Hybrid chemo-enzymatic heterogeneous catalyst for selective hydroxylation of biomass-derived molecules

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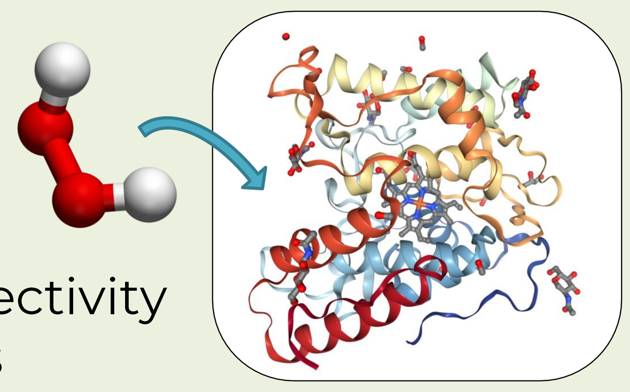
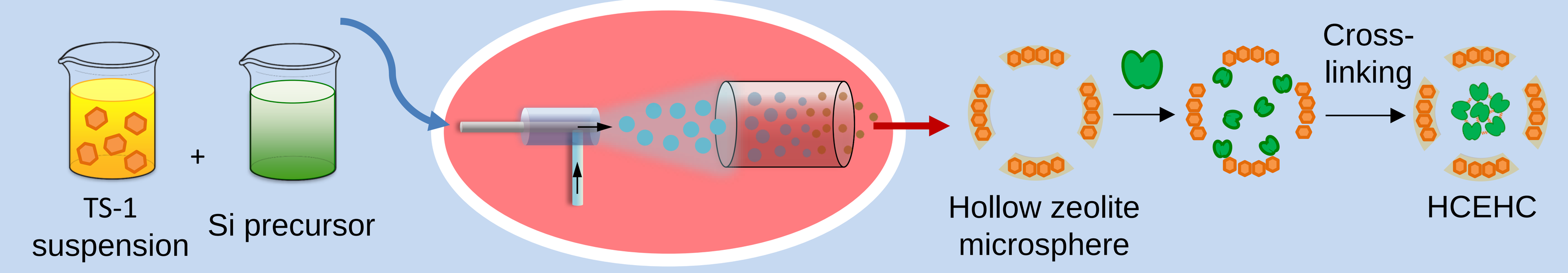
Introduction:

- **Unspecific peroxygenases** (**UPO**) allow selective **C–H oxyfunctionalization** at **room temperature** [1]
- ↗ Require **no cofactors**, H_2O_2 for oxygen atoms & final electron acceptor
- ↗ Unspecific: endless **substrate** choices
- ↗ **Regio** + **enantio selective**
- ↗ Highly engineered protein: [2]
  - \+ Resistant to **organic solvents**
  - \+ **Customizable** specificity & selectivity
- ↘ **Sensitive to high H_2O_2** concentrations



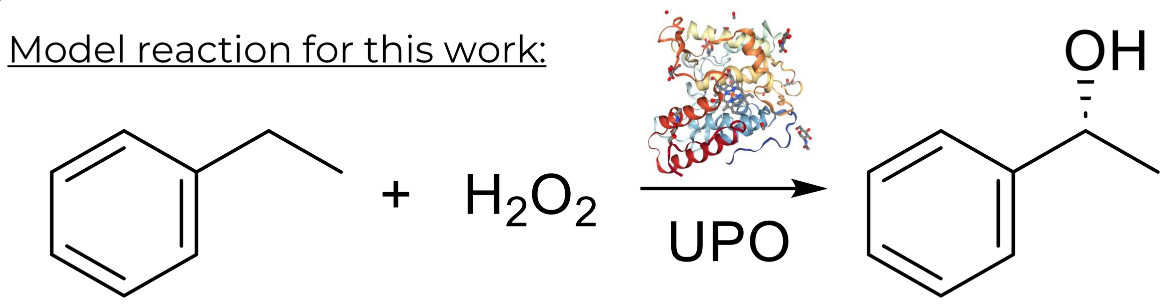
- **H_2O_2** can be generated in room temperature water under low H_2 pressure using supported **Au-Pd nanoparticles**, typically on **TiO_2**
- Au-Pd/TiO_2 have been proven to be a suitable source of H_2O_2 for UPOs in a one-pot system [3]



