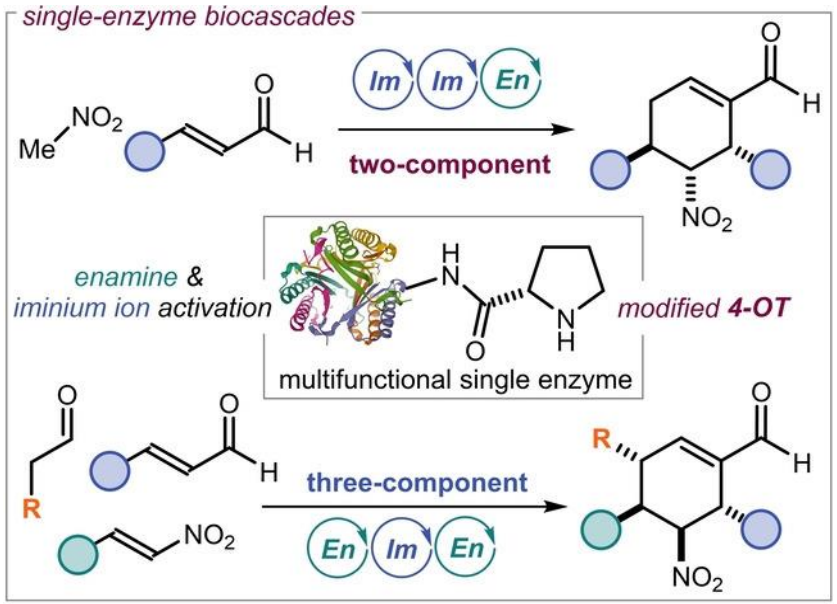
BIOTRANS
LA ROCHELLE 2023

Enantioselective Biocascade Catalysis With a Single *Multifunctional* Enzyme



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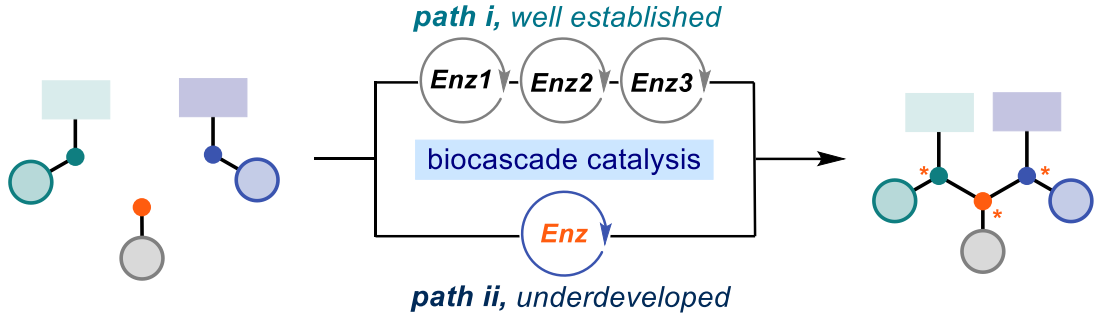
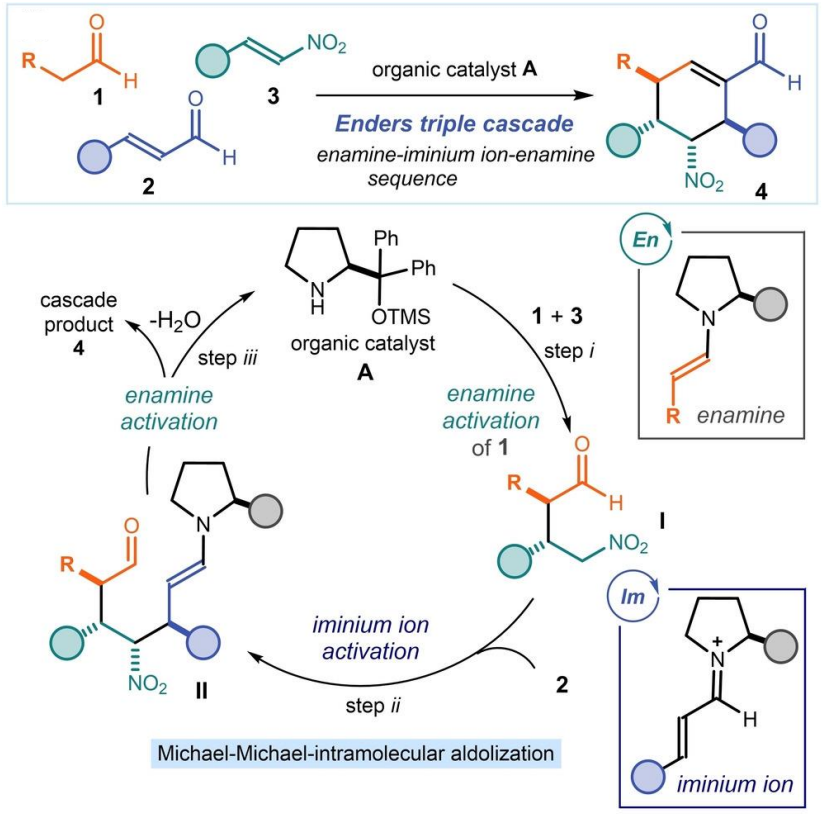
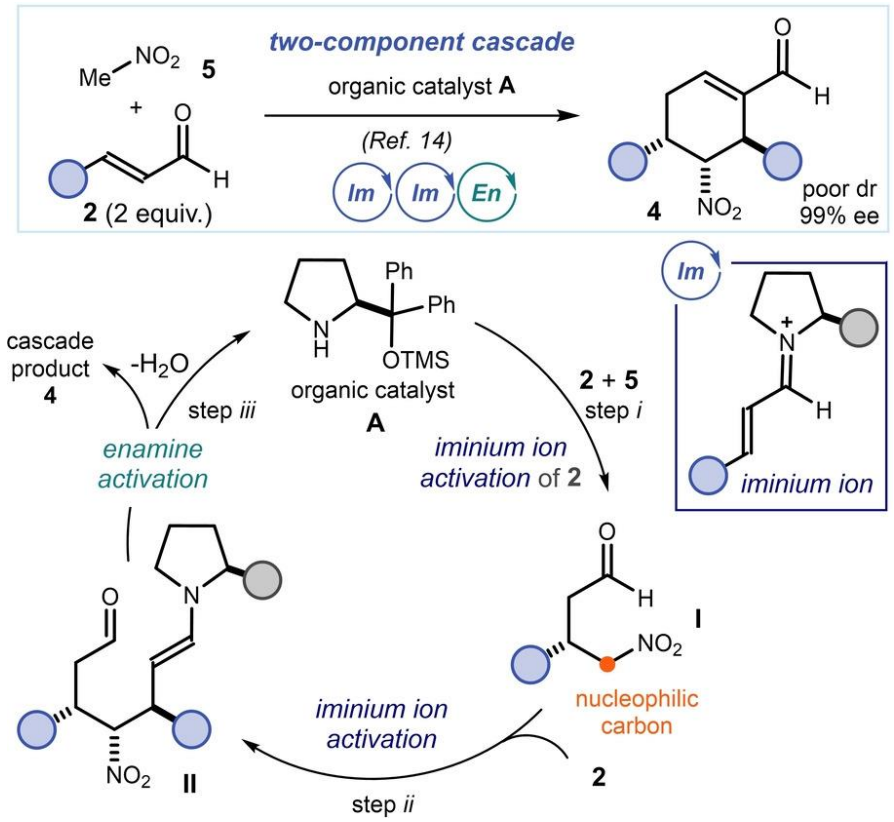
