Stabilization and application of a Baeyer-Villiger monooxygenase as protein scaffold in photochemical reactions

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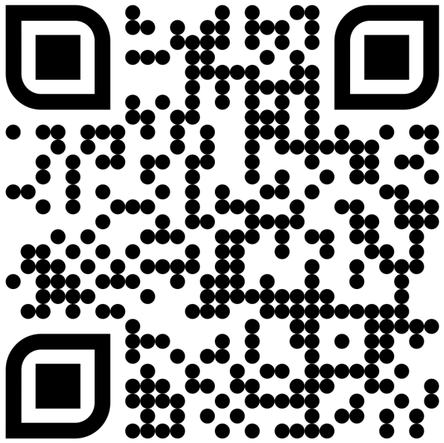

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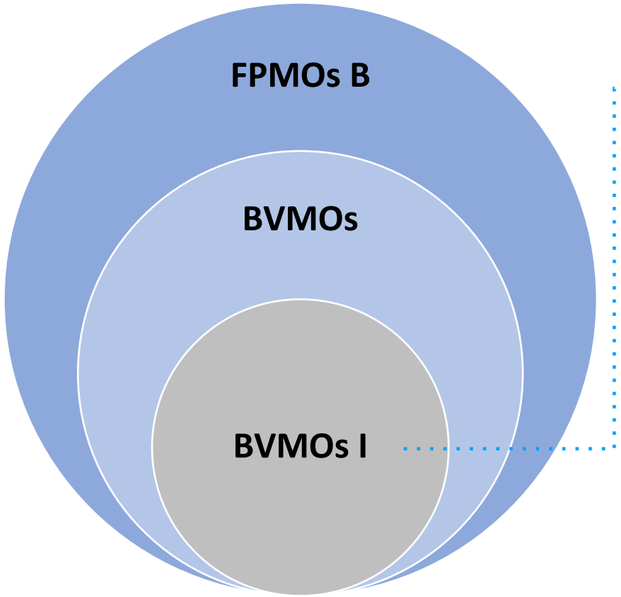
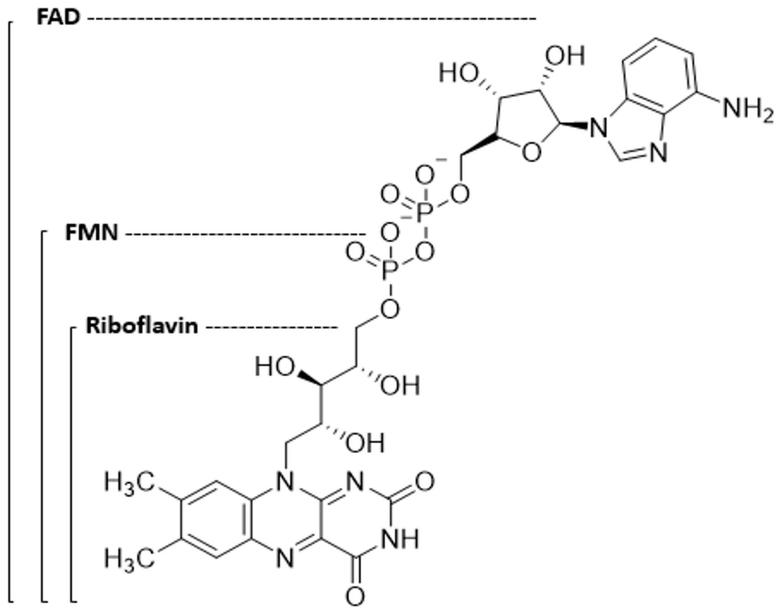
Photochemical reactions with BVMOs

BVMOs belong to the family of **Flavoproteins** and catalyze the **Baeyer-Villiger oxidation**



FAD in the active site

Coenzyme **NADPH**



Aim: **Hijack the protein scaffold to drive singlet oxygen reactions, using FAD as a photosensitizer**

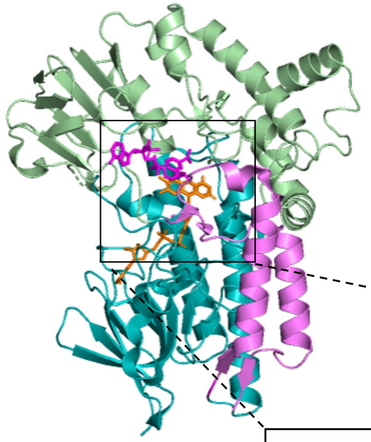
Biochemical characterization of BVMOs

OTEMO	*CHMO Acineto*	*CHMO Arthro*
Expression -Solubility		
Good Solubility	Good Solubility	Poor Solubility (even in presence of chaperones)

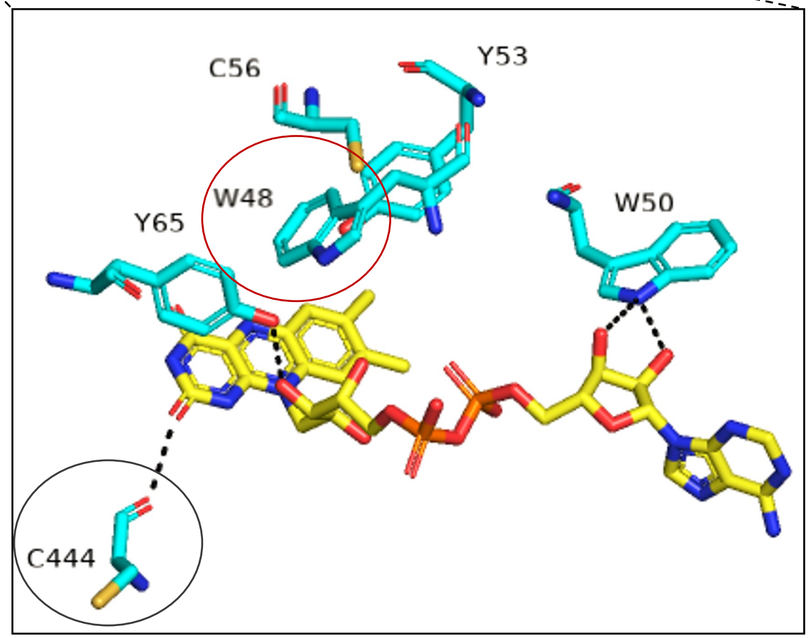
Balke et al., ACS Chem. Biol., 2016, 11, 38-43.

Rational design mutagenesis

OTEMO
from *Ps. putida*



PDB ID: 3UP4

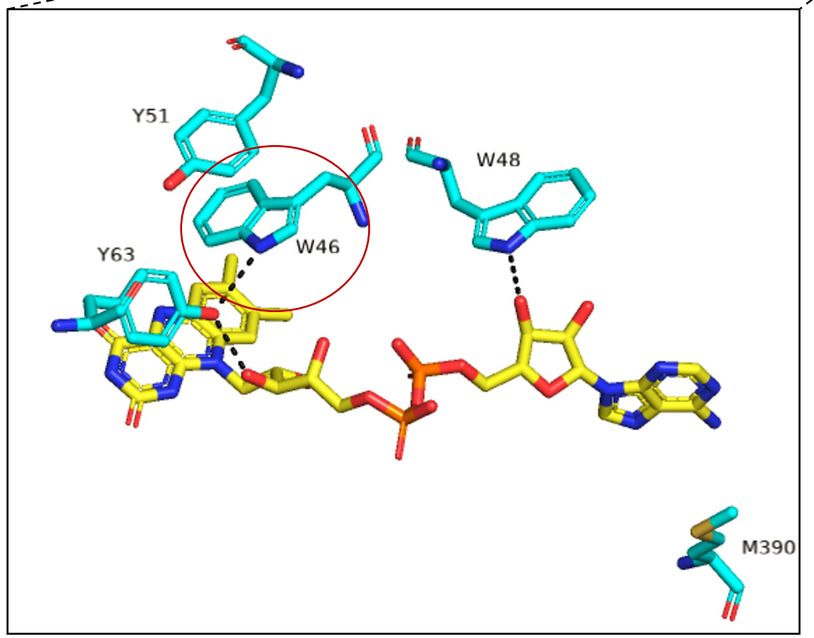


Targeting residues labile to oxidation may lead to

the construction of a more stable BVMO

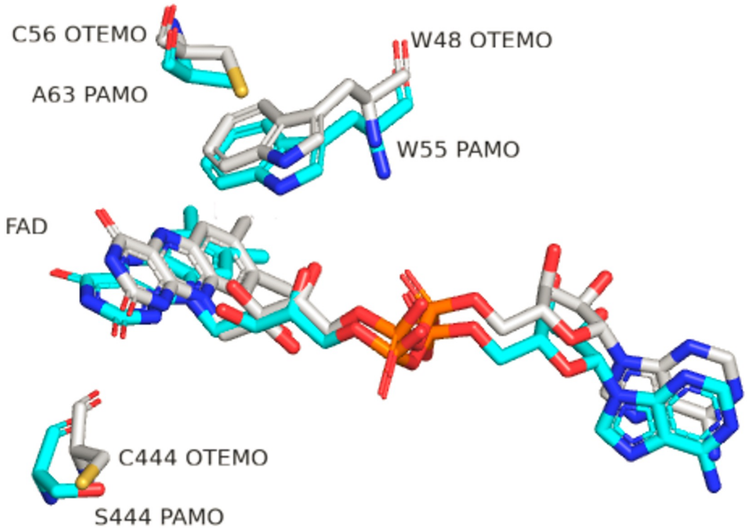
CHMO Acineto
from *Acinetobacter sp.*

Homology model



Biochemical characterization

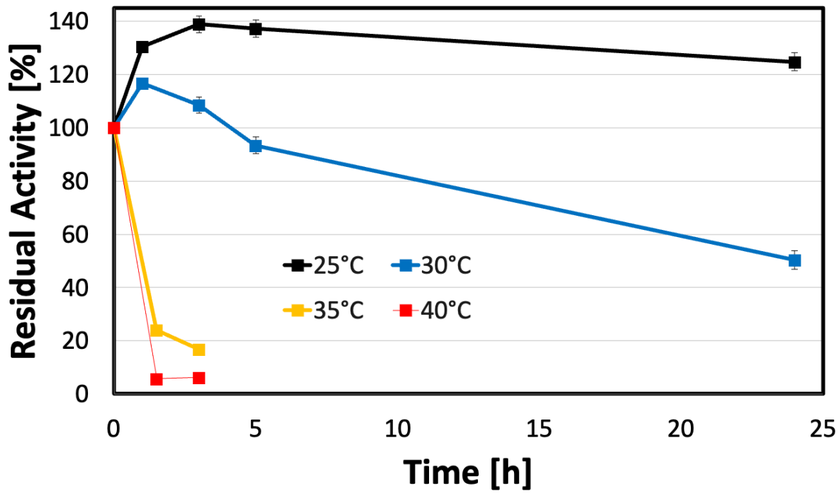
Variant	Color
OTEMO	yellow
OTEMO C444S	yellow
OTEMO W48L	colorless
CHMO Acineto	yellow
CHMO Acineto W46L	colorless



PAMO PDB ID: 1W4X

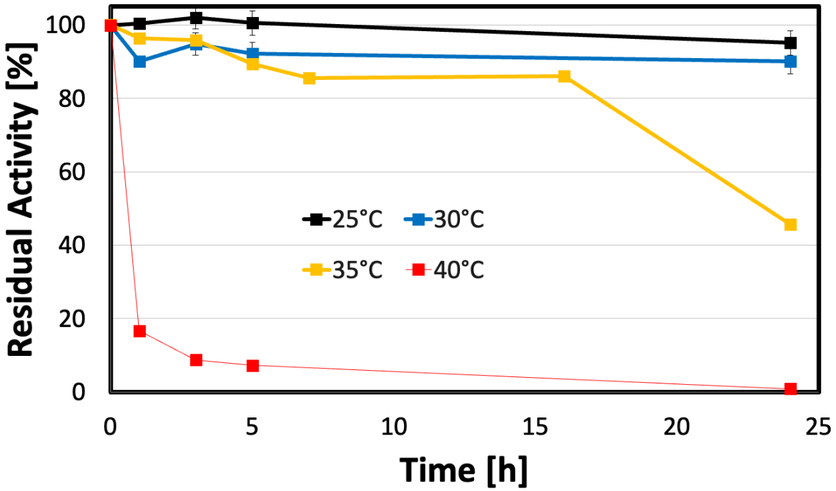
OTEMO WT

Tm = 39,7 ± 0,3 °C



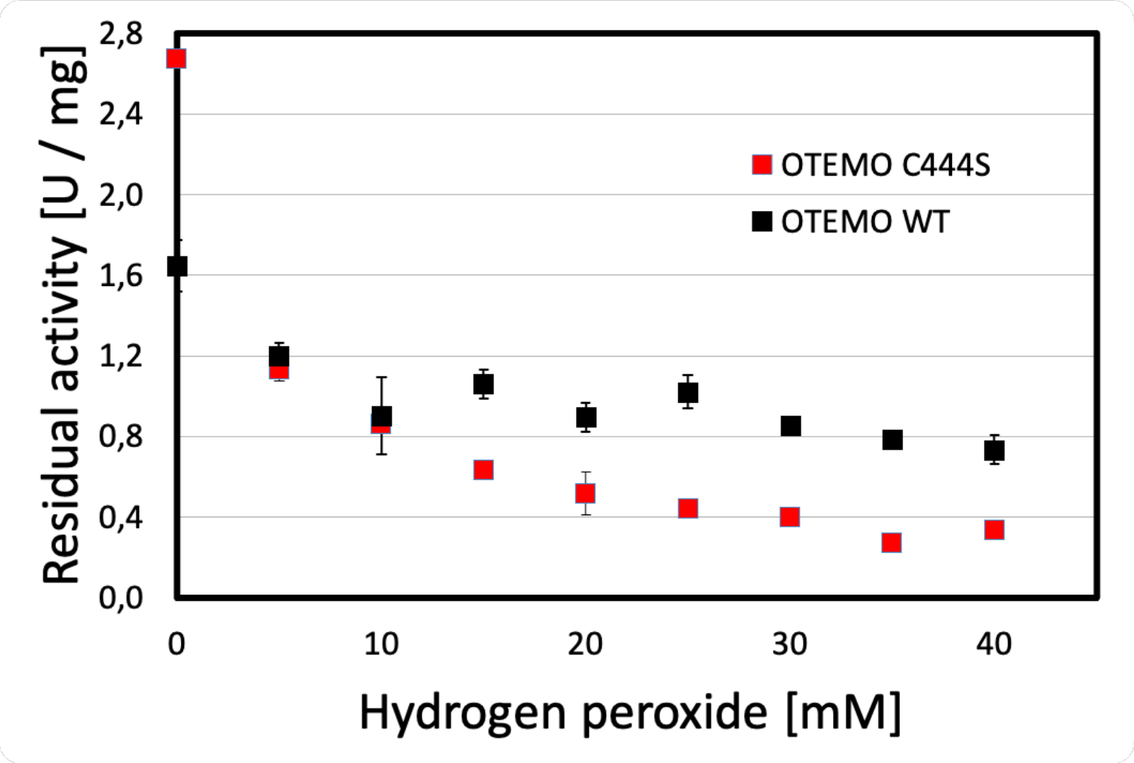
OTEMO C444S

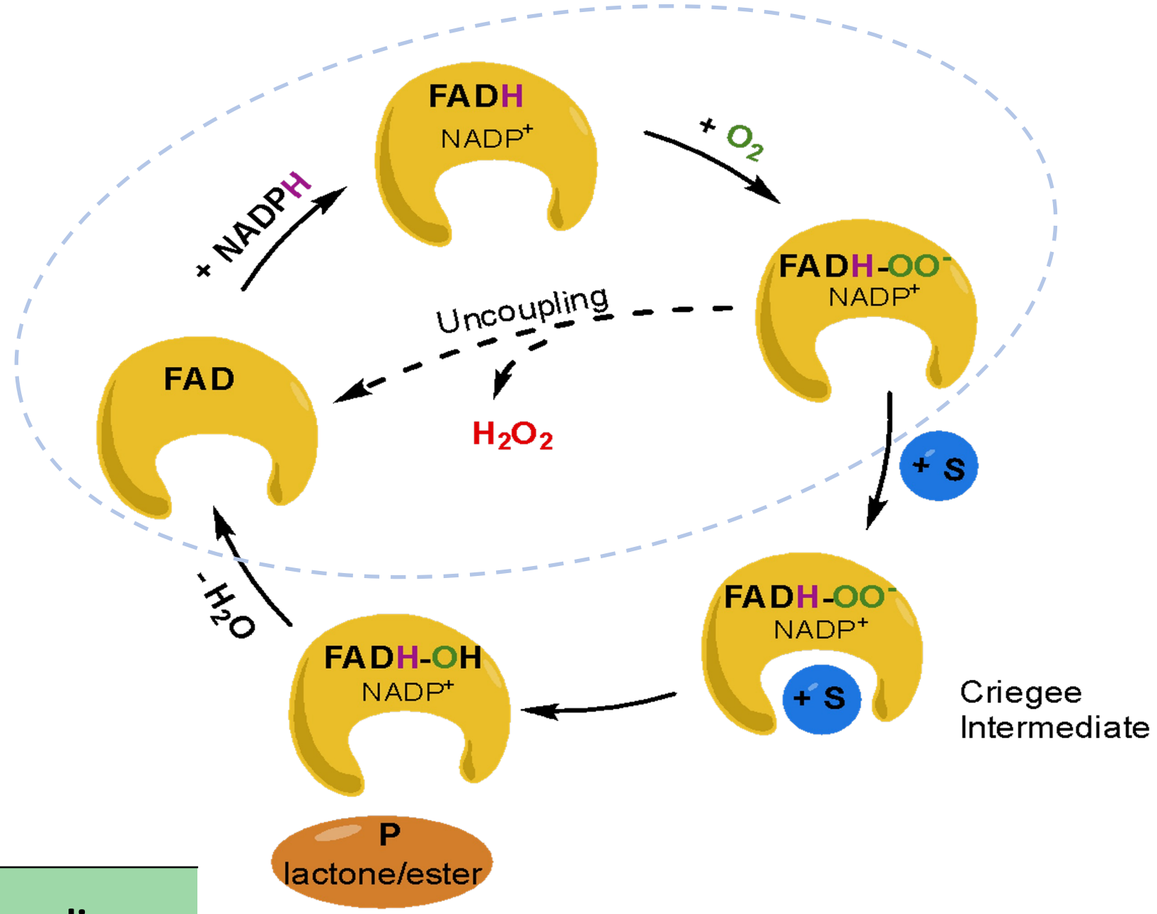
Tm = 40,3 ± 0,1 °C



Oxidative stability

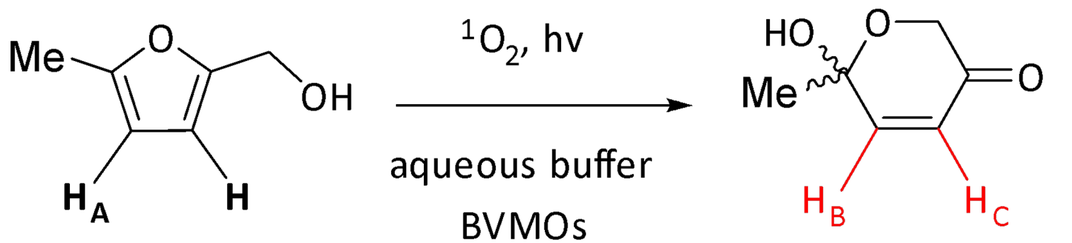
Incubation for 1h @ 25°C





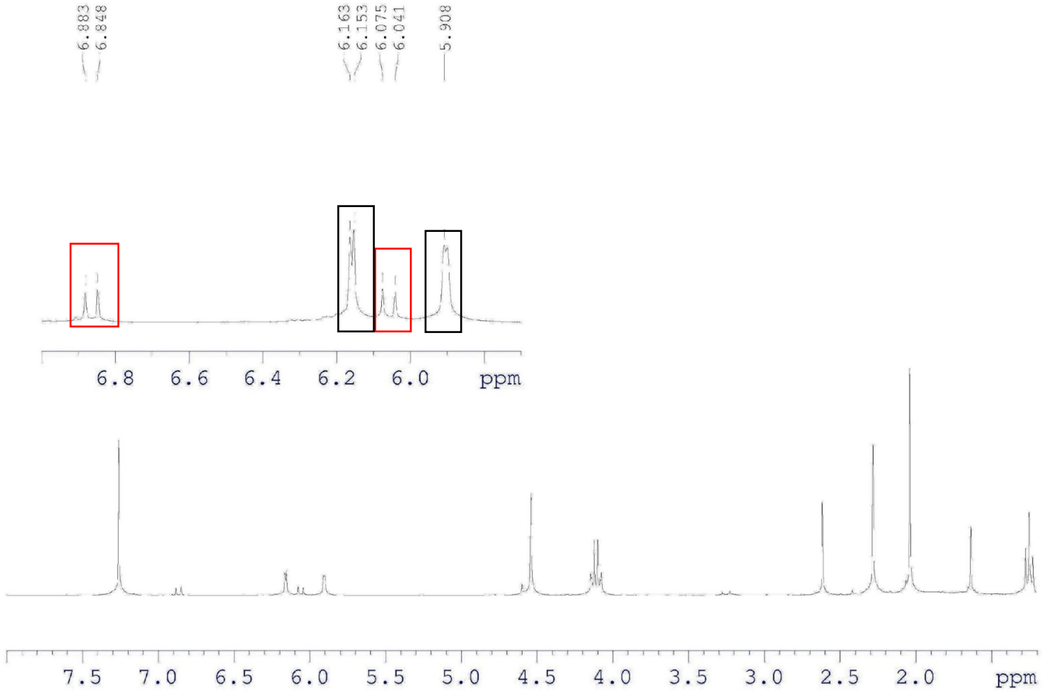
Enzyme	Specific activity [U/mg] (with substrate)	Specific activity [U/mg] (without substrate)	Uncoupling [%]
OTEMO	1.10 ± 0.02	0.03 ± 0.02	2.7
OTEMO C444S	2.30 ± 0.03	0.5 ± 0.1	21.7

