Ischia Advanced School of Organic Chemistry
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UNIVERSITÄT BERN

Enzymes in Hybrid Catalytic Systems

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Integrated catalysis

*The **combination** of biocatalysis and chemocatalysis can be more powerful than either technique alone.*

C. Heckmann

Chem. Eur. J. ***2021****, 27, 16616 – 16620*

What we try to do:

1. Biocatalysts immobilization → Increased stability and versatility
2. Integration of biocatalysis with chemical reactions → Compatibility
3. Flow chemistry set up → Modularity and easier transition

Immobilisation of the biocatalyst

Many options and variables:

1. Type of support and functionalization
2. Type of immobilization (covalent or not)
3. Type of chemistry

Increased Enzyme Stability

(Ir)reversible bond

Lower catalytic efficiency

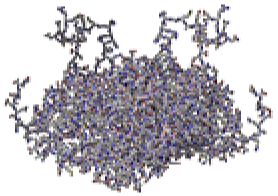
CapiPy

inseit

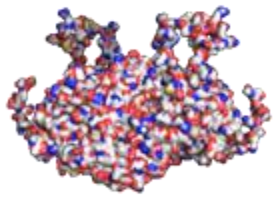
BIOCATALYST IMMOBILIZATION

GMQTQDYQALDRAHHLHPFTDFKALGEEGSRVVTHAEGVYI
HDSEGNRILDGMAGLWCVNLGYGRRELVEAATAQLEQLPY
YNTFFKTTHPPAVRLAEKLCDLAPAHINRVFFTGSGSEANDT
VLRMVRRYWALKGQPDKQWIIGRENAYHGSTLAGMSLGGM
APMHAQGGPCVPGIAHIRQPYWFGEGRDMSPEAFGQTCAE
ALEEKILELGEEKVAAFIAEPVQGAGGAIMPPESYWPAVKKV
LAKYDILLVADEVICGFGRLGEWFGSQHYGLEPDLMPIAKGL
SSGYLPIGGVLVGDRVAETLIEEGGEFFHGFTYSGHPTCAAV
ALKNLELLEAEGVVDRVRDDLGPYLAERWASLVDHPIVGEA
RSLGLMGALELVADKTTGQRFDKSLGAGNLCRDLCFANGLV
MRSVGDTMIISPPLVIRREEIDELVELARRALDETARQLTQVP
HTQEEPTA

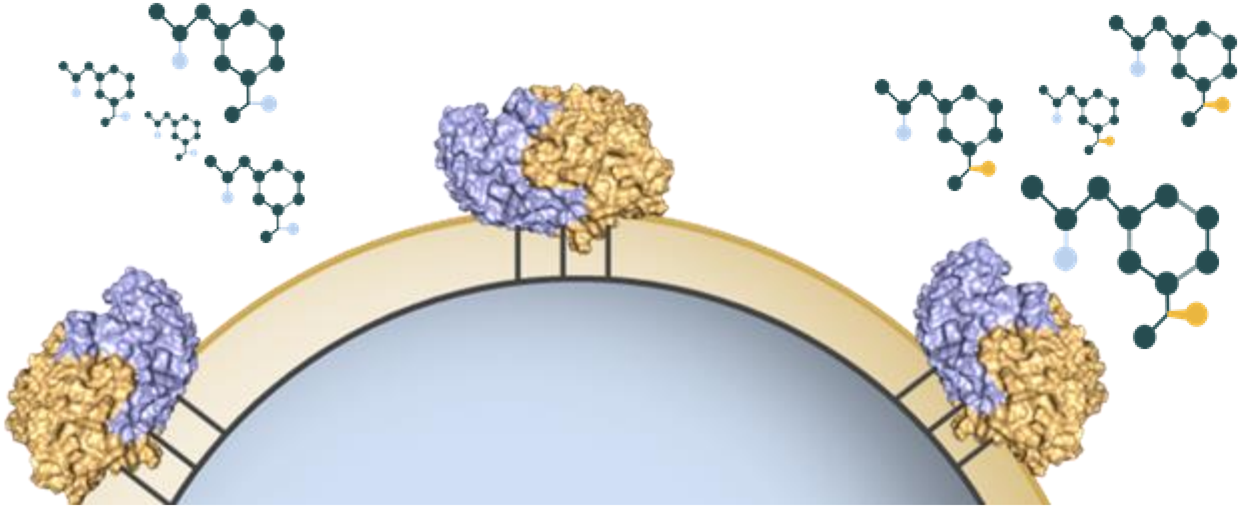
Sequence



Modelling

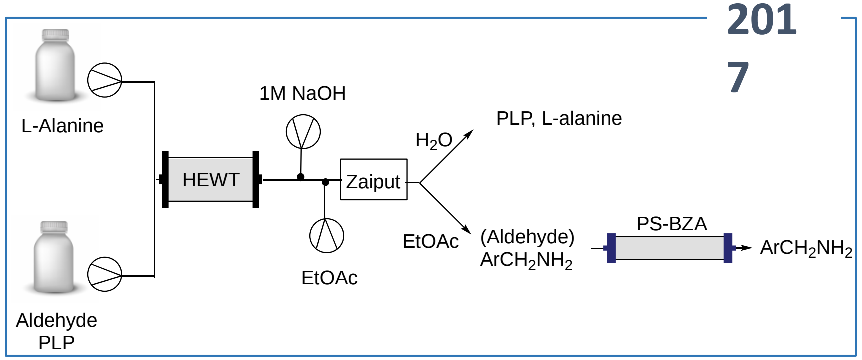


Simulation



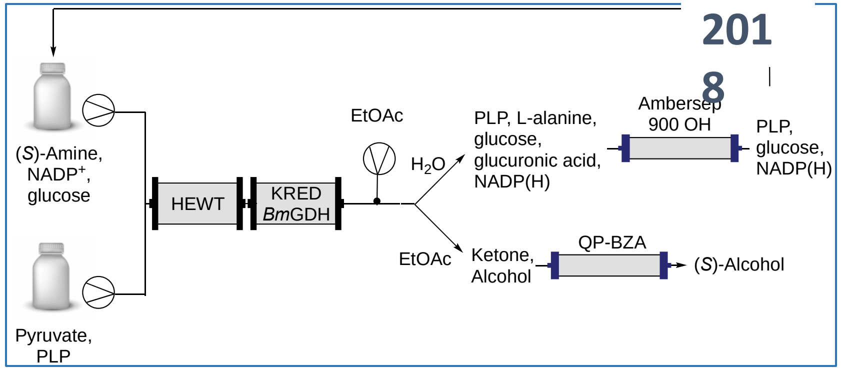
Immobilized enzymes in flow

2017



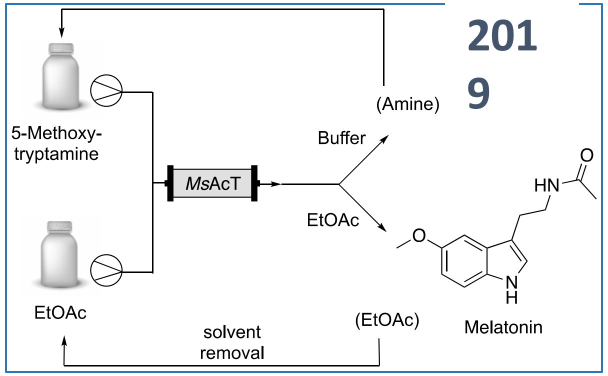
Green Chemistry **2017**, 19, 372-375

2018



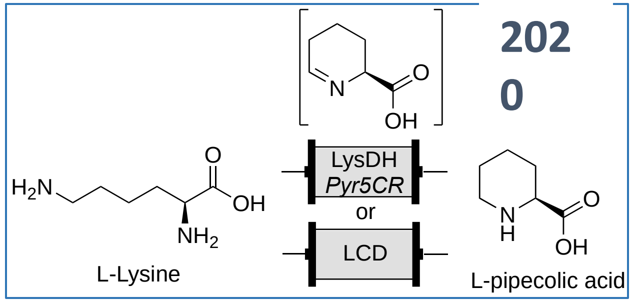
Nature Catalysis **2018**, 1, 452-459

2019



Green Chemistry **2019,** 21, 3263-3266

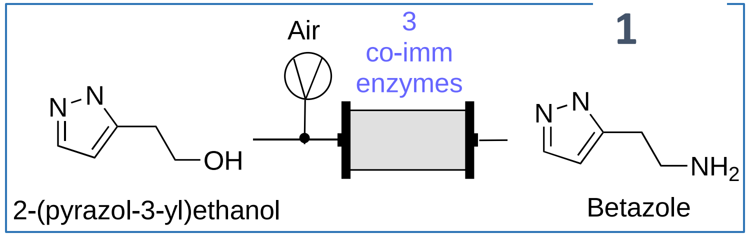
2020



Green Chemistry **2020,** 22, 5310- 5316

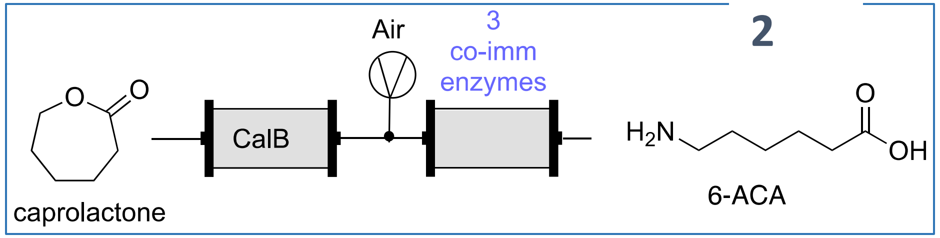
ChemCatChem **2024,** e202301671

2021



Green Chemistry **2021**, 23, 4595-4603

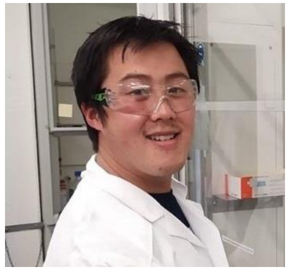
2022

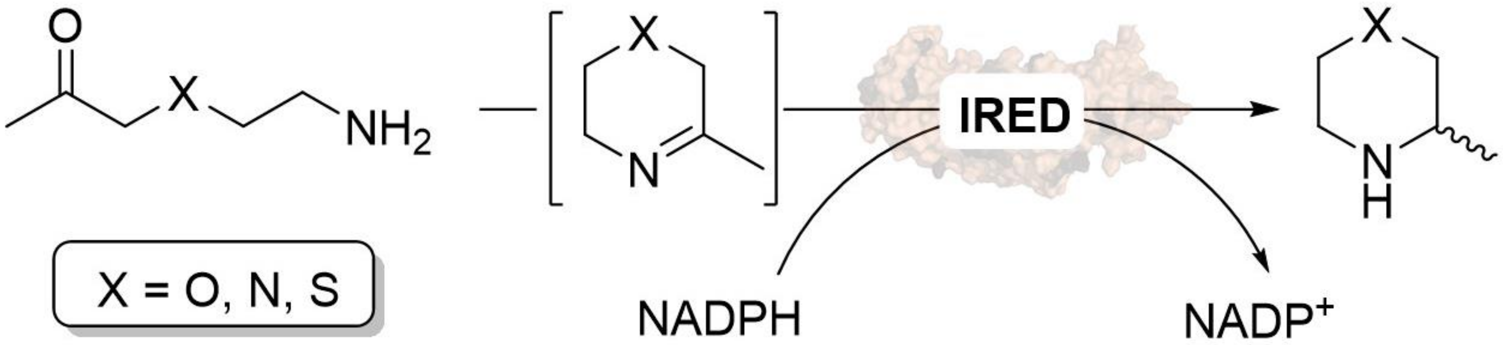


ChemSusChem **2022**, 16, e202200811

…purely biocatalytic cascades…

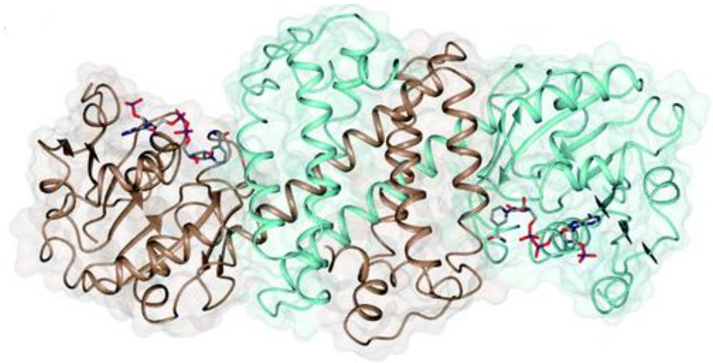
A recent example

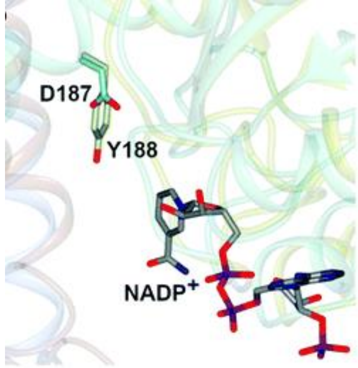


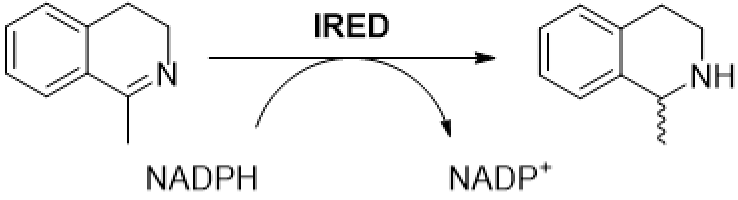


Manuscript in preparation **2024**

Imine reductases (IREDs)







- Dimeric proteins
- Easy heterologous expression in *E. coli*
- High enantioselectivity (>99%)
- High conversions (70-100% at 5 mM scale)

- Reduction of hydrophobic cyclic imines
- Cofactor-dependent enzymes
- Very poor enzyme immobilization (<5% efficiency)
- No flow reactions reported so far

Gilio *et al.* **2022**, *Chem. Sci.*,13: 4697-4713

Selection of biocatalysts

Enzyme	Enantioselectivity
IRED-1	(*S*)
IRED-2	(*S*) and (*R*)
IRED-3	(*S*)
IRED-4	putative (*S*)
IRED-5	putative (*R*)
IRED-6	(*R*)

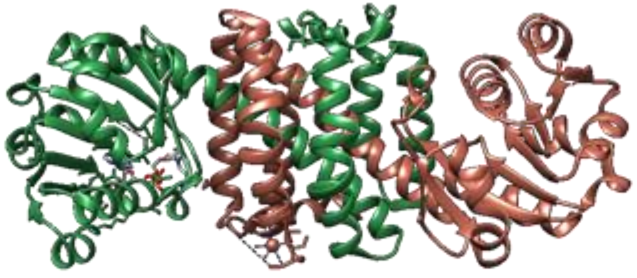
New enzymes!

IRED-4 → 71% sequence homology to IRED-2

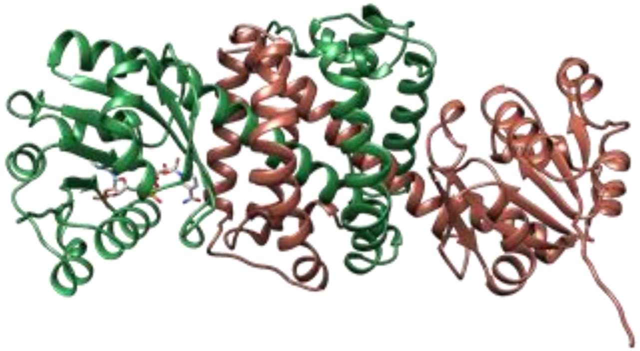
IRED-5 → 76% homology to IRED from *C. cellulans*

Protein homology models

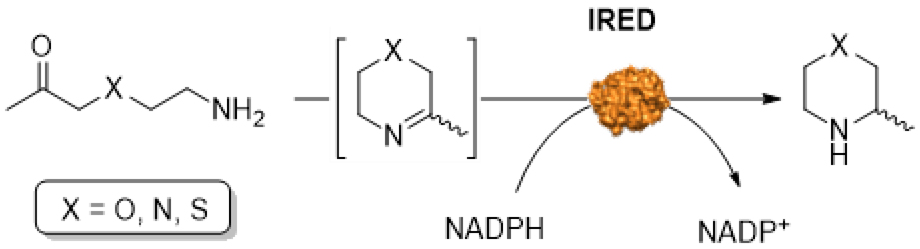
IRED-2
PDB: 5A9S



IRED-4

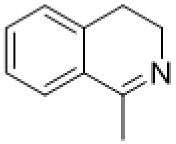
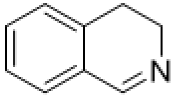
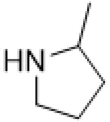
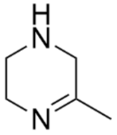
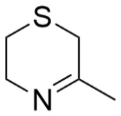
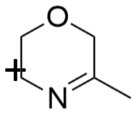


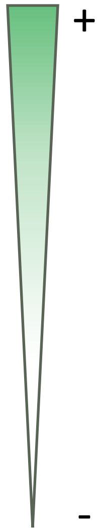
Activity assay: substrate scope



Reaction conditions
- **Substrate:** 5 mM
- **NADPH:** 0.3 mM
- **Buffer**: 100 mM phosphate pH 7.5 with 1% DMSO
- **T°**: 37°C
- **Volume**: 0.2 mL

Specific activity (U/mg)

						
IRED-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IRED-2	0.24	0.12	0.06	n.d.	62.3	13.6
IRED-3	0.1	0.1	0.12	n.d.	9.8	2.5
IRED-4	0.1	0.21	0.1	n.d.	39.4	8.6
IRED-5	1.4	0.8	0.2	n.d.	5.9	4.5
IRED-6	0.3	0.07	0.1	n.d.	14.8	10.8



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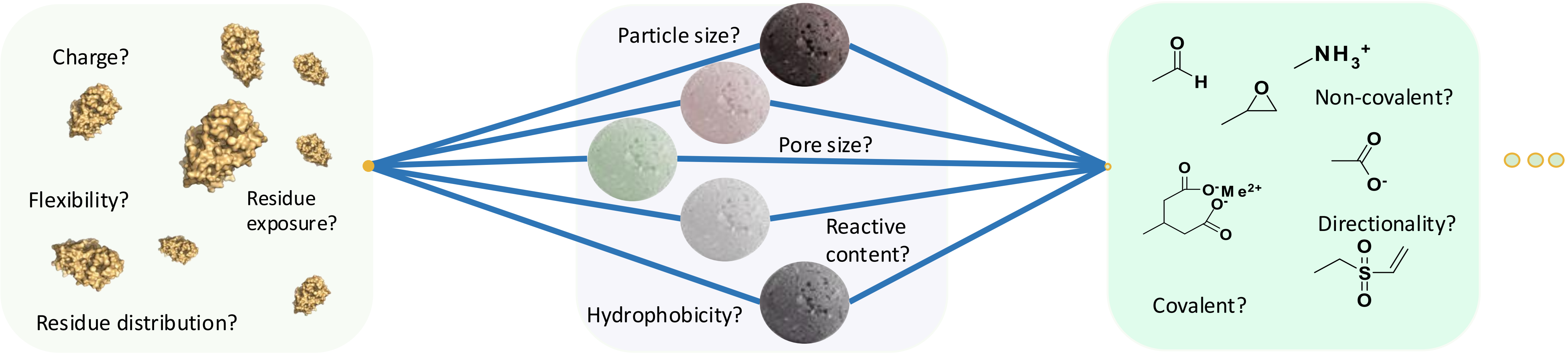
Paradisi Research

Protein immobilization

inseit
BIOCATALYST IMMOBILIZATION

Protein of interest | **Material of the support** | **Binding chemistry**



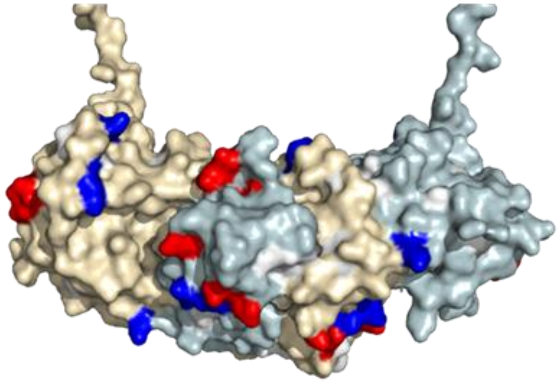
CapiPy: Relevant Clusters

Legend:

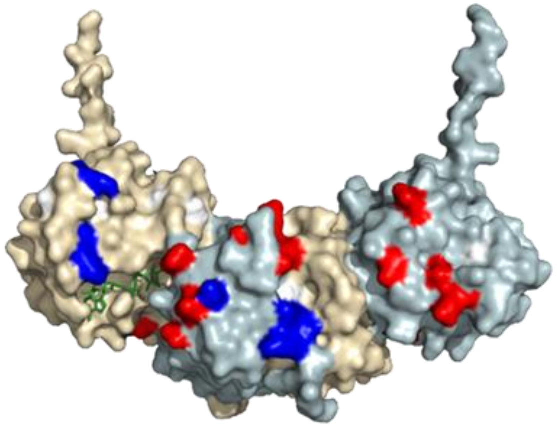
Monomer 1
Monomer 2

Cluster of Lys
Cluster of Asp and Glu

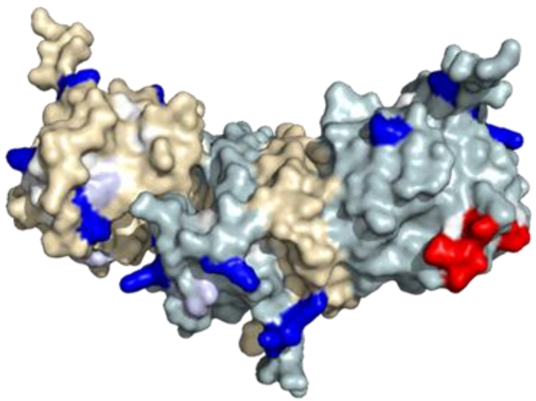
IRED-3



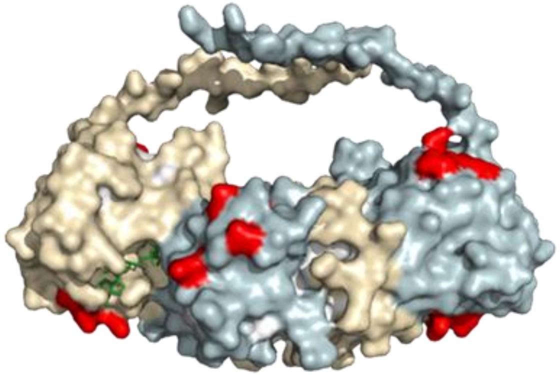
IRED-4



IRED-5



IRED-6



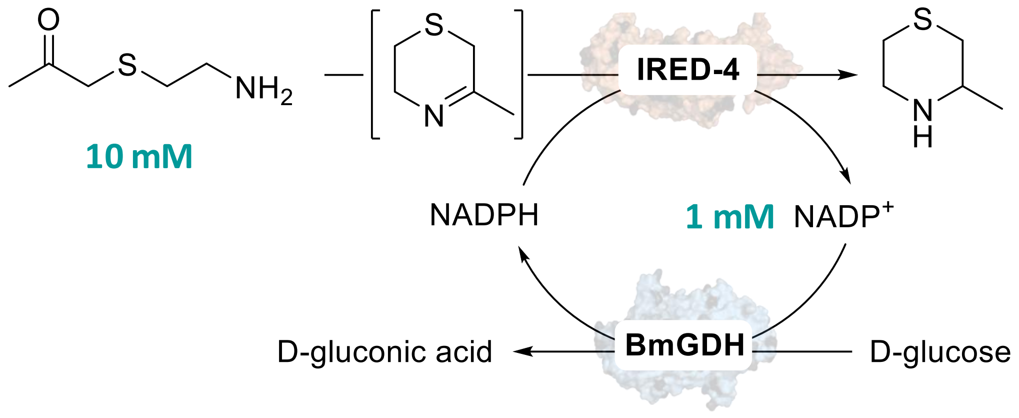
Paradisi Research

Enzyme Immobilization Screening

Enzyme	Chemistry	Immobilization Yield (%)	Recovered Activity (%)	Reusability (%)
IRED-3	Ag/Epoxy-Amino	52	24	35
	Ag/Epoxy-Amino	84	8	93
	Ag/Epoxy-Amino	99	26	89
	Ag/PEI	100	95	20
	Ag/PEI-GA	**90**	**19**	**100**
IRED-4	Ag/Epoxy-Amino	10	24	100
	EP400SS/Epoxy-Amino	**60**	**9**	**100**
	EP403S/Epoxy-Amino	25	8	74
IRED-5	**Ag/Epoxy-Metal**	**90**	**39**	**100**
	Ag/Epoxy-Amino	19	21	88
IRED-6	**Ag/Metal**	**94**	**17**	**63**

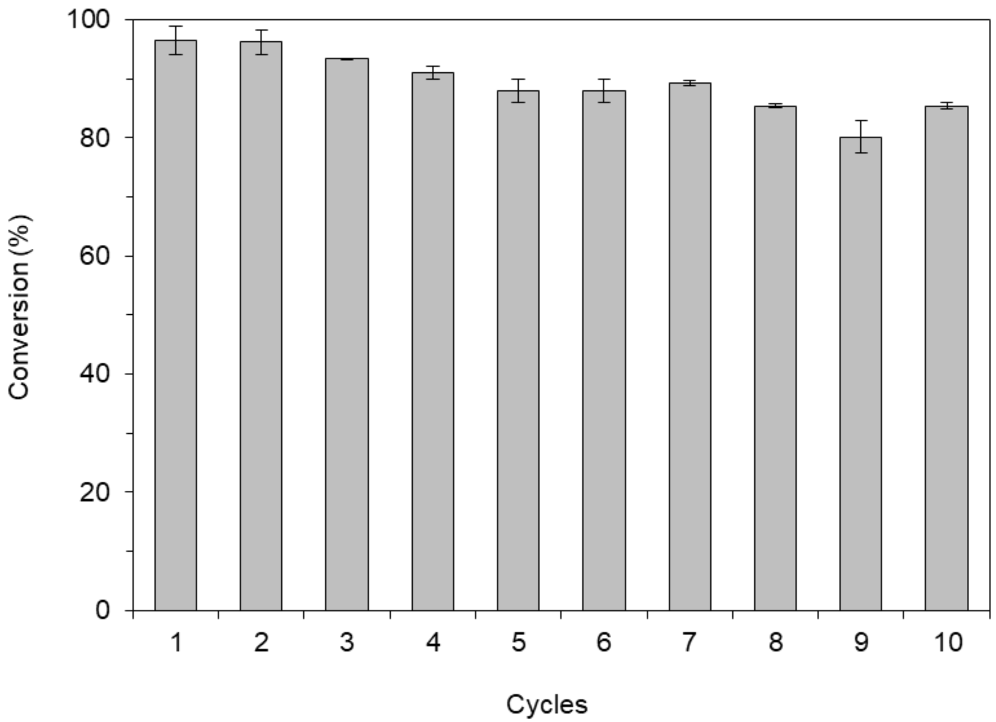
Cofactor Recycling and Reusability

Cofactor regeneration



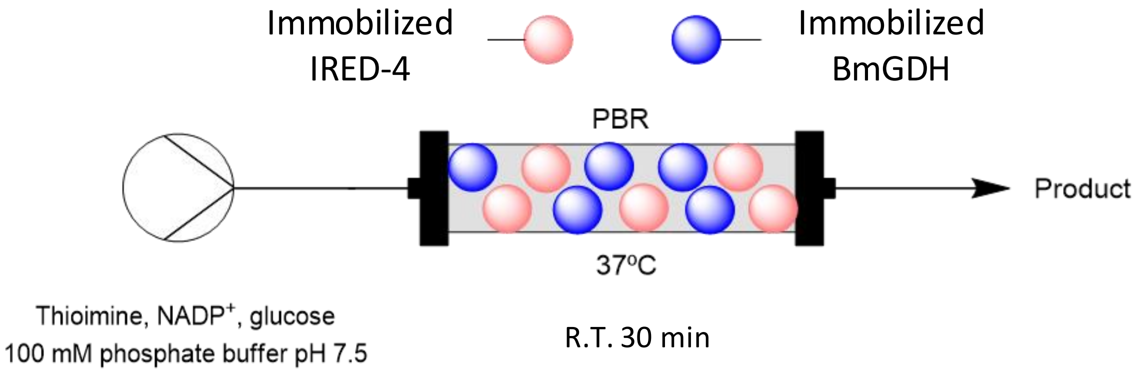
BmGDH: glucose dehydrogenase from *Bacillus megaterium*

Reusability of immobilized biocatalysts

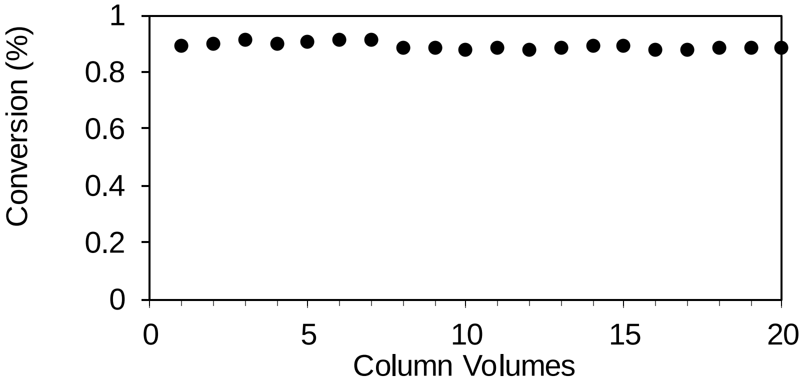


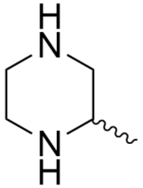
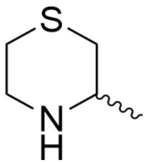
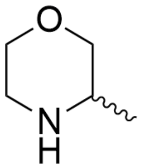
10 mM thioimine, 1 mM NADP+, 40 mM glucose.
Tº: 37ºC. Cycle time: 2 h.

Continuous Flow



Operational Stability



Heterocyclic amine: product	Substrate concentration (mM)	Conversion (IRED-4) (%)	Conversion (IRED-5) (%)
	10	3.4	n.d.
	10	98	91
	50	88	91
	100	61	46
	10	46	69
	50	19	50
	100	14	28

Summary (1)

⭐ Choice of the enzyme to target a specific reaction

⭐ Immobilization efficiency

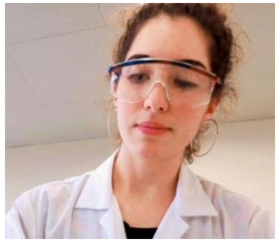
⭐ Compatible recycling system

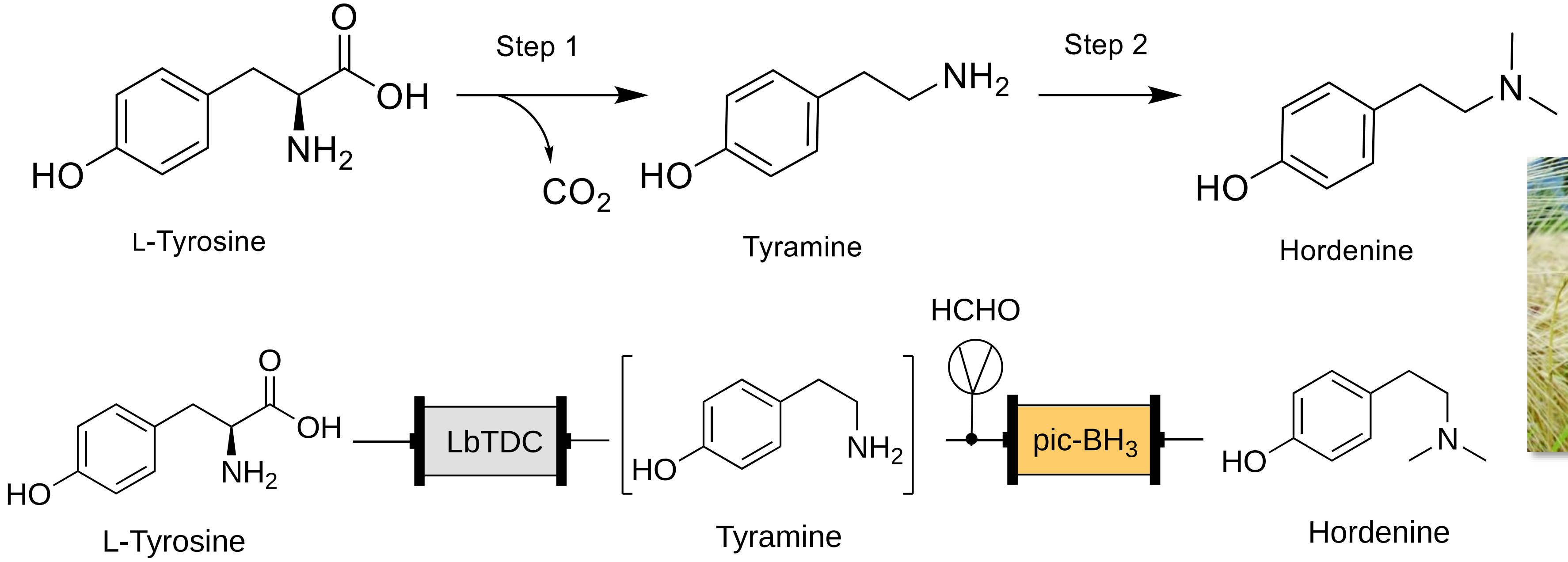
⭐ Implementation in continuous flow

Integrated (bio)catalysis

Integrating chemical steps

2022





Calculated E-factor (Environmental Factor = kg of waste per kg of desired product): **36**

Starting material value raised by **200-fold** (L-tyrosine disodium salt hydrate 1.72 €/g, hordenine 382 €/g)

Molar conversion 92%

Isolated yield 77%

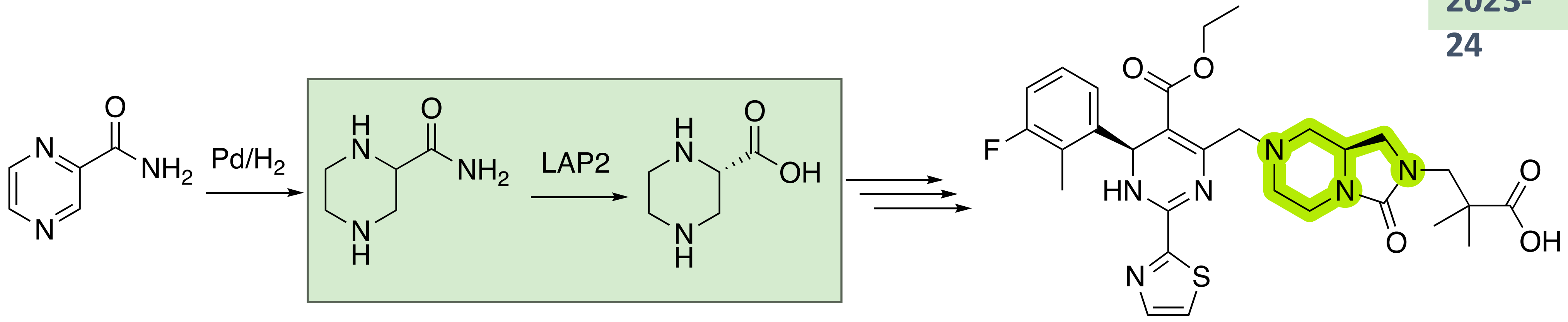
Residence time 2.5 min

130 mL in 4h

An industrial challenge

With Hans Iding and team at Roche

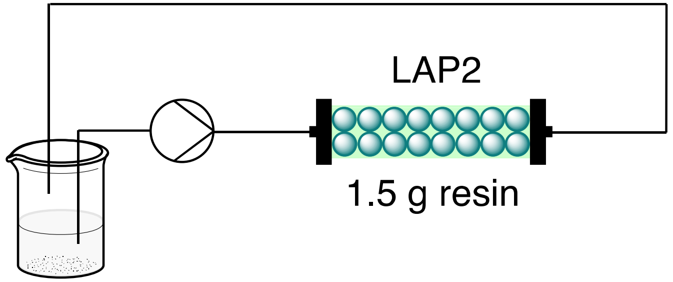
2023-24



Linvencorvir

Challenges:

1. Substrate load 20% w/v
2. High K_M for the substrate
3. Product inhibition
4. High concentration of NH_3 is generated
5. The enzyme is heavily glycosylated



Amide
20% w/v

Contact time: 5 x 20 min

Molar conversion: 41%

Product inhibition still a problem

NH_3 ↑ through open vessel

Metrics:

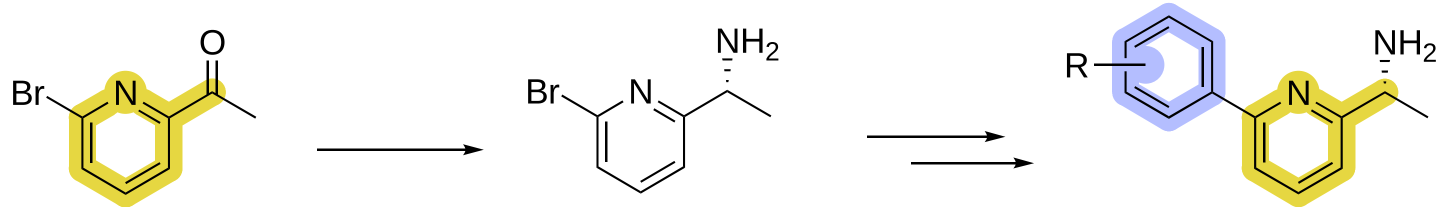
E-factor: 14.5 → 3.6

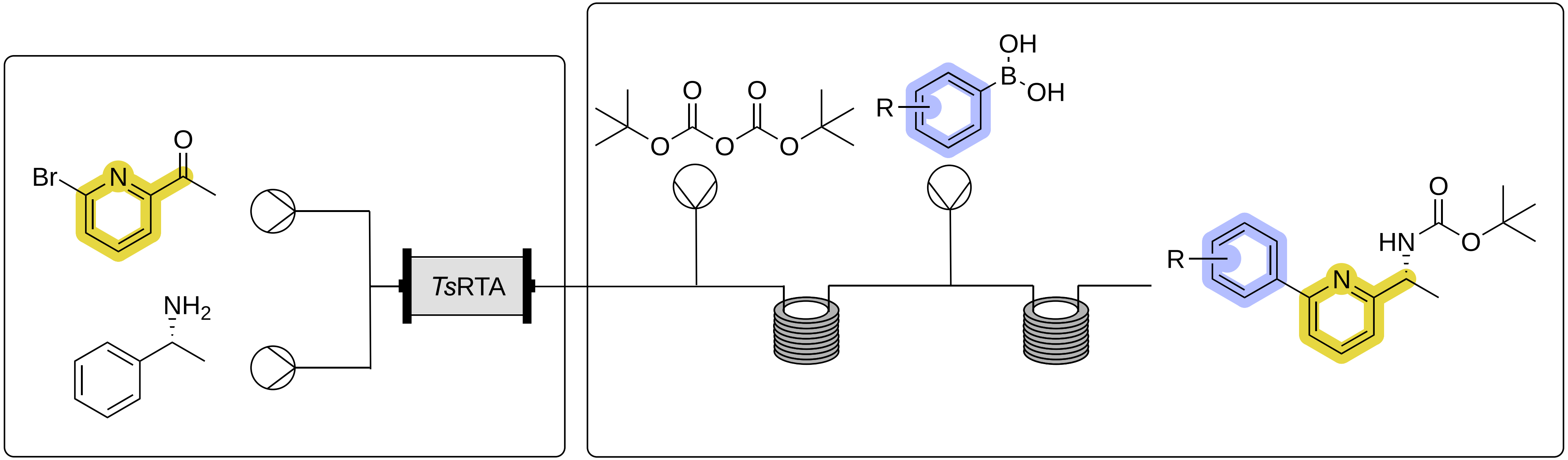
STY: 2.9 → 50.4

Solvent switching for integrated catalysis

With Radka Snajdrova and Hanjoerg Lehmann at Novartis

2023-24





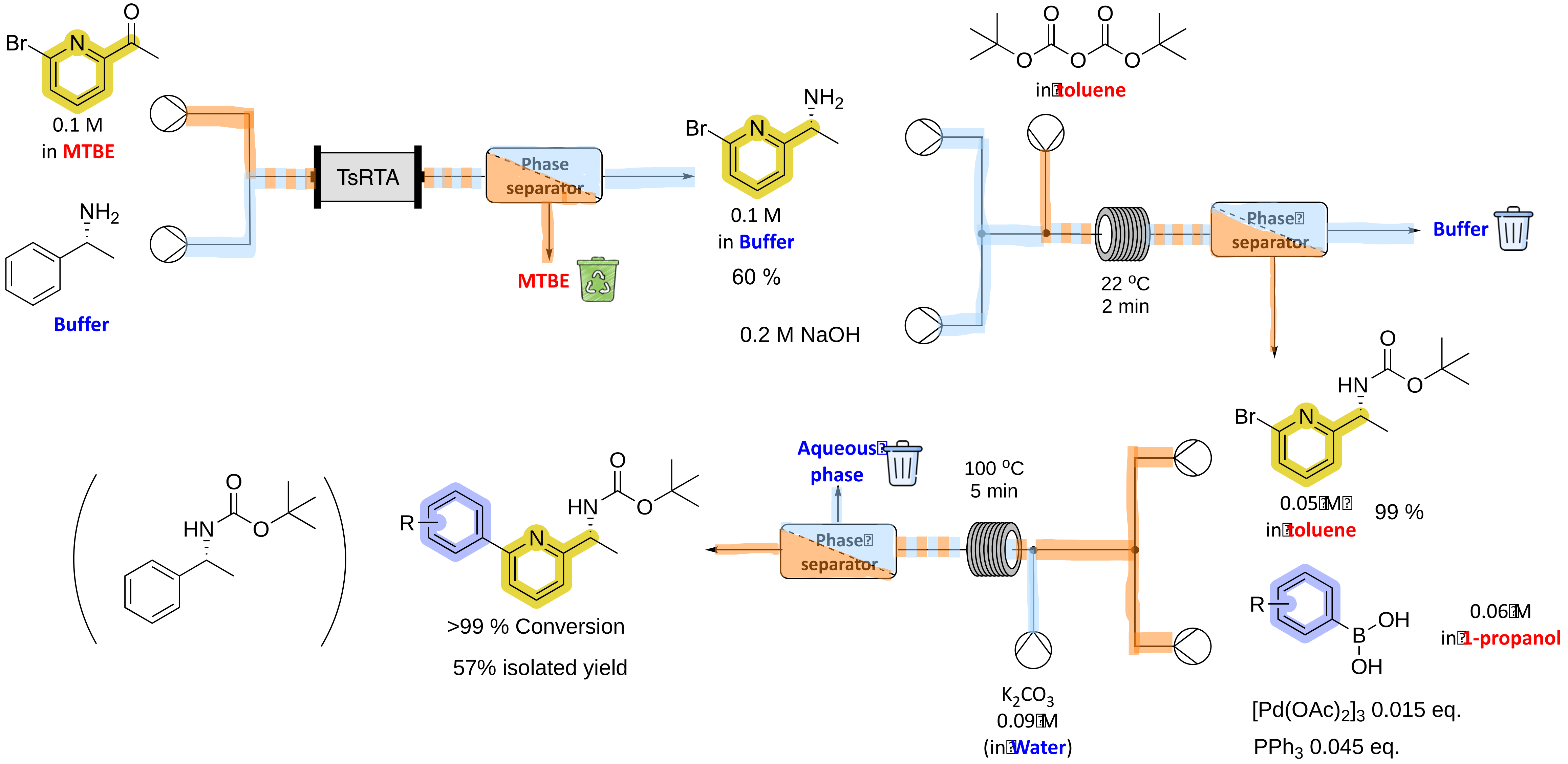
Biocatalytic transamination

Downstream chemical process

Aqueous solvent systems for biocatalytic step is incompatible with the Suzuki-Miyaura cross-coupling

with Díaz-Kruik, *et al*. *OPRD* **2024**, DOI: 10.1021/acs.oprd.4c00080

Solvent switching for integrated catalysis



with Díaz-Kruik, *et al*. *OPRD* **2024**, DOI: 10.1021/acs.oprd.4c00080

Paradisi Research

Summary (2)

- There are several challenges in integrating catalytic approaches
- Compatible chemistry (may be limited)
- Flow enables optimal conditions
- Solvent switching: rethink standard chemistry

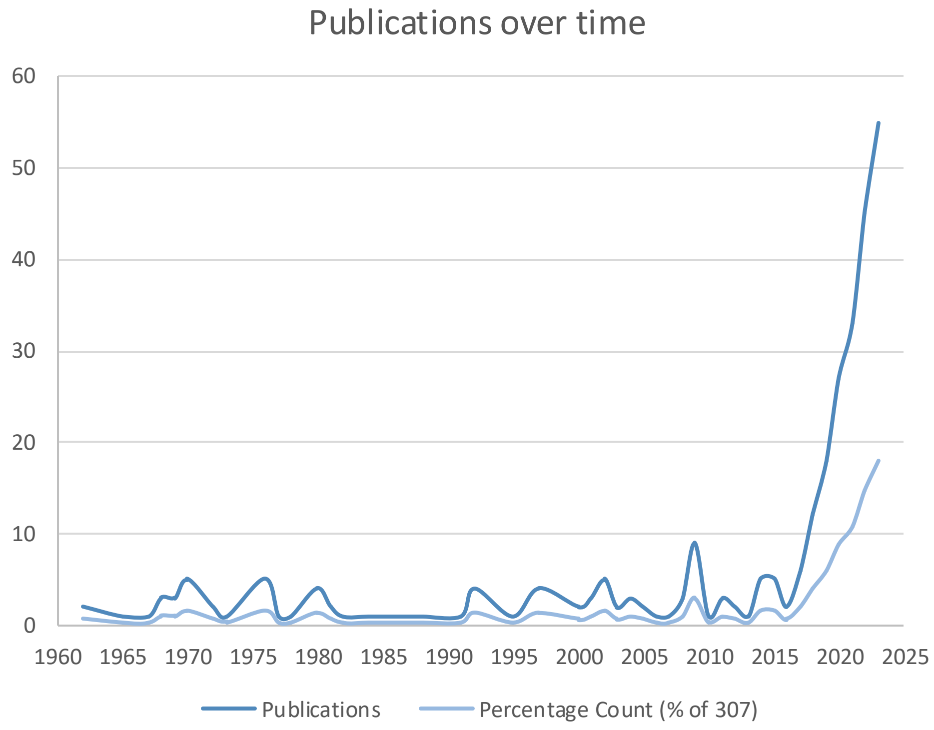
Smart materials for enzyme immobilization

Photobiocatalysis

- Photons as traceless catalysts
- Powered by the sun

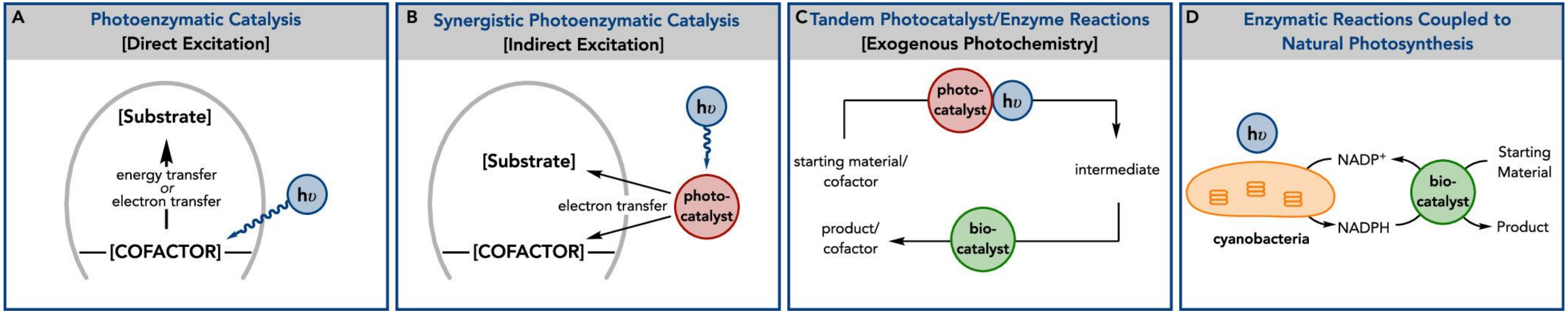
- Mild reaction conditions
- Specificity and selectivity

The popularity of the subject has been increasing in recent years:

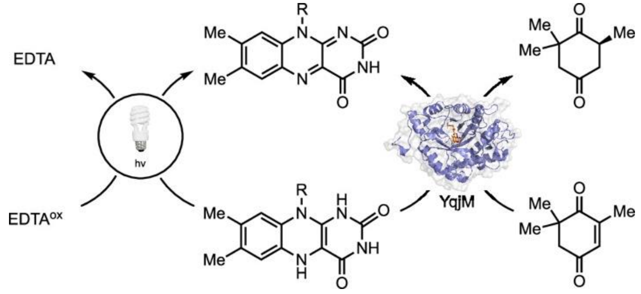


Web of Science Database search terms: “Photobiocatalysis” or “Photoenzymatic”