Enzyme Cascade Reactions in Biocatalysis



Targeted Cascade Reactions: precedents in Organocatalysis

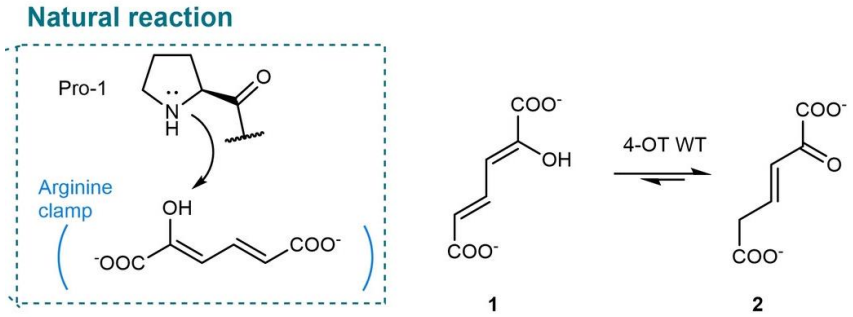


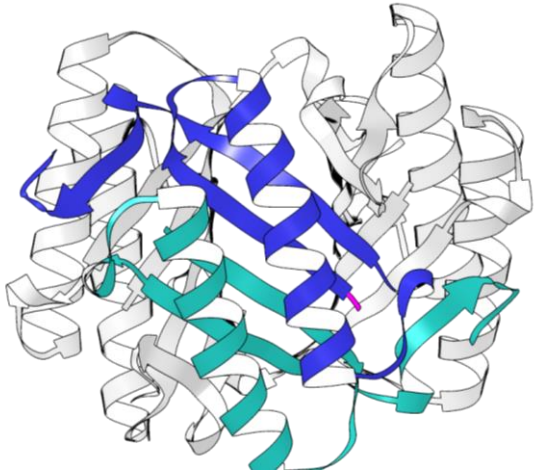


a) D. Enders and co-workers, *Nature* **2006**, *441*, 861-863. b) D. Enders and co-workers, *Synlett* **2009**, *19*, 3175-3178.

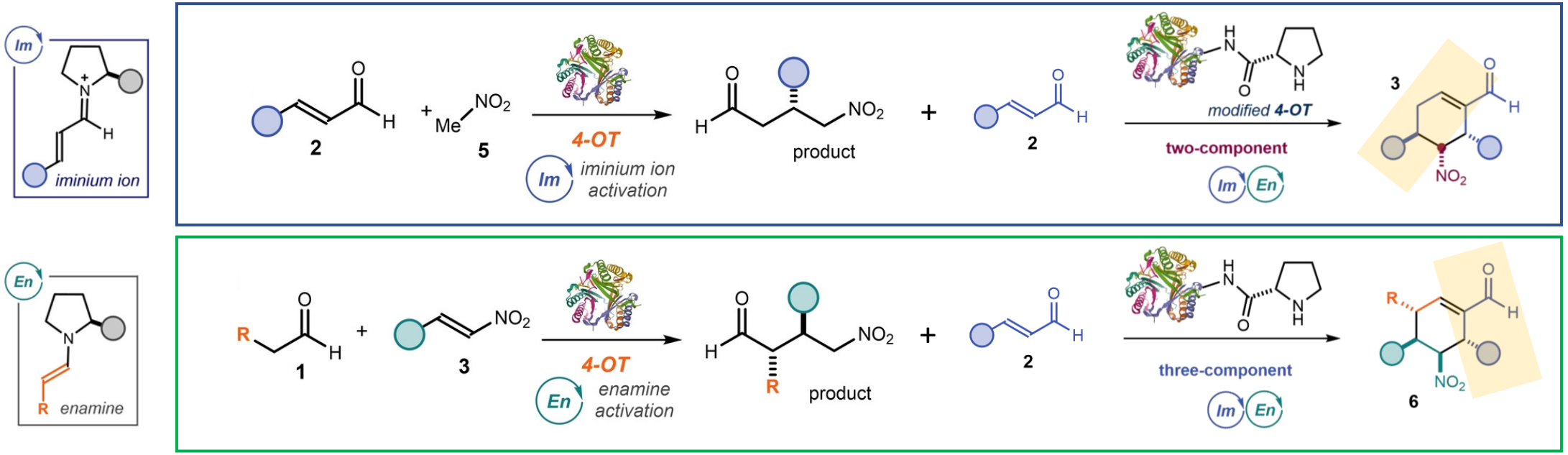
Enzyme Identification and plan design

- **Pp-4OT 4-OT from Pseudomonas sp.**
- **β1-α-β2 monomers (blue and light green) form a dimer**



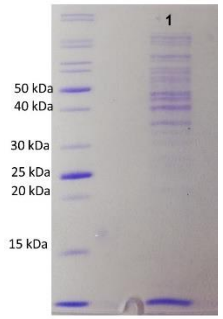


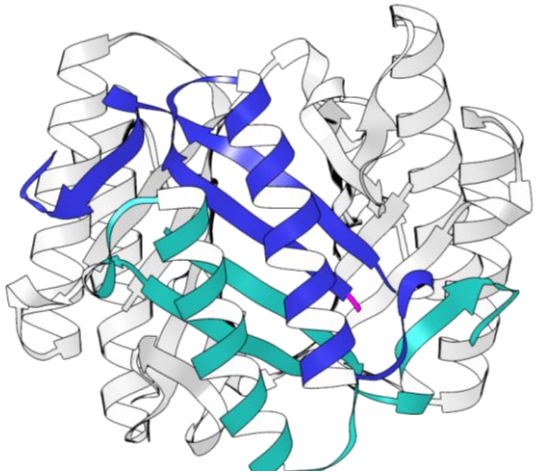
Wild type Pp-4OT (PDB: 4X19)



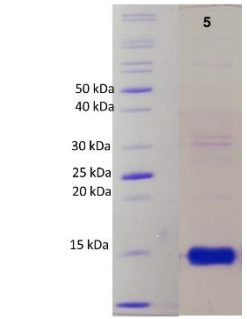
Poelarends and co-workers, *Angew. Chem. Int. Ed*. **2012**, 51, 1240-1243.
Poelarends and co-workers, *ACS Catal.* **2019**, 9, 4369-4373.

