1st Call for H.F.R.I. Research Projects for the Support of Faculty Members and Researchers and the Procurement of high-cost Research Equipment

Title:	Development of sustainable chemoenzymatic processes for optically pure amines from alcohols or alkynes
Project Acronym:	CEPOPA
Project No:	664
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Deliverable	D4.1 - Optimized protocol on the one-pot chemoenzymatic reactions

The envisioned chemoenzymatic process could not be materialized as planned from the beginning. Many approaches have been tested and new mitigation plans aroused.

Obstacles:

- Graphene oxide didn't work as expected for the oxidation, in mild conditions. All tested substrates gave no or traces of desired **ketone.**
- Carbon nitride porous sheets produce **acetophenone from phenylethanol.** However, they work as photocatalyst, dimerizing **PLP,** leading to protein denaturation
- All oxidative (nano)materials tested didn't give the benchmark acetophenone product, even when tested with various conditions (solvent free, organic solvent, high temperatures, H_2O_2 or O_2 as oxidants)
- When enzyme was immobilized (even reductive aminases), the cascade trial with either **alcohol** or **alkyne** gave no amine of interest neither the expected **ketone**. Even in raised temperatures.
- Transaminases are inactivated with even a small amount of **hydrogen peroxide,** which could be used as a green oxidation agent

Mitigations:

The oxidation part of the cascade was modified to be done with enzymes (either monooxygenases or peroxygenases).

- BVMOs as alternative oxidation plan, from **ketones** to **lactones**. BVMO was mutated for oxidative stability purposes
- UPOs as oxidation plan using **hydrogen peroxide** to produce acetophenone from **ethylbenzene (alkane).**
- Reaction engineering of the UPO reaction gave mainly **acetophenone** instead of **phenylethanol**

- One pot-one step (UPO-ATA) didn't work, probably due to hydrogen peroxide remaining in the reaction mixture even when used as a substrate.

Optimized protocol:

- 2μM UPO, 5% acetonitrile, 25mM Ethylbenzene, 50mM phosphate buffer pH 7 and 0.5 M H_2O_2 provided with a syringe pump with flow 140 μL/h. At room temperature for 4 hours.
- Reaction was checked through HPLC or GC, to monitor acetophenone production
- When significant amount was produced, the reaction mixture was subjected into high temperature (60 ºC) for 30 mins, in order to break down the remaining hydrogen peroxide.
- Buffered isopropylamine (0.5 M) at HEPES buffer 50 mM pH 8 was added in the mixture after left to room temperature. Also, pH was adjusted to 8 and the appropriate amount of PLP was added (1 mM).
- Then, 1 mg of 3HMU was added and the mixture was left at 35 ºC for 48 h in the dark

Reaction scheme: