*, 27380–27389. https://doi.org/10.1021/jacs.3c08291
2. Fang, S.; Acevedo, L.D.; Solivais, A.; Zhou, X.; Delfosse, E.S.; Frey, B.L.; Smith, L. M.; Martell, J.D. Directed Evolution of APOX for Proximity Labeling Using Phenols with High Redox Potentials. *ChemRxiv* **2025.** https://chemrxiv.org/engage/chemrxiv/article-details/67c0ea95fa469535b93b1f29
3. Ogorek, A.N.†; Pani, S.†; Mertick-Sykes, E.J.; Dickinson, B.C.*; Martell, J.D*. *In Preparation*.

Protein-Scaffold-Diverse Artificial Photoenzymes Powered by a Reversibly Binding BpAD Cofactor

Hui-Jie Pan, *Nanjing University, CN*

Enzymes are nature's most efficient and selective catalysts, yet their utility is often constrained by the limited scope of reactions they catalyze. To unlock new possibilities, artificial enzymes offer a compelling solution. However, current methods for constructing artificial enzymes typically rely on the irreversible incorporation of non-natural active sites into protein scaffolds, complicating design and severely limiting scaffold diversity. Here, inspired by natural cofactors like NAD(P)$^+$, we present a reversible binding strategy for artificial enzyme development. We introduce BpAD, a photoactive general cofactor that can seamlessly integrate into a wide range of NAD$^+$-dependent protein scaffolds. This cofactor enables the efficient catalysis of both intermolecular and intramolecular [2+2] cycloaddition reactions with exceptional enantioselectivity, demonstrating a broad substrate scope and remarkable enantiodivergence. Computational studies confirm the precise, dynamic binding of BpAD within these scaffolds and unveil a key exo-attack pathway in the stepwise C–C bond formation mechanism. Furthermore, BpAD displays strong orthogonality with NAD$^+$, allowing both cofactors to operate simultaneously without interference. This reversible cofactor binding strategy not only simplifies artificial enzyme design but also opens the door to leveraging a diverse array of protein scaffolds for tailored catalytic applications.



Photoenzymatic Hydroaminations and Hydroarylations via an Emergent Mechanistic Pathway

Felix Raps, *Princeton University, USA*

Translation of small molecule reactivity into biocatalytic frameworks offers access to highly precise catalysts for selective synthesis. These systems can overcome limitations often innate to small molecule catalysts, frequently focusing on stereo- and regiocontrol of transformations. In addition, biocatalysts can offer unexpected results, harnessing emergent mechanisms that in some cases are unprecedented in organic synthesis.

This talk will feature the discovery of an emergent mechanism for a Markovnikov-selective, photoenzymatic hydroamination in a Baeyer-Villiger Monooxygenase that was amplified by directed evolution. The development allowed for the preparation of highly-congested α-tertiary amines difficult to access with traditional methodologies. Mechanistic investigations using small molecule probes, DFT, and QM/MM revealed the behavior within the enzyme active site. Furthermore, the more recent development of a hydroarylase will be presented, focusing on the requirements of the enzyme to facilitate the reaction, as well as studies of the changes to the active site by crystallography and computational tools.

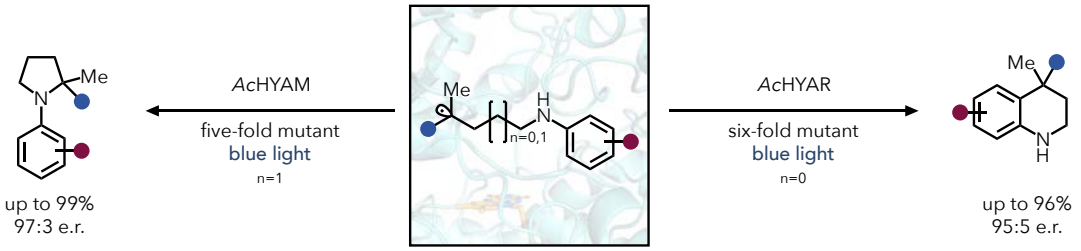


Figure *Generation of a benzylic radical in the enzyme active site of evolved Baeyer Villiger Monoogyenases enables divergent transformation into hydroamination and hydroarylation products.*

References

1. Raps, F. C.; Rivas-Souchet, A.; Jones, C. M.; Hyster, T. K., Emergence of a distinct mechanism of C–N bond formation in photoenzymes. *Nature* **2025,** *637* (8045), 362–368.
2. Emmanuel, M. A.; Bender, S. G.; Bilodeau, C.; Carceller, J. M.; DeHovitz, J. S.; Fu, H.; Liu, Y.; Nicholls, B. T.; Ouyang, Y.; Page, C. G.; Qiao, T.; Raps, F. C.; Sorigué, D. R.; Sun, S.-Z.; Turek-Herman, J.; Ye, Y.; Rivas-Souchet, A.; Cao, J.; Hyster, T. K., Photobiocatalytic Strategies for Organic Synthesis. *Chem. Rev.* **2023,** *123* (9), 5459-5520.

Artificial Enzymes Featuring Genetically Encoded Thiophenolate-Based Non-Canonical Amino Acids for Catalysis

Gerard Roelfes, *University of Groningen, NL*

Genetic code expansion, in particular stop codon suppression, has proven to be a valuable approach towards artificial enzymes displaying new-to-nature catalytic activity.[1,2] We have used SCS to introduce non-canonical amino acid, e.g. bipyridyl alanine, that bind a catalytic metal ion,[3] or that function directly as catalytic residue, e.g. para-aminophenylalanine and p-boronophenylalanine.[4,5]

Now we introduce two new classes of artificial enzymes that have been created by the incorporation of non-canonical amino acids containing a thiophenol-derivative as side chain, such as 4-mercaptophenylalanine (pSHF) and 3-mercaptotyrosine (SHY), at various positions into the protein scaffolds LmrR and RamR.These thiophenolates were exploited as soft ligands for noble metals, in particular Au(I). The new artificial gold enzymes proved to be active in intramolecular hydramination reactions and heterocyclization reactions.[6] Active enzymes have been characterized structurally as well as kinetically. Currently we are evolving these enzymes towards higher activity and (enantio-)selectivity

Thiophenolates also are attractive for application as nucleophilic catalysts. We found especially some of the RamR-based enzymes to be good biocatalysts for enantioselective intramolecular Morita-Baylis-Hillman reactions. Depending on the position of the pSHF or SHY residue within RamR, up to 50 % ee has been obtained thus far. Further rounds of directed evolution are currently underway.

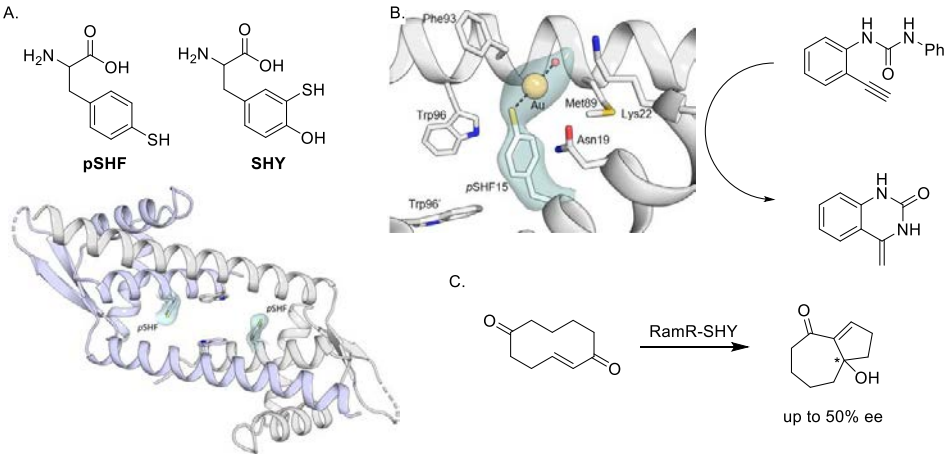


Figure *A. Non-canonical amino acids pSHF and SHY and crystal structure of LmrR_V15pSHF; B. zoom of active site of LmrR-pSHF-Au(I) and catalyzed intramolecular hydroamination. C. Asymmetric intramolecular Morita-Bayliss-Hillman reaction catalyzed by RamR-SHY.*

References

1. I. Drienovská, G. Roelfes, *Nat. Catal.* **2020**, *3*, 192-202.
2. Z. Birch-Price, F.J. Hardy, T.M. Lister, A.R. Kohn, A.P. Green, *Chem. Rev.* **2024**, *124*, 8740-8786.
3. I. Drienovská, A. Rioz-Martínez, A. Draksharapu, G. Roelfes, *Chem. Sci.* **2015**, *6*, 770-776.
4. I. Drienovská, C. Mayer, C. Dulson, G. Roelfes, *Nat. Chem.* **2018**, *10*, 946-952.
5. L. Longwitz, R.B. Leveson-Gower, H.J. Rozeboom, A.-M.W.H. Thunnissen, G. Roelfes, *Nature* **2024**, *629*, 824–829.
6. M.J. Veen, F.S. Aalbers, H.J. Rozeboom, A.-M.W.H. Thunnissen, D.F. Sauer, G. Roelfes, *Angew. Chem. Int. Ed.* **2024** (early view), e202421912.

Accurate Structure Prediction with AlphaFold 3

Zachary Wu, *Google DeepMind, UK*

AlphaFold 3 has expanded the frontiers of biomolecular modeling, including in the prediction of protein-ligand interactions. This presentation will cover the core principles of AlphaFold 3, and then focus on the methods developed for accurate ligand prediction. Finally, we will present key results showcasing the model's performance, accuracy, and practical utility.

Repurposing Old Yellow Enzymes to Desaturases and Michaelases

Yuxuan Ye, *Westlake University, CN*

Flavin-dependent old yellow enzymes (OYEs) are privileged biocatalysts with extensive utilization in both academic and industrial settings.[1] Natural OYEs catalyze the asymmetric reduction of activated C=C bonds using the reduced form of the flavin cofactor. We have successfully harnessed the promiscuity of OYEs[2-4] to catalyze two underexplored new-to-nature reactions, desaturation and Michael addition, with complementary reactivity and selectivity to existing chemical methods.

Desaturation: Guided by the principle of microscopic reversibility, OYEs have been utilized to facilitate carbonyl dehydrogenation, the reverse process of their native reduction.[5-7] This biocatalytic desaturation platform has achieved desymmetrizing desaturation of cyclohexanones[8-10] and site-divergent late-stage functionalization of cyclic ketones. Furthermore, with the merger of photoenzymatic catalysis, selective β-C–H alkylation of carbonyl compounds has been realized.

Michael Addition: OYEs are well-known to catalyze reactions via hydride transfer mechanism (H^-). Recently, OYEs are employed in photoenzymatic reactions via hydrogen atom transfer pathway ($H\cdot$). We have repurposed OYEs to catalyze asymmetric Michael addition via proton transfer mechanism (H^+), an underdeveloped activation mode with great potentials.[11,12] A variety of chiral allenoates and cabonyl compounds with a quaternary stereogenic center have been prepared efficiently.

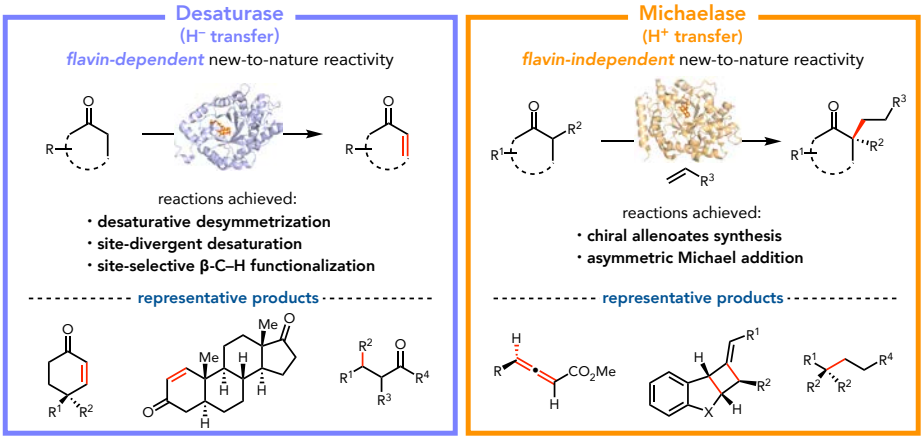


Figure *Flavin-dependent desaturases and flavin-independent Michaelases repurposed from old yellow enzymes.*

References

1. H. S. Toogood, N. S. Scrutton, *ACS Catal.* **2018**, *8*, 3532.
2. K. Durchschein, M. Hall, K. Faber, *Green. Chem.* **2013**, *15*, 1764.
3. M. Hall, *RSC Chem. Biol.*, **2021**, *2*, 958.
4. T. K. Roy, R. Screedharan, P. Ghosh, T. Gandhi, D. Maiti, *Chem. Eur. J.*, **2022**, *28*, e202103949.
5. Y. V. S. N. Murthy, Y. Meah, V. Massey, *J. Am. Chem. Soc.* **1999**, *121*, 5344.
6. M. Schittmayer *et al.*, *Adv. Synth. Catal.* **2011**, *353*, 268.
7. G. Jiang *et al.*, *Angew. Chem. Int. Ed.* **2023**, *62*, e202302125.
8. H. Wang *et al*. *Nat. Chem.* **2024**, DOI: 10.1038/s41557-024-01671-1.
9. Q.-Q. Zeng *et al*. *Nat. Synth.* **2024**, DOI: 10.1038/s44160-024-00596-4.
10. J. M. A. van Hengst *et al*. *ChemCatChem.* **2025**, *17*, e202401447.
11. M. S. Bobescu *et al*. *ACS Catal.* **2022**, *12*, 7396.
12. L. Wang *et al*. *Nat. Commun.* **2024**, *15*, 5753.

Posters

Decoupling Cytochrome P450 Monooxygenase from Ferredoxin and Ferredoxin Reductase: A Fast Approach to Substrate Scope Screening

Fatemeh Aziziyan[1], Francesca Paradisi[1]

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Cytochrome P450 (CYP) enzymes, as bioreactors, play a key role in biocatalysis.[1] In natural systems, they require association with their redox partners for electron transfer to attain high efficiency.[2] In recombinant systems, the entire operon, which includes CYPs and their redox partners, is cloned, and activity is assessed using a whole-cell system.[3] Whole-cell biotransformation offers a promising approach for producing high-value molecules. However, engineering microorganisms for this purpose faces challenges, including the metabolic burden from enzyme overexpression in host cells and the difficulty of balancing cell survival with production yield.[4]

In contrast, cell-free systems have emerged as promising alternatives. A typical feature of these systems is the separation of cell growth and product synthesis, which results in high yield conversion. Due to the nature of these systems, the reaction conditions can be easily manipulated and optimized. By lacking cellular barriers, these systems can overcome transfer limitations. [4] Therefore, designing efficient systems for biotransformation and maximizing product yields is extremely pursued. Previously, the application of CYPs in whole-cells was significantly hindered by their dependence on compatible electron-transport chains, inefficient electron utilization, and low enzyme efficiency.[5]

In this study, we proposed the design of two separate constructs to decouple the redox partner from CYP monooxygenase homologues, aiming to improve electron transfer and coupling efficiencies. This design improves control, flexibility, scalability, and wider potential applications. It also facilitates the pathway reassembly, potentially leading to more efficient biocatalytic processes. Additionally, an efficient redox pair could be coupled with other CYP monooxygenase homologues, simplifying the cloning process for new biocatalysts within this family. We also explored the wide substrate scopes to determine the potential of this design in developing an efficient biocatalytic system for producing high-value pharmaceutical compounds.

References

1 C. A. Martinez and S. G. Rupasinghe, *Curr. Top. Med. Chem.*, 2013, **13**, 1470–1490.
2 X. Zhang, Y. Peng, J. Zhao, Q. Li, X. Yu, C. G. Acevedo-Rocha and A. Li, *Bioresour. Bioprocess.*, 2020, **7**, 1–18.
3 S. Cornelissen, M. K. Julsing, J. Volmer, O. Riechert, A. Schmid and B. Bühler, *Biotechnol. Bioeng.*, 2013, **110**, 1282–1292.
4 C. Yang, Y. Liu, W. Q. Liu, C. Wu and J. Li, *Front. Bioeng. Biotechnol.*, DOI:10.3389/FBIOE.2021.730663.
5 P. Cannazza, M. Rabuffetti, S. Donzella, V. De Vitis, M. L. Contente, M. da C. F. de Oliveira, M. C. de Mattos, F. G. Barbosa, R. P. de Souza Oliveira, A. Pinto, F. Molinari and D. Romano, *AMB Express*, 2022, **12**, 48.

Poster abstracts are as submitted and were not edited or typeset.

The natural redox cofactor PQQ enables photocatalytic radical cyclizations

Srishti B. Bahukhandi[1]; Andreas S. Klein[1]; Ghulam Mustafa[1]; Maria Weyh[1]; Alexandra Walter[2]; Erling Thyrhaug[2,3]; Jürgen Hauer[2,3]; Golo Storch[2,3]; Cathleen Zeymer[1,3]

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[3]TUM Catalysis Research Center, Technical University of Munich (TUM), 85748 Garching, Germany

Photoenzymatic catalysis facilitates stereoselective new-to-nature chemistry under mild conditions. In addition to the rational design of artificial photoenzymes, naturally occurring redox enzymes have been repurposed for this approach. Most prominently, flavin-containing cofactors can promote photoredox catalysis in the chiral protein environment, with several examples of enantioselective C–C bond forming reactions reported in recent years.[1] Here, we add another class of natural enzymes, which utilize the pyrroloquinoline quinone (PQQ) cofactor, to the toolbox of photobiocatalysis. Although structurally distinct from flavin, PQQ exhibits mechanistic similarities, as it also absorbs visible light and is capable of single-electron transfer.[2] First, we established the trimethyl ester $PQQMe_3$ as a stand-alone photoredox catalyst in pure organic solvent. Upon excitation, $PQQMe_3$ enables the redox-neutral radical cyclization of an N-(bromoalkyl)-substituted indole. We then tested a panel of PQQ-dependent sugar and alcohol dehydrogenases for photoenzymatic catalysis in aqueous buffer, focusing on a redox-neutral radical reaction to form oxindoles. Under optimized reaction conditions, we obtained 69% yield and an 82:18 enantiomeric ratio. Our work thus demonstrates that PQQ enzymes are capable of stereoselective photoredox catalysis.[3] Future enzyme engineering efforts based on computational modelling and directed evolution will fully unlock their synthetic potential.

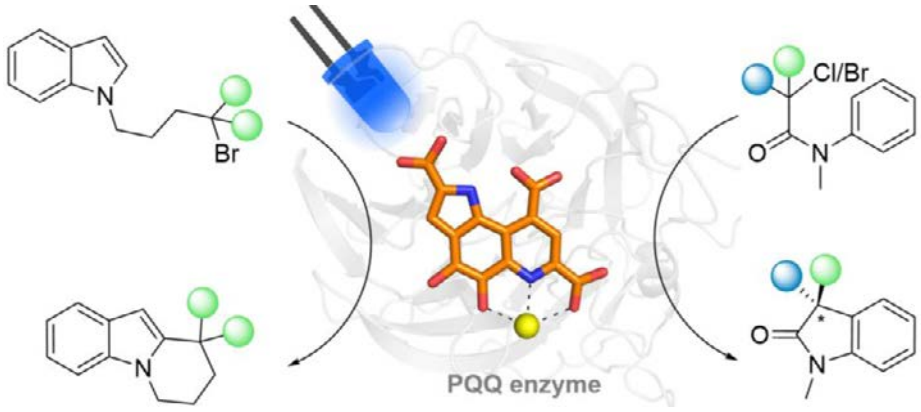

Figure 1: Photoenzymatic catalysis of redox-neutral radical cyclizations

Selected publications

[1] H. Fu, T. K. Hyster, *Acc. Chem. Res*. **2024**, 57 (9), 1446-1457.
[2] S. Fukuzumi, S. Itoh, T. Komori, T. Suenobu, A. Ishida, M. Fujitsuka, O. Ito, *J. Am. Chem. Soc.* **2000**, 122 (35), 8435-8443.
[3] S. B. Bahukhandi, A. S. Klein, G. Mustafa, M. Weyh, A. Walter, E. Thyrhaug, J. Hauer, G. Storch, C. Zeymer, *ChemRxiv* **2025** (DOI: 10.26434/chemrxiv-2025-cz860).

Identification and Engineering of Promiscuous Amidases for Multi-Purpose Applications

Thomas Bayer[1]; Hannes Meinert[1]; Ina Somvilla[1]; Louis Schmidt[2]; Florian Oehlschläger[1]; Hannah Meier[1]; Patrick Ihrle[1]; Marco Seifert[1]; Clemens Cziegler[1]; Ulrike Garscha[2]; Uwe T. Bornscheuer[1]

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[2] Department of Pharmaceutical & Medicinal Chemistry, Institute of Pharmacy, University of Greifswald, Friedrich-Ludwig-Jahn-Str. 17, 17489 Greifswald, Germany

The sequencing of (meta)genomes results in the deposition of novel protein sequences in public databases at a rate that outpaces their functional annotation through direct biochemical characterization or complementary *omics* studies. [1] In this work, we used the sequences of recently discovered metagenomic urethanases [2–4] to mine public databases. Bioinformatic analysis yielded novel enzymes of the amidase signature (AS) superfamily with superior activity on notoriously stable *N*-aryl amides and carbamates, motifs that are also present in environmental pollutants including synthetic polymer waste. [5]

The engineering of AS family members through site-directed mutagenesis was assisted by a unique biosensor-based screening platform, repurposed for the rapid assessment of amidase activity. [6, 7] The combination of beneficial mutations not only yielded enzyme variants with greatly improved specific activities against small molecules; the release of up to 10% of monomeric building blocks from synthetic polymers – including a thermoplastic polyester-polyurethane and the polyamide nylon-6 [3] – is the highest reported to date by the action of a single enzyme. [5]

Together, the customized AS family enzymes offer unprecedented versatility as demonstrated by the biocatalytic hydrolysis of low-molecular-weight compounds and the cleavage of Cbz-protecting groups, which are routinely used for the protection of amines in organic and medicinal chemistry [8], under mild reaction conditions in aqueous media. Finally, the valorization of chemical (plastic) waste advances the applications of promiscuous amidases and addresses current socioeconomic and environmental challenges. [1, 5]

Selected publications

[1] T. Bayer, U.T. Bornscheuer *et al.*, *Angew. Chem. Int. Ed.* **2025**, e202505976
[2] Y. Branson, U.T. Bornscheuer *et al.*, *Angew. Chem. Int. Ed.* **2023**, *20*, e202216220
[3] T. Bayer, U.T. Bornscheuer *et al.*, *Angew. Chem. Int. Ed.* **2024**, *63*, e202404492
[4] L. Rotilio, J.P. Morth *et al.*, *Angew. Chem. Int. Ed.* **2025**, *64*, e202216220
[5] T. Bayer, U.T. Bornscheuer *et al.*, *submitted* (**2025**)
[6] T. Bayer, R. Wei *et al.*, *iScience* **2022**, *25*, 104326
[7] T. Sakoleva, T. Bayer *et al.*, *ChemBioChem* **2025**, *26*, e202400712
[8] J. Omprakash Rathi, G. Subray Shankarling, *ChemistrySelect* **2020**, *5*, 6861

Poster Session

In vivo catalysis by artificial metalloenzymes for the production of aldehydes

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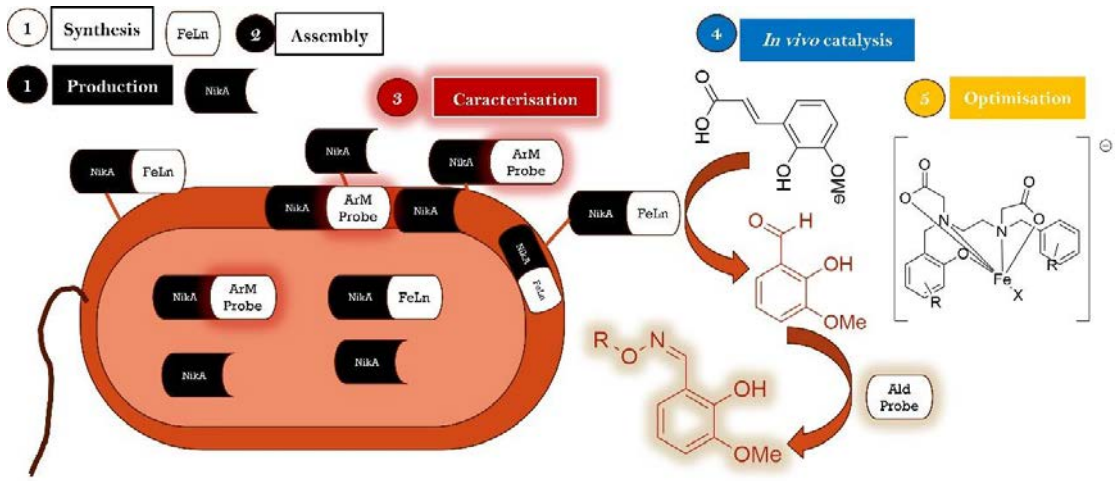
Improving the environmental impact of chemistry is increasingly becoming one of its core aspects. The search for greener alternatives to key steps in processes is becoming increasingly relevant and urgent. When it comes to catalysis, the design of bioinspired catalysts has focused attention on Artificial Metalloenzymes (ArM). The versatility and sustainability of these systems has been steadily improved, to the point where *in vivo* catalysis is now more achievable than ever. Our aim is to propose an alternative for the synthetic biology of aldehydes production using ArMs. We hereby report the design of a *de novo in vivo* catalytic system capable of producing aldehydes from oxidative cleavage of alkenes issued from biomass. This catalytic system uses an iron based inorganic complex inserted into the NikA protein cavity to form an active ArM for the O_2-mediated oxidative cleavage of alkenes.[1]



The cleverness of this system lies in the use of *E. coli* to produce the NikA protein at the outer membrane. This enables continuous production of the protein and catalysis under mild conditions. *In vivo* construction with NikA at the outer membrane has been successfully obtained and alternative constructs with periplasmic or cytoplasmic NikA are expected. Vanillin production using this *in vivo* catalytic system was achieved under aqueous conditions at room temperature, whereas it was absent in a ΔNikA strain. Currently, a high throughput screening method and a method for detecting ArM formation are being investigated by UV and fluorescence with original fluorescent probes. These results show great potential for a more sustainable way of producing aldehydes from biomass.

