

Mid-term seminar of the projects
from cofunded call of ERA
CoBioTech



Fabrication of hierarchically organized multi-functional heterogeneous biocatalysts for the modular synthesis of ω -amino acids from renewable feedstocks

Project acronym: HOMBIOCAT

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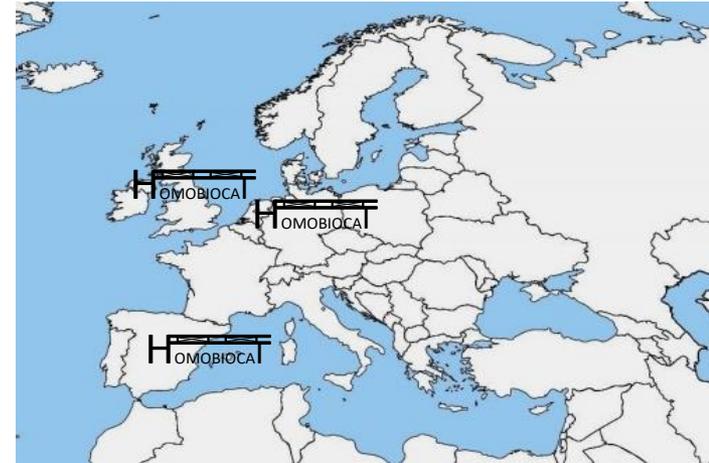


- CIC biomaGUNE (research institute), Spain (CIC BMG)
- Bioassays (company), Spain (BIOA)
- University of Nottingham (university), UK (UNO)
- Ruhr-Universität Bochum (university), Germany (RUB)

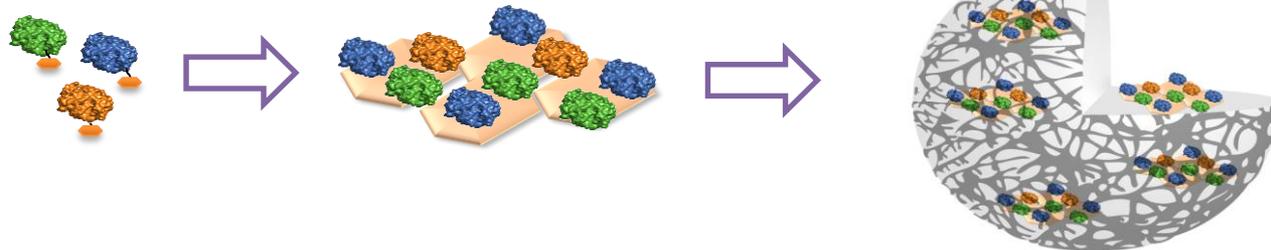


- Total project budget: **1.130.000 €**
- Project start: 01/05/2018 (RUB, 06/2018, UNO 08/2018, CIC BMG 01/2019)

- Duration: May 2018-April 2021
- Partners: 4 partners (1 industrial), 3 EU countries



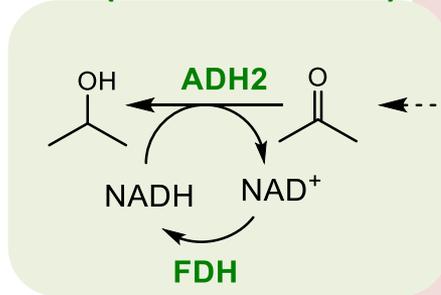
- Aim: Development of a **tool-box** for fabrication of **efficient multifunctional biocatalysts**



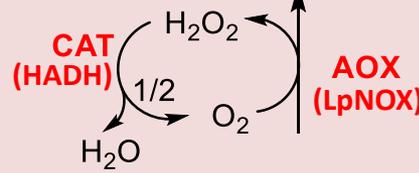
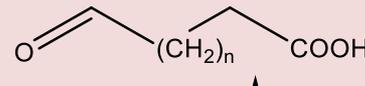
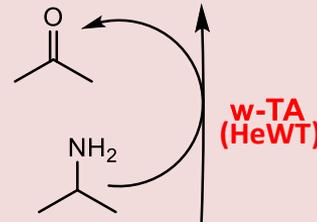
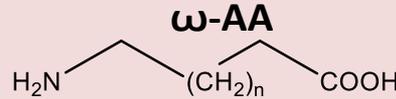
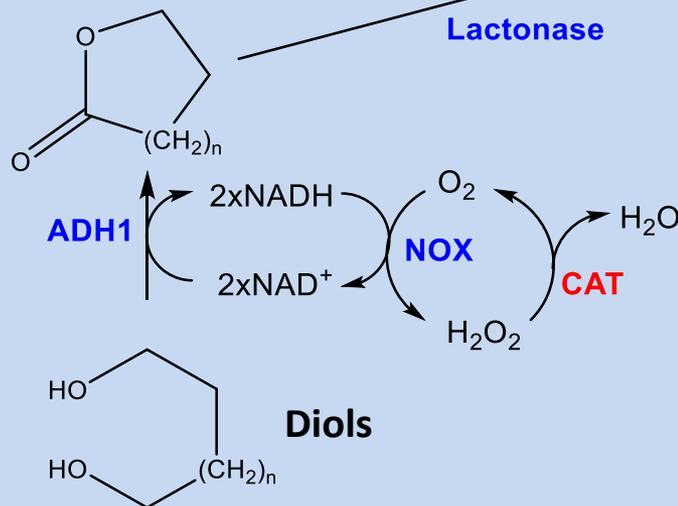
- **Project main objective:** *Developing a tool-box for the fabrication of hierarchically organized heterogeneous multi-functional biocatalysts*
- **Specific aims:**
 - ✓ **WP1** Design and functional expression of 4 enzymatic modules
 - ✓ **Wp2** Design and functional expression of orthogonal scaffolding protein units
 - ✓ **WP3** Solid-phase assembly of multi-enzyme systems into scaffolds *in vitro*
 - ✓ **WP4** Co-immobilization of different enzymatic assemblies on solid materials
 - ✓ **Wp5** Synthesis of ω -amino acids from renewable sources (diols or bio-oils) catalyzed by multi-functional heterogeneous biocatalysts
 - ✓ **Wp5** 10 L scale-up production under industrially relevant conditions and use real biorenewable feedstocks
 - ✓ **WP6** Preparation of a genetic toolbox encoding different enzymes fused with distinct scaffolding units