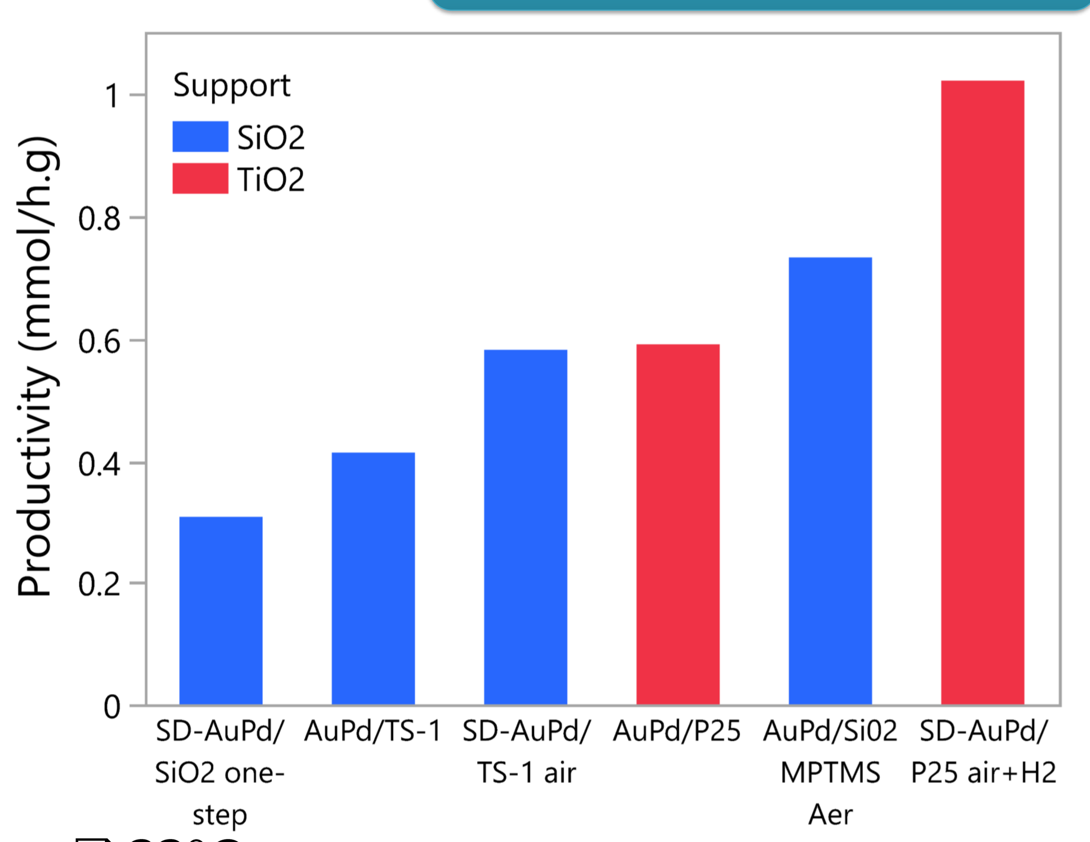
Method for creating **Hybrid Chemo-Enzymatic Catalysts** (**HCEHC**) developed by our team [4,5]

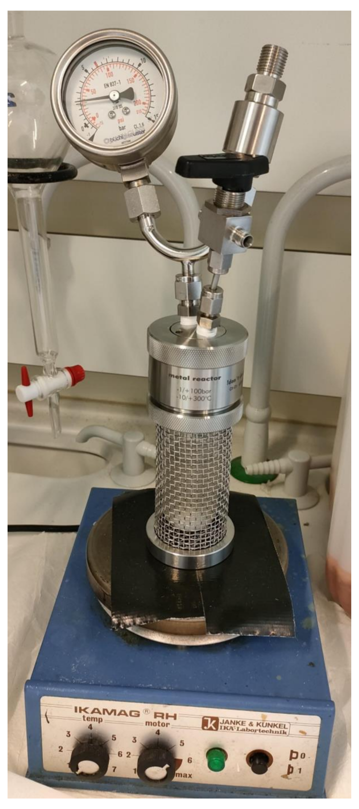
- → Allows immobilization of enzymes on supports with few/weak binding sites
- → Retains activities of both inorganic and biological catalysts
- → Great for **cascade reactions**!