Acknowledgements: We kindly thank the Agence Nationale de la Recherche for funding this project as well as our collaborators from the Résonance Magnétique group of the Laboratoire Modélisation et Exploration des Matériaux for their interest in our work.

1. S. Lopez, L. Rondot, C. Leprêtre, C. Marchi-Delapierre, S. Ménage, and C. Cavazza, *J. Am. Chem. Soc.*, 2017, 139 (49), 17994-18002.

Design of Artificial Catalytic Cobalt Enzymes using SpyTag/SpyCatcher Technology

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Metalloproteins promote several of the most complex biomolecular processes in Nature. The design of new metalloproteins is therefore of interest in the field of the development of new efficient biocatalysts which can carry out reactions that are not relevant for biological systems but are important for applications in bio- and nanotechnology.[1] In the redesign of metalloproteins one of the major challenges is the introduction of metal binding sites in specific position of the construct.
Here we present a new cobalt protein designed using the SpyCatcher/SpyTag construct.[2] The Spy complex is an artificial protein system in which a peptide (SpyTag, ST) binds to a protein (SpyCatcher, SC) through an isopeptide bond, to give rise to a recombined Spy protein.[2] Using this technology, the metal binding site can be designed on the peptide and grafted on the protein component without redesigning the entire construct. Moreover, the peptides can be synthesized by solid-phase synthesis, making the SpyTag a playground where we can easily introduce different binding site for different metal ions, expanding the space of redesign of the Spy protein toward the use of non-natural amino acids.
We provided the SpyTag peptide with an ATCUN fragment, and introduced a Co(III) ion in the binding site. The metallopeptides were characterized by UV-visible spectrophotometry. The metallopeptides and the reconstituted metalloproteins were tested for the catalytic reduction of aqueous H^+ into gaseous H_2. At the current stage of development of these constructs, gas-chromatographic data demonstrate that these assemblies promote hydrogen evolution under photocatalytic conditions using ruthenium bipyridyl as the photosensitizer. The spectroscopic investigations and the study of the catalytic properties of our systems allowed us to establish that hydrogen production under photostimulated conditions actually occur. The aspects related to the design of the protein construct and of the catalytic site will be discussed.

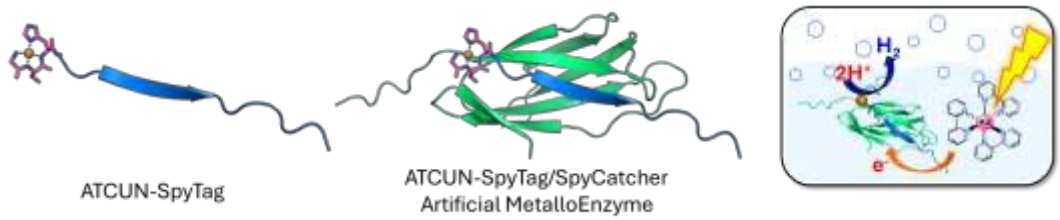

Figure Artificial metalloenzymes result from anchoring ATCUN site to a SpyTag (ST) (left) that can bind a cobalt ion, forming Co-ST. Co-ST recombined with SpyCatcher (SC) (middle) catalyze the reduction of aqueous H^+ into gaseous H_2 (right) The resulting hybrid catalyst can be optimized by modifing SpyTag sequence by de novo design, instead to modifing the protein.

Project "Artificial enzymes for the photocatalytic production of hydrogen in photosynthetic bacteria" National Recovery and Resilience Plan (NRRP), M2 C2 Inv. 3.5 funded by the European Union – NextGenerationEU. Project RSH2A_000009, C.D. 445 29/12/2022 Italian Ministry of Environment and Energy Security. "Bando di Ateneo per la Ricerca 2022" – A Way Towards Artificial Metalloenzymes - University of Parma

Selected publications

[1] F. Yu, V.M. Cangelosi, M.L. Zastrow, M. Tegoni, J.S. Plegaria, A.G. Tebo, C.S. Mocny, L. Ruckthong, H. Qayyum, V.L. Pecoraro, *Chem. Rev.* **2014** 114, 3495.
[2] S.C. Reddington, M. Howarth, *Curr. Opin. Chem. Biol.* **2015**, 29, 94.

Installation of an organocatalyst into a protein scaffold creates an artificial Stetterase (ArtiSt)

Alice MacAulay[1], Eva Klemencic[1], Richard C. Brewster[1], Süleyman Mert Ünal[2], Evangelia Notari[2], Christopher W. Wood[2], Amanda G. Jarvis[1]* and **Dominic J. Campopiano**[1]*
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The synthesis of 1,4 dicarbonyl compounds remains a challenging transformation in synthetic chemistry due to the innate polarity mismatch of carbonyl fragments when forming even-numbered dicarbonyls [1]. One synthetic route, reported by Stetter in 1973, uses a nucleophilic catalyst to catalyse C–C bond formation between an aldehyde and an α,β-unsaturated carbonyl [2]. Such reactions can be catalysed by N-heterocyclic carbenes (NHCs), a class of compounds inspired by the vitamin B derived cofactor thiamine pyrophosphate (TPP) [3]. The seminal work of Breslow described the innate catalytic properties of TPP [4] and has inspired subsequent generations of researchers in organocatalysis. The award of the Novel prize in chemistry in 2021 for the development of asymmetric organocatalysis should further inspire the artifical enzymes field to harness natural and man-made organocatalysts for synthesis.

There are a surprisingly few natural Stetterases (e.g. MenD and PigD [5]), so we set out to generate an artificial enzyme with this target function. Using an inactive protein scaffold (human steroid carrier protein, hSCP) covalently functionalised with a synthetic, TPP-inspired N-heterocyclic carbene (NHC), we created an artificial Stetterase (ArtiSt) which catalyses a stereoselective, intramolecular Stetter reaction. We demonstrate that ArtiSt functions under ambient conditions with low catalyst loading. Furthermore, activity can be increased >20 fold by using the SCP template from a hyperthermophilic bacterium *Thermus thermophillus* (TTSCP). To our knowledge, this represents the first time that a NHC-modified protein has displayed Stetterase activity. This talk will highlight our initital concepts/design, our choice of template/reagents, and our successful characterisation of this exciting new biocatalyst.

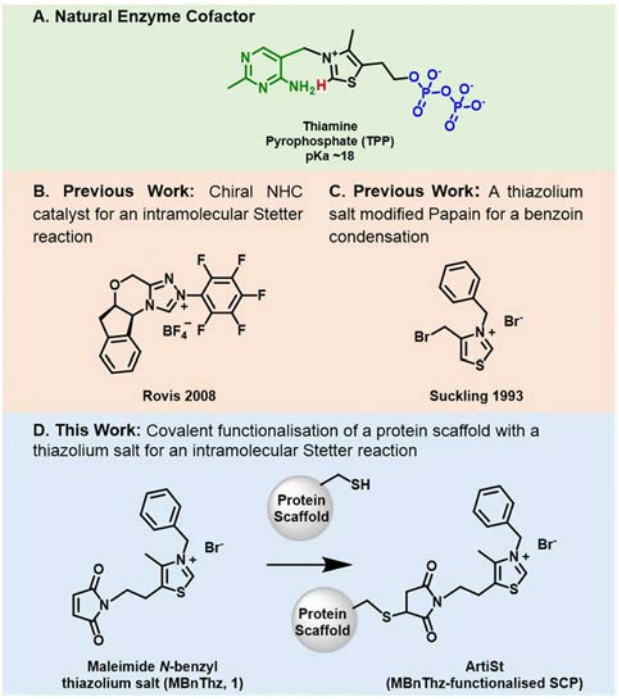

Figure. The chemical structure of the cofactor thiamine pyrophosphate (TPP) showing the C2-proton (red), pyrophosphate (PPi, blue) and pyrimidine (PYR, green). (B) The structure of a chiral triazolium salt used by Rovis to catalyse an intramolecular Stetter reaction. (C) The structure of N-benzyl-4-bromomethylthiazolium bromide prepared by Suckling to modify papain for use in an intramolecular benzoin condensation. (D) A scheme of this work showing the structure of the thiazolium salt (MBnThz) and its functionalisation of a cysteine-containing protein scaffold to create the artificial Stetterase (ArtiSt).

Selected publications

[1] M. Lemmerer, M. Schupp, D. Kaiser, N. Maulide, *Nat. Synth.*, **2022**, *1*, 923.
[2] H. Stetter, M. Schreckenberg, *Angew. Chem.*, **1973**, *12*, 81.
[3] M. N. Hopkinson, C. Richter, M. Schedler, F. Glorius, *Nature*, **2014**, *510*, 485
[4] R. Breslow, *J. Am. Chem. Soc.*, **1958**, *80*, 3719.
[5] M. Müller, G. A. Sprenger, M. Pohl, *Curr. Opin. Chem. Biol.*, **2013**, *17*, 261.

Artificial Metalloenzymes For Radical Biocatalysis

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Radical biocatalysis is a rapidly growing field aiming to achieve better stereocontrol of reactions featuring reactive radical intermediates.[1-3] However, there are limited examples of designer enzymes catalyzing these challenging transformations.[4-5] We have developed a strategy to assemble an artificial enzyme for metalloredox radical reactions based on the Lactococcal multidrug resistance regulator (LmrR) featuring a genetically incorporated non-canonical amino acid, bipyridyl-L-alanine (BpyA).[6] In this work, these unnatural residues were positioned close to each other in a homodimeric LmrR scaffold, providing a coordination sphere where two ligands can bind one first-row transition metal ion. This system was tested in catalysis of atom-transfer radical cyclization (ATRC) and a promising activity was observed with cobalt, albeit with a modest yield (23%) and lack of enantioselectivity. These initial results have spurred further investigations, including molecular dynamics simulations, directed evolution, and substrate engineering, to enhance both catalytic efficiency and selectivity of this new-to-nature biocatalytic system.

Reactions implemented:
Atom-transfer radical cyclization

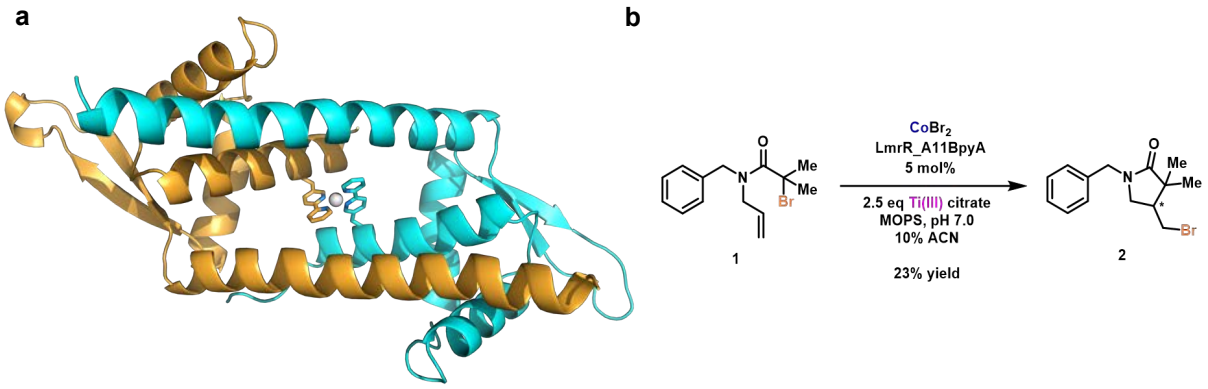

Figure 1. a) Co-based artificial metalloenzyme featuring two BpyA metal-binding residues (LmrR_A11BpyA_Co); **b)** ATRC catalyzed by LmrR_A11BpyA_Co system

Selected publications

[1] J. Li, A. Kumar, J.C. Lewis, *Angew. Chem. Int. Ed.* **2023**, e202312893
[2] K. F. Biegasiewicz, S. J. Cooper, X. Gao, D. G. Oblinsky, J. H. Kim, S. E. Garfinkle, L. A. Joyce, B. A. Sandoval, G.D. Scholes, T. K. Hyster , *Science* **2019**, 364, 1166-1169.
[3] Q. Zhou, M. Chin, Y. Fu, P. Liu, Y. Yang, *Science* **2021**, 374, 1612-1616.
[4] K. Renggli, M. G. Nussbaumer, R. Urbani, T. Pfohl, N. Bruns, *Angew. Chem. Int. Ed.* **2013**, 53, 1443-1447.
[5] Chen, D., Zhang, X., Vorobieva, A. A., Tachibana, R., Stein, A., Jakob, R. P., Zou, Z., Graf, D. A., Li, A., Maier, T., Correia, B. E., & Ward, T. R. *Nat. Chem.* **2024**,16, 1656–1664.
[6] I. Drienovska, A. Rioz-Martinez, A. Draksharapu, G. Roelfes, *Chem. Sci.* **2015**, 6, 770-776.

Predicting Temperature induced Protein Unfolding via Structural Attribute trained Algorithms

Bastian Daniel[1], Kamela Myrtollari[2, 3], Elia Calderini[4], Tobias Schöngaßner[2], Daniel Kracher[2], Stela Galusic[2], Anita Slavica[2], Andreas Taden[2], Daniel Mokos[1], Anna Schrüfer[1], Gregor Wirnsberger[1], Robert Kourist[2], Gruber Karl[1]

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[2] *Graz University of Technology, Institute of Molecular Biotechnology*
[3] *Henkel AG & Co. KGaA, Adhesive Research/Bioconjugates*
[4] *Bayer AG*

Enzyme catalysis continues to significantly contribute to the transition of the chemical and pharmaceutical industries towards sustainable production and a circular economy. Low thermal stability or lifetimes do often hamper the use of enzymes in biocatalytic processes. To better understand and ultimatively to predict thermal unfolding we have created an pipeline to extract structural attributes from protein structures to train algorithms with them in combination with the respective unfolding temperatures to create models for the prediction of thermal unfolding of unknown related proteins. Calculated was for example the amount of salt bridges, hydrogen bonds and hydrophobic clusters. The distribution of respective structural attributes in a protein are depicted in Figure 1.

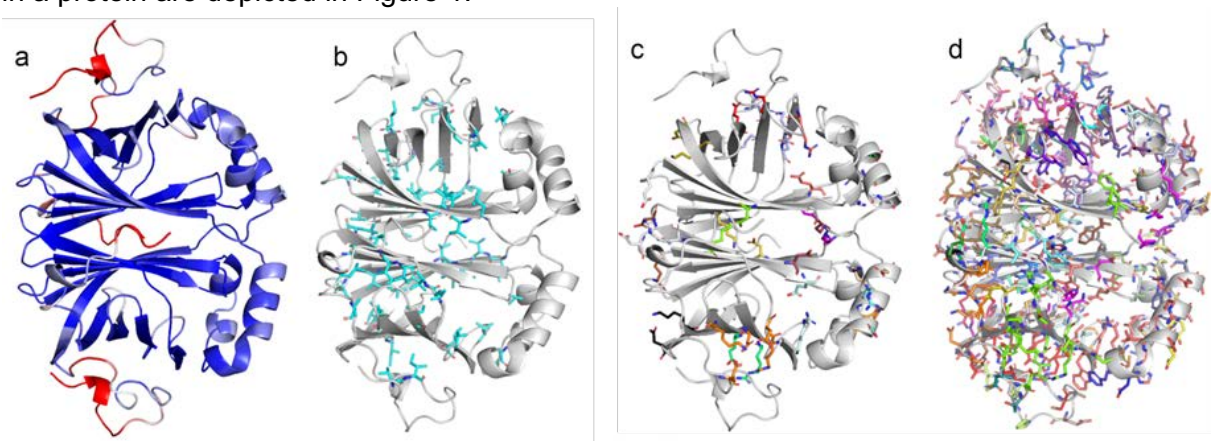

Figure 1. *Analysis of the structural properties of phenolic acid decarboxylases with N55 as an example. N55 depicted as cartoon and colored by RMSF (**a**), Residues in: Hydrophobic clusters (**b**), salt bridges (**c**), hydrogen bonds (**d**).*

To achieve the best prediction of the unfolding temperature, all possible 4-fold combinations of all single attributes were tested by running a multiple linear regression, a random forest regression, and a k-nearest neighbours' regression (KNN) for each using leave-one-out to determine the mean absolute error (MAE). Subsequently, the performance of the algorithms was tested with a data set derived from an additional set of proteins. The method was found to correctly recall the proteins with the highest melting temperatures and to outperform currently available global melting temperature prediction tools.[1]

Selected publications

1. Myrtollari, K. *et al.* Stability Increase of Phenolic Acid Decarboxylase by a Combination of Protein and Solvent Engineering Unlocks Applications at Elevated Temperatures. *ACS Sustain Chem Eng* **12**, 3575–3584 (2024).

Metalloenzyme engineering with non-canonical amino acid incorporation

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[†]These authors contributed equally.

Metalloenzymes are rich targets for protein engineering. While traditional mutagenesis with the 20 canonical amino acids has enabled significant advances, its chemical scope remains limited. The incorporation of non-canonical amino acids (ncAAs) into proteins has the potential to expand the chemistry accessible through mutagenesis and e enable precise control through mutations that are more finely tuned and rationally designed than those achieved with canonical amino acid mutagenesis. To demonstrate this potential, we examined ncAA-based mutagenesis of a classical metalloenzyme: a laccase. Laccases are multicopper oxidases that catalyze the one-electron oxidation of various substrates. Fungal laccases exhibit high redox potentials, high activity, and broad substrate scope. In contrast, bacterial laccases are much easier to express, are thermally stable, and tolerate a wider range of reaction conditions. However, bacterial laccases have low activity and low redox potentials. Thus, bacterial laccases present many advantages, but their lower activity precludes them from more widespread use.
Based on fundamental chemical principles, we posited that ncAA-based protein engineering could be used to improve the activity of bacterial laccases. Based on these principles, we designed and synthesized several ncAAs that we posited could improve catalysis, engineered the corresponding genetic code expansion tools (aminoacyl-tRNA synthetase/tRNA pairs) for incorporation of these ncAAs, and validated these tools in a classical model protein (GFP). Using these tools, we expressed laccases variants with site-specific incorporation of the target ncAAs and performed a comparative study to assess the changes endowed by ncAA-based mutagenesis. Our results demonstrate that rationally designed ncAA variants can significantly improve catalytic performance. Furthermore, the enhancements derived from ncAA-based mutagenesis provide a foundation for additional optimization using traditional directed evolution. Overall, this work highlights the power of ncAA-based mutagenesis to fine-tune catalytic sites beyond the capabilities of conventional approaches.

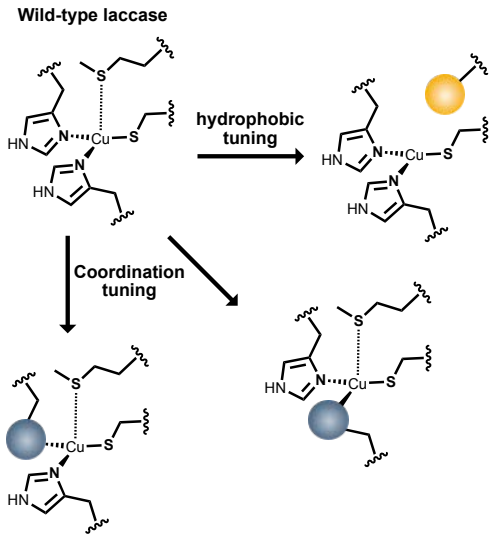

Figure Schematic of ncAA-based engineering of a bacterial laccase.

Selected publication:

S Fischer, A Natter Perdiguero, K Lau, A Deliz Liang, Hydrophobic tuning with non-canonical amino acids in a copper metalloenzyme, BioRxiv, **2025**, https://doi.org/10.1101/2025.02.09.636911

Reprogramming Enzymes: Engineering TOYE with Secondary Amine-Functionalized ncAAs

Stefanie Hanreich; Emilia Iglesias-Moncayo; Alejandro Gran-Scheuch; Ivana Drienovská

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Demand for sustainable, high-performance industrial processes is driving the exploration of novel biocatalytic strategies that extend well beyond nature's inherent limits. Although natural enzymes excel in selectivity and environmental compatibility, their catalytic repertoire is confined by the limited set of canonical amino acids and cofactors. Advances in genetic code expansion now enable the precise incorporation of noncanonical amino acids (ncAAs) with unique functional groups, offering an innovative opportunity to engineer enzymes with entirely new catalytic activities. Several pioneering examples have already underscored the tremendous potential of this approach.[1]

In our work, we focus on incorporating various ncAAs bearing secondary amine moieties into evolvable protein scaffolds.[2,3] Complementary structural insights obtained via X-ray crystallography and computational modeling have guided our selection of optimal protein frameworks. In the example presented here, we engineered a designer enzyme based on the thermophilic Old Yellow Enzyme (TOYE). Variants of TOYE, modified to contain secondary amines at key positions, have been evaluated in Michael addition and cyclopropanation reactions featuring a range of nucleophiles (Figure 1). The most promising candidates are currently undergoing directed evolution to further enhance their catalytic performance and selectivity.

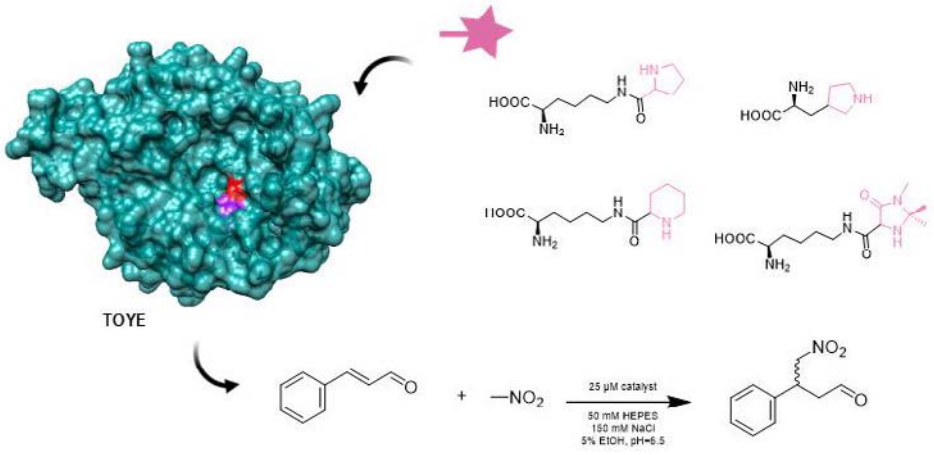

Figure 1. A general representation of TOYE-based artificial enzymes incorporating noncanonical amino acids with secondary-amine moieties. The figure highlights the enzyme's catalytic role in facilitating the Michael addition reaction.