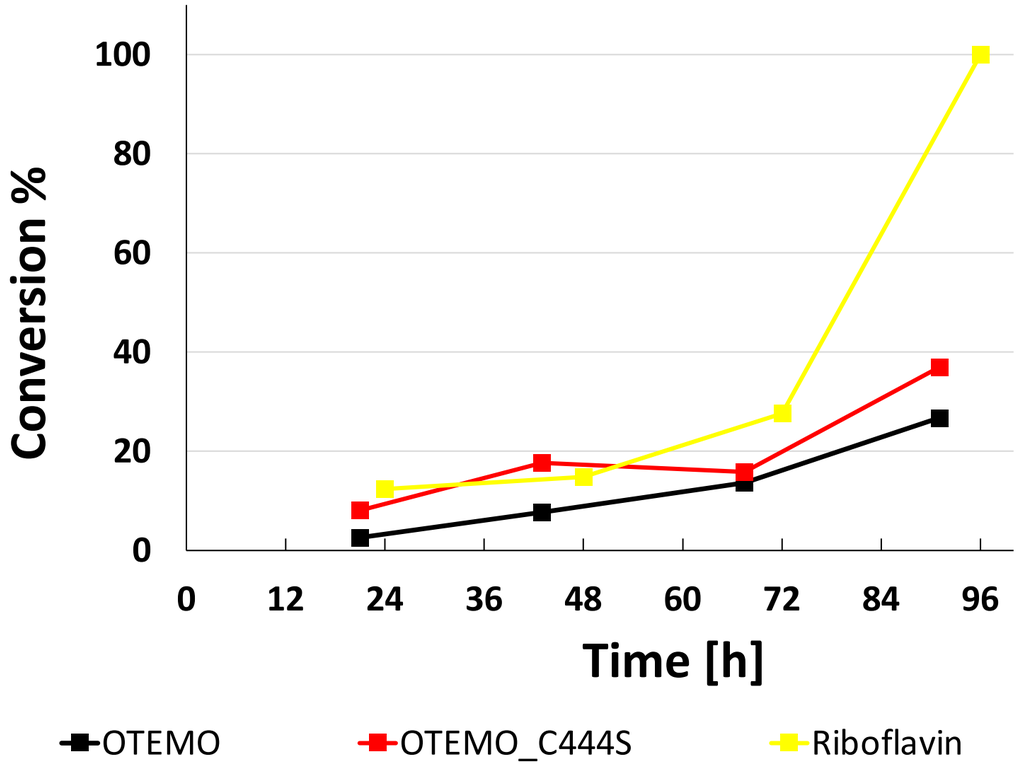
The control photochemical reaction



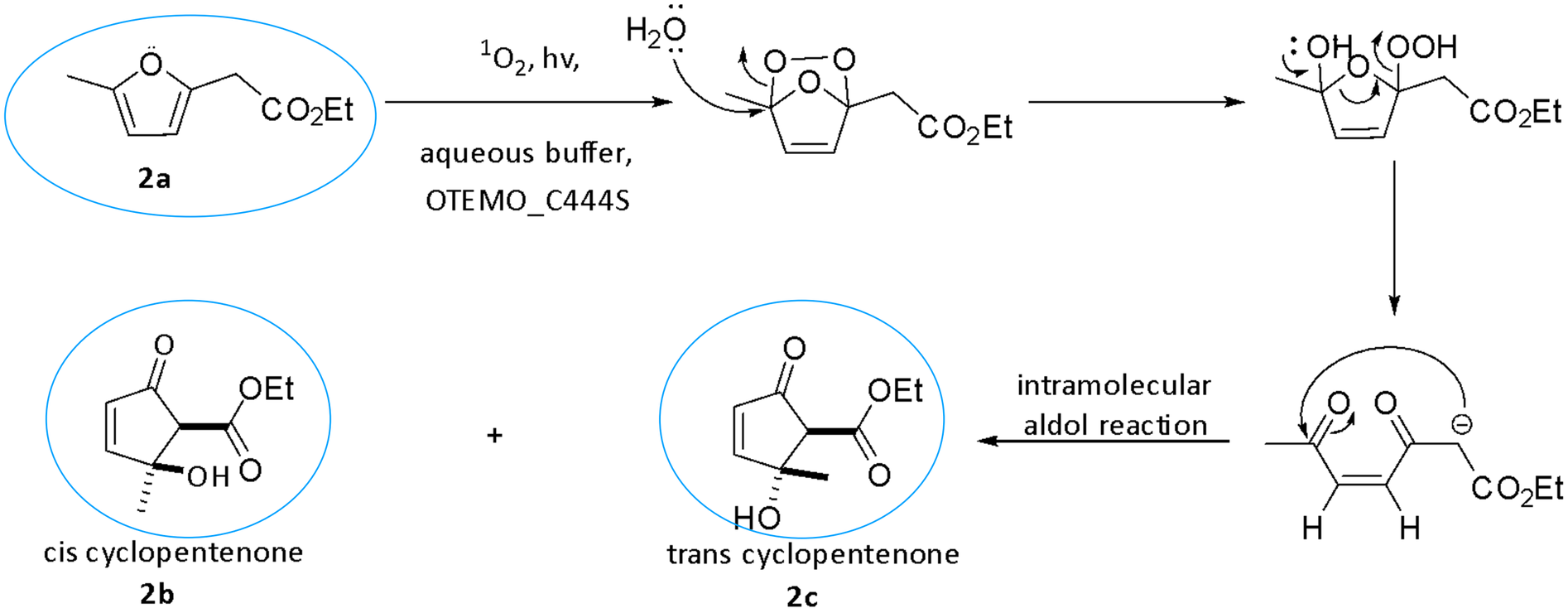
5-methyl-furfuryl-alcohol

pyran compound





Extending the range of furan substrates

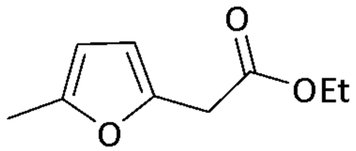


Conditions:

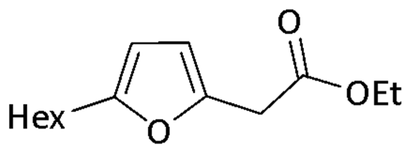
KPi, 50 mM
Oxygen saturation
40 mg substrate
2.2 nmol enzyme / PS
5% DMSO
Blue light irradiation
24 h @ 25 °C