Model reaction for this work:



H_2O_2 production





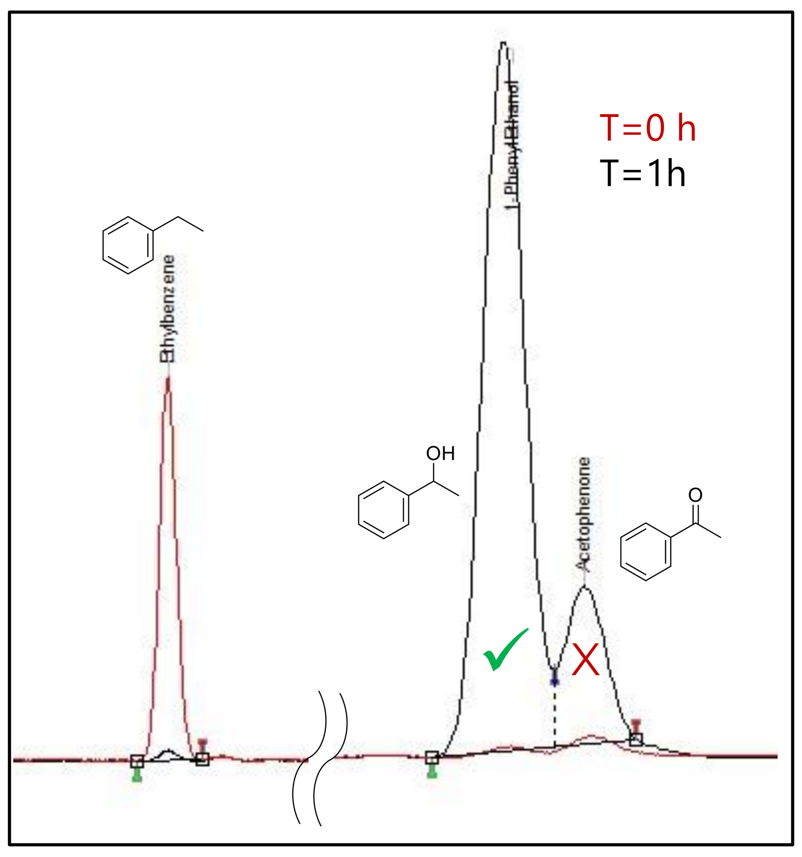
- ❑ 22°C
- ❑ 0.3 bar H_2
- ❑ 2.7 bar dry air
- ❑ pH 7.6 - Tris-HCl 50mM

✓ Silicon-based supports are relevant
✓ Spray-drying (**SD**) **increases activity**

Cascade reaction

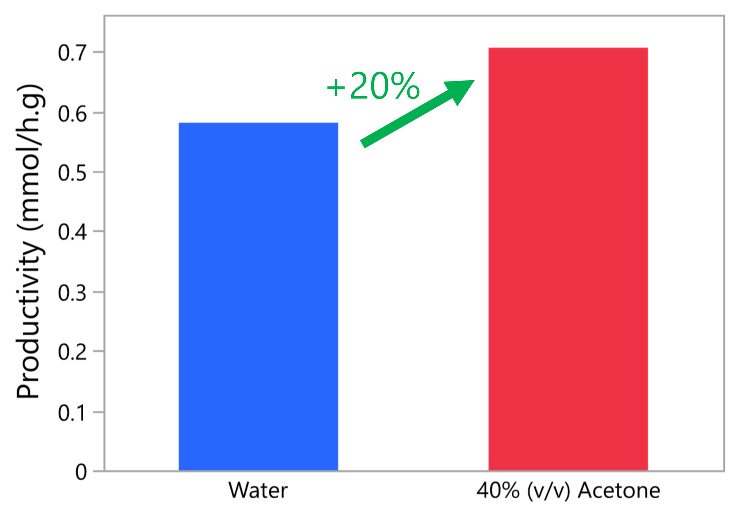
- ✓ One-pot cascade works as intended
- ✓ **Full conversion** of saturated solution after 1h with 1 mg_{cat}/mL
- ✗ Poor substrate miscibility with water: **low** and unreliable **concentrations**
- ✗ Low concentration favors overoxidation of product

→ Substrate **miscibility** needs to be **increased**!