Photobiocatalysis

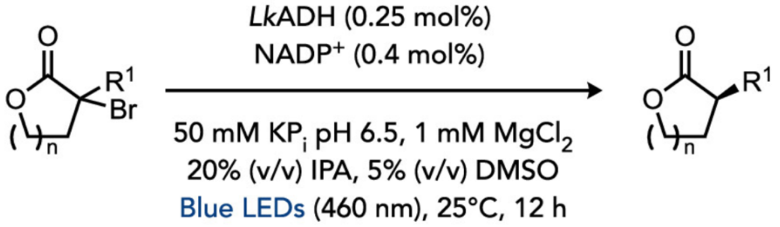


Cofactor regeneration



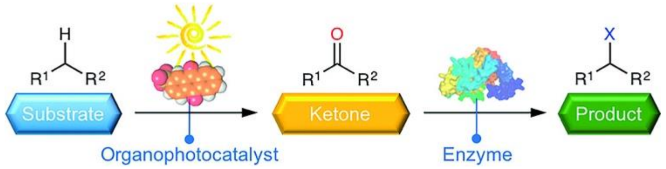
Hollmann, *Adv. Synth. Catal.* **2009**, *351, 3279*

New reactivity



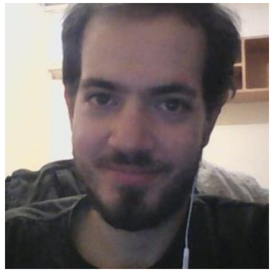
Hyster, *Nature*, **2016**, 540, 414

Tandem reactions



Höhne, Schmidt, *EuJOC*, **2019**, 1, 80

Photoactive supports for biocatalysis

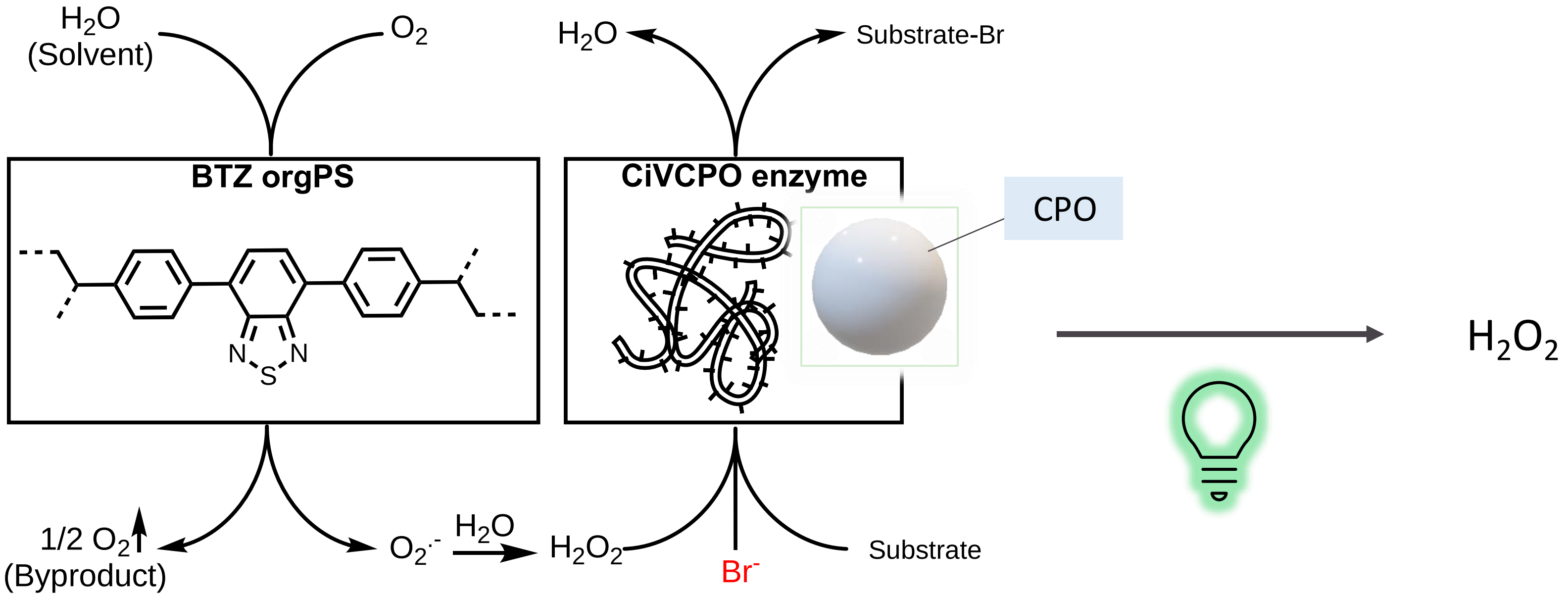


Haloperoxidases

- Most abundant halogenases (Heme or V dependent)
- Consume H_2O_2 to generate XOH (‘electrophilic’ X^+)
- Can withstand high T, organic solvents
- **Sensitive to high [H_2O_2]**

Organic Photosensitiser (OrgPS)

- Able to generate H_2O_2
- Photostable
- **Polymerisable**
- Easy to obtain



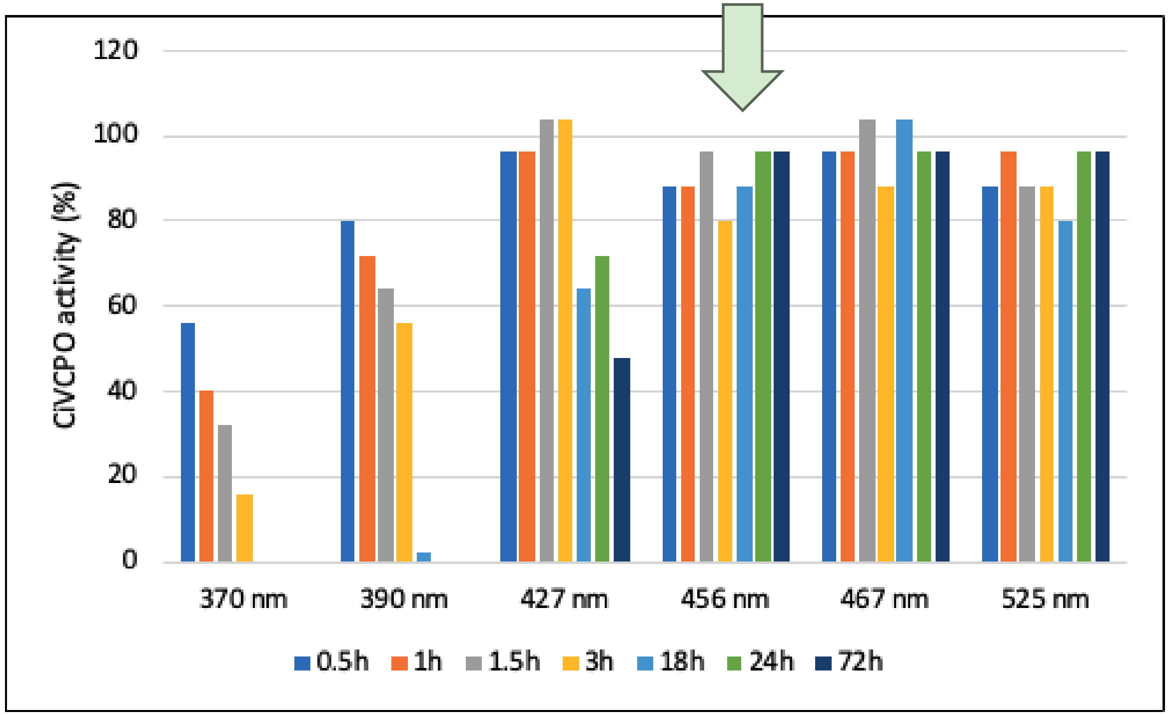
Broumidis, Paradisi *Angew. Chem. Int. Ed.* **2024**, 63, e202401912

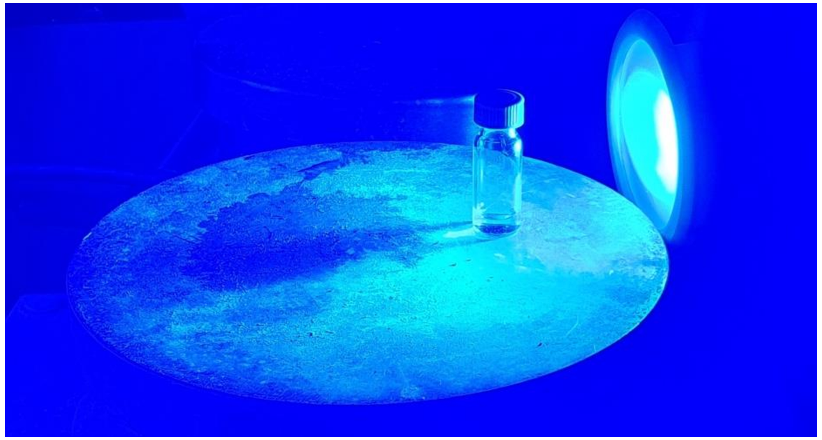
Photostability of CiVCPO

Vanadium-dependent chloroperoxidase

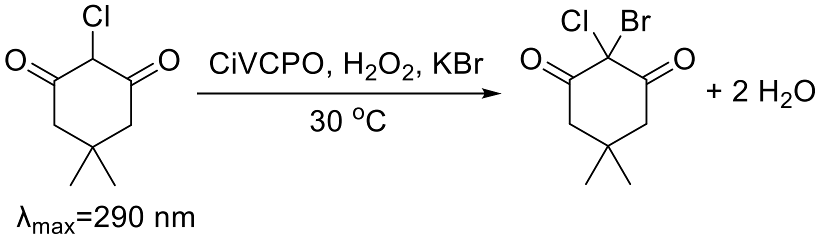
from *Curvularia inaequalis* (CiVCPO)

(Thanks to Frank Hollmann for the plasmid!)

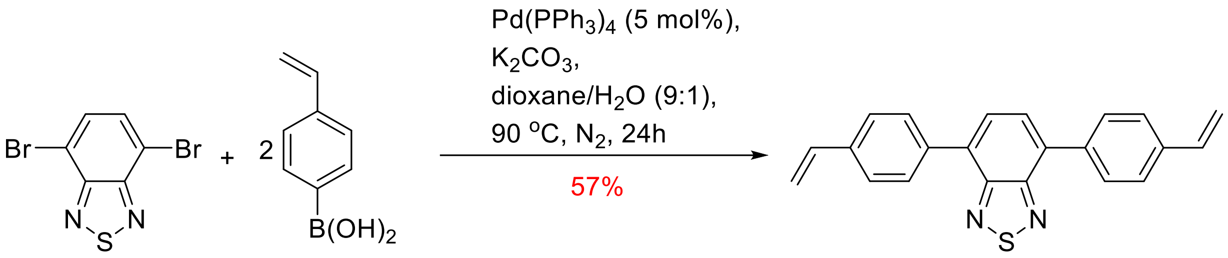


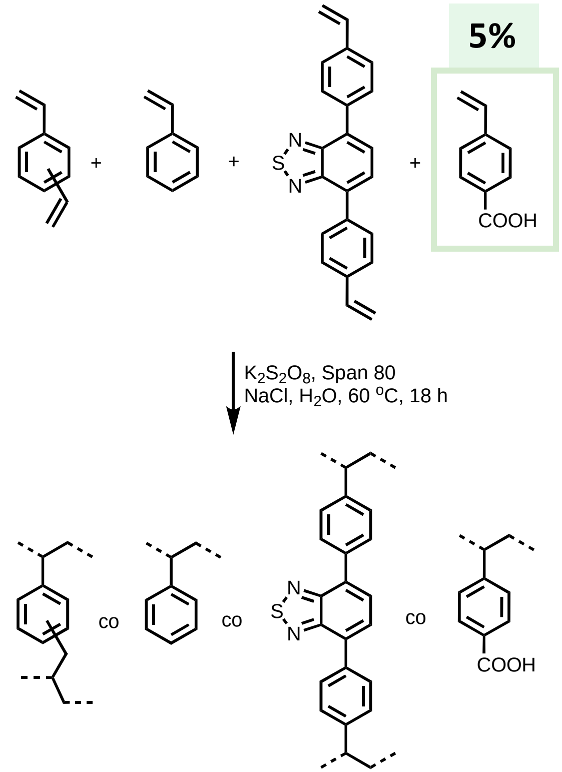


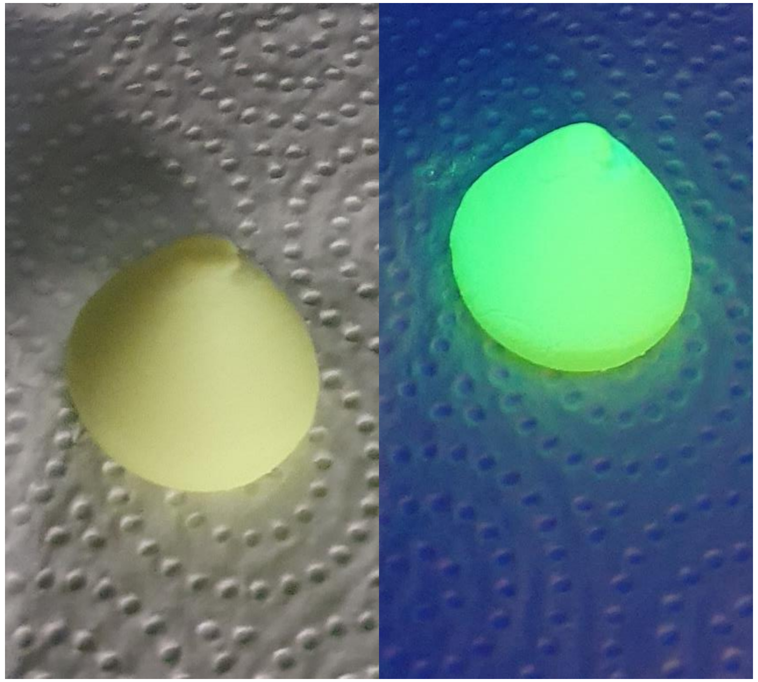
Activity Assay (MCD):

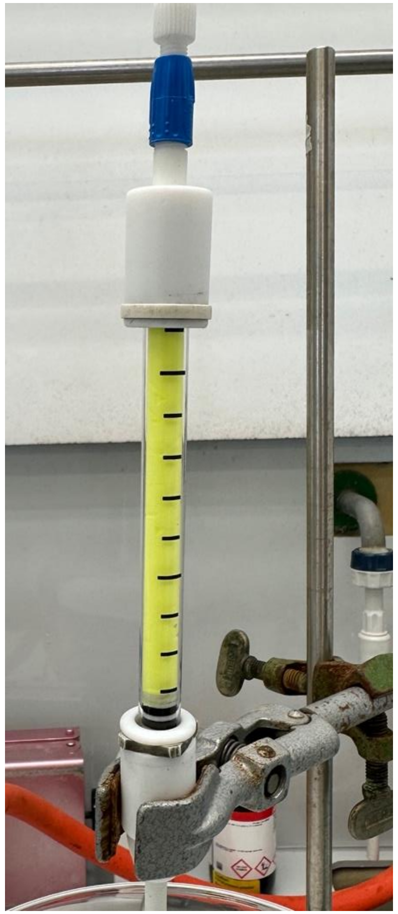


pHIPE-BTZ/COOH Monolith Synthesis



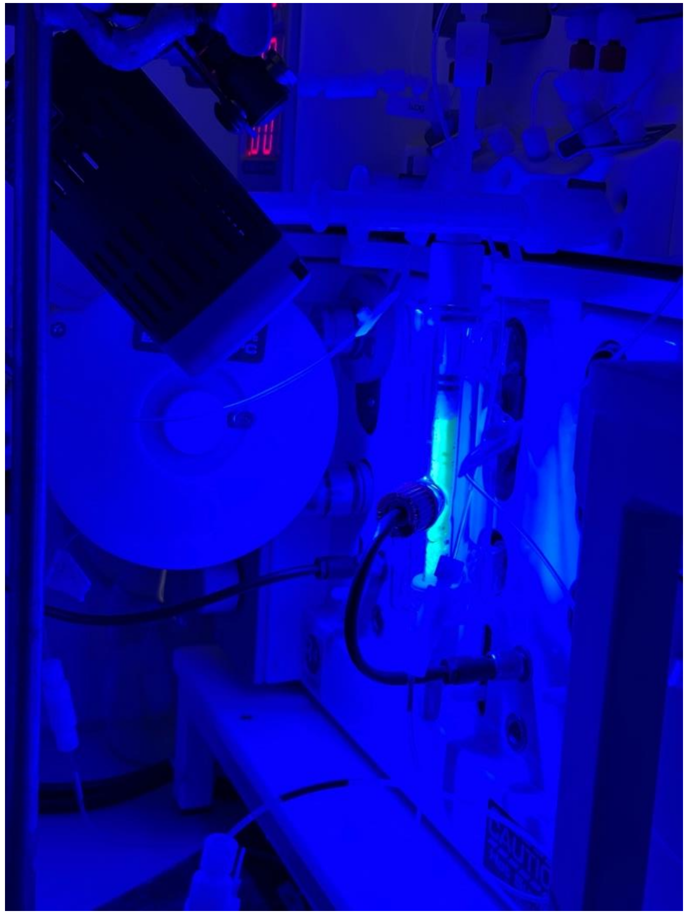






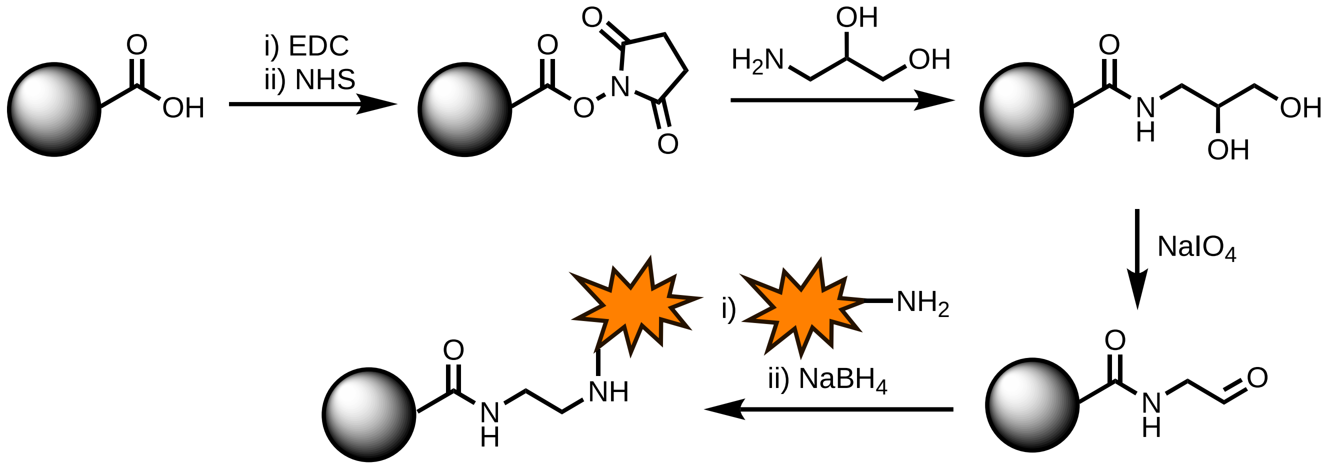
H_2O_2 generation efficiency

$$^{3}O_2 \xrightarrow[\text{Solvent system, } R_t = 10\text{ min, } 26\ ^{o}C]{\textbf{pHIPE-BTZ}\text{, hv (456 nm)}} H_2O_2$$