Selected publications

[1] B. Brouwer, F. Della-Felice, J. H. Illies, E. Iglesias-Moncayo, G. Roelfes, I. Drienovská, *Chem. Rev.,* **2024**, *124*, 10877.
[2] A. Gran-Scheuch, E. Bonandi, I. Drienovská, *ChemCatChem,* **2024**, *16*, e202301004.
[3] A. Gran-Scheuch, S. Hanreich, I. Keizer, J. W. Harteveld, E. Ruijter, I. Drienovská, *Faraday Discuss*., **2024**, *252*, 279.

Photobiocatalysis: Expanding Reactivity Through Non-Biological Cofactors

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Photobiocatalysis enables new transformations by combining light with enzyme catalysis, but is largely limited to native flavin cofactors. Here, we expand the cofactor scope of flavin-dependent oxidoreductases (FDORs) to include synthetic photocatalysts such as methylene blue, safranin O, and eosin Y. Using docking, fluorescence binding, and rational design, we identify FDOR variants with high affinity and broad cofactor tolerance. This work provides a foundation for engineering artificial photoenzymes with enhanced functionality and non-natural reactivity.

Computational Design of a Hyperstable Protein Platform for Multifunctional Biocatalytic Applications

Wael Elaily[1,2], David Stoll[2], Morakot Chakatok[2], Matteo Aleotti[3], Birgit Grill[2], Markus Braun[2], Horst Lechner[2], Mélanie Hall[3] and Gustav Oberdorfer[2]

[1]Institute of Molecular Biosciences, University of Graz, Austria.
[2]Institute of Biochemistry, Graz University of Technology, Austria.
[3]Institute of Chemistry, University of Graz, Austria.

Catalysis is at the core of overcoming current environmental challenges to help transform into a more sustainable society. Several catalytic strategies have emerged over the last couple of decades with biocatalysis gaining lots of attention in this respect. However, this branch of catalysis would be even more thriving if generation of stable and customizable protein scaffolds would be readily available. Such highly stable and asymmetric environments could act as reaction chamber in catalyzing a wide range of reactions. In this study, we are detailing the design and experimental characterization of computationally designed de novo proteins with a non-natural fold, which act as biocatalysts. The initial design 6H5L and several of its variants catalyze the aldol condensation and retro-aldol reaction. All designs form a helical barrel structure comprised of six antiparallel straight helices connected by five loops, resulting in an open channel with two accessible cavities. All designs exhibit high thermal stability and an excellent overall fit between measured and calculated scattering profiles from small-angle x-ray scattering experiments. An experimentally determined crystal structure of a surface redesigned variant shows close to perfect agreement to the design. To highlight the versatility of this fold as a biological reaction chamber, we computationally designed variants in which the active site is placed throughout the central cavity of the parent design, demonstrating the high mutational tolerance of the fold. This approach resulted in several enhanced variants, with 6H5L_199K achieving a 10-fold increase in retro-aldolase activity and showing catalytic promiscuity by effectively catalyzing other reactions, including the Michael addition. Remarkably, 6H5L also exhibited alanine transaminase activity in its holo-protein form bound to PLP. Finally, we showcase that the initial design as well as variants exhibit significant aldolase/retro-aldolase activity in whole cell biotransformations, making this design the first de novo biocatalyst to be tested in this form. With a tolerance of up to 20% of organic solvent, these designs hold promise for further utilization and optimization in the field of white biotechnology.

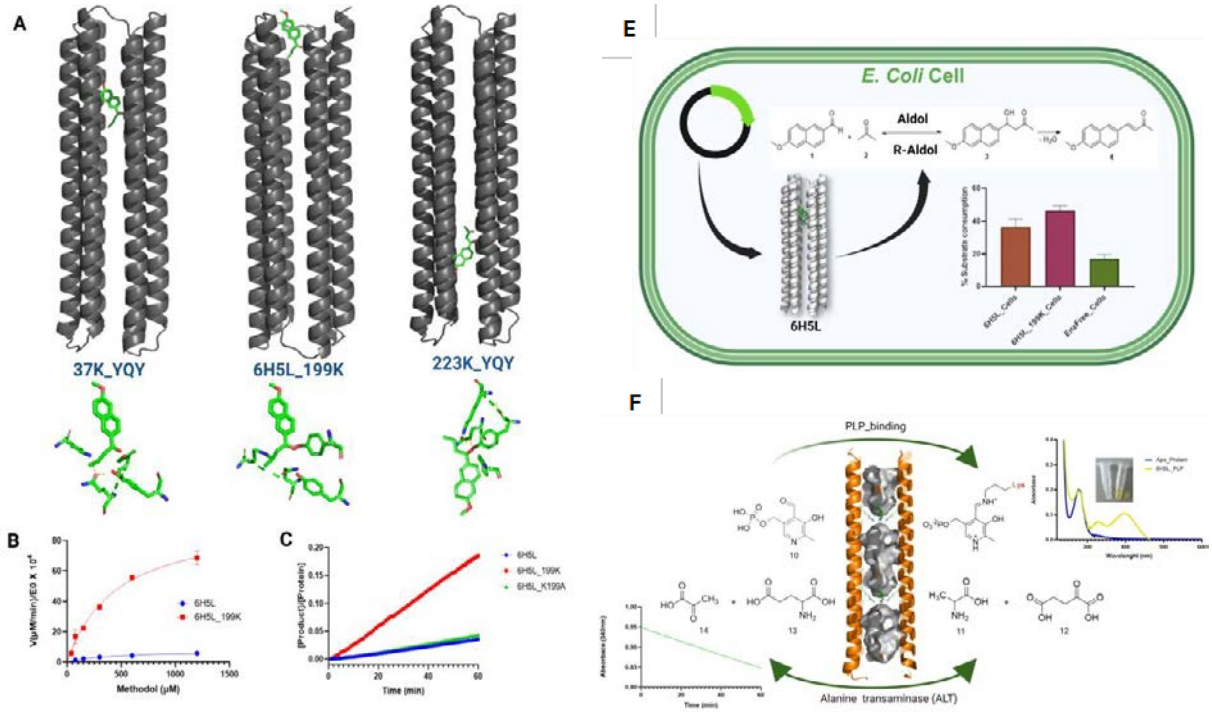

Figure 1. A) 6H5L active site redesign variants over the hydrophobic channel were generated using Rosetta and docked with Methodol 3 substrate. **B)** Michaelis–Menten plots of 6H5L_199K (red) show a ten-fold increase in activity compared to the original 6H5L (blue). **C)** Retro-aldolase progress curves for 6H5L_199K (red) compared to the original 6H5L (blue) and the knockout variant 6H5L_K199A (green). **E)** Overexpressed cells containing 6H5L and 6H5L_199K proteins catalyzed aldol reaction achieving significant activity, with substrate consumption rates of 36% and 46%, respectively. **F)** 6H5L designs exhibited clear binding with PLP coenzyme and demonstrated significant alanine transaminase activity.

Selected publication:

Wael Elaily, David Stoll, Morakot Chakatok, Matteo Aleotti, Birgit Grill, Horst Lechner, Mélanie Hall, Gustav Oberdorfer. **Computational design of a thermostable de novo biocatalyst for whole cell biotransformations.** bioRxiv 2024.10.07.617055 (2024) doi:10.1101/2024.10.07.617055.

Protein Nanomaterials for Solar Energy Conversion

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[2] Swiss Nanoscience Institute, University of Basel, Basel, CH
[3] Max Planck Institute for Terrestrial Microbiology, Marburg, DE

Computational protein design has advanced significantly, enabling the design of complex nanoscale assemblies, such as cages and sheets, with high accuracy.
However, most efforts have focused on structural novelty rather than functional, biocatalytic assemblies. Here, we harness emergent nanoscale properties by building photocatalytic protein nanomaterials for biohybrid solar cells and light-driven redox catalysis.
To create 2D nanosheets, we will engineer protein–protein interfaces on photoenzyme's[1] surfaces to drive self-assembly into well-defined, densely packed layers. These nanosheets will be designed to coat electrodes in biohybrid solar cells, where their dense packing and controlled orientation will enhance light harvesting and directional electron transfer. For 3D nanospheres, we attach photoenzymes to highly symmetrical protein cages via designed linkages and interfaces to minimize flexibility. These nanospheres will serve as confined photoreactors when redox enzymes are co-encapsulated inside the capsid. Additionally, their defined geometry enables structural characterization of the attached enzymes via cryogenic electron microscopy.
Our work demonstrates how nanoscale protein design can harness emergent structural properties to improve enzyme function. Here, we use this approach to engineer light-driven protein materials with enhanced biocatalytic activity.

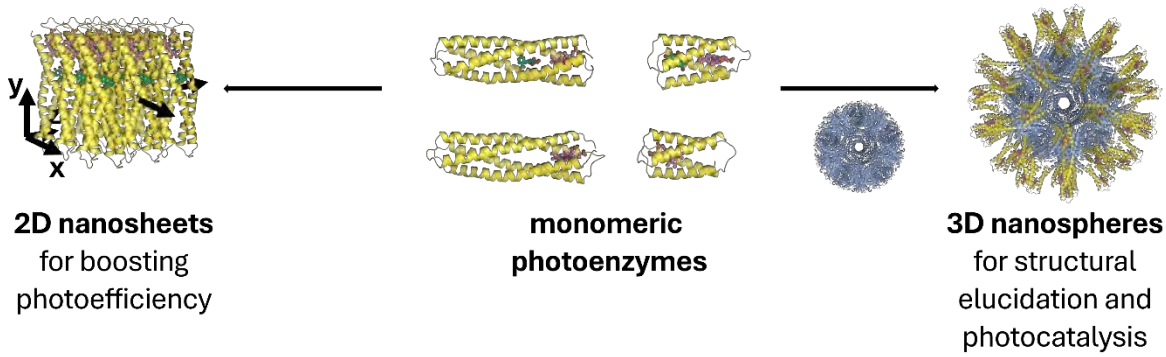

Figure Starting from previously designed photoenzymes (middle), 2D nanosheets (left) will be designed to enhance light capture and suppress unwanted side reactions. The design of 3D nanospheres (right) will enable structure determination using cryo-EM and the construction of modular photo-biocatalysts.

Selected publications

[1] H. A. Bunzel, J. A. Smith, T. A. A. Oliver, M. R. Jones, A. J. Mulholland, J. L. R. Anderson *bioRxiv*, 2022.2012. 2020.521207 (2022).

Effects of Superflavination on NOX Oligomerization and Activity

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This study investigates flavin-associated enzymatic tuning of four distinct NAD(P)H oxidase (NOX) enzymes, examining both activation and inhibition effects. Flavins are essential for NOX-catalyzed electron transfer from NADPH to oxygen, where their redox properties influence enzyme function through complex structural and mechanistic interactions.[1–3] Mechanistic studies have demonstrated that flavin redox states and structural dynamics regulate enzymatic activity and substrate specificity,[2–5] while Sieber et al. have highlighted their role in tuning oxygen-dependent enzymes for industrial applications.[1] Given that industrial biocatalysis often involves fluctuating cofactor availability, understanding how flavins influence enzyme stability, oligomerization, and activity is crucial. Despite their importance, the precise influence of flavin binding on NOX enzyme stability and function remains unclear.
To address this, we conducted enzyme kinetics assays under varying flavin concentrations to assess how flavins modulate catalytic activity. Additionally, we performed protein stability and structural analyses using nano-differential scanning fluorimetry (NanoDSF), circular dichroism (CD) spectroscopy, and mass photometry (Refeyn) to investigate flavin-induced conformational changes and their effect on NOX oligomeric states. Our findings indicate that FAD binding can influence enzyme oligomerization, as well as enzymatic efficiency.
Understanding the structural and functional consequences of flavin binding provides valuable insights for enzyme engineering. Our results show that FAD affects both the oligomeric state and activity of NOX enzymes, suggesting that controlling flavin-dependent assembly could be relevant for optimizing electron transfer efficiency in biocatalysts. Furthermore, stabilizing specific oligomeric states through protein engineering or cofactor modifications may enhance enzyme robustness in industrial and biotechnological applications.

Selected publications

[1] Al-Shameri, A., Schmermund, L., Sieber, V., ChemCatChem 2023, 15, e202301109.
[2] Romero, E., Castellanos, J. R. G., Gadda, G., Fraaije, M. W., Mattevi, A., Chem. Rev. 2018, 118, 1742–1769.
[3] Sheng, D., Ballou, D. P., Massey, V., Biochemistry 2001, 40, 11156–11167.
[4] Entsch, B., Ballou, D. P., Massey, V., J. Biol. Chem. 1976, 251, 2550–2563.
[5] Ziegler, D. M., Trends Pharmacol. Sci. 1990, 11, 321–324.

NHC Metal Peptide Conjugates for Abiotic Catalysis

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N-heterocyclic carbene (NHC) ligands have fundamentally transformed homogeneous catalysis, with impact in particular on catalysis with first row transition metals and precious platinum group metals. Considering these advances, it is remarkable that only few attempts have been demonstrated to incorporate NHCs into artificial metalloenzymes.[1–4] We therefore sought to develop non-canonical amino-acids (ncAAs) for site-specific incorporation into a peptide chain. For example, we have demonstrated the introduction of the NHC-like ncAA Hum into a small peptide to produce a minimalistic iridium peptide conjugate for hydrogenation reactions as a miniaturized prototype of an artificial hydrogenase.[5] Here, we will present our latest advances in developing NHC precursor ncAAs and their scope in binding a diverse set of transition metals for new-to-nature catalysis

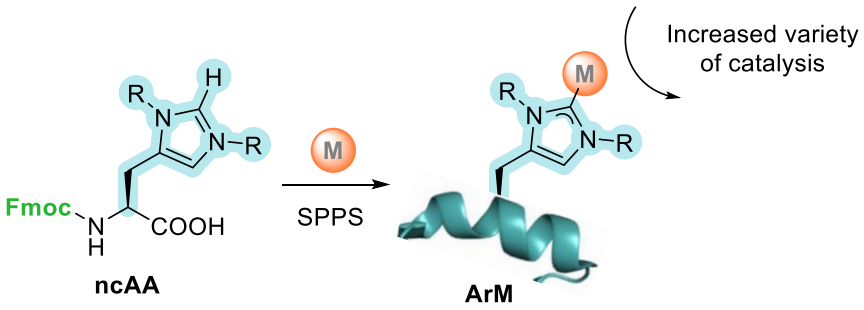

Figure Non-canonical amino-acids with a NHC-like side-chain which can be site-specifically included into a peptide sequence through Solid Phase Peptide Synthesis (SPPS).

Selected publications

(1) Mayer, C.; Gillingham, D. G.; Ward, T. R.; Hilvert, D. An Artificial Metalloenzyme for Olefin Metathesis. *Chem. Commun.* **2011**, *47* (44), 12068. https://doi.org/10.1039/c1cc15005g.
(2) Planchestainer, M.; McMaster, J.; Schulz, C.; Paradisi, F.; Albrecht, M. Carbene-Induced Rescue of Catalytic Activity in Deactivated Nitrite Reductase Mutant. *Chemistry A European J* **2020**, *26* (66), 15206–15211. https://doi.org/10.1002/chem.202002444.
(3) Basauri-Molina, M.; Riemersma, C. F.; Würdemann, M. A.; Kleijn, H.; Klein Gebbink, R. J. M. Lipase Active Site Covalent Anchoring of Rh(NHC) Catalysts: Towards Chemoselective Artificial Metalloenzymes. *Chem. Commun.* **2015**, *51* (31), 6792–6795. https://doi.org/10.1039/C4CC09700A.
(4) Köhler, V.; Wilson, Y. M.; Dürrenberger, M.; Ghislieri, D.; Churakova, E.; Quinto, T.; Knörr, L.; Häussinger, D.; Hollmann, F.; Turner, N. J.; Ward, T. R. Synthetic Cascades Are Enabled by Combining Biocatalysts with Artificial Metalloenzymes. *Nature Chem* **2013**, *5* (2), 93–99. https://doi.org/10.1038/nchem.1498.
(5) Lenzen, K.; Planchestainer, M.; Feller, I.; Padrosa, D. R.; Paradisi, F.; Albrecht, M. Minimalistic Peptidic Scaffolds Harbouring an Artificial Carbene-Containing Amino Acid Modulate Reductase Activity. *Chem. Commun.* **2021**, *57* (72), 9068–9071. https://doi.org/10.1039/D1CC03158A.

FOCUSING METAGENOMIC SCREENING ON STABLE AND FUNCTIONALLY DIVERSE ENZYME REPRESENTATIVES

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Metagenomic databases provide a huge resource for finding biocatalysts. However, functional redundancy among homologs and low stability of naturally occurring enzymes limit the usefulness of metagenomic mining. We present a new computational pipeline that integrates AlphaFold structure prediction, evolutionary analysis, and Rosetta atomistic calculations to generate stable variants of enzymes that span the natural diversity of an enzyme family. Our approach focuses on the active site, clustering natural sequences according to sequence diversity in the active-site pocket. We then apply PROSS stability design[1] to each enzyme to stabilise it and improve its heterologous expression levels. This workflow focuses the experimental screening effort on a small fraction of the enzymes in any family most likely to exhibit functional differences and increases the likelihood that the enzymes are expressible and stable. We anticipate that this will accelerate the discovery of stable, high-efficiency enzymes in basic and applied biocatalysis.

References

1) Goldenzweig, A. et al. Automated Structure- and Sequence-Based Design of Proteins for High Bacterial Expression and Stability. Mol. Cell 63, 337–346 (2016).

Small Molecule-Directed Metal-Catalyzed Prodrug Therapy (MCPT)

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Most traditional cytotoxic anticancer drugs used in chemotherapy indiscriminately target rapidly proliferating cells. Their mode of action mainly relies on either impairment of mitosis or induction of DNA damage and, therefore, affects both rapidly proliferating cancer cells and healthy cells. By utilizing a prodrug therapy, where a less cytotoxic form of the anticancer drug is administered, the undesirable and unspecific death of healthy cells can be minimized. Yet the accumulation and site-selective release of the cytotoxic drug in the vicinity of the diseased tissue is key to a successful outcome of the cancer treatment. The prerequisite for the accumulation is the over-expression of a membrane-bound receptor that can be actively targeted by its natural ligand, antagonist, or antibody (ADC, SMDC, ADEPT).[1,2,3]
Our project is based on the development of an innovative therapy consisting of an organometallic catalyst conjugated to a targeting unit with a high affinity for a receptor over-expressed on the surface of cancer cells. Following selective accumulation at the surface of target cancer cells, the catalyst will site-specifically uncage a subsequently-administered non-toxic prodrug to release a potent cytotoxic drug. Notably, the small molecule-directed metal catalyst prodrug therapy (MCPT) is expected to be more selective than current chemotherapies. Further, compared to antibody-drug conjugates (ADC), the small molecule components are more straightforward and inexpensive to produce and tailored for a given target. They are also anticipated to exhibit greater tissue diffusion rates due to their low molecular weight.

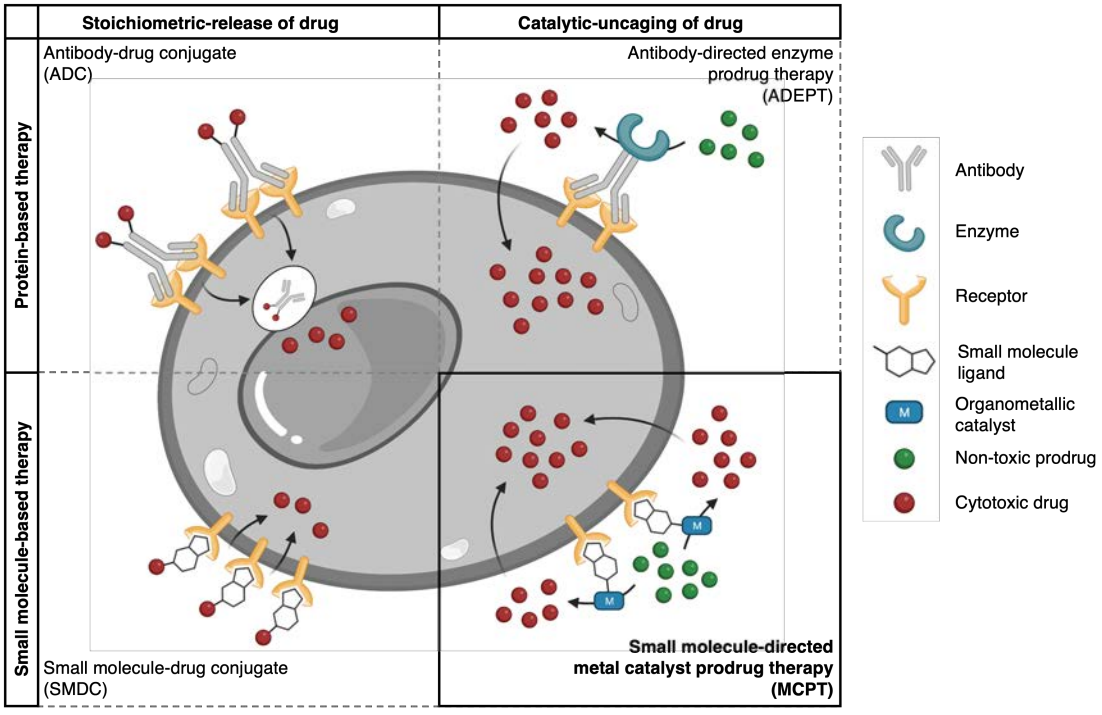

Figure Active targeting strategies to selectively accumulate chemotherapeutic drugs on cancer cells.

Selected publications

[1] J. Z. Drago, S. Modi, S. Chandarlapaty, *Nat. Rev. Clin. Oncol*. **2021**, *18*, 327–344.
[2] G. Casi, D. Neri, *J. Med. Chem*. **2015**, *58*, 8751–8761.
[3] S. K. Sharma, K. B. Bagshawe, *Expert Opin. Biol. Ther*. **2017**, *17*, 1–13.

Discovery and Development of Cytochrome P450BM3-Activating Agents Derived from Dicarboxylic Acids

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Cytochrome P450BM3 (P450BM3) is a heme enzyme that catalyzes the sub-terminal hydroxylation of long-chain fatty acids (Figure a). Due to its high turnover frequency, P450BM3 has attracted considerable interest as a promising biocatalyst. However, its substrate specificity limits its applicability to non-native substrates. Mutagenesis is the most established method for altering substrate specificity, but it typically requires screening large libraries of variants for each target substrate, which is time-consuming. To address this limitation, we previously developed a novel approach termed the “substrate misrecognition system” (Figure b). This strategy employs decoy molecules that can co-occupy the substrate pocket of P450BM3 along with non-native substrates. As a result, the enzyme can oxidize otherwise unreactive compounds such as benzene. A key advantage of this system is that it uses wild-type P450BM3 without requiring any genetic modification; simply adding a decoy molecule is sufficient to broaden its substrate scope. However, nearly all effective decoy molecules reported thus far have been derived from amino acids, especially phenylalanine, as exemplified by C9-Phe shown in Figure b. This reliance on amino acid-based scaffolds imposes limitations on the structural and functional diversity of decoy molecules.

In this study, we developed novel decoy molecules completely free of amino acid motifs. Using dicarboxylic acid derivatives as building blocks, we designed a core skeleton that can be synthesized via a one-pot reaction. Through this approach, we successfully identified several potent decoy molecules that promote the oxidation of small organic compounds. Moreover, by comparing structurally related decoy molecules and conducting X-ray crystallographic analysis of co-crystals with P450BM3 using the newly developed decoys, we identified key structural features essential for the activation of P450BM3 by dicarboxylic acid-based decoy molecules. Notably, the newly developed decoy molecules are cell-permeable and enhance intracellular P450BM3-catalyzed whole-cell biotransformations of benzene to phenol. Overall, this work demonstrates the unique advantages and essential characteristics of dicarboxylic acid-based decoy molecules, offering new avenues for expanding the utility of P450BM3 in biocatalysis.

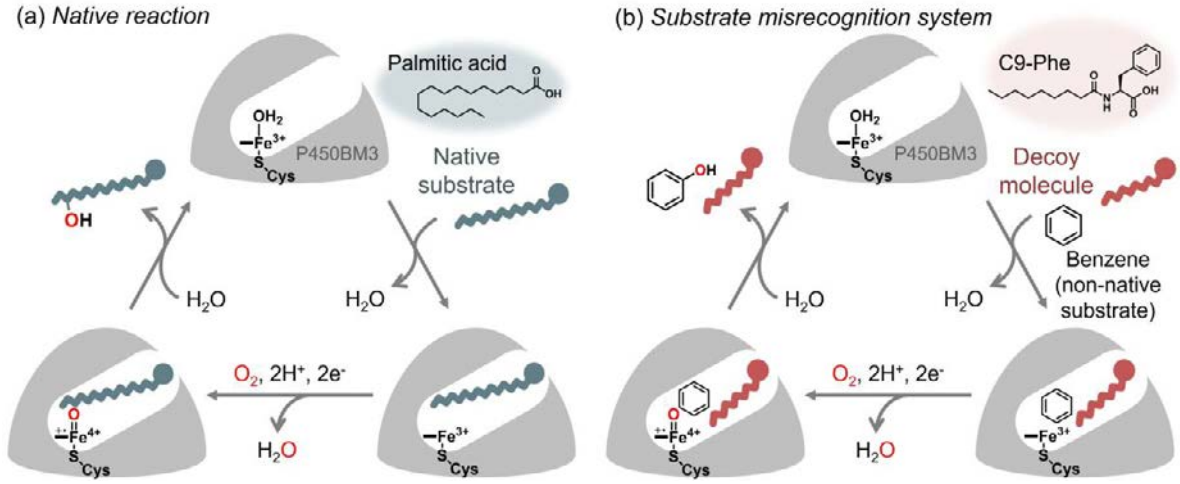

Figure (a) Hydroxylation reaction of long-chain fatty acids by P450BM3 (b) Substrate misrecognition system enabling hydroxylation of benzene using a decoy molecule.

Selected publications

1. C. J. C. Whitehouse, S. G. Bell and L. Wong, *Chem. Soc. Rev.* **2012**, *41*, 1218-1260.
2. O. Shoji, S.Yanagisawa, J. Kyle Stanfield, K. Suzuki, Z. Cong, H. Sugimoto, Y. Shiro and Y. Watanabe, *Angew. Chem. Int. Ed.* **2017**, *56*, 10324-10329.
3. M. Karasawa, J. K. Stanfield, S. Yanagisawa, O. Shoji and Y. Watanabe, *Angew. Chem. Int. Ed.* **2018**, *57*, 12264-12269.
4. M. Ishigami, S. Ariyasu, C. Kasai, Y. Aiba, H. Sugimoto and O. Shoji, *Manuscript in preparation.*

Engineered and Artificial Myoglobins: electrochemical and catalytic studies

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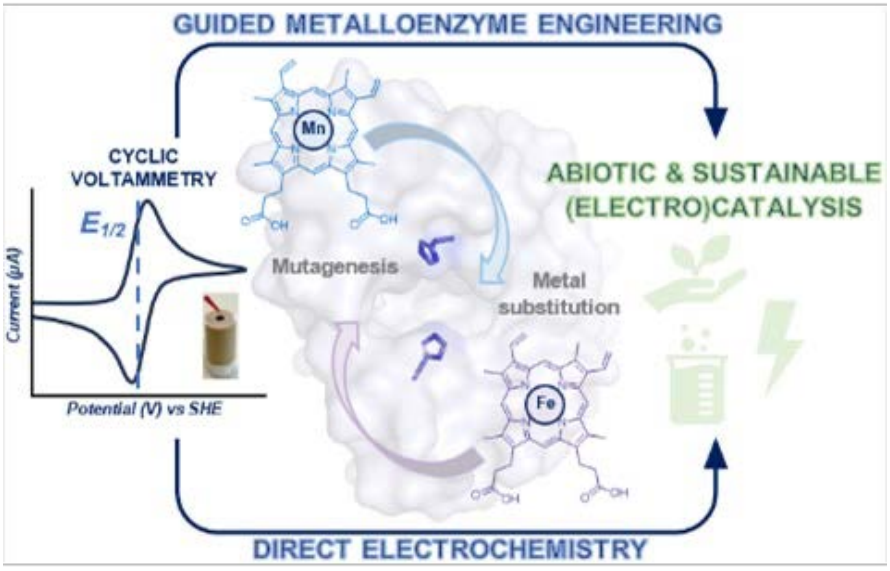
Myoglobin (Mb) is a 18 kDa monomeric globular hemoprotein with a native function in O2 storage. Its malleable architecture makes it an ideal scaffold for artificial metalloenzyme (ArMs) development.[1] Unnatural cofactors such as metalloprophyrins and salophen complexes can be introduced to these scaffolds giving rasie to enzymes for abiotic reactions, such as carbene transfer.[2] It has been shown that electrochemical tools can be used to screen and tune the redox activity profile of Mb ArM mutant systems.[3] These tools demonstrate great potential in guiding further engineering of ArM libraries for target applications in redox transformations. We have developed a robust method for non-covalent immoblisation of myoglobin-based ArMs onto pyrolytic graphite electrodes allowing direct electrochemical measurements to determine the redox potentials of both metal-PPIX and salophen Mbs. Here we will present our method for direct electrochemical characterisation alongside catalytic studies focusing on oxidation reactions and carbene transfer which probed how changes in redox potential and the active site residues of the systems influence catalytic activity and product selectivity.



Selected publications

[1] K. Oohora and T. Hayashi, *Dalton Trans.,* **2021**, *50*, 1940.
[2] a) H. Key, P. Dydio, D. S. Clark, J. F. Hartwig, *Nature*, **2016**, *534*, 534. b) J. R. Carey *et al. J. Am. Chem. Soc.* **2004**, *126*, 10812. c) G. Sreenilayam, E. J. Moore, V. Steck, R. Fasan *Adv. Synth. Catal.* **2017**, *359*, 2076.
[3] A. Bhagi-Damodaran, I. D. Petrik, N. M. Marshall, H. Robinson, Y. Lu, *J. Am. Chem. Soc.*, **2014**, *136*, 11882.

An Artificial Copper-carbene Transferase for the Asymmetric Synthesis of Chiral Organoboranes

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The genetic incorporation of non-canonical metal-binding amino acids via stop codon suppression provides an effective strategy for constructing the artificial metalloenzymes (ArMs), enabling the site-specific incorporation of metal complexes at desired positions within protein scaffolds[1].

In this study, we developed a novel copper(I)-dependent artificial carbene transferase (Cu-carbene transferase) featuring a genetically encoded metal-binding unnatural amino acid (2,2′-bipyridin-5-yl)alanine (BpyA)[2]. For the first time, an *in-situ* reduction strategy was employed to convert a copper(II)-containing metalloprotein into a functional copper(I)-dependent artificial metalloenzyme (Fig. **1a**). Furthermore, TnmS3 (an antibiotic binding protein)[3] was repurposed as an artificial protein scaffold, which proved highly effective in constructing an efficient carbene transferase (Fig. **1b**). Through directed evolution using a magnetic bead-based screening assay, this Cu-carbene transferase was optimized, achieving high reactivities and enantioselectivities in asymmetric B–H carbene insertion reactions to produce diverse chiral organoboranes (Fig. **1c**). This work not only expands the toolbox of artificial metalloenzymes for asymmetric catalysis, but also introduces a versatile in situ copper(I) generation strategy and a new efficient artificial protein scaffold, offering a general platform for engineering redox-active metalloenzymes.

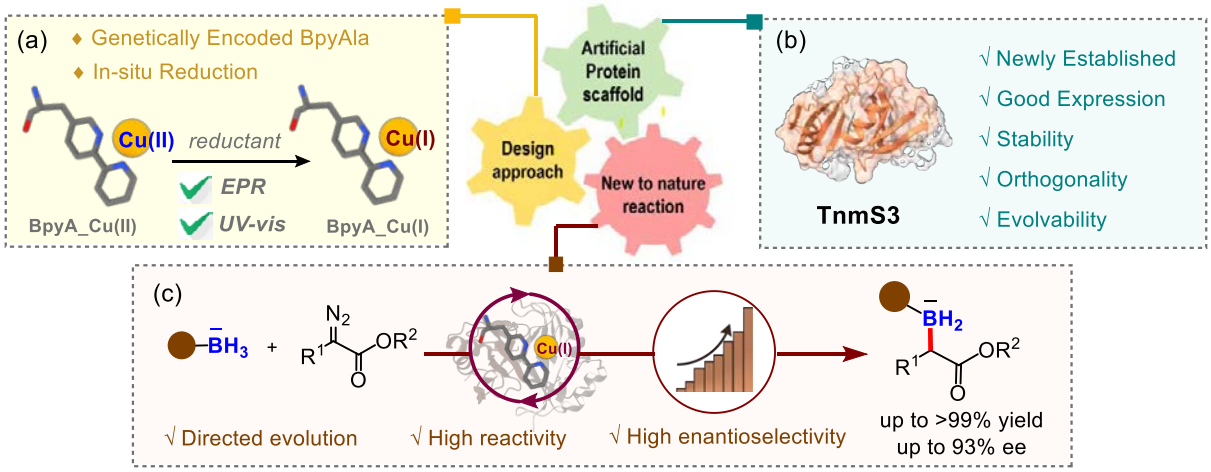

Figure 1 An artificial copper-carbene transferase for the asymmetric synthesis of chiral organoboranes

Selected References

[1] (a) Y. Yu, C. Hu, L. Xia, J. Wang, *ACS Catal.* **2018**, *8*, 1851. (b) G. Roelfes, *Acc. Chem. Res.* **2019**, *52*, 545. (c) B. Brouwer, F. Della-Felice, J. H. Illies, E. Iglesias-Moncayo, G. Roelfes, I. Drienovská, *Chem. Rev.* **2024**, *124*, 10877.

[2] (a) J. Xie, W. Liu, P. G. Schultz, *Angew. Chem. Int. Ed.* **2007**, *46*, 9239. (b) I. Drienovská, A. Rioz-Martínez, A. Draksharapu, G. Roelfes, *Chem. Sci.* **2015**, *6*, 770. (c) R. Jiang, F. Casilli, A-M. W.-H. Thunnissen, G. Roelfes, *Angew. Chem. Int. Ed.* **2025**, e202423182.

[3] C.-Y. Chang, et al. *Cell Chem Biol* **2018**, *25*, 1075.

Photovoltaic enzymes by design and evolution

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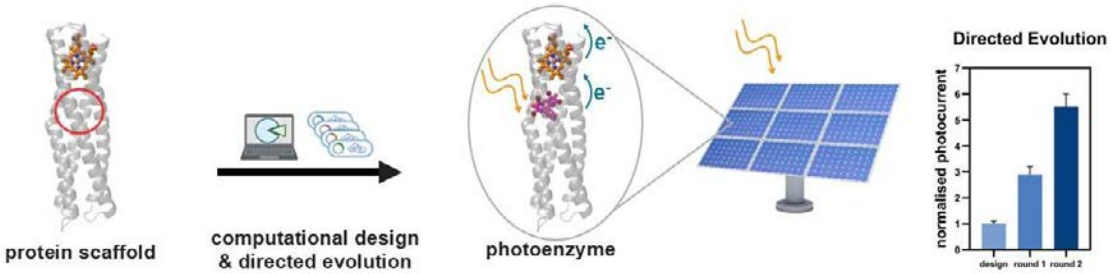
Meeting the growing global energy demand requires innovative strategies for sustainable energy conversion. Solar energy is abundant, yet conventional photovoltaic technologies are limited in efficiency. Natural photosystems achieve outstanding quantum yields, but they are too complex and fragile for direct integration into photovoltaic devices. To overcome this challenge, we combine computational protein design and directed evolution to engineer stable, efficient photoenzymes for biohybrid photovoltaic systems.

Using computational design, we introduced a binding pocket for an organic photosensitizer into a heme-binding protein scaffold. The resulting photoenzyme stably incorporates a small-molecule chromophore into its electron transfer chain, enabling light harvesting and photoinduced electron transfer [1]. This integration enhances photovoltaic efficiency by controlling the photoredox chemistry of the bound cofactors and promoting the injection of photoexcited electrons into a photoanode. To boost performance, we then applied two rounds of directed evolution, yielding ~6-fold higher activity. To our knowledge, this is the first demonstration of evolving a protein explicitly for a photovoltaic function. In parallel, we are expanding the modular helical-bundle scaffold by adding binding sites for additional photosensitizers and redox mediators to enhance light capture and electron flow further.

By integrating protein design and directed evolution with biological catalysis and photovoltaic materials, our approach offers a promising route to address current limitations in solar energy conversion.



Selected publications

[1] Bunzel, H. A. et al. *bioRxiv* 2022.12.20.521207 (2022).

Biocatalysis Under Pressure; driving reactions in a reduced atmosphere

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Recent advancements in biocatalysis have expanded the applicability of enzymes under non-conventional conditions, particularly for compatibility in industrial processes. In many cases, immobilisation techniques have markedly enhanced enzyme performance, improving stability in non-aqueous media and enabling reusability.[1] Novel strategies are increasingly focused on overcoming equilibrium limitations, enabling unnatural reactions and integrating biocatalysts with chemical processes.[2]

This work explores an innovative method for performing enzymatic reactions under reduced pressure, employing a rotary evaporator as the reaction vessel. This strategy aims to accommodate reactions generating volatile by-products, facilitating their in situ evaporation and shifting the reaction equilibrium towards the desired product(s). This is demonstrated through lipase-catalysed kinetic resolutions (KR) of amines, yielding chiral amines and amides of high enantiopurity. Additionally, a two-step, one-pot biocatalytic cascade is developed, coupling two immobilised biocatalysts, a pyridoxal 5'-phosphate (PLP)-dependant transaminase (TA) and a lipase (Figure 1). Despite the challenges associated with pairing these two biocatalysts,[3] optimisating reaction conditions – such as immobilisation support, solvent choice, water content, and the application of the reduced pressure methodology – enables a sequential one-pot transaminase/lipase synthetic cascade, achieving promising yields and high stereoselectivity.

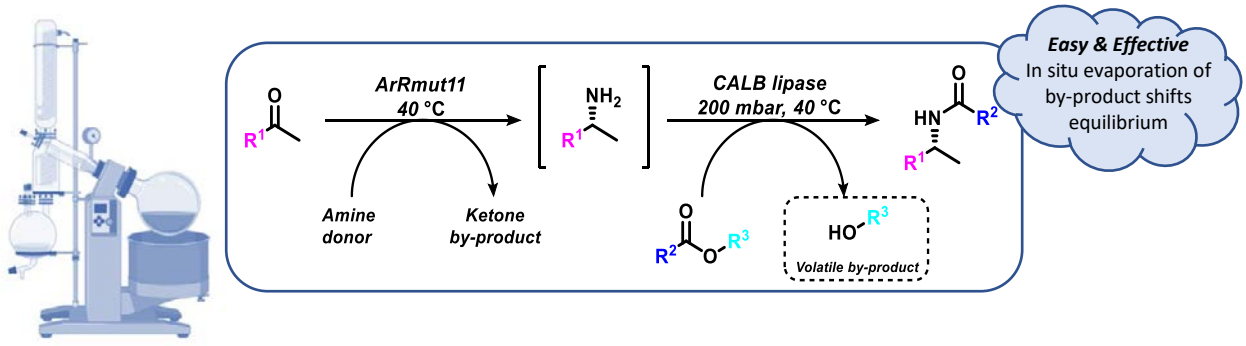

Figure 1. Reaction scheme of the transaminase-lipase cascade for the formation of chiral amides. The rotary evaporation methodology is used to remove excess amine donor after the TA step and to drive product formation in the lipase-catalysed step.

References

1. Sheldon, R. A.; Basso, A.; Brady, D., New frontiers in enzyme immobilisation: robust biocatalysts for a circular bio-based economy. *Chemical Society Reviews*, **2021**, *50*, 5850-5862.
2. Winkler, C. K.; Schrittwieser, J. H.; Kroutil, W., Power of Biocatalysis for Organic Synthesis. *ACS Central Science*, **2021**, *7*, 55-71.
3. Pintor, A.; Mattey, A. P.; Lavandera, I.; Gotor-Fernández, V.; Volkov, A., Development of an amine transaminase-lipase cascade for chiral amide synthesis under flow conditions. *Green Chemistry*, **2023**, 25, 6041-6050.

Harnessing Photoenzymatic Systems for Intermolecular C-H Fluorination

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Organofluorine compounds are vital in pharmaceuticals, and enzymes, nature's most efficient catalysts, offer tremendous potential for precise fluorination. However, no enzymatic strategies for intermolecular C-H fluorination have been realized—until now. We present a novel radical photoenzymatic system (InterFase2) enabling intermolecular C-H fluorination using an unnatural amino acid within a robust de novo protein scaffold. This system achieves chemoselective benzylic monofluorination in aqueous solutions with Selectfluor, driven by hydrogen atom transfer from the photoexcited amino acid (**Fig. 1**). It successfully fluorinated various aromatic compounds and enabled the biosynthesis of fluorinated polyketides and chiral fluorinated alcohols. These results establish radical photoenzymatic systems as a powerful new approach for efficient, selective biocatalytic fluorination, with direct relevance to pharmaceuticals

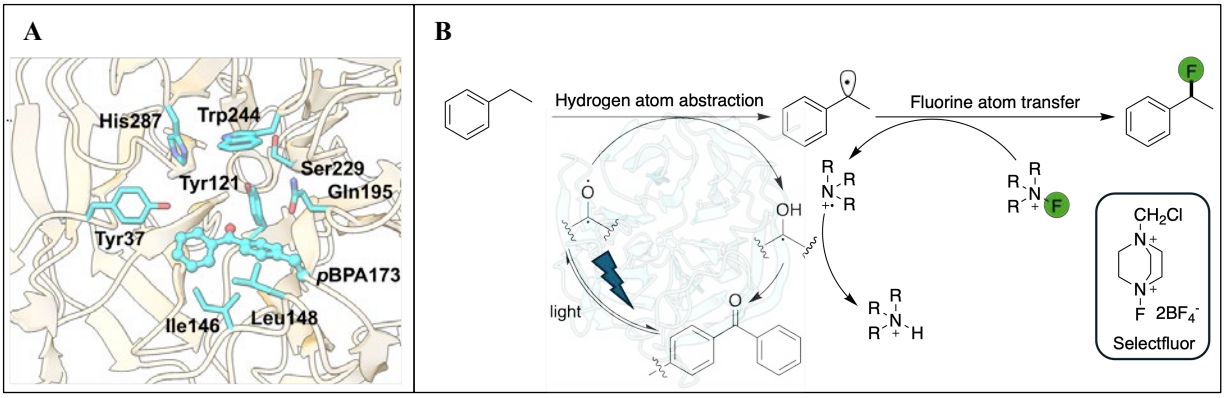

Figure 1: A, Design of artificial metalloenzyme, InterFase2, guided by alanine screening and site saturation mutagenesis. **B**, The proposed photoenzymatic intermolecular C-H fluorination reaction mechanism.

Selected publications

[1] J-B. Xia, C. Zhu, C. Chen, *JACS*, **2013**, 135(46), 17494-17500.
[2] J. S. Trimble, R. Crawshaw, F. J. Hardy, C. W. Levy, M. J.B. Brown, D. E. Fuerst, D. J. Heyes, R. Obexer, A. Green, *Nature*, **2022**, 611(7937), 709-714
[3] N. Sun, J. Huang, J. Qian, T-P. Zhou, J. Guo, L. Tang, W. Zhang, Y. Deng, W. Zhao, G. Wu, R-Z. Liao, X. Chen, F. Zhong, Y. Wu, *Nature*, **2022**, 611(7937), 715-720
[4] Q. Zhao, Z., Chen, J. Soler, X. Chen, J. Rui, N. T. Ji, Q. E. Yu, Y. Yang, M. Garcia-Borras, X. Huang, *Nat. Synth.*, **2024**, 3, 958-966.
[5] L. P. Zhao, B. K. Mai, L. Cheng, F. Gao, Y. Zhao, R. Guo, H. Wu, Y. Zhang, P. Lui, Y. Yang, *Nat. Synth*., **2024**, 3, 967-975.

A Fiber Forming Artificial Metalloenzyme for Heterogeneous Biocatalysis

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The field of Artificial Metalloenzymes (ArMs) is expanding, in terms of designs and in reactions catalyzed by them, and in the recent years, it has witness notworthy achievements such as ArMs showing catalytic properties that challenge natural enzymes.[1,2] Yet, to translate these achievements into industrial applications, some improvements may be required.
Among these, we aim here at tackling 3 properties of an ArMs that industries may be interested into : its stability towards a wide range of conditions (co-solvent, temperature, etc), its recyclability and its ease to engineer. Indeed, we propose here to use the Type I left-handed β–helix (LβH) as a scaffold to develop ArMs, as β–solenoids have been shown to form very robust fibers (ie they can constitute stable heterogeneous catalysts) and as they are tandem repeat proteins, they are well-suited for protein design.[3]
This presentation will briefly introduce the sequence-structure analysis[4] of natural LβH-containing proteins that was used to rationally design a 35-aa peptide that self-assemble into well-defined and robust fibers. We will present their characterizations by spectroscopic (IR, CD) and microscopic (AFM, TEM, CryoEM) means. Finally, we will present how such model peptide can serve as scaffold for the design of catalytic fibers, performing Cu-catalyzed reactions.

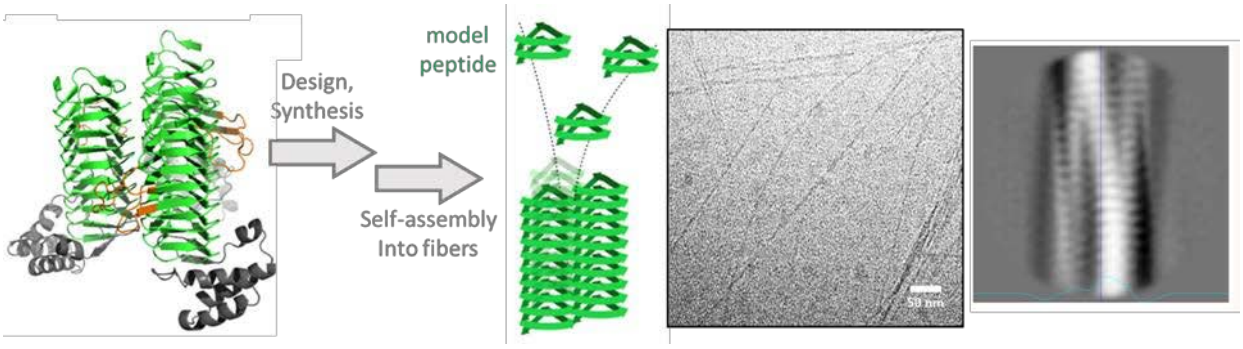

Figure 1. Model peptides able to fold and assemble as β–helix were designed based on analysis of crystal structures of natural examples of this fold (left). These peptides were shown to form stable and well-defined fibers, of which structure was determined by CryoEM.

Selected publications

[1] F. Schwizer, Y. Okamoto, T. Heinisch, Y. Gu, M. M. Pellizzoni, V. Lebrun, R. Reuter, V. Köhler, J. C. Lewis, T. Ward, *Chem. Rev.* **2018**, *118*, 142.
[2] T. Vornholt, F. Leiss-Maier, W. J. Jeong, C. Zeymer, W. J. Song, G. Roelfes, T. Ward, *Nat. Rev. Methods Primers*, **2024**, *4*, 78.
[3] F. Parmeggiani, P. S. Huang, Curr. Opin. Struct. Biol. 2017, 45, 116–123.
[4] M. Naude, P. Faller, V. Lebrun, *Proteins: Struct., Funct., Bioinf.*, **2024**, 92(11), 1318–1328.

Designing fully computational and efficient *de novo* enzymes: insights into catalysis and foldability

Dina Listov[1], Eva Vos[2], Gyula Hoffka[3,4], Shlomo Yakir Hoch[1], Andrej Berg[5], Shelly Hamer-Rogotner[6], Orly Dym[6], Shina Caroline Lynn Kamerlin[2,3], Sarel J. Fleishman*[1]

Designing enzymes from scratch is a rigorous test of our understanding and control of protein structure and function. Despite significant advances in computational protein optimization, creating efficient enzymes without relying on in vitro evolution remains a formidable challenge.

To address this challenge, I developed a fully computational workflow for the *de novo* design of efficient Kemp eliminases without recourse to iterative experimental optimization. The most efficient design features over 140 mutations relative to any natural protein, high stability (> 85°C), and unprecedented catalytic efficiency for a fully computationally designed protein (12,700 $M^{-1}s^{-1}$). By breaking down the key steps of the computational workflow, I identified factors necessary for catalysis, revealing that mutations both within and outside the active site contribute synergistically to the observed high activity and stability. Additionally, mutating a residue used in all Kemp elimination design studies of the past two decades increases efficiency to > 10^5 $M^{-1}s^{-1}$. Our approach overcomes critical limitations in enzyme design, generating stable, highly efficient, new-to-nature enzymes with natural protein folds and it enables the testing of hypotheses about the fundamentals of biocatalysis.

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Developing Myoglobin-Based Artificial Fluorinases

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Artificial Metalloenzymes (ArMs) are novel biocatalysts built by binding a synthetic metal complex to a natural protein scaffold.[1] They offer the advantages of enzymatic catalysis including mild condition and high enantioselectivity, and broaden the reaction scope to abiotic transformations. Myoglobin has been proved to a versatile ArM scaffold in the past for various transformations such as carbene transferase[2], nitrogen oxide reductase[3] and peroxidase[4].

Hereby myoglobin is investigated as a potential fluorinase that can conduct fluorination reaction at mild aqueous conditions. Radical fluorine transfer reaction of N-fluoroamide is selected based on literature reports[5]. A series of salophen complexes with different substituents were designed, binding predicted by molecular docking and synthesised. The recombinant protein was obtained by protein expression and cofactor exchange, and characterised with native mass spectrometry and circular dichroism. Investigation into the catalytic activity of the recombinant protein in an intramolecular C-H fluorination reaction (Figure 1) is underway.

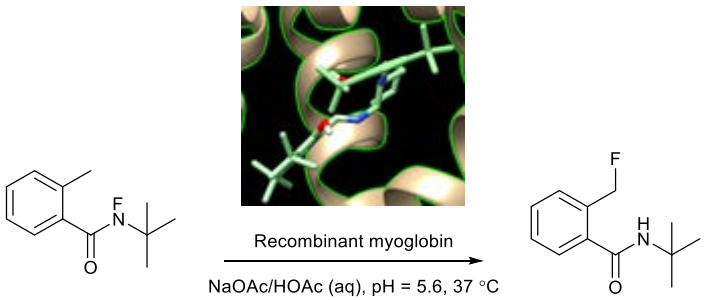

Figure 1. Proposed Fluorination Reaction

References

1. F. Schwizer, *et al.*, *Chem. Rev.*, 2018, **118**, 142-231.
2. (a) M. Bordeaux, V. Tyagi and R. Fasan, *Angew. Chem. Int. Ed.,* 2015, **54**, 1744-1748. (b) H. M. Key, P. Dydio, D. S. Clark and J. F. Hartwig, *Nature,* 2016, **534**, 534-537.
3. V. kohler and T. R. Ward. *ChemBioChem*, 2010, **11**, 1049-1051.
4. T. Hayashi, *et al., J. Am. Chem. Soc.,* 1999, **121**, 7747-7750.
5. (a) Q. Zhao, *et al., Nat. Synth.,* 2024, **3**, 958-966. (b) L.-P. Zhao, *et al., Nat. Synth.,* 2024, **3**, 967-975.

Exploring Bacterial Hydrazine Biosynthetic Pathways Featuring Cupin/Methionyl tRNA Synthetase-like Enzymes

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Nitrogen–nitrogen (N–N) bond-containing functional groups in natural products and synthetic drugs play significant roles in biological activity. Over the decades, the mechanisms of N–N bond formation in natural organic molecules have garnered increasing attention[1]. Recent advances have unveiled various enzymatic and nonenzymatic strategies, rapidly expanding our understanding of natural N–N bond construction. A group of didomain proteins possessing metal-binding cupin/methionyl-tRNA synthetase (MetRS)-like domains—also known as hydrazine synthetases—generate amino acid-based hydrazines that serve as key biosynthetic precursors for diverse N–N bond-containing functionalities, such as hydrazone, diazo, triazene, pyrazole, and pyridazinone groups[2]. In this presentation, I will discuss genome-mining[3], the elucidation of biosynthetic pathways[4], and the biocatalytic applications of this unique bond-forming machinery.

References

[1] (a) A. J. Waldman, T. L. Ng, P. Wang, E. P. Balskus, *Chem. Rev.* **2017**, *117*, 5784–5863. (b) Y. Katsuyama, K. Matsuda, *Curr. Opin. Chem. Biol.* **2020**, *59*, 62–68. (c) L. Chen, Z. Deng, C. Zhao, *ACS Chem. Biol.* **2021**, *16*, 559–570. (d) H. Y. He, H. Niikura, Y. L. Du, K. S. Ryan, *Chem. Soc. Rev.* **2022**, *51*, 2991–3046. (e) C. Angeli, S. Atienza-Sanz, S. Schröder, A. Hein, Y. Li, A. Argyrou, A. Osipyan, H. Terholsen, S. Schmidt, *ACS Catal.* **2024**,*15*, 310–342.
[2] K. Matsuda, T. Wakimoto, *ChemBioChem* **2024**, e202300874.
[3] (a) K. Matsuda, K. Arima, S. Akiyama, Y. Yamada, Y. Abe, H. Suenaga, J. Hashimoto, K. Shin-Ya, M. Nishiyama, T. Wakimoto, *J. Am. Chem. Soc.* **2022**, *144*, 12954–12960. (b) K. Matsuda, Y. Nakahara, A. R. Choirunnisa, K. Arima, T. Wakimoto, *ChemBioChem* **2024**, e202300838.
[4] K. Arima, S. Akiyama, K. Shin-ya, K. Matsuda, T. Wakimoto, *Angew. Chem. Int. Ed.* **2023**, 62, e202305155.

The use of SPY system for artificial photo-metalloenzymes

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There is an ongoing demand on making greener the synthetic processes of the modern chemical industry. Moreover, the synthesis of chiral products with high optical purity is also desired in various fields, such as agrochemicals and pharmaceutics.[1,2] Enzyme catalysis fulfills both, as they are able to catalyze enantioselective processes under ambient conditions.[3] Although a plethora of enzymes are in use nowadays in biocatalytic processes, their use is limited, as not all types of desired reactions are covered by the used enzymes. Important reactions, such as enantiomer-selective C—C bond formation, can be achieved by combining metal catalysts with proteins, resulting in artificial metalloenzymes. Tailoring the first and second coordination spheres of the metal centre is a huge challenge because of the presence of several side chains able to coordinate. This challenge can be overcome by the Trojan horse method.

In this work the Trojan horse method is achieved by the SPY tagging system. The SPY protein is constructed from the SpyTag peptide (ST) and the SpyCatcher protein (SC), which bind to each other spontaneously.[4] In our systems the catalytically active metal centre is bound to the ST, which will react with the SC in a well-defined position, thus the metalation step is simplified: the metal complex reacts only with a peptide instead of a whole protein, avoiding possible side-reactions. This technology has the possibility to control the secondary coordination sphere, which is crucial for the catalysis of enantioselective processes.

The planned artificial metalloenzymes contain Cu(I/II) and Ru(II) metal centres surrounded by substituted 2,2'-bipyridine or 1,10-phenanthroline ligands with the ability of enantioselective (photo)catalysis of Diels-Alder reaction, atom transfer radical addition and isomerization reactions. The planned computational models of the SPY systems will show amino acids present in the second coordination sphere, which will be modified by point mutations in order to improve their catalytic activity. The library of the enzymes will provide an insight on the effect of the second coordination sphere on the reaction.

Acknowledgement: Funded by the European Union. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

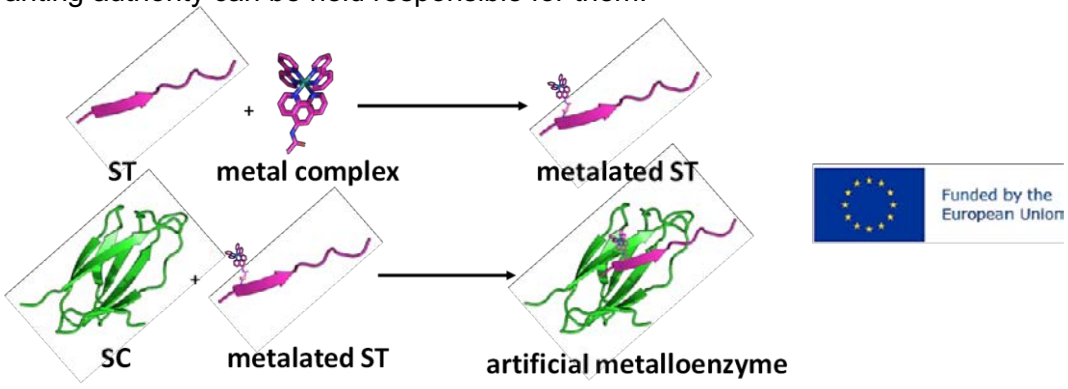

Figure 1 The Trojan horse method in order to devise SPY based artificial metalloenzymes.

Selected publications

[1] P. Jeschke, *Pest Manag. Sci.*, **2018**, *74*, 2389.
[2] N. Senkuttuvan et al., *RSC Adv.*, **2024**, *14*, 33429.
[3] U.T. Bornscheuer et al., *Nature*, **2012**, *485*, 185.
[4] D. Hatlem et al., *Int. J. Mol. Sci.,* **2019**, *20*, 2129

Trying to catch the manganese: a SpyTag/SpyCatcher approach

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Artificial metalloenzymes are synthetic constructs designed to mimic and extend the function of natural enzymes. Typically, they are composed of an abiotic metal cofactor incorporated into a protein scaffold. [1] The interest in these systems is still growing due to their diverse potential applications. They can act as model systems, used for understanding complex processes involving natural enzymes, and also their precise design can allow to catalyze non-biological, new-to-nature reactions, which is a particularly attractive feature in the context of biotechnology and biocatalysis. [1, 2]

This project aims to develop an artificial metalloenzyme capable of promoting oxidation reactions, which would ultimately serve to deplete ROS.

The first step involves introducing an abiotic fragment into the protein. Among the various strategies developed, one of the most popular is the irreversible covalent interactions of the metal-containing fragment with the protein. For this purpose, the Spy strategy would be used here. This strategy is based on the two key components: a short peptide (SpyTag, ST) and a protein (SpyCatcher, SC), which precisely and spontaneously form a covalent isopeptide bond between the Lys (SC) and Asp (ST) residues, resulting in a stable Spy protein. [3]

By using this approach, the complexity of manipulating the protein architectures is reduced due to the remarkable flexibility provided by the design and synthesis of two separate molecules, precisely conjugating in the later stage. This strategy not only simplifies the whole process by avoiding the genetic modifications, but also expands the possibilities of designed systems by introducing specific, non-biological groups to the SpyTag fragment.

In this project, to enable the redox activity of the entire system, the SpyTag is functionalized with an artificial, tripodal Mn-binding site. Manganese, a well-known metal cofactor found in plenty of natural enzymes, is an ideal candidate for ensuring the redox activity of this new compound.

After the synthesis, the obtained artificial metalloenzyme will be further examined in terms of the kinetics of the oxidation reaction.

To the best of our knowledge, this is the first work using the Spy strategy to introduce an unnatural metal-coordinating fragment into a protein, creating a truly artificial metalloenzyme.

Figure Binding of SpyTag and SpyCatcher to obtain the SpyProtein [4]

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Selected publications

[1] H. J. Davis, T. R. Ward, *ACS Cent. Sci.*, **2019**, *5*, 1120.
[2] D. J. DiPrimio, P. L. Holland, *J. Inorg Biochem.* **2022**, *219*, 111430.
[3] D. Hatlem, T. Trunk, D. Linke, J. C. Leo, *IJMS*, **2019**, *20*, 2129.
[4] https://www.rcsb.org/3d-view/4MLI/1

Discovery and evolution of enzymes for catalysis and chemical biology

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Enzymes found in Nature perform incredible chemistry under mild reaction conditions and with high selectivity. Compared to traditional chemical strategies for manufacturing small molecules, enzymatic strategies have the potential to reduce waste, eliminate costly separation steps, and lower energy requirements. We combine expertise in biochemistry, biosynthesis, structural biology, and protein evolution to discover and to engineer enzymes for biocatalysis and chemical biology. In one project area, we use bioinformatics to identify and characterize unexplored metalloenzymes. In another, we are developing a high-throughput platform to evolve enzymes for site-selective bioconjugation. We use evolution not only to develop useful tools for synthesis and biological discovery, but also to probe the basic mechanisms by which enzymes achieve selectivity in substrate recognition and reactivity.

Dual-Functional Designer Enzyme: Installing a Catalytically Active Multi-nuclear Metal Center while Preserving the Protein's Native Function

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[3]Institute of Fluid Science, Tohoku University
[4]Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology

Designer enzymes have successfully achieved abiotic reactivity and high catalytic performance through various engineering approaches.[1] Traditional strategies usually focus on maximizing new catalytic functions, which can sometimes reduce the intrinsic bioactivity of the original protein scaffold.

Here, we present an alternative strategy to introduce non-native functions while maintaining native protein functions. By strategically incorporating a synthetic trinuclear zinc center[2] into the inner pore of human macrophage migration inhibitory factor (MIF), we created a dual-functional enzyme that achieves non-native catalysis while maintaining native functions (Figure a and b).

We performed a computational geometry search to list suitable sites for introducing histidine residues as ligands for the trinuclear zinc center, excluding sites important for native functions of MIF, followed by DFT calculations to further narrow down the mutated sites.

X-ray crystallography confirmed the successful formation of a trinuclear zinc center containing bridging carbonate ions coordinated only by amino acid side chains, validating our rational design approach. As designed, this designer metalloenzyme exhibits hydrolytic activity as a non-native function while maintaining native activities.[3]

This study demonstrates the design of a multifunctional metalloenzyme that adds catalytic ability to biologically active proteins while maintaining natural functions. Given that proteins generally retain biological roles, this approach paves the way for the development of synthetic biological tools relevant to life phenomena.

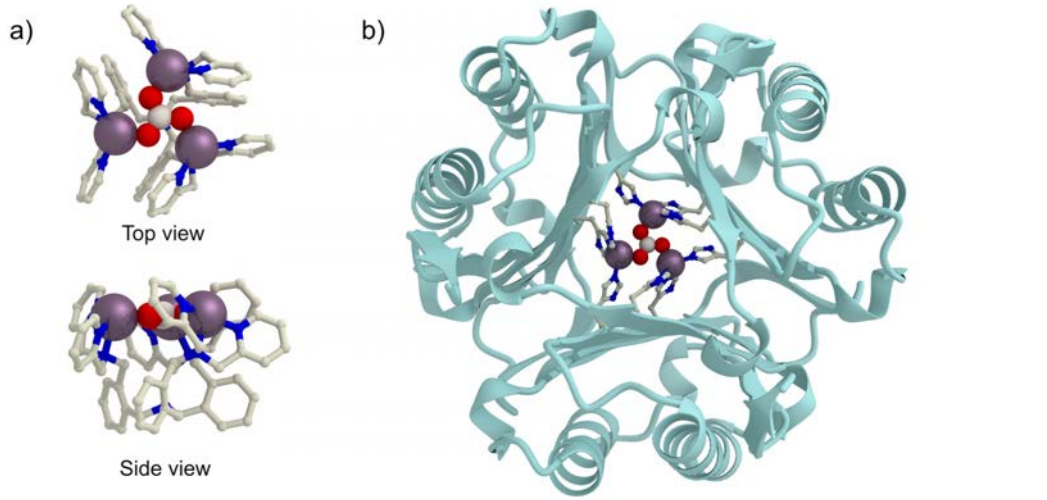

Figure. Design and structure of the dual-functional designer metalloenzyme. (a) Crystal structure of the synthetic trinuclear zinc complex with bridging carbonate used as a template for the design.[2] (b) Crystal structure of the designer tri-zinc enzyme.

References

[1] F. Schwizer, Y. Okamoto, T. Heinisch, Y. Gu, M. M. Pellizzoni, V. Lebrun, R. Reuter, V. Köhler, J. C. Lewis, T. R.Ward, *Chem. Rev.* **2018**, *118*, 142.
[2] X. Liu, P. Du, R. Cao, *Nat. Commun.*, **2013**, *4*, 2375.
[3] A. Ueno, F. Takida, T. Kita, T. Ishii, T. Himiyama, T. Mabuchi, Y. Okamoto *submitted*.

Enzyme Engineering to Access Biaryl Natural Products

Daniel J. Ong[1]; Cole C. Meyer[2]; Di Yang[2]; Lauren M. Murray[2]; Lara E. Zetzsche[1]; Alison R. H. Narayan[1, 2, 3]

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Biaryl motifs are prevalent in ligands for asymmetric catalysis, bioactive natural products, and pharmaceutically relevant scaffolds. Established synthetic methods provide powerful approaches for forging biaryl bonds, however, existing methods can require pre-functionalization of coupling partners and can face limited yields and selectivity challenges. In contrast, Nature has evolved cytochrome P450 enzymes capable of a wide breadth of oxidation chemistry, including oxidative C–C bond formation. A biocatalytic oxidative coupling approach offers an efficient alternative strategy for catalyst-controlled selective biaryl C–C bond formation under mild conditions. Still, there remains untapped potential in leveraging biocatalytic methods to generate a wider range of complex biaryl natural products, such as naphthylisoqiuinoline alkaloids (NIQs). This diverse class of natural products exhibits promising bioactivities against tropical diseases and often showcases atropisomerism due to a biaryl axis that can be rotationally hindered by steric strain between *ortho*-substituents. The structural complexity of NIQs presents a challenge for stereoselective synthesis. Here, we share our progress in optimizing a wild-type P450 enzyme's promiscuous oxidative cross-coupling activity via a semi-rational engineering approach to become a viable biocatalyst for the synthesis of NIQs. We anticipate this work will showcase the utility of P450s in chemoenzymatic syntheses of NIQs and inspire the development of complementary biocatalysts to access a broader scope of biaryl natural products.

Artificial Enzymes with Non-natural Active Centers Incorporated into Protein Matrices

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Hemoprotein containing heme, an iron porphyrin, as a cofactor is a promising scaffold toward artificial metalloenzymes due to the unique characteristics derived from the synergetic combination of the metal cofactor and protein matrix. In this context, our group has demonstrated artificial metalloenzymes constructed by insertion of an artificial metal cofactor into the heme-binding site of a simple hemoprotein [1].

These engineered proteins show catalytic activities including C–H bond hydroxylation, C–H bond amination, and olefin cyclopropanation. In the case of olefin cyclopropanation, redox potential of metal center is critical for both of formation and activation of the active species. A porphycene iron complex as a cofactor largely accelerates the formation of the metal carbenoid species in a protein matrix and shows 26-fold times higher turnover frequency for styrene substrate [2]. Recently, we found that an iron complex of electron deficient porphyrin in a protein matrix demonstrates catalytic cyclopropanation of octene as a more inert alkane [3]. In this work, we hypothesized that investigation using myoglobins reconstituted with synthetic cofactors possessing various redox potentials reveals the correlation of redox potential and cyclopropanation reactivity. We employed iron porphyrin with two and one trifluoromethyl groups at peripheral sites (FePor$(CF_3)_2$ and FePorCF_3, respectively), native heme and iron porphycene (FePc) as cofactors. The range of Fe(II)/Fe(III) redox potentials of the four myoglobins exceeds 340 mV. It was found that myoglobin with more positive redox potential shows higher reactivity toward inert alkenes. In particular, myoglobin reconstituted with FePor$(CF_3)_2$ (rMb(FePor$(CF_3)_2$)) exhibits a 165-fold higher turnover number for 1-octene cyclopropanation compared to native myoglobin. Mechanistic studies indicate that rMb(FePor$(CF_3)_2$) generates an active species with a radical character. In contrast, myoglobin reconstituted with FePc provides a detectable iron–carbene species [2] with an electrophilic character. This work highlights the significance of redox-focused design of the iron porphyrinoid cofactor in hemoproteins to enhance cyclopropanation reactivity.

A series of findings show that the engineering of cofactor in hemoproteins is useful strategy for this-type of catalysis design. The concept using non-natural active centers for the protein is further expanded to the artificial enzyme containing a photosensitizer toward photo reaction.

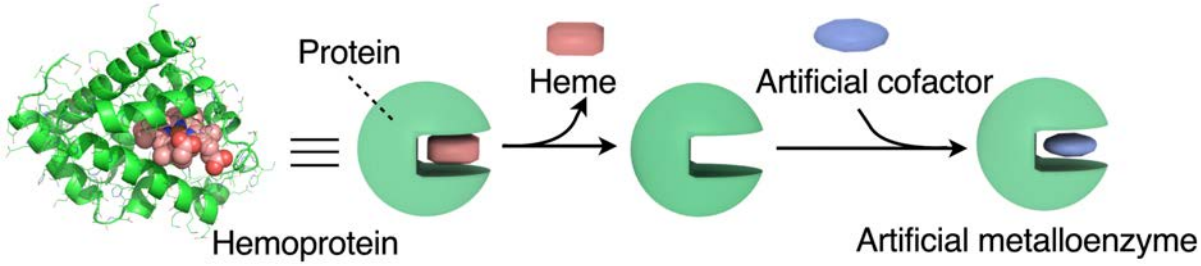

Figure Shcemeatic representation of reconstituted hemoprotein with artificial cofactor.

References

[1] K. Oohora K., T. Hayashi, *Dalton Trans.*, **2021**, *50*, 1940-1949.
[2] K. Oohora, H. Meichin, L. Zhao, M. W. Wolf, A. Nakayama, J. Hasegawa, N. Lehnert, T. Hayashi, *J. Am. Chem. Soc.*, **2017**, *139*, 17265-17268.
[3] Y. Kagawa, K. Oohora, T. Himiyama, A. Suzuki, T. Hayashi, *Angew. Chem. Int. Ed.*, **2024**, *63*, e202403485.

Photoenzymatic Hydroaminations and Hydroarylations via an Emergent Mechanistic Pathway

Felix C. Raps[1], Ariadna Rivas-Souchet[1], Chey M. Jones[2], Chufan Jin[1], Alexandra C. Brown[1], Damien Sorigué[1], Todd K. Hyster[1]

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Translation of small molecule reactivity into biocatalytic frameworks offers access to highly precise catalysts for selective synthesis. These systems can overcome limitations often innate to small molecule catalysts, frequently focusing on stereo- and regiocontrol of transformations. In addition, biocatalysts can offer unexpected results, harnessing emergent mechanisms that in some cases are unprecedented in organic synthesis.
This talk will feature the discovery of an emergent mechanism for a Markovnikov-selective, photoenzymatic hydroamination in a Baeyer-Villiger Monooxygenase that was amplified by directed evolution. The development allowed for the preparation of highly-congested α-tertiary amines difficult to access with traditional methodologies. Mechanistic investigations using small molecule probes, DFT, and QM/MM revealed the behavior within the enzyme active site. Furthermore, the more recent development of a hydroarylase will be presented, focusing on the requirements of the enzyme to facilitate the reaction, as well as studies of the changes to the active site by crystallography and computational tools.