Substrate	Photosensitizer	Solvent	Ratio cis/trans product	Conversion 2a to 2b	Conversion 2a to 2c	Total Conversion
2a	rose Bengal	MeOH	2.0	-	-	-
2a	OTEMO_C444S	KPi 50 mM, pH 8.0	0.9	11.1%	12.5%	23.6%
2a	OTEMO_C444S	KPi 50 mM, pH 9.0	1.2	14.6%	12.5%	27.1%

Extending the range of furan substrates

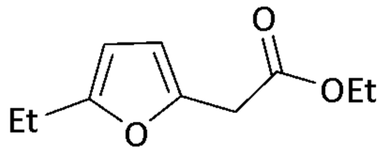


2a

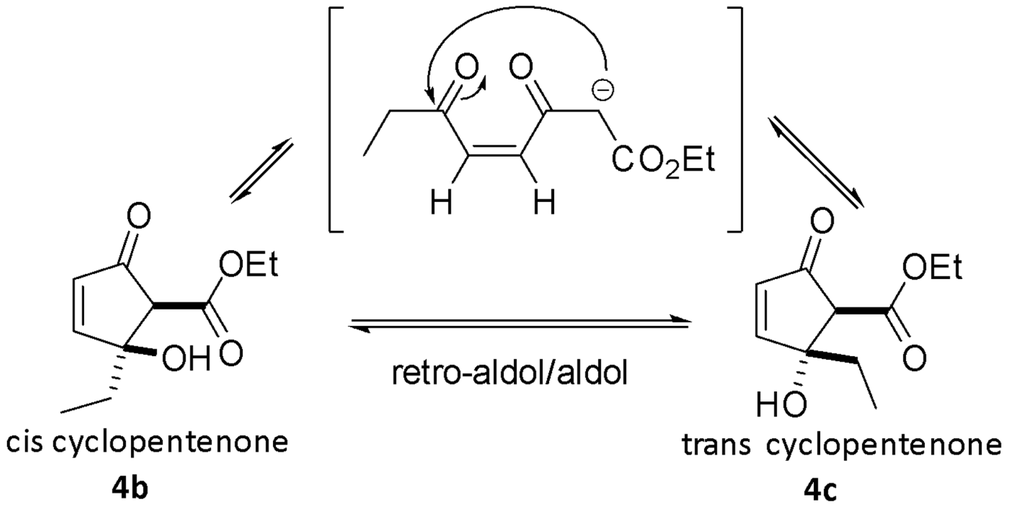


3a

Hex = hexyl (C_6H_{13})
Et = ethyl (C_2H_5)



4a



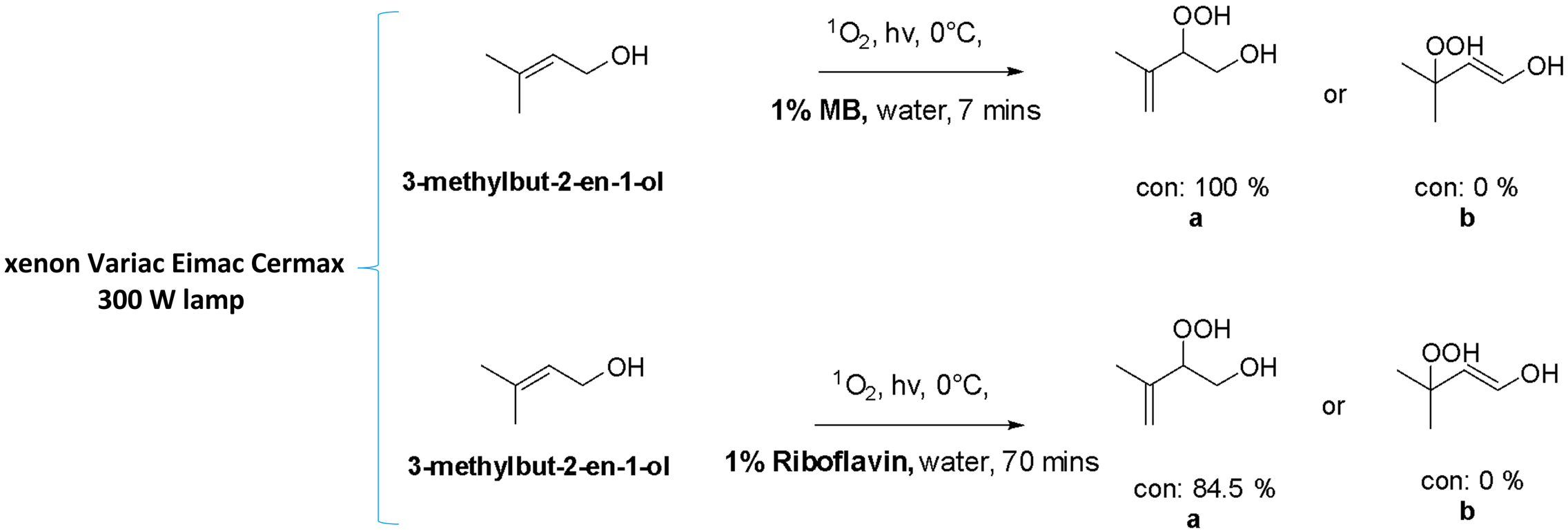
Substrate	Photosensitizer	Solvent	Conversion 2a to 2b	Conversion 2a to 2c	Total Conversion
2a	OTEMO_C444S	KPi 50 mM, pH 8.0	11.1%	12.5%	23.6%
2a	OTEMO_C444S	KPi 50 mM, pH 9.0	14.6%	12.5%	27.1%
Substrate	**Photosensitizer**	**Solvent**	**Conversion 4a to 4b**	**Conversion 4a to 4c**	**Total Conversion**
4a	OTEMO_C444S	KPi 50 mM, pH 8.0	12.5%	0%	12.5%
4a	OTEMO_C444S	KPi 50 mM, pH 9.0	15.7%	0%	15.7%

Conditions:
KPi, 50 mM
Oxygen saturation
40 mg substrate
2.2 nmol enzyme
5% DMSO
Blue light irradiation
24 h @ 25°C

Performing ene photoreactions with OTEMO C444S

The **ultimate goal** was the usage of enzyme-generated $^1O_2$ to produce chiral building blocks, exploiting the coordination abilities and the steric environment of the active site.

OTEMO C444S was not active in such reactions.



Conclusions

Successful proof-of-principle for the concept of enzyme-mediated singlet oxygen reactions

The mutation C444S **increased the specific enzymatic activity** of OTEMO by 2-fold

Thermostability increase of OTEMO_C444S at 30°C and 35°C

Trp residues are essentials for the effective engagement of FAD in the active site of BVMOs

Flavin is a weak photosensitizer and with the extra limitations by the use of enzymes, it could not deliver synthetically useful levels of reaction (so far)

Acknowledgement

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "1st Call for H.F.R.I. Research Projects to support Faculty Members & Researchers and the Procurement of High-Cost Research Equipment grant" (Project Number: 664).

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