Acetone could serve as a non-competitive cosolvent for UPO!

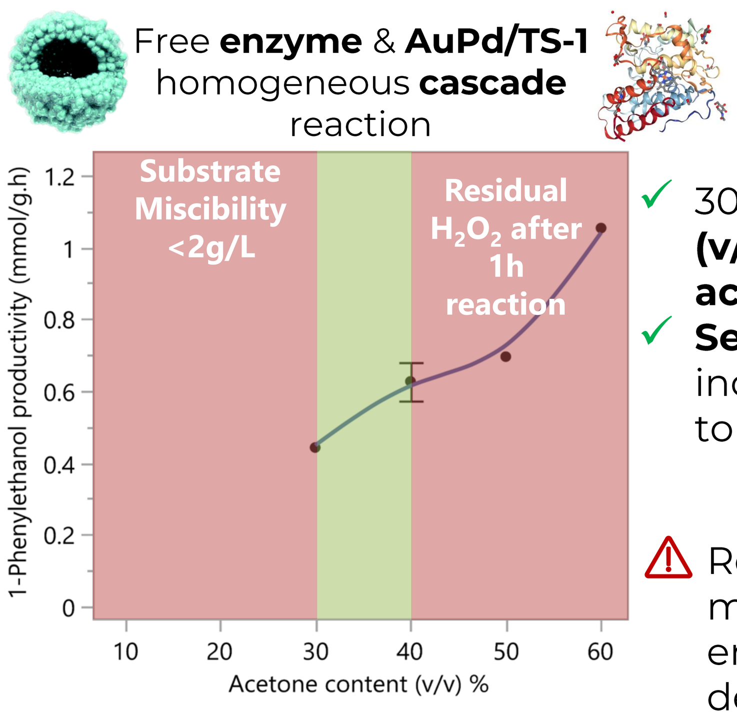
High concentration of acetone **increases H_2O_2** productivity



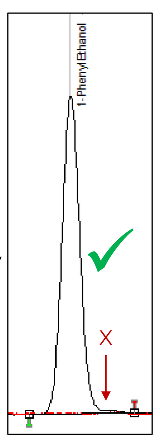
Same conditions, using SD AuPd/TS-1 catalyst, 1mg/mL

But how far can we push UPO in water:Acetone mixtures?

Free **enzyme** & **AuPd/TS-1** homogeneous **cascade** reaction

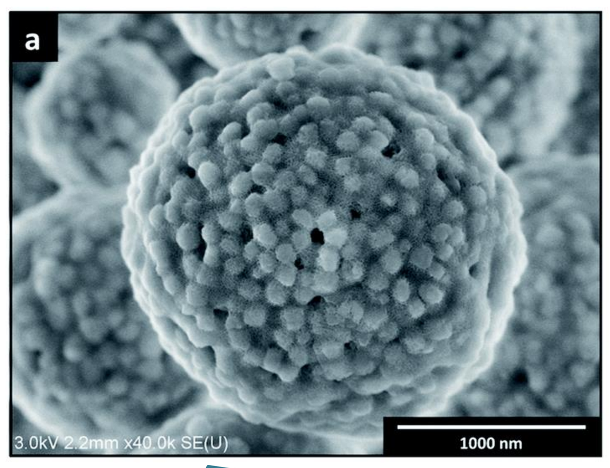


- ✓ 30 to **40 % (v/v)** of **acetone**
- ✓ **Selectivity** increases to **~99%**