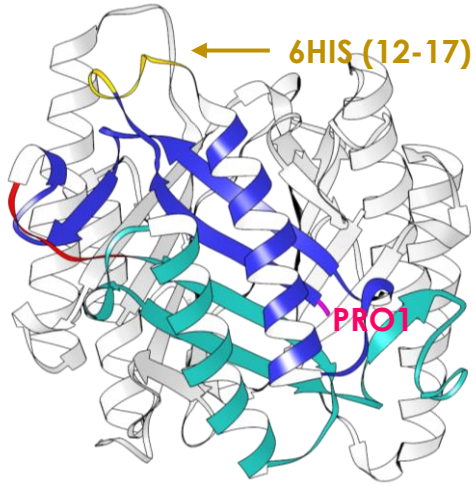
Genetic modifications and reactivity test



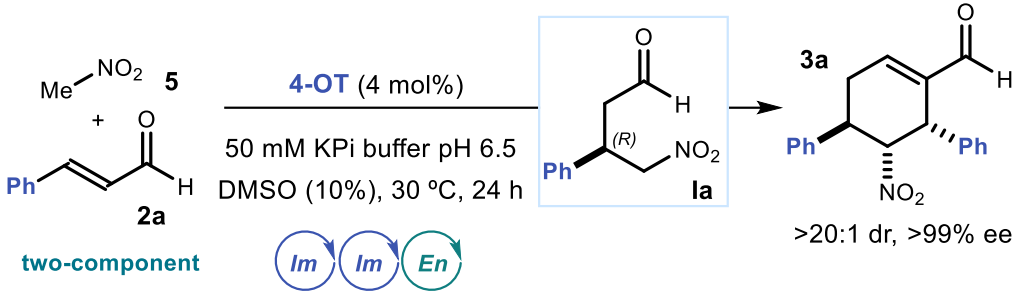


Wild type Pp-4OT (PDB: 4X19)



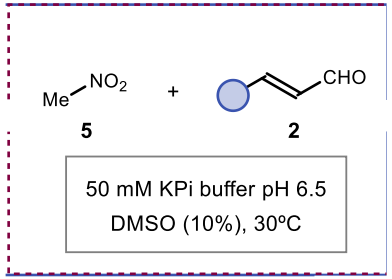


Genetically modified Pp-4OT
Linker (red), His-Tag (yellow)

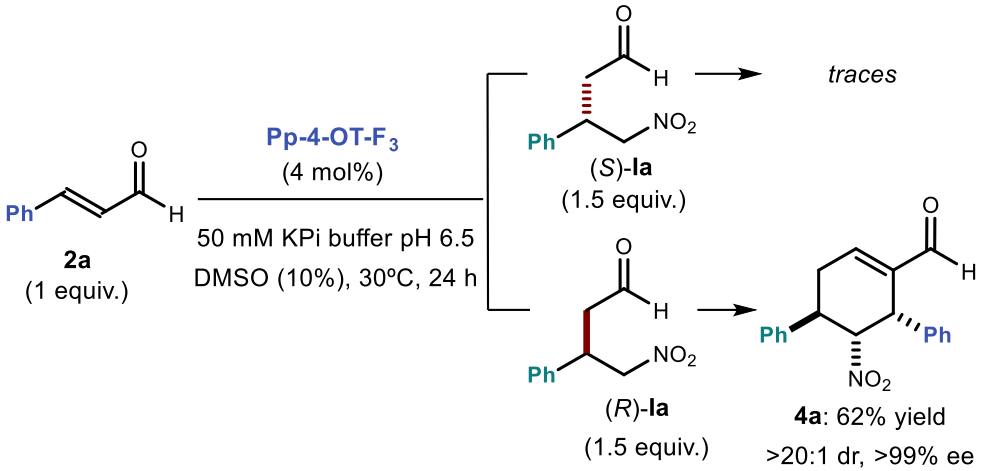


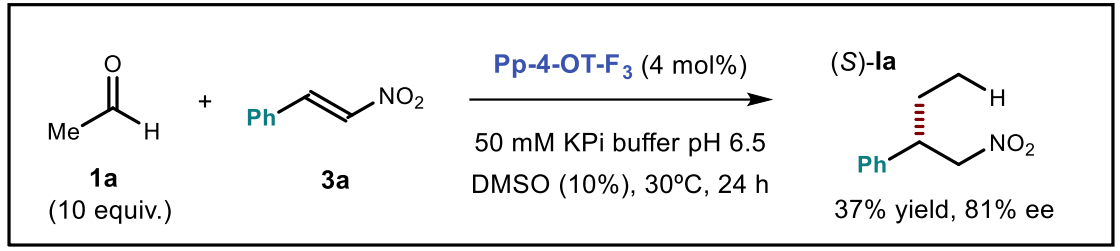
entry	enzyme	deviation	yield (%) of **Ia** (ee %)	yield **3a** (%)
1	**Pp**-4OT	10 equiv. of **2a**	44 (99)	<5