Entry	Solvent system	Rt (min)	H_2O_2 Output (µM)
1	H_2O	10	18.6
2	H_2O/MeOH (9:1)	10	1.96
3	H_2O/MeOH (6:4)	10	1.00
4	H_2O/DMF (9:1)	10	7.42
5	H_2O/DMF (9:1)	10 (air)	13.6
6	H_2O/DMF (9:1)	30	7.05
7	H_2O/DMF (3:1)	10	5.60
8	H_2O/DMF (3:1)	10 (air)	9.05
9	H_2O/DMF (3:1)	30	6.0
10	**H_2O/2-MeTHF (1:1)**	**10**	**115.7**
11	H_2O/2-MeTHF (1:1)	10 (air)	97.9
12	H_2O/2-MeTHF (1:1)	30	89.8

VCPO immobilization

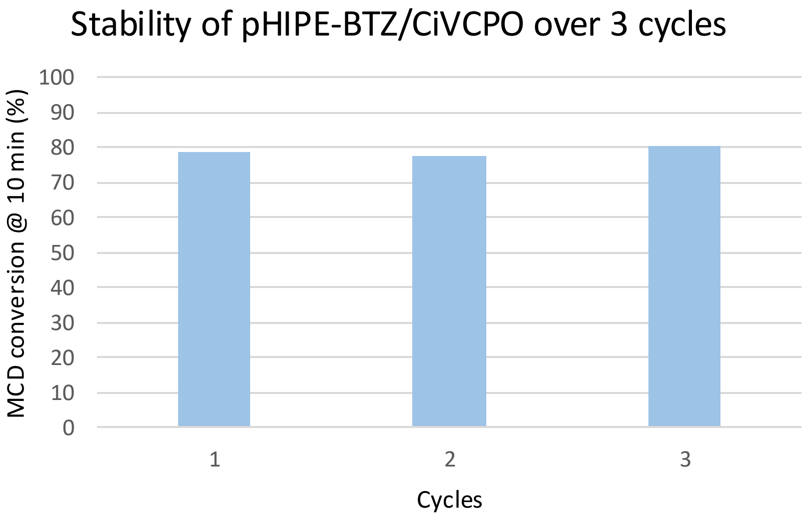


Protein Loading: 2.5 mg/g

Immobilization yield: 98%

Recovered activity: 82%

Immobilized activity: 1.7 U/mg



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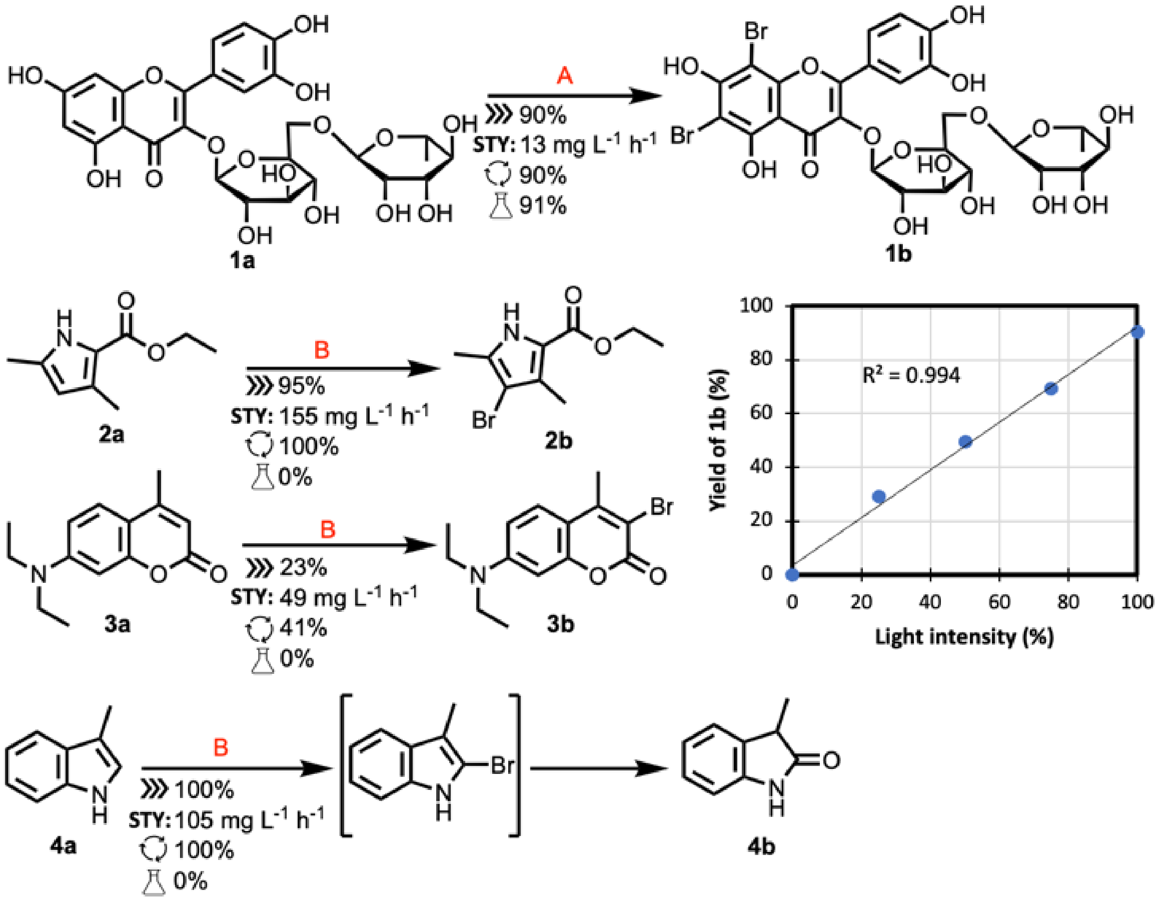
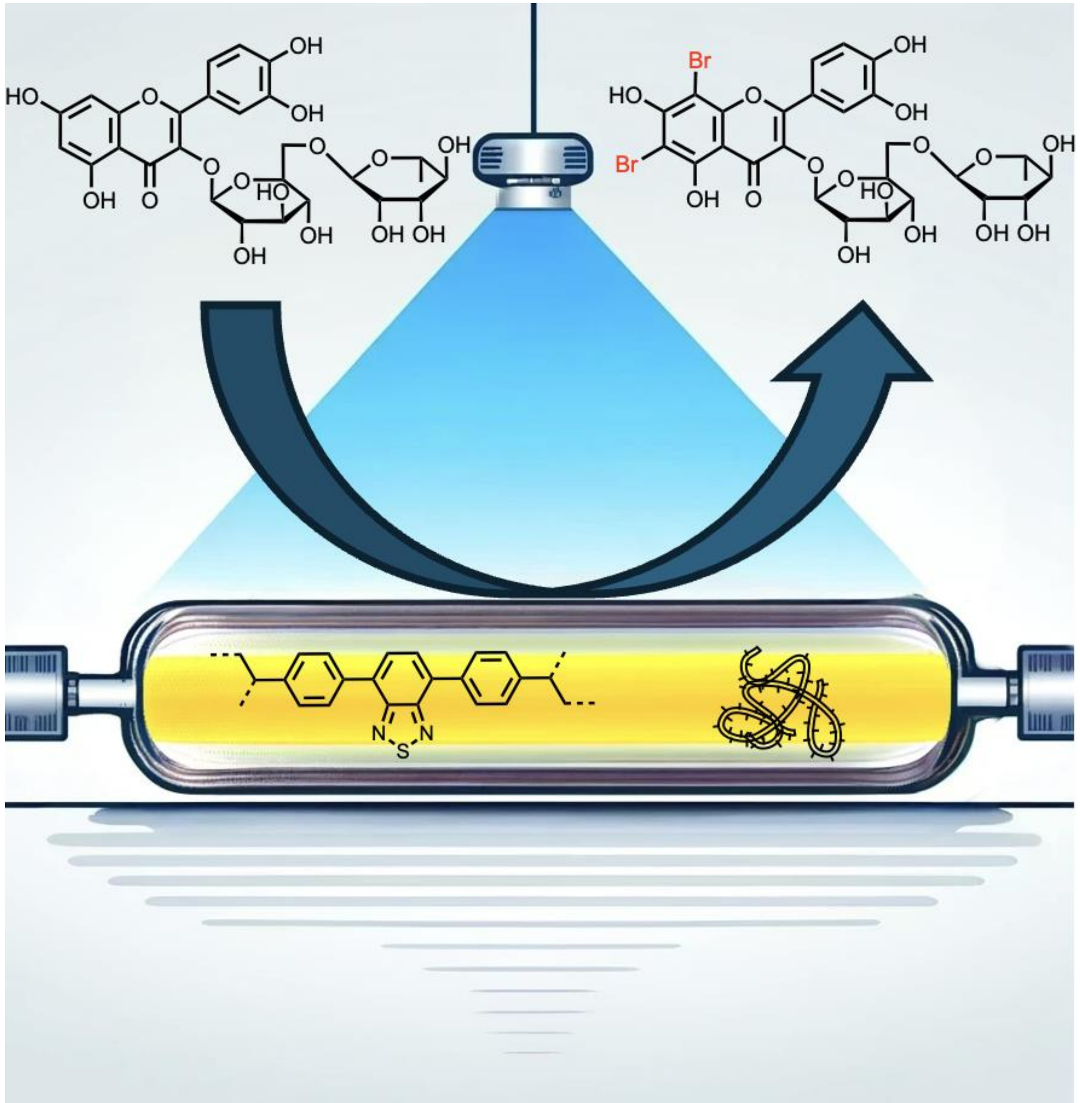
Reaction Scope

Substrate (**1-4a**) → **BTZ-pHIPE/CiVCPO**, hv (456 nm, 50 W), A: Substrate (0.1 mM in Citrate (50 mM, pH 6, 10 mL)), 25 mM NaBr, 1 mM Na_3VO_4, R_t = 10 min, 26 °C → Product (**1-4b**)



Summary (3)



- ⭐ First example of an integrated photobiocatalytic resin
- ⭐ Excellent single pass & recirculation yields
- ⭐ Stable and reusable system
- ⭐ Offers easy product separation

Acknowledgements

Roche

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Thank you for your attention!