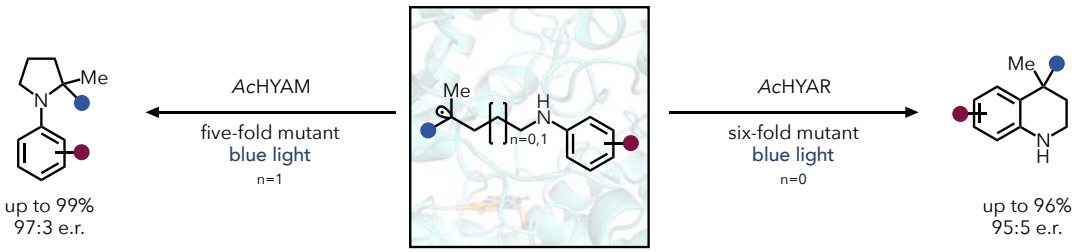

Figure Generation of a benzylic radical in the enzyme active site of evolved Baeyer Villiger Monoogyenases enables divergent transformation into hydroamination and hydroarylation products.

Selected Publications

[1] Raps, F. C.; Rivas-Souchet, A.; Jones, C. M.; Hyster, T. K., Emergence of a distinct mechanism of C–N bond formation in photoenzymes. *Nature* **2025,** *637* (8045), 362–368.

[2] Emmanuel, M. A.; Bender, S. G.; Bilodeau, C.; Carceller, J. M.; DeHovitz, J. S.; Fu, H.; Liu, Y.; Nicholls, B. T.; Ouyang, Y.; Page, C. G.; Qiao, T.; Raps, F. C.; Sorigué, D. R.; Sun, S.-Z.; Turek-Herman, J.; Ye, Y.; Rivas-Souchet, A.; Cao, J.; Hyster, T. K., Photobiocatalytic Strategies for Organic Synthesis. *Chem. Rev.* **2023,** *123* (9), 5459-5520.

Making metathesis essential

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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 accelerated continuous evolution 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 (*dn*TRP). Directed evolution revealed the improved 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 the recently reported E.coli based orthogonal replication system (EcORep)[2] through ring-closing metathesis triggered release of indole as a selection system. In vivo hypermutation of the *dn*TRP R5 under selective conditions promises a vast improvement in covered sequence space, more closely mimicking natural evolution.

References

[1] Z. Zou, I. Kalvet, B. Lozhkin, E. Morris, K. Zhang, D. Chen, M. Ernst, X. Zhang, D. Baker, T. Ward, **2025** PREPRINT available at Research Square /10.21203/rs.3.rs-5849532/v1
[2] R. Tian, F. Rehm, D. Czernecki, Y. Gu, J. Zürcher, K. Liu, J. Chin, *Science* **2024**, *383*, 6681.

Development of Artificial Metalloenzymes Based on Copper-Binding β-Helix Mimicking Peptides for C-C Bond Forming Reactions

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Artificial metalloenzymes (ArMs) offer a promising strategy for expanding the catalytic repertoire of biomolecules by integrating metal centers into designed peptide and protein scaffolds.[1] In this work we focus on developing β-helix mimicking peptides as stable and structurally well-defined scaffolds for metal site engineering. Building on previous sequence-structure analysis of left-handed β-helices,[2] we rationally designed self-assembling β-helix peptides with coordinating residues to form metal-binding upon folding and assembly. The peptides' ability to self-assemble into fibrillar architectures was confirmed by computational methods and is aimed to facilitate both catalytic function and recyclability. A small library of peptides was generated, varying the coordination environment of the metal. The peptides were synthesized using solid-phase peptide synthesis (SPPS), purified, and characterized using electron microscopy in order to confirm the peptides' ability to assemble into fibers. Their catalytic activity was explored on Atom Transfer Radical reactions. These first assays demonstrated the potential of these peptides as a platform for developing heterogeneous catalysts, as stereoselectivity was observed. This work contributes to advancing the design of bioinspired catalysts, demonstrating how rationally engineered self-assembling peptide scaffolds can provide efficient, potentially recyclable ArMs for C-C bond forming transformations, with potential applications in sustainable catalysis and green chemistry.

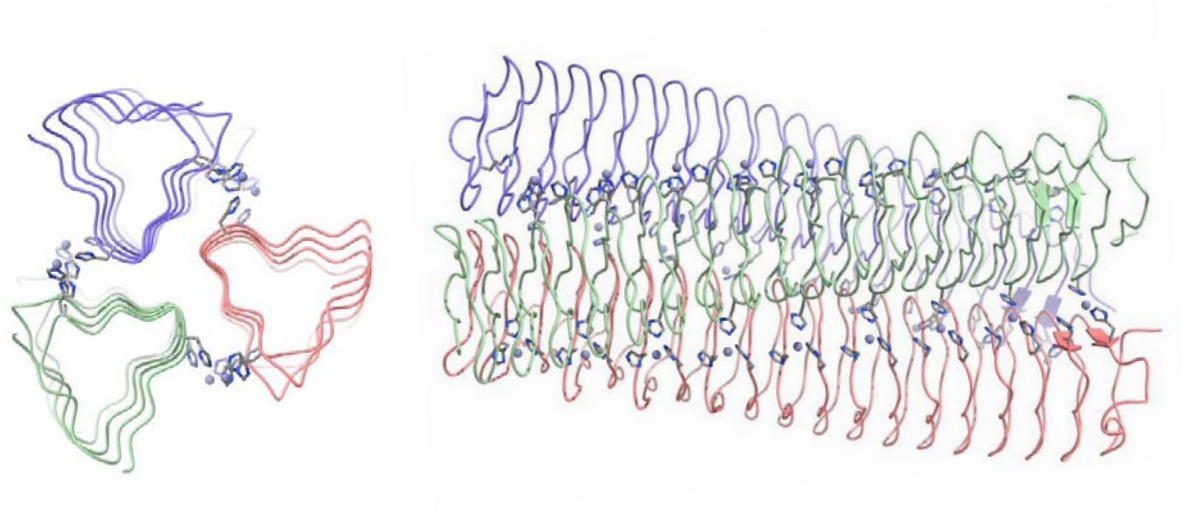

Figure 1 Homotrimeric helical structure of the Zn^{II}-bound peptide, created by Alpha Fold 3 with nine copies of the peptide [with N-terminal and C-terminal caps added to facilitate modeling]. Left : cross section view, Rigth : side view.

Selected publications

[1] F. Schwizer, Y. Okamoto, T. Heinisch, Y. Gu, M. M. Pellizzoni, V. Lebrun, R. Reuter, V. Köhler, J. C. Lewis, L. Nobody, *Chem. Rev.* **2017**, *118*, 142.

[2] M. Naude, P. Faller, V. Lebrun, *Proteins Structure Function and Bioinformatics*, **2024**, 92(11), 1318–1328. https://doi.org/10.1002/prot.26726

Lighting Up Biocatalysis: New-to-Nature Photoenzymes Through Engineering of a Thioxanthonylalanine-Specific Aminoacyl-tRNA Synthetase

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Photoenzymes represent a promising frontier in biocatalysis, combining the selective nature of protein scaffolds with the reactivity of photocatalysts. In recents years, the activities of natural photoenzymes like the fatty acid photodecarboxylase from *Chlorella variabilis*[1] have been succesfully complemented by various artificial photoenzymes. Approaches to design these synthetic biocatalysts include the repurposing of natural cofactors such as flavins and nicotinamides as photocatalysts[2], as well as the introduction of photoactive metal ions[3] or organic photosensitizers[4] into protein scaffolds.
In this context, genetic code expansion via stop codon suppression has facilitated the site-specific incorporation of photocatalytic noncanonical amino acids (ncAAs). Notably, this strategy has recently led to the development of photoenzymes containing the ncAA benzoylphenylalanine (BzF), capable of catalyzing [2+2] photocyclization reactions[5,6].
In this work, we report the synthesis of a new photoactive ncAA bearing a thioxanthone moiety (thioX), an efficient visible-light photosensitizer[7]. The engineering of a specific aminoacyl-tRNA synthetase (thioXRS), based on the system from *Methanocaldococcus jannaschii*[8], enabled the incorporation of thioX at defined positions within the protein LmrR, a well-established scaffold for abiological catalysis[9]. The resulting photoenzyme, LmrR_96thioX, efficiently catalyzed the *E*/*Z* isomerization of a hydroxycinnamate ester, outperforming both other protein variants and the free photocatalyst.
Together, the successful engineering of thioXRS and the proven high efficiency of LmrR_96thioX advance the development of efficient new-to-nature photoenzymes.

References

[1] D. Sorigué, B. Légeret, S. Cuiné, S. Blangy, S. Moulin, E. Billon, P. Richaud, S. Brugière, Y. Couté, D. Nurizzo, P. Müller, K. Brettel, D. Pignol, P. Arnoux, Y. Li-Beisson, G. Peltier, F. Beisson, Science 2017, 357, 903–907.
[2] M. A. Emmanuel, S. G. Bender, C. Bilodeau, J. M. Carceller, J. S. DeHovitz, H. Fu, Y. Liu, B. T. Nicholls, Y. Ouyang, C. G. Page, T. Qiao, F. C. Raps, D. R. Sorigué, S.-Z. Sun, J. Turek-Herman, Y. Ye, A. Rivas-Souchet, J. Cao, T. K. Hyster, Chem. Rev. 2023, 123, 5459–5520.
[3] A. S. Klein, F. Leiss-Maier, R. Mühlhofer, B. Boesen, G. Mustafa, H. Kugler, C. Zeymer, J. Am. Chem. Soc. 2024, 146, 25976–25985.
[4] J. Guo, J. Qian, D. Cai, J. Huang, X. Yang, N. Sun, J. Zhang, T. Pang, W. Zhao, G. Wu, X. Chen, F. Zhong, Y. Wu, J. Am. Chem. Soc. 2024, 146, 19030–19041.
[5] J. S. Trimble, R. Crawshaw, F. J. Hardy, C. W. Levy, M. J. B. Brown, D. E. Fuerst, D. J. Heyes, R. Obexer, A. P. Green, Nature 2022, 611, 709–714.
[6] N. Sun, J. Huang, J. Qian, T.-P. Zhou, J. Guo, L. Tang, W. Zhang, Y. Deng, W. Zhao, G. Wu, R.-Z. Liao, X. Chen, F. Zhong, Y. Wu, Nature 2022, 611, 715–720.
[7] N. F. Nikitas, P. L. Gkizis, C. G. Kokotos, Org. Biomol. Chem., 2021, 19, 5237-5253.
[8] I. Sungwienwong, Z. M. Hostetler, R. J. Blizzard, J. J. Porter, C. M. Driggers, L. Z. Mbengi, J. A. Villegas, L. C. Speight, J. G. Saven, J. J. Perona, R. M. Kohli, R. A. Mehl, E. J. Petersson, Org. Biomol. Chem. 2017, 15, 3603–3610.
[9] G. Roelfes, Acc. Chem. Res. 2019, 52, 545–556.

In Vivo Continuous Evolution of an Artificial Metalloenzyme Toward Sustaining Cellular Metabolism

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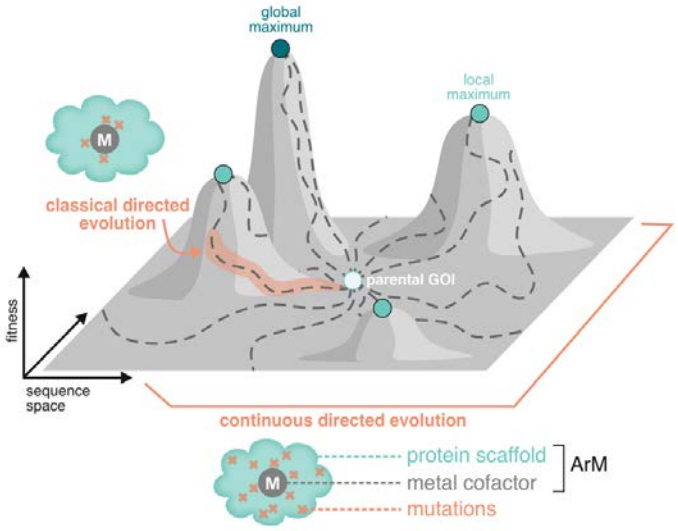
Artificial metalloenzymes (ArMs) combine the broad reaction scope of organometallic catalysis with the advantages of enzymatic catalysis (e.g., selectivity, evolvability, mild reaction conditions)[1]. Such non-natural biocatalysts may allow the incorporation of new-to-nature reactions into the metabolic pathways of living cells, potentially enabling biomanufacturing and synthetic biology applications. Nonetheless, metabolic engineering with ArMs remains a largely unexplored area, partly due to their limited catalytic efficiencies compared to natural enzymes[2]. To increase the efficiency of ArMs, directed evolution has been used extensively. However, due to its dependence on manually staged mutagenesis and relatively low-throughput screening, the possible explored sequence space is constrained in classical directed evolution[3]. As such, these efforts have mostly led to only modest improvements in ArM efficiency. Recently, **continuous evolution** methods based on *in vivo* gene hypermutation have emerged, in which evolution occurs rapidly and autonomously during cell replication. Such methods allow long mutational trajectories to be traversed on laboratory timescales, and could potentially accomplish the required evolutionary remodelling of ArM protein scaffolds presumably needed to achieve the catalytic efficiencies of natural enzymes. In this work, we will apply a powerful continuous evolution system **OrthoRep**[4] to evolve streptavidin-based ArMs. A metalloenzyme catalyzing an indole-forming reaction was integrated into the metabolic pathways of *S. cerevisiae*, and its performance was coupled to cell growth by complementing tryptophan auxotrophy. After an extensive continuous evolution coupled with selection, we hope to engineer, for the first time, an ArM capable of sustaining cellular metabolism.



Selected publications

[1] Vornholt, T. *et al.* Artificial metalloenzymes. *Nat. Rev. Methods Primer* **4**, 1–17 (2024).
[2] Zou, Z., Higginson, B. & Ward, T. R. Creation and optimization of artificial metalloenzymes: Harnessing the power of directed evolution and beyond. *Chem* **10**, 2373–2389 (2024).
[3] Molina, R. S. *et al.* In vivo hypermutation and continuous evolution. *Nat. Rev. Methods Primer* **2**, 1–22 (2022).
[4] Ravikumar,s A., Arzumanyan, G. A., Obadi, M. K. A., Javanpour, A. A. & Liu, C. C. Scalable, Continuous Evolution of Genes at Mutation Rates above Genomic Error Thresholds. *Cell* **175**, 1946-1957.e13 (2018).

Design, synthesis and characterisation of artificial metallopeptides based on the Spy System

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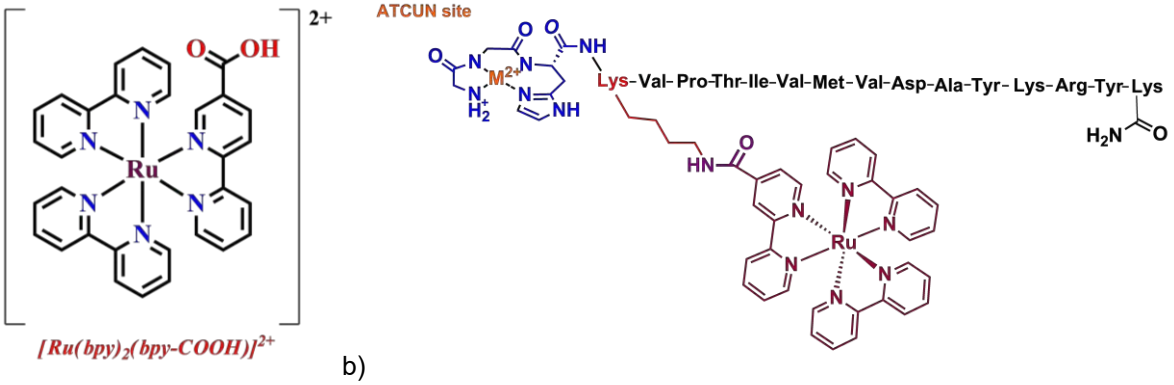
SpyTag is a short peptide that forms an isopeptide bond upon encountering its protein partner SpyCatcher. This covalent peptide interaction is a simple and powerful tool for bioconjugation and extending what protein architectures are accessible.[1] The isopeptide bond formed between the two components happens between the side chains of a lysine (Lys) on SpyCatcher and the carboxyl of an aspartate side chain (Asp) on SpyTag.[2] Tris(bipyridyl)ruthenium(II) chloride, $[Ru(Bpy)_3]Cl_2$, is a complex with interesting photochemical characteristics; which absorbs light in the visible region, (~ 450 nm), leading to an electronic transition from the ground state to a metal-to-ligand charge transfer (MLCT) excited state. This excited state is characterized by a relatively long lifetime, allowing for various photochemical reactions, including photocatalysis.[3]
For this reason, we are looking to graft the $[Ru(Bpy)_3]^{2+}$ group to the SpyTag. The same SpyTag will be designed to bear also a catalytic site (e.g. an ATCUN (Amino Terminal Copper (II) and Nickel (II) Motif) to coordinate a redox active metal ion (i.e. Co^{2+}). Through this strategy, it will be possible to exploit $[Ru(Bpy)_3]^{2+}$ photochemical properties to promote Co^{2+} redox activity, giving a new artificial metalloenzyme.
This poster will highlight our recent progress towards the design, synthesis and characterisation of an artificial metallopeptide, with a focus on different synthesis strategies to covalently bind a photoactive metal complex, including the synthesis of ruthenium-functionalized fmoc-amino acids for its using in solid phase peptide syntehsis.



a) b)

Figure 1: a) Photocatalytic group: Tris(bipyridyl)ruthenium(II) with carboxylate group on one of the ligands; b) Spy Tag peptide containing the catalytically active site (ATCUN site; in blue) and a lysine (in red) with the photocatalytic complex (dark red).

Project “Artificial enzymes for the photocatalytic production of hydrogen in photosynthetic bacteria” National Recovery and Resilience Plan (NRRP), M2 C2 Inv. 3.5 funded by the European Union – NextGenerationEU. Project RSH2A_000009, C.D. 445 29/12/2022 Italian Ministry of Environment and Energy Security. Project PRIN of National Interest (PRIN) 2022 “Bioinspired systems for ROS regulation: metalloporphyrinoids in neurodegeneration and artificial biocatalysis”prot. 2022RCRWE5 - Italian Ministry of University and Research (MUR).

Selected publications

[1] S. C. Reddington and M. Howarth,” Curr Opin Chem Biol, vol. 29, pp. 94–99. **2015**.
[2] D. Hatlem, T. Trunk, D. Linke, and J. C. Leo, *Int J Mol Sci*, vol. 20, no. 9, **2019**.
[3] S. Pitre, C. McTiernan, W. Vine et al. Sci Rep **5**, 16397 (**2015**).

Guiding Generative Protein Language Models Towards High Fitness Regions with Reinforcement Learning

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[6]ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain

Protein language models have transformed protein design by generating functional, remarkably diverse proteins. However, their generative distributions tend to mirror common properties of the training data, limiting their ability to sample high-fitness sequences outside of natural diversity (Figure 1a). Reinforcement learning (RL) has emerged as a powerful tool to align language models with desired features. We introduce ProtRL, an iterative RL strategy that guides protein language models toward user-defined properties such as enhanced stability or thermostability. ProtRL aligns the model by generating sequences, scoring them through interactions with an oracle (environment), and feeding the resulting sequence-score pairs back to the agent (Figure 1b). ProtRL effectively design proficient protein–protein [1] and protein–macromolecule binders [2]. We have also applied ProtRL to steer the model toward generating sequences with higher predicted activity (Figure 1d), offering promising perspectives in enzyme design.

Unlike methods that rely on fixed input sequences, protein language models can efficiently explore a vast sequence space [3], unconstrained by natural biodiversity or the limitations of bootstrapping from a single sequence (Figure 1c). RL can effectively navigate the complex trade-offs of protein design, addressing the challenging task of identifying the Pareto frontier in multi-objective optimization, potentially generalizing to any protein design problem.

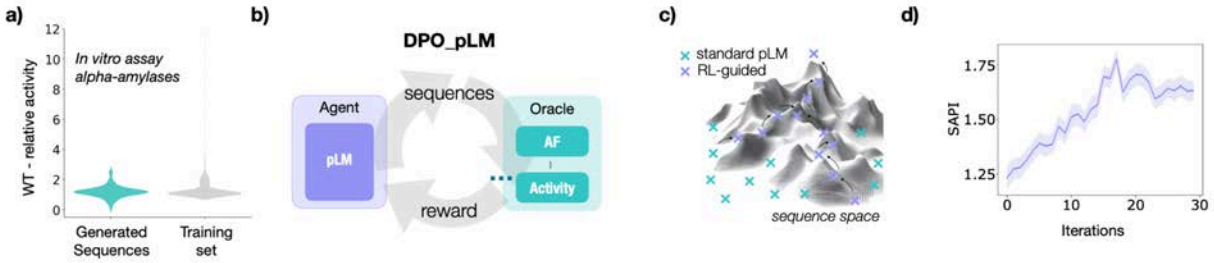

Figure 1 pLMs mirror the distributions of their underlying training sets for multiple properties, including experimental data on the activity of generated and natural alpha amylases (a). A visualization of the ProtRL approach that can concomitantly sample large regions and maximize specific properties by iteratively receiving feedback from an oracle (b). This approach can guide the agent towards the region with highest fitness (c). ProtRL can optimize, among many other features, the predicted Specific Activity Performance Index (SAPI) for a given sequence.

Selected publications

[1] Stocco, F. *et al.* Guiding Generative Protein Language Models with Reinforcement Learning. Preprint at https://doi.org/10.48550/arXiv.2412.12979 (2025)
[2] Artigues, M. Suñé E. et al. SYNEVO: towards synthetic evolution of biomolecules via aligning protein language models to biological hardware. [Manuscript in preparation] (2025)
[3] Merlicek, L. P. *et al.* AI.zymes – A modular platform for evolutionary enzyme design. Preprint at https://doi.org/10.1101/2025.01.18.633707 (2025)

Engineering Limonene Epoxide Hydrolases for the Transformations of Chiral Oxetanes

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Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences

Enzymes are key drivers for the development of synthetic biology and widely utilized as catalysts in industrial sectors including pharmaceuticals, healthcare, energy, materials, foods and environmental science. However, the types of natural enzyme-catalyzed reactions are relatively limited. Breaking through the existing catalytic reaction types and endowing enzymes with new catalytic activities to synthesize useful novel functional molecules remains a significant challenge. Rational enzyme design has been proven to be an effective strategy for the biocatalysis of new reactions and synthesis of new molecules.[1,2] Herein we present our recent work on the development of biosynthesis and transformation of chiral oxetanes,[3,4] a class of four-membered cyclic ethers that possess unique chemical and biological activities. Stereoselective protein engineering of Limonene Epoxide Hydrolases were conducted to access both (*R*)- and (*S*)-configured oxetanse via desymmetrization or kinetic resolution (Figure 1). The variants display high stereoselectivity and activity, yielding a broad range of functional chiral oxetanes. Our approaches involve combinatorial active-site saturation test/iterative saturation mutagenesis (CAST/ISM), single-code saturation mutagenesis (SCSM) and triple-code saturation mutagenesis (TCSM) strategies, which were demonstrated to be highly efficient and effective in the biosynthesis chiral oxetanes.