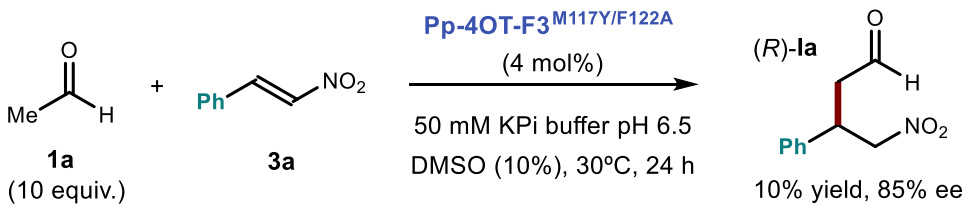
Reaction scope and product desymmetrization

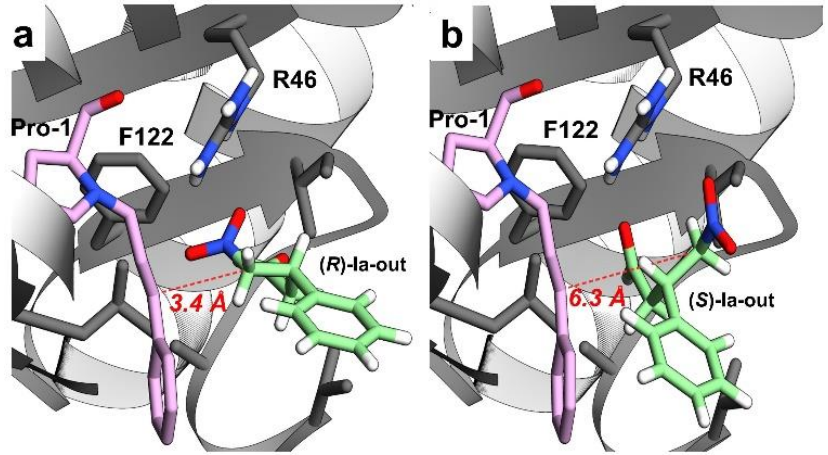


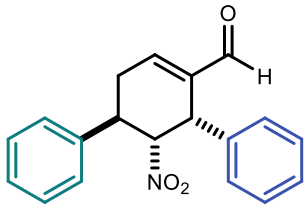
Enable the biocatalytic version of Enders triple cascade



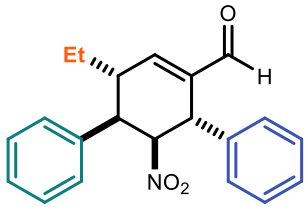




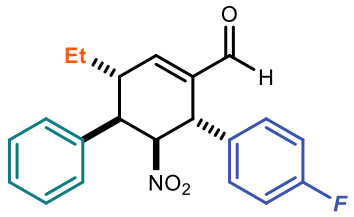




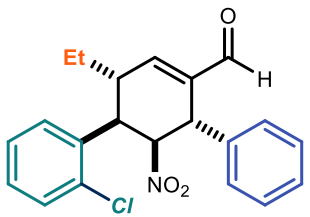
3a: 28% yield
>20:1 dr, >99% ee
(with 6 equiv. of **1a**)



6a: 50% yield
3.7:1 dr, >99% ee



6b: 43% yield
6:1 dr, >99% ee



6c: 51% yield
4.7:1 dr, >99% ee
(23% yield isolated)

Conclusions

- Use of multifunctional enzymes for biocascade processes characterized by different reaction sequences
- entry into thermodynamically unfavored trisubstituted products
- Protocol for non-symmetric trisubstituted products
- Genetic modifications for easier catalyst preparation
- Biocascades can match and even surpass in efficiency the potential of organocatalytic cascade catalysis

Interested in more ?

Angew. Chem. 2022, 134, e202212176.

Acknowledgements

- *Dr Adriana Faraone*
- *Laura Kqiku*
- *Dr Jan Vilim*
- *Gianluca Simionato*

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