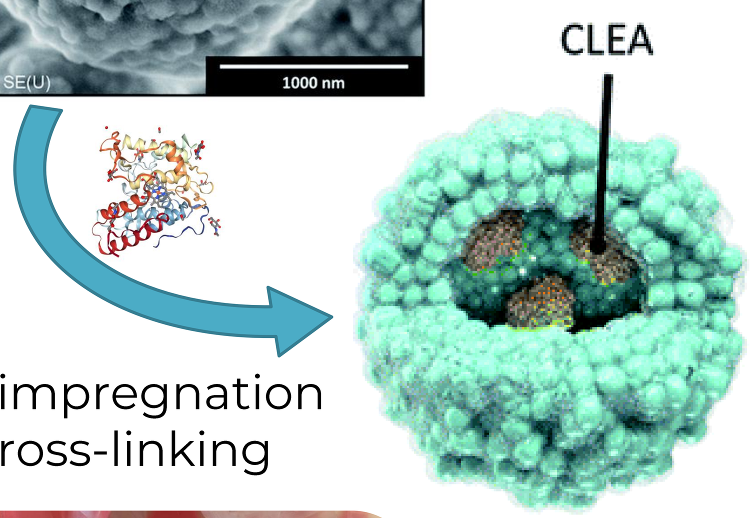


⚠ Residual H2O2 may indicate enzyme deactivation!

HCEHC



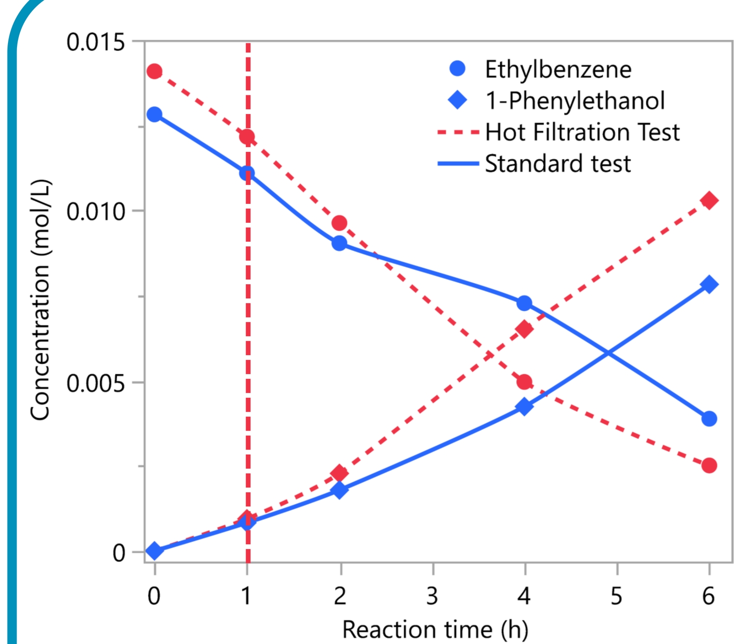
TS-1 hollow microsphere



UPO impregnation & cross-linking

UPO @ SD-AuPd/TS-1

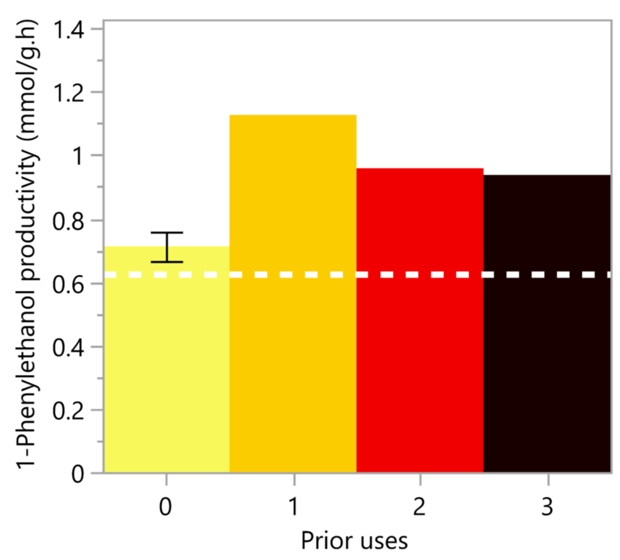
Catalytic performance of HCEHC



- ❑ 22°C
- ❑ 0,3 bar H_2
- ❑ 2,7 bar dry air
- ❑ pH 7,6 – Acetone + Tris-HCl 50mM (40/60 % v/v)
- ❑ ~1 mg_{cat}/mL
- ❑ HFT: catalyst removal @t=1h, replaced with SD AuPd/TS-1

Evident **leaching** of **UPO** in the medium **after 1h**

Performance **increases** after **subsequent uses**

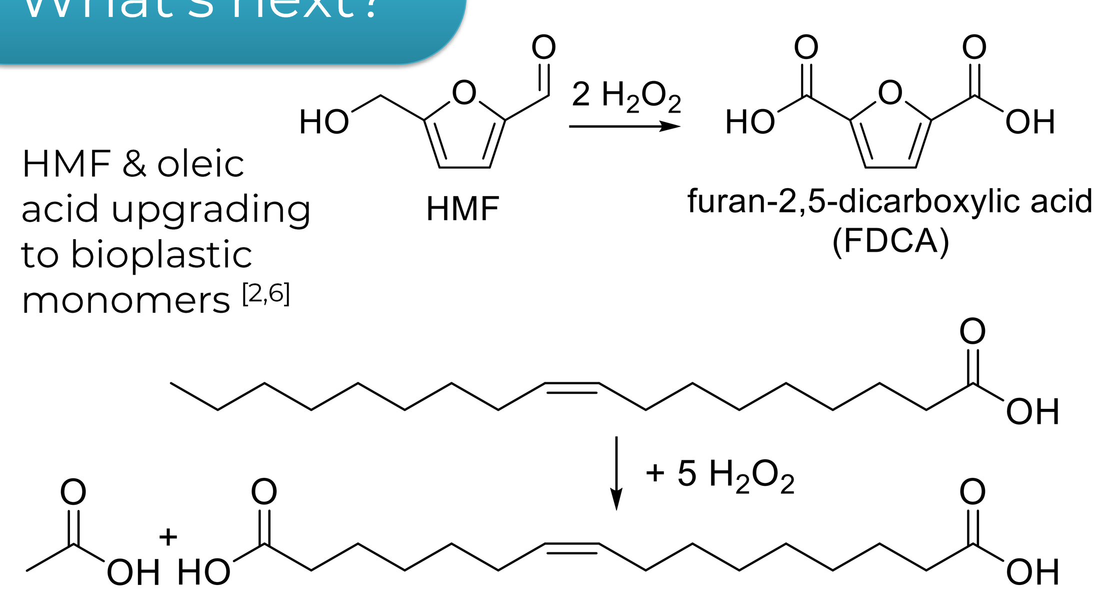


- ❑ Catalyst filtered and washed 3x with buffer between uses
- ✓ **Better** than homogeneous cascade (white dotted line)
- ✓ Argument for **active site proximity** effect

HCEHC overloaded with **UPO**? Surface may be encumbered?

What's next?

- ❑ HMF & oleic acid upgrading to bioplastic monomers [2,6]



➢ First tests show **57% conversion** of **oleic acid** after 24h; products yet to be identified

[1] Hobisch, M.; Holtmann, D.; Gomez de Santos, P.; Alcalde, M.; Hollmann, F.; Kara, S. Biotechnol. Adv. 2021, 51, 107615. [2] Martin-Diaz, J.; Molina-Espeja, P.; Hofrichter, M.; Hollmann, F.; Alcalde, M., Biotechnol. Bioeng. 2021, 118 (8), 3002–3014. [3] Freakley, S. J.; Kochius, S.; van Marwijk, J.; Fenner, C.; Lewis, R. J.; Baldenius, K.; Marais, S. S.; Opperman, D. J.; Harrison, S. T. L.; Alcalde, M.; Smit, M. S.; Hutchings, G. J., Nat. Commun. 2019, 10 (1), 4178. [4] Smeets, V.; Baaziz, W.; Ersen, O.; Gaigneaux, E. M.; Boissière, C.; Sanchez, C.; Debecker, D. P., Chem. Sci. 2020, 11 (4), 954–961. [5] Debecker, D. P.; Smeets, V.; Van der Verren, M.; Meersseman Arango, H.; Kinnaer, M.; Devred, F., Curr. Opin. Green Sustain. Chem. 2021, 28, 100437. [6] Olmedo, A.; Ullrich, R.; Hofrichter, M.; del Río, J. C.; Martínez, Á. T.; Gutiérrez, A., Antioxidants 2022, 11 (4), 744.