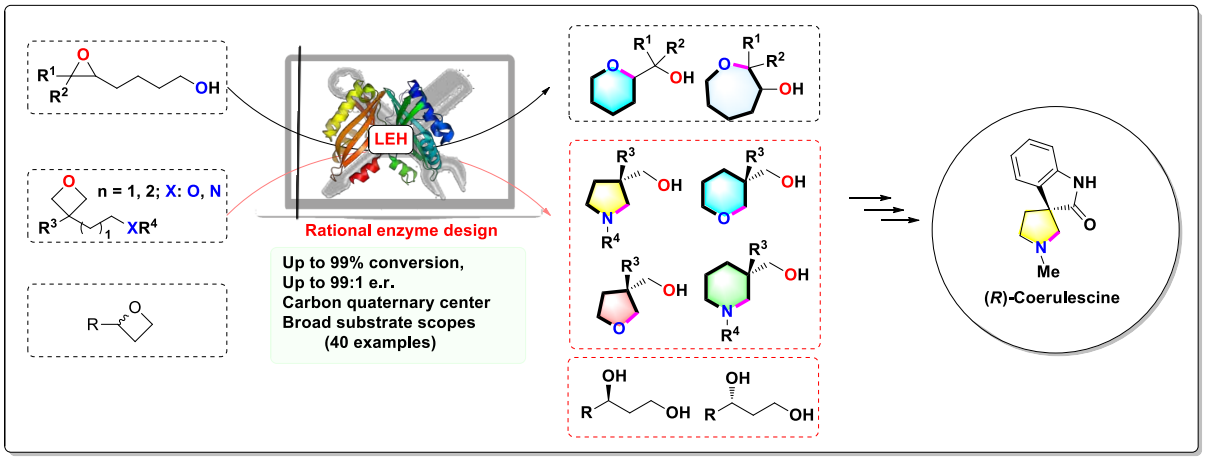

Figure 1 Rational enzyme design enables the synthesis of chiral oxetanes and 1,3-diols

Selected publications

[1] M.T. Reetz, G. Qu, Z. Sun, *Nat. Synth*, **2024**, 3, 19-32.
[2] B. Yuan, D. Yang, G, Qu. N, Turner, Z, Sun. *Chem. Soc. Rev.,* **2024**, 53, 227-262.
[3] J. Li, G. Qu, X. Li, Y. Tian, C. Cui, F. Zhang, W. Zhang, J. Ma, M.T. Reetz, Z. Sun, *Nat. Commun.*, **2022**, 13:7813.
[4] J. Li, B. Yuan, C. Li, Z. Zhao, J. Guo, P. Zhang, G. Qu, Z. Sun, *Angew. Chem. Int. Ed*, **2024**, e202411326.

Engineering a Light-responsive Synthase for Improved Optogenetic Control of Cyclic-di-GMP Dynamics

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The use of biofilm-mediated bioprocesses for biotechnological applications such as biocatalysis and bioremediation are being explored due to the easier process control of immobilized biofilms and the increased tolerance to physicochemical stresses conferred by the biofilm matrix. To utilise these beneficial biofilms effectively, it is necessary to engineer a controllable biofilm so that an optimal biofilm thickness can be maintained in these engineered systems. This can be achieved with optogenetic tools that target the intracellular dynamics of a ubiquitous second messenger, bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP), that plays a vital role in mediating the switch between the motile and sessile modes of life in bacteria. However, to control intracellular c-di-GMP dynamics with high spatiotemporal resolution, it is necessary to engineer the repertoire of optogenetic proteins currently available to improve their activity and photosensitivity. Therefore, in this study, a three-step directed evolution approach was used to engineer a near-infrared (NIR) light-responsive diguanylate cyclase (DGC), BphS, to increase its activity under NIR light. After two rounds of directed evolution, an improved variant, BphS-13, with approximately 13 times greater activity than the wild-type BphS was obtained. The engineered BphS-13 was able to synthesise much greater amounts of c-di-GMP than the wild-type BphS after just 16 h of exposure to NIR light and its ability to significantly improve biofilm response to NIR light was further demonstrated in a flow cell setup. Overall, this study demonstrates the promising application of directed evolution methods in improving and expanding the repertoire of tools available in the field of optogenetics.

Optimisation of a Periplasmic Binding Protein (PBP) based Artificial Metalloenzyme (ArM) for Biocatalysis

Seán A. Thompson [a,b], Alex H. Miller [a], Elena V. Blagova [b], Gideon J. Grogan* [b] and Anne-K. Duhme-Klair* [a]

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Artificial Metalloenzymes (ArMs) combine the broad reaction scope and versatility of organometallic catalysts with the selectivity and biocompatibility of proteins.[1] ArMs are produced by anchoring synthetic metal-based catalysts in a protein scaffold via key methods including covalent, dative, and supramolecular binding. This, in turn, enhances various properties of the synthetic catalyst through interactions with the protein which acts as a highly defined secondary coordination sphere.[2] The ArM design developed in the Duhme-Klair group utilises periplasmic binding proteins (PBPs) involved in the iron-uptake pathway of Gram-negative bacteria. By incorporating a synthetic iridium transfer hydrogenation catalyst inside a PBP by attaching it to a natural iron chelator, azotochelin, an imine reductase ArM was obtained, which was capable of redox-reversible assembly.[3] The initial ArM design saw a moderate catalytic rate and selectivity; however, by employing an analogous thermophilic protein, the overall stability of the ArM towards organic solvents was improved, along with the catalytic activity increasing 6-fold. Additionally, the overall selectivity of the ArM was greatly improved by 40% by altering the design of the synthetic catalyst. Chiral ligands with different configurations were synthesised and anchored inside the ArM. The protein scaffold further enhanced the selectivity of these catalysts, improving the overall ArM performance towards the reduction of 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline to (R/S)-salsolidine and added the ability to control the major enantiomer of the product. Lastly, this system was applied to produce crosslinked ArM agglomerates that are capable of redox-triggered cofactor release and subsequent recharging with alternative cofactors for diverse selectivity.

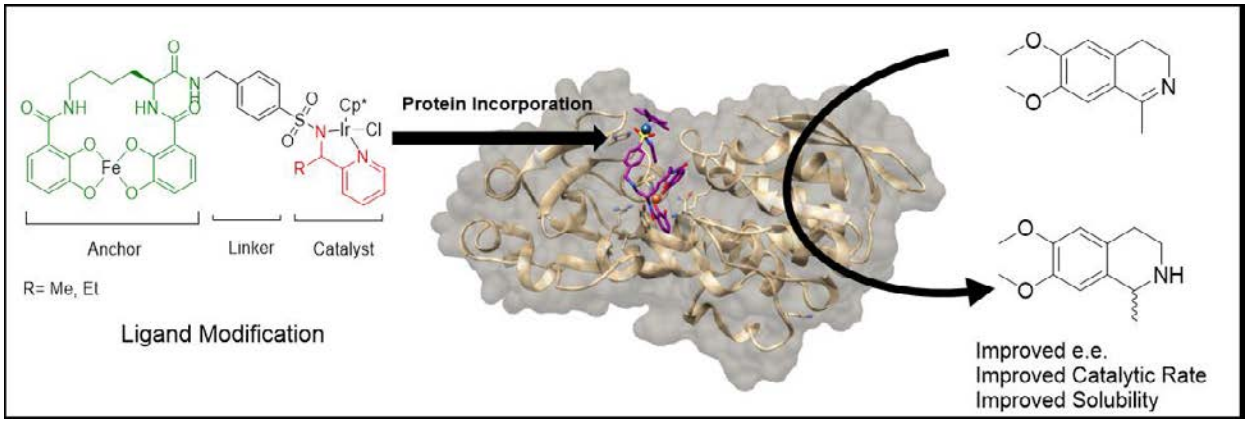

Figure 1:Modifaction and incorporation of a synthetic catalyst in a PBP protein and the catalytic reduction of a prochiral imine by the ArM

[1] F. Schwizer, Y. Okamoto, T. Heinisch, Y. Gu, M. M. Pellizzoni, V. Lebrun, R. Reuter, V. Köhler, J. C. Lewis and T. R. Ward, *Chemical Reviews*, **2018**, 118, 142-231.
[2] T. Himiyama and Y. Okamoto, *Molecules*, **2020**, 25.
[3] D. J. Raines, J. E. Clarke, E. V. Blagova, E. J. Dodson, K. S. Wilson and A.-K. Duhme-Klair, *Nature Catalysis*, **2018**, 1, 680-688.

Genetic Code Expansion with Novel Metal-Binding Unnatural Amino Acids for the Development of Artificial Metalloenzymes

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Artificial metalloenzymes (ArMs) have the potential to provide highly-selective, new-to-nature, chemical synthesis pathways for challenging transformations in aqueous environments.[1] To date, ArMs development has largely relied on bioconjugation strategies to install pre-formed transition metal complex catalysts within a protein scaffold.[2] However, the incorporation of metal-binding unnatural amino acids directly within a protein through genetic code expansion (GCE) presents several significant advantages, such as specific control over the incorporation site with complete selectivity, and enabling directed evolution.

This poster will present our groups recent work in this area, covering the synthesis of non-cannonical amino acids (ncAAs) with metal-binding functionalities, including bipyridyl alanine (**BpyAla**) and a pyridyl triazine ncAA (**Trz**),[3] and their subsequent incorporation within the human steroid carrier protein (SCP-2L) using GCE. The resulting artificial apoproteins are capable of selectively binding abiotic metal co-factors for a diverse range of applications, including imaging agents and enantioselective Lewis-acid catalysis.[4]

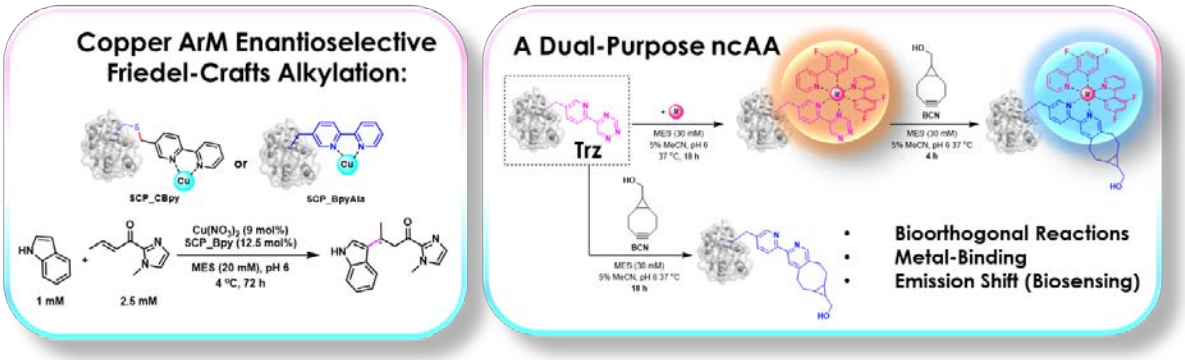

Figure 1. Graphical abstracts for some of the GCE work presented in this poster: (Left) Enantioselective Friedel-Crafts alkylation with **Cu@BpyAla**, and (Right) Inverse Electron-Demand Diels-Alder cycloaddition with **Ir@Trz**.

Selected publications

[1] H. J. Davis, T. R. Ward, *ACS Cent. Sci.,* **2019**, *5*, 1120-1136.
[2] A. G. Jarvis, *Curr. Opin. Chem. Biol.,* **2020**, *58*, 63-71.
[3] G. J. Day, A. V. Zaytsev, R. C. Brewster, V. N. Kozhevnikov, A. G. Jarvis, *Angew. Chem. - Int. Ed.,* **2024**, e202413073.
[4] E. Klemencic, R. C. Brewster, H. S. Ali, J. M. Richardson, A. G. Jarvis, *Catal. Sci. Tech.,* **2024**, *14*, 1622-1632.

A novel atom transfer radical polymerization initiation activity of galactose oxidase

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Department of Chemistry and Centre for Synthetic Biology, Technical University of Darmstadt

In nature, the enzyme galactose oxidase (GalOx) catalyzes the oxidation of *D*-galactose into *D*-galacto-hexodialdose with formation of hydrogen peroxide. Here, we report a novel activity of GalOx as ATRP-initiase. Usually, ATRP (Atom Transfer Radical Polymerization) is catalyzed by transition metal complexes, which are toxic, environmentally unfavorable and can contaminate the products. These factors limit the application of ATRP derived polymers. We found that GalOx can initiate ATRP, a reactivity previously known for heme-metalloenzymes and laccases. To screen reaction conditions and engineered variants of GalOx for their polymerization activity, we developed an assay that is based on the copolymerization of dimethylacrylamide (DMA) with coumarine acrylamide (CA), a molecule that exhibits weak fluorescence in it's monomer form and intense fluorescence, when polymerized.

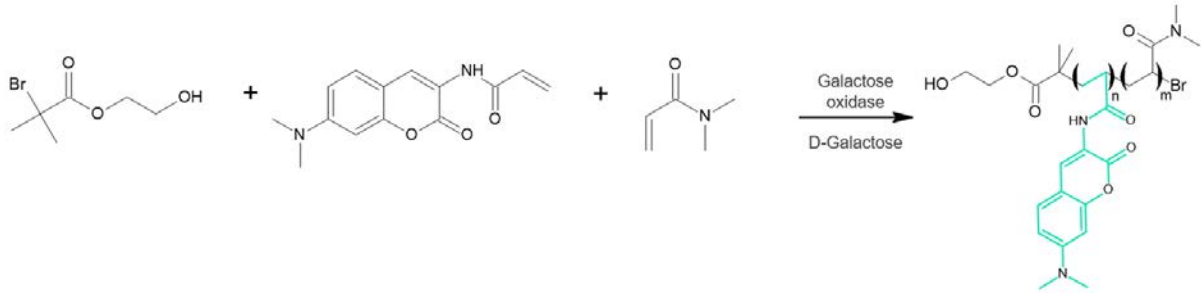

Figure 1. Reaction scheme of an assay for screening of GalOx polymerization activity.

Figure 2. Mixture of DMA and CA monomers (on the left) and a poly(DMA-*co*-CA) copolymer (on the right) under UV-light.

Reactivity Studies of Artificial Metalloenzyme MspA-[Fe^{III}(TAML)]$^-$ Using Nanopore Technology

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To guide the design of more efficient biocatalysts, understanding the kinetic mechanisms of enzymes is essential to control their intricate structure-function relationship. A key challenge in this process is the identification of transient chemical species, which often remain elusive in ensemble measurements due to their short lifetimes. To overcome this limitation, we harness nanopore technology to observe intermediates of a biomimetic peroxidase complex in oxidation reactions at the single-molecule level. We designed an artificial metalloenzyme (ArM) by conjugating the peroxidase mimic [Fe^{III}(TAML)]$^-$ to the protein nanopore MspA (Figure). The resulting ArM is inserted into a lipid bilayer to enable nanopore detection in the presence of organic substrate molecules and sacrificial oxidants. By monitoring changes in flow of ionic through-pore current under an applied voltage, this approach enables the direct real-time observation of enzyme catalysis at the single-molecule level, revealing lifetimes of reactive intermediates. This study will provide insights on interaction of substrate and reactive intermediates, providing stepwise monitoring of the catalytic cycle.
Overall, nanopore technology offers a powerful platform for elucidating reaction mechanisms and advancing our understanding of enzyme function.

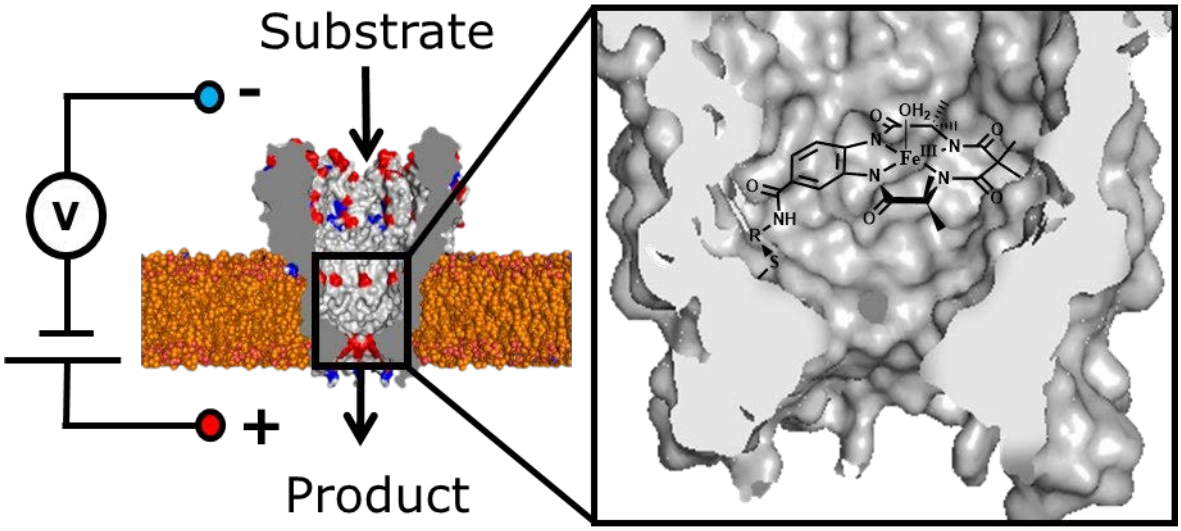

Figure: A nanopore protein modified with [Fe^{III}(TAML)]$^-$ is inserted in a lipid bilayer membrane, across which a voltage is applied. The formation of chemical intermediates in the nanopore can be monitored by the changes in current measured over the nanopore system.

A Dual Cofactor Artificial Metalloenzyme for Synergistic Asymmetric Catalysis

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Abstract:

Artificial metalloenzymes (ArMs) offer promising tools to integrate abiotic activities into biocatalysis. Although synergistic catalysis has been widely applied in small-molecule catalysis, it has rarely been implemented in biocatalysis—including ArMs—in part due to the challenge of engineering cofactors that work synergistically within a single protein scaffold. Here we show that the biotin-streptavidin technology can be exploited to anchor two distinct synthetic cofactors in the neighbouring binding sites of homotetrameric streptavidin. Capitalizing on the micromolar affinity of the Strep-tag II peptide for streptavidin, we combined a catalytic peptide for iminium catalysis with a biotinylated nickel-based Lewis acid within streptavidin. The resulting synergistic ArM catalyzed the asymmetric Michael addition of ketones to enals, and its catalytic performance was improved by chemo-genetic means: i) biotinylated cofactor engineering, ii) automated solid-phase peptide synthesis, and iii) iterative saturation mutagenesis of streptavidin. Mechanistic studies revealed the molecular basis of the synergistic mechanism, as well as the role of key mutations in stabilizing the cofactors and active site geometry. Accordingly, two complementary catalytic systems have been developed for enantiodivergent synthesis of small molecule building blocks. This work outlines a general approach for the development of synergistic ArMs, as well as the utility of peptide cofactors in ArMs.

Computational Strategies for Engineering Non-Heme and Heme Iron-Dependent Enzymes

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Enzymes are macromolecules that function as biological catalysts, facilitating over 5,000 biochemical reactions through enzymatic catalysis. These reactions occur under relatively mild conditions and exhibit high efficiency and selectivity. Iron, the most abundant transition metal in biological systems, serves as a cofactor for many enzymes. Based on the structure of their active sites, iron-dependent enzymes can be classified into different types.

In this study, we conducted an *in-silico* investigation of two non-heme iron-dependent dioxygenases, *Novosphingobium aromaticivoran* (NOV1) and *Caulobacter segnis* Dioxygenase (CsO2) [1], both of which have similar and relatively narrow substrate scopes. Our aim is to perform computational approaches to identify the potential mutants capable of processing substrates not recognized by wild-type NOV1 and CsO2. Firstly, we utilized the deep learning-based protein design tool, proteinMPNN [2], to redesign the enzymes sequences, enhancing their ligand binding affinity and catalytic activity. Secondly, we employed the Rosetta protein design suite, a physics-based approach, to redesign NOV1 and CsO2 active sites for stabilizing reaction transition states [3]. This process involved: i) identifying reaction transition states through quantum mechanics calculations, ii) constructing a theozyme based on the transition states and key residues, and iii) subsequently using RosettaMatch algorithm to refine the enzyme active site.

CYP153A6, a heme iron dependent monooxygenase that catalyzes allylic hydroxylation with highly regioselectivity [4]. However, due to the inherent structural flexibility of cytochrome P450 enzymes and the presence of multiple substrate and water tunnels, we applied an ensemble docking strategy to study this enzyme's molecular recognition. We then identified and resigned key hotspot residues within these tunnels. Through tunnel engineering, CYP153A6 can be optimized to improve catalytic efficiency and broaden its substrate scope.

We hope our computational strategies can provide potential applicability to general enzymatic systems for enhancing catalytic activity and expanding substrate specificity.

Acknowledgment
This work was supported by the European Union's Horizon Europe research and innovation program under the Marie Skłodowska-Curie grant agreement No. 101073546 (MSCA Doctoral Network Metal-containing Radical Enzymes – MetRaZymes), and grants from MIUR - "Progetto Eccellenza 2023 – 2027".

Selected publications

[1] P. Cannazza, M. Rabuffetti, S. Donzella, V. De Vitis, M. L. Contente, M. D. de Oliveira, M. C. de Mattos, F. G. Barbosa, R. P. de Souza Oliveira, A. Pinto, F. Molinari, *AMB Express*, **2022**, 12 (1), 48.

[2] J. Dauparas, I. Anishchenko, N. Bennett, H. Bai, R. J. Ragotte, L. F. Milles, B. I. Wicky, A. Courbet, R. J. de Haas, N. Bethel, P. J. Leung, *Science*, **2022**, 378 (6615), 49–56.

[3] F. Richter, A. Leaver-Fay, S. D. Khare, S. Bjelic, D. Baker, *PLoS One*, **2011**, 6 (5), e19230.

[4] V. De Vitis, P. Cannazza, L. Mattio, D. Romano, A. Pinto, F. Molinari, T. Laurenzi, I. Eberini, M. L. Contente, *ChemBioChem*, **2023**, 24 (21), e202300477.

Synergistic photobiocatalysis for enantioselective triple radical sorting

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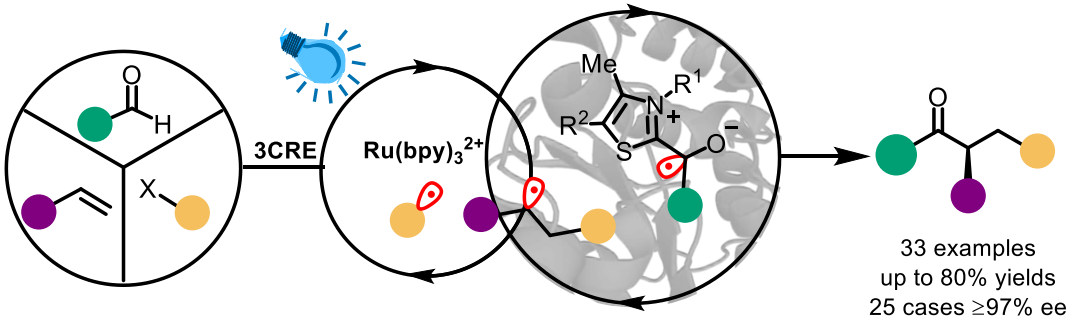
Harnessing enzymes for non-natural asymmetric transformations, which are challenging for traditional chemocatalysis, holds great significance[1-3]. Despite the notable benefit of multicomponent reactions in broadening chemical space and enhancing molecular complexity[4], achieving enzymatic conversion of three variable substrates into enantioenriched compounds via a single reaction has remained rare.[5] This limitation primarily arises because an enzyme's active site cannot concurrently tame multiple substrates or intermediates, especially in cases involving multiple radical intermediates[6]. Recently, chemocatalytic radical sorting has emerged as an enabling strategy for a variety of appealing reactions[7]. However, directing such processes in an enantioselective manner is highly challenging due to the inherent difficulty in the stereocontrol of radicals[8]. Herein, we repurpose a thiamine-dependent enzyme[9,10] through directed evolution and synergy with photoredox catalysis, to facilitate an unprecedented photobiocatalytic enantioselective three-component radical cross-coupling. Mechanistic investigations have provided crucial insights into how this dual photo-/enzyme system precisely directs the three distinct radicals involved in the transformation, unlocking new enzyme reactivity and enabling access to a variety of enantioenriched carbonyl compounds. Our approach has achieved exceptional stereoselectivity, with 25 out of 33 examples achieving ≥97% enantiomeric excess. This work not only expands the repertoire of biocatalysis but also provides a unique strategy for sorting multiple radicals complementing existing chemical tools.



key challenges: an enzyme for three-component reactions; native enzyme reactivity; chemo-/enantio-selectivities involving triple radical.

Figure Photoenzymatic three-component radical cross-couplings.

Selected publications

[1] Chen, K., Arnold, F. H. *Nat. Catal*. **2020**, *3*, 203-213.
[2] Reetz, M. T. *J. Am. Chem. Soc*. **2013**, *135*, 12480-12496.
[3] R. Buller, S. Lutz, R. J. Kazlauskas, R. Snajdrova, J. C. Moore, U. T. Bornscheuer. *Science*. **2023**, *382*, eadh8615.
[4] Dömling, A., Wang, W., Wang, K. *Chem. Rev*. **2012**, *112*, 3083-3135.
[5] Thorpe, T. W., Marshall, J. R., Turner, N. J. *J. Am. Chem. Soc.* **2024**, *146*, 7876-7884.
[6] Leifert, D., Studer, A. *Angew. Chem. Int. Ed.* **2020**, *59*, 74-108.
[7] Wang, J. Z., Lyon, W. L., MacMillan, D. W. C. *Nature* **2024**, *628*, 104-109.
[8] Shovan Mondal, Frédéric Dumur, Didier Gigmes, Mukund P. Sibi, Michèle P. Bertrand, Malek Nechab. *Chem. Rev.* **2022**, *122*, 5842-5976.
[9] Breslow, R. *J. Am. Chem. Soc.* **1958**, *80*, 3719-3726.
[10] Gabriel S. Brandt, Natalia Nemeria, Sumit Chakraborty, Michael J. McLeish, Alejandra Yep, George L. Kenyon, Gregory A. Petsko, Frank Jordan, Dagmar Ringe Biochem. **2008**, *47*, 7734-7743.

Repurposing ThDP-dependent Enzyme for Enantioselective Radical Acylation

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Nanjing University

Thiamine diphosphate (ThDP)-dependent enzymes are ubiquitously distributed in all domains of life. Typically, they catalyse a variety of C–C bond-forming (or bond-breaking) reactions through nucleophilic addition (or the reverse) of the umpolung carbanion-enamine (Breslow intermediate) with different electrophiles. These well-characterized biocatalysts have inspired the development of *N*-heterocyclic carbenes (NHCs). Although a few early studies revealed that pyruvate: ferredoxin oxidoreductase (PFOR)-catalysed acetylation of coenzyme A (CoA) through single-electron oxidation pathway, the ability of ThDP-dependent enzymes in stereocontrolled radical chemistry remains unknown.

The Huang group pioneered a dual-catalyst system consisting of a ThDP-dependent enzyme and an organophotoredox catalyst to achieve an unnatural enantioselective radical acylation (Figure). In this system, a ThDP-dependent BAL is repurposed to be an unprecedented radical acyl transferase (RAT) by merging green-light induced single-electron transfer and directed evolution, catalysing acylation of carboxylic-acid-derived prochiral radicals with a plethora of aldehydes in an enantioselective fashion. This work introduces a new class of radical enzymes and a distinct acyl radical-transfer mechanism, which not only expands the toolbox of biocatalysts but also represents an alternative superior to chemo-NHC catalysts for the stereocontrol of prochiral radicals.

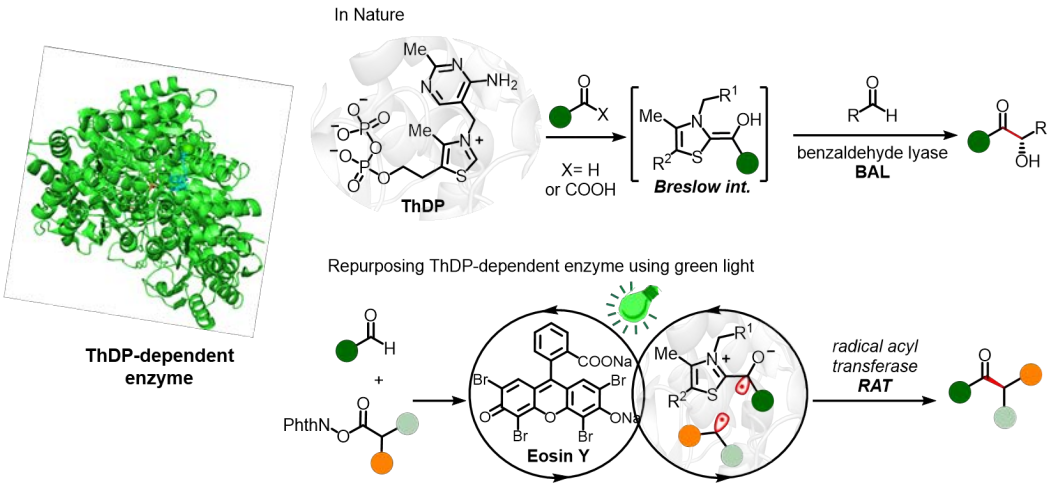

Figure The natural reactivity catalysed by ThDP-dependent benzaldehyde lyase and repurpose benzaldehyde lyase into an unnatural radical acyl transferase using green light.

Selected publications

[1] Xu, Y.; Chen, H.; Yu, L.; Peng, X.; Zhang, J.; Xing, Z.; Bao, Y.; Liu, A.; Zhao, Y.; Tian, C.; Liang, Y.; Huang, X., A light-driven enzymatic enantioselective radical acylation. *Nature* **2024**, *625* (7993), 74-78.

Stereoselective Radical Reactions Enabled by Metalloenzyme Design and evolution

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Department of Chemistry and Biochemistry, University of California Santa Barbara

Radical reactions have found widespread applications in both small molecule and macromolecule synthesis. However, it remains challenging to control the stereochemistry of radical transformations and to discover new modes of radical catalysis. Combining synthetic chemistry, enzymology and protein engineering, our group has been developing stereoselective free radical processes through the design and evolution of metalloenzymes. In our previous work, by capitalizing on the innate redox properties of first-row transition-metal cofactors, we repurposed and evolved natural metalloproteins to catalyze unnatural radical reactions in a stereocontrolled fashion. Through a metalloenzyme-catalyzed halogen atom transfer mechanism (XAT, X = F, Cl, Br and I), a range of radical C–C, C–Br, and C–F bond forming reactions proceeded with excellent total turnover numbers (up to 20,000) and outstanding stereocontrol. In this talk, I will present the latest work from our group over the past year in the area of metalloenzyme design and engineering.

Repurposing hemoproteins for metal-catalyzed H atom transfer in asymmetric radical biocatalysis

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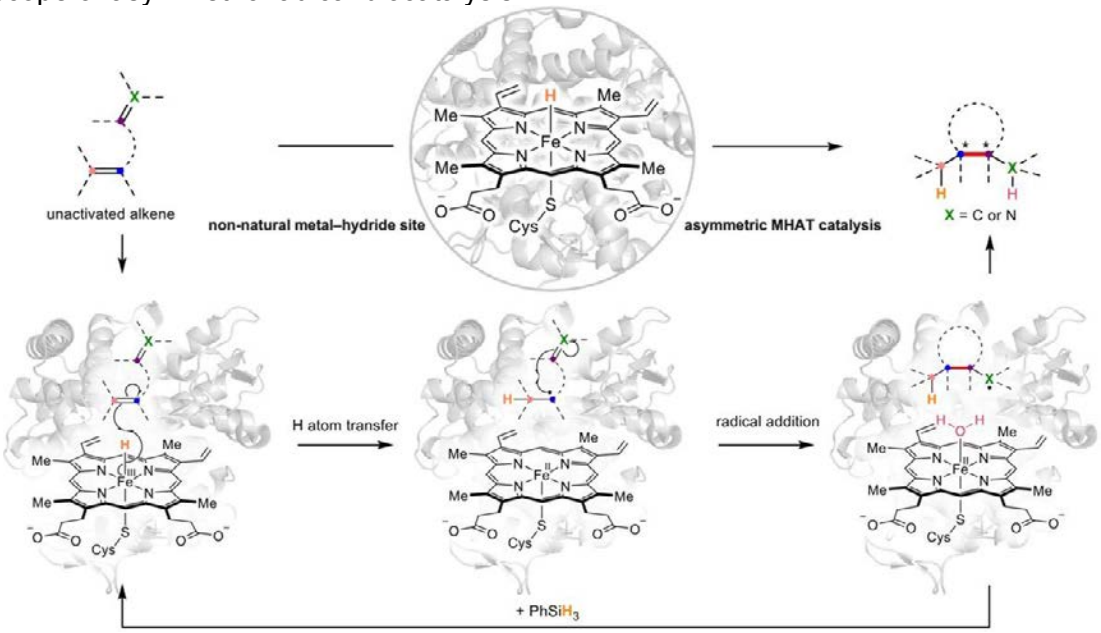
Transition metal–hydrides have been widely exploited in homogenous catalysis for hydrofunctionalization of unsaturated moieties, including carbonyls, alkenes and alkynes[1,2]. As a complement to the well-established chemistry of these complexes involving heterolytic metal–hydride bond cleavage, metal–hydride hydrogen atom transfer (MHAT) has attracted increased interest, as it offers a promising strategy for radical hydrofunctionalization of unactivated alkenes[3] thus enabling late-stage diversification of complex molecules[4,5]. However, due the weak interactions between the prochiral organic radical species and the enantiopure metal catalyst[6], achieving asymmetric MHAT[7] remains challenging. Herein, we report our efforts to repurpose cytochrome P450 enzymes to catalyze asymmetric MHAT, a new-to-nature reaction. Directed evolution of the well-studied $P450_{BM3}$ (CYP102A1) enzyme led to the identification of a triple mutant that catalyzes asymmetric MHAT radical cyclization of unactivated alkenes to afford diverse cyclic compounds, including pyrrolidines and piperidines, in up to a 98:2 enantiomeric ratio under aerobic whole cell conditions. In addition to electron-deficient alkenes, alternative radical acceptors–including hydrazone, oxime and nitrile–were accepted by the repurposed $P450_{BM3}$ to afford the corresponding enantioenriched cyclization products. Mechanistic investigations support an MHAT mechanism proceeding via homolytic cleavage of a fleeting iron(III)–hydride species[3,7]. Directed evolution using CYP119 as hemoprotein led to the identification of a stereocomplementary MHATase, highlighting the potential of repurposed hemoproteins for MHAT biocatalysis. Our study showcases the feasibility of integrating abiotic metal–hydride activity into native metalloenzymes to expand the scope of asymmetric radical biocatalysis.



References:

[1] Maurizio Peruzzini, R. P. *Recent advances in hydride chemistry*. 1 edn, (Elsevier Science, 2002).
[2] Norton, J. R. Introduction: Metal hydrides. *Chem. Rev.* **116**, 8315-8317 (2016).

[3] Crossley, S. W. M., Obradors, C., Martinez, R. M. & Shenvi, R. A. Mn-, Fe-, and Co-catalyzed radical hydrofunctionalizations of olefins. *Chem. Rev.* **116**, 8912-9000 (2016).
[4] Wu, J. & Ma, Z. Metal-hydride hydrogen atom transfer (MHAT) reactions in natural product synthesis. *Org. Chem. Front.* **8**, 7050-7076 (2021).
[5] Green, S. A., Crossley, S. W. M., Matos, J. L. M., Vásquez-Céspedes, S., Shevick, S. L. & Shenvi, R. A. The high chemofidelity of metal-catalyzed hydrogen atom transfer. *Acc. Chem. Res.* **51**, 2628-2640 (2018).
[6] Sibi, M. P., Manyem, S. & Zimmerman, J. Enantioselective radical processes. *Chem. Rev.* **103**, 3263-3296 (2003).
[7] Shevick, S. L., Wilson, C. V., Kotesova, S., Kim, D., Holland, P. L. & Shenvi, R. A. Catalytic hydrogen atom transfer to alkenes: a roadmap for metal hydrides and radicals. *Chem. Sci.* **11**, 12401-12422 (2020).

Strategies for Robust Electroenzymatic Integration: Electricity-driven enzymatic dynamic kinetic oxidation

Beibei Zhao, Xiaoqiang Huang
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Electrochemical synthesis has emerged as a rapidly advancing innovative tool in chemical synthesis, offering distinct advantages including cost-effectiveness, tunable electrode potentials, and sustainability. The current integration of electrochemical synthesis with enzyme catalyzed primarily encompasses two approaches: (1) electrochemical regeneration of enzyme cofactors to facilitate the enzyme's native catalytic activity[1], and (2) electrochemically generated intermediates or substrates participating in subsequent enzyme-catalyzed native transformations (electrochemical/enzyme cascades)[2,3,4]. In contrast, electricity-driven new enzyme reactivity remains elusive.
Here we report the reshaping of thiamine-dependent enzymes with ferrocene-mediated electrocatalysis to unlock an unnatural dynamic kinetic oxidation of racemic aldehydes. This work represents a unique electroenzymatic system that unlocks new-to-nature enzyme reactivity, demonstrating the potential of combining enzymes with electricity for innovative catalysis design and practical applications.

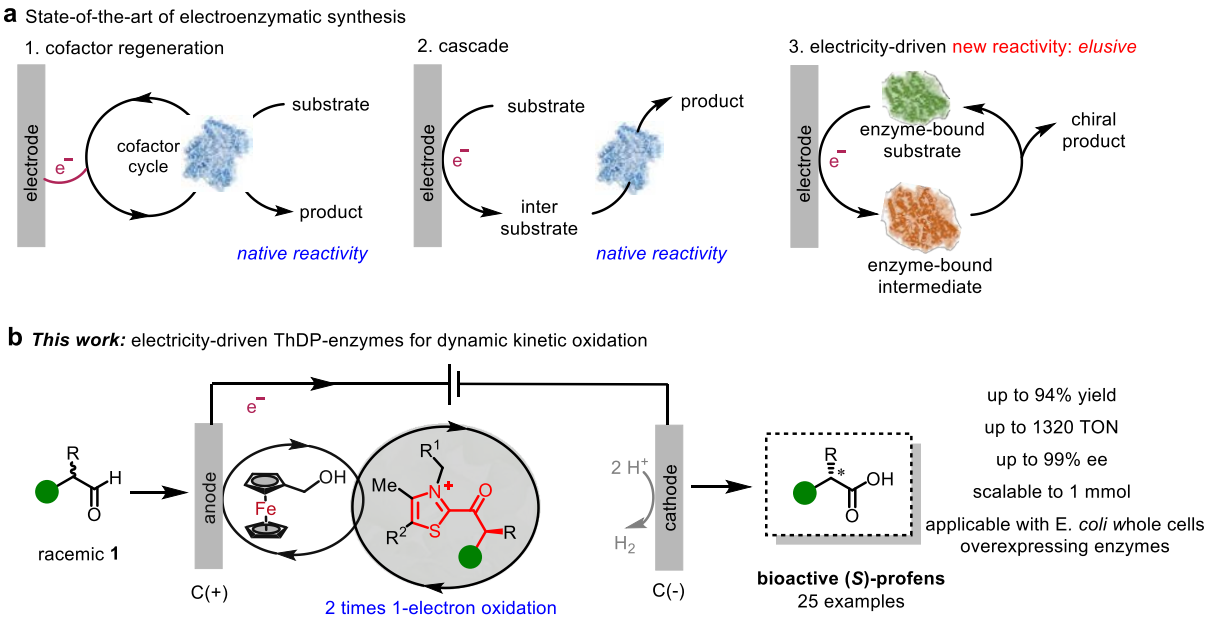

Figure *Harnessing electricity to unlock ThDP-dependent-enzyme-catalyzed dynamic kinetic oxidation.*

Selected publications

[1] Hollmann, F., Hofstetter, K., Habicher, T., Hauer, B. & Schmid, A. *J. Am. Chem. Soc.* **2005,** 127, 6540-6541.
[2] Chen, H., Tang, T., Malapit, C. A., Lee, Y. S., Prater, M. B., Weliwatte, N. S., Minteer, S. D. *J. Am. Chem. Soc.* **2022**, *144*, 4047-4056.
[3] Wu,R., Li, F., Cui, X., Li, Z., Ma, C., Jiang, H., Zhang, L., Zhang,Y.-H. P. J., Zhao, T., Zhang, Y., Li, Y., Chen, H., Zhu, Z. *Angew. Chem. Int. Ed.* **2023**, *62*, e202218387.
[4] Jayathilake, B. S., Bhattacharya, S., Vaidehi, N. &Narayanan, S. R., *Acc. Chem. Res.* **2019**, *52*, 676-685.

Unlocking Enamine Catalysis for Ketone Functionalization by Artificial Enzyme Design and Evolution

Zhi Zhou

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Developing novel enzymes for diverse asymmetric transformations to synthesize potential bioactive compounds, is therefore of high academic and industrial interest.[1] Absorbing catalytic modes from organocatalysis may be fruitful for designing new-to-nature enzymes with novel functions. Recently, researchers have developed several new-to-nature enzymes for iminium and enamine catalysis by repurposing natural enzymes and artificial enzymes. While most of these researches involve the use of α,β-unsaturated aldehydes or acetaldehyde. By contrast, expanding the repertoire of enzymes via aminocatalysis for ketone functionalization will be of great significance but so far remains rare.[2]

Here we report a newly designed artificial enzyme harboring a catalytic sencondary amine residue by covalent modification that catalyze the asymmetric Michael additions of cyclic ketones to nitroolefins via enamine activation with high efficiency and excellent stereoselectivity. In the subsequent investigation, we successfully achieved the asymmetric Mannich and Aldol reaction by this type of artificial enzymes with promising results. Our work provides an efficient biocatalytic strategy for cyclic ketone functionalization, and highlights the usefulness of artificial enzymes for expanding the biocatalytic repertoire for non-natural reactions.[3]

Reactions implemented:
Michael Addition, Aldol Reaction, Mannich Reaction

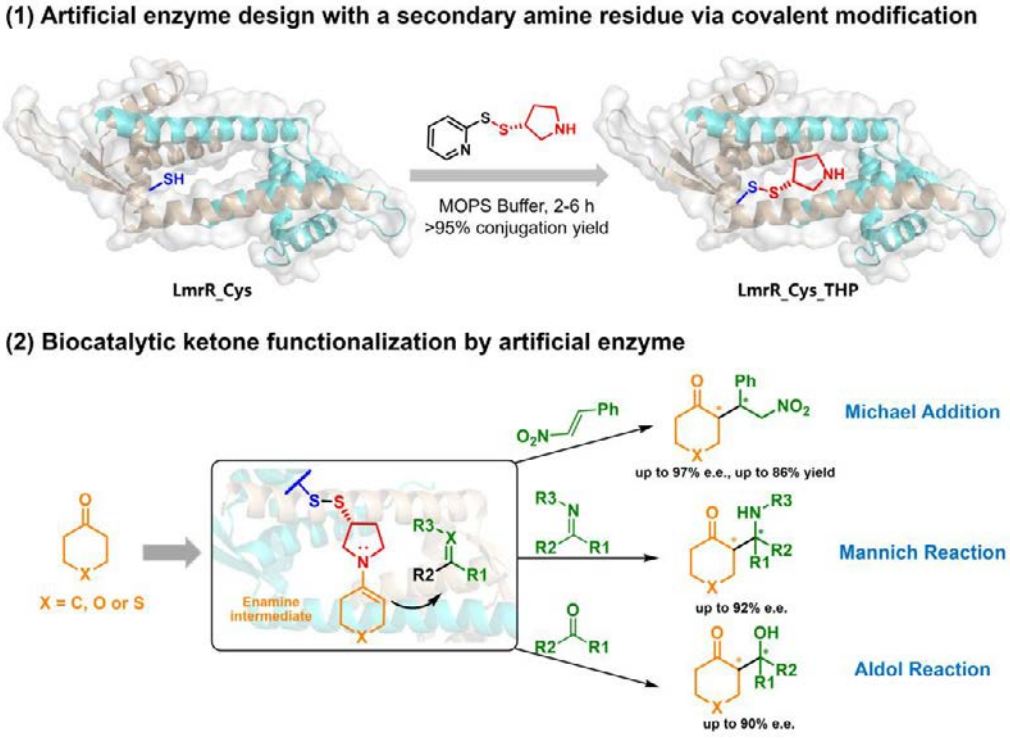

Figure. Enzymatic ketone functionalization reactions by a designer artificial enzyme harboring a catalytic pyrrolidine residue via enamine catalysis.

Selected publications

[1] Y. Yang, F. H. Arnold, *Acc. Chem. Res.* **2021**, *54*, 1209.
[2] X. Garrabou, R. Verez, D. Hilvert, *J. Am. Chem. Soc.* **2016**, *139*, 103.
[3] Z. Zhu, Q. Hu, Y. Fu, Y. Tong, Z. Zhou, *Angew. Chem. Int. Ed.* **2024**, *e202404312.*

Design and Evolution of Artificial Diels–Alderase by Covalent Incorporation of Artificial Secondary Amine Cofactor

Zhixi Zhu; Zeying Zhi; Zhi Zhou*

School of Life Sciences and Health Engineering, Jiangnan University, China

The Diels–Alder (D-A) reaction is a [4+2] cycloaddition process that occurs between conjugated dienes and substituted alkenes. As a pivotal method for constructing carbon frameworks, the D-A reaction has been widely employed in the synthesis of natural products. However, these natural D-A reactions typically depend on the precise recognition of specific substrates[1]. While previously developed artificial metalloenzymes have successfully achieved asymmetric D-A reactions, their mechanisms predominantly rely on the activation of Lewis acid properties by artificial cofactors such as ferrocyanide copper[2]. To date, the design of non-metallic artificial Diels–Alderase remains scarce.

Despite numerous examples of D-A reactions in organic chemistry via amine catalysis, limited analogous reactions catalyzed by amines in biocatalytic systems have been reported to date. Based on the artificially designed cofactor THP and the protein scaffold LmrR, our group engineered the artificial non-metallic enzyme LmrR_Cys_THP through covalent modification of a specific cysteine residue within LmrR. Using this enzyme, we previously demonstrated the asymmetric Michael addition reaction between cyclic ketones and nitroolefins[3]. Herein, we reported the first biocatalytic asymmetric D-A reaction of cyclic enones and substituted alkenes catalyzed by LmrR_Cys_THP via the cross dienamine intermediate by amino catalysis. Following directed evolution optimization, the exo-selective product was obtained with up to 95% enantiomeric excess (e.e.) and up to 90% yield. Our study highlights the usefulness of artificial enzymes for expanding the biocatalytic repertoire for non-natural reactions.

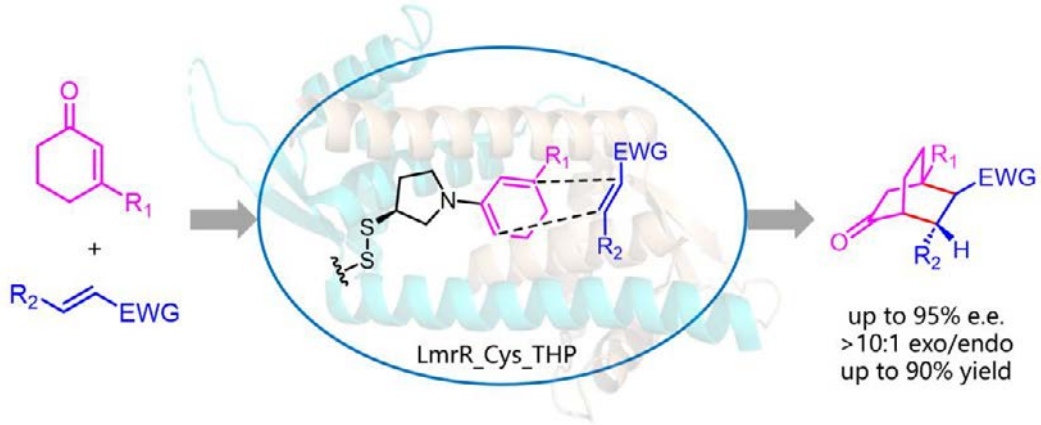

Figure. The asymmetric D–A reactions catalyzed by artificial enzyme

[1] L. Gao, Y. Zou, X. Liu, J. Yang, X. Du, J. Wang, X. Yu, J. Fan, M. Jiang, Y. Li, K. N. Houk, X. Lei, *Nat. Catal.* **2021**, 4, 1059-1069.
[2] J. Bos, F. Fusetti, A. J. M. Driessen, G. Roelfes, *Angew. Chem. Int. Ed.* **2012**, 51, 7472-7475.
[3] Z. Zhu, Q. Hu, Y. Fu, Y. Tong, Z. Zhou, *Angew. Chem. Int. Ed.* **2024**, 63, e202404312.