WP1 Design and Fabrication of multi-enzymes systems (RUB)

MODULE D: keto-alcohol conversion (>95 % conversion)

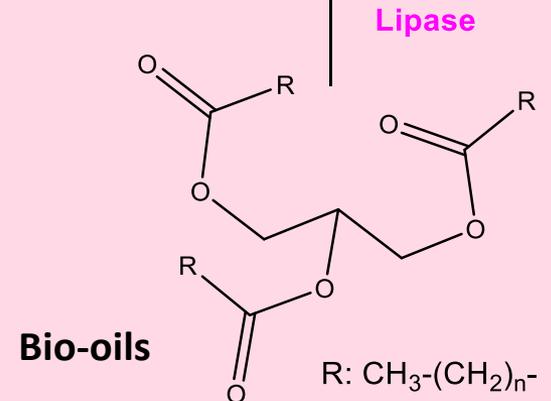
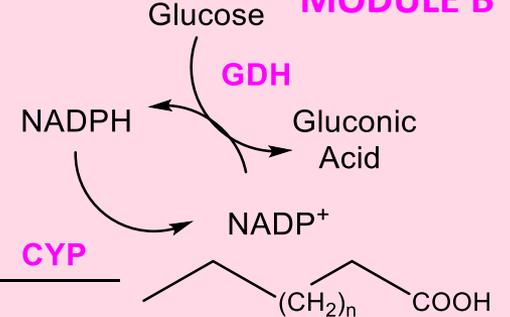


MODULE A: 5-hydroxy pentanoic acid 100mM (70% conversion)



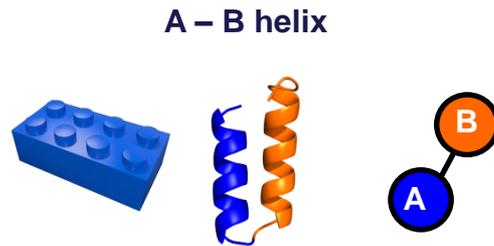
MODULE C: 6-amino hexanoic acid 50mM (80% conversion)

MODULE B

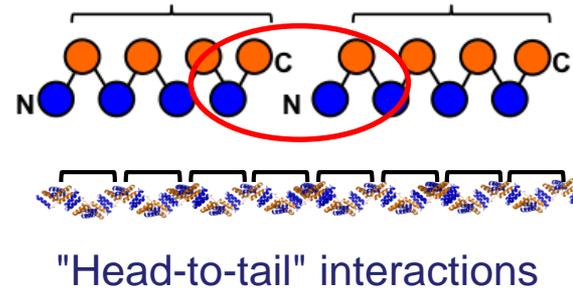


- WP2 Design of scaffolding units for the ordered assembly of enzymes (CIC BMG)

Building block: CTPR Unit

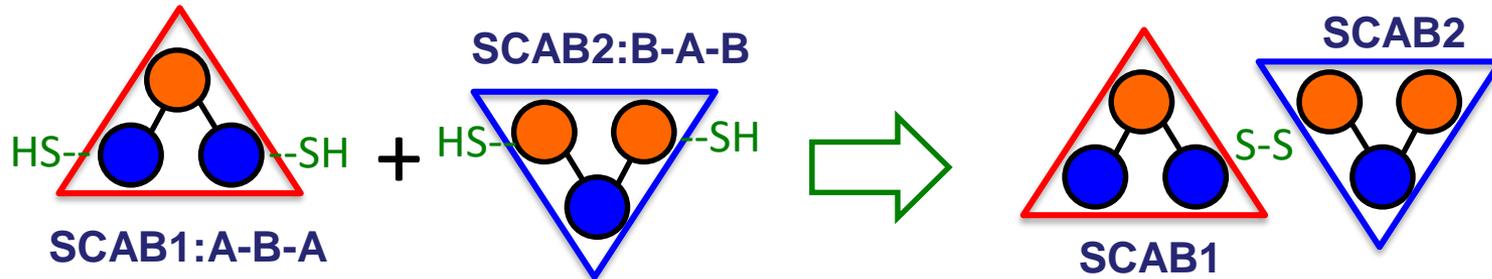


Linear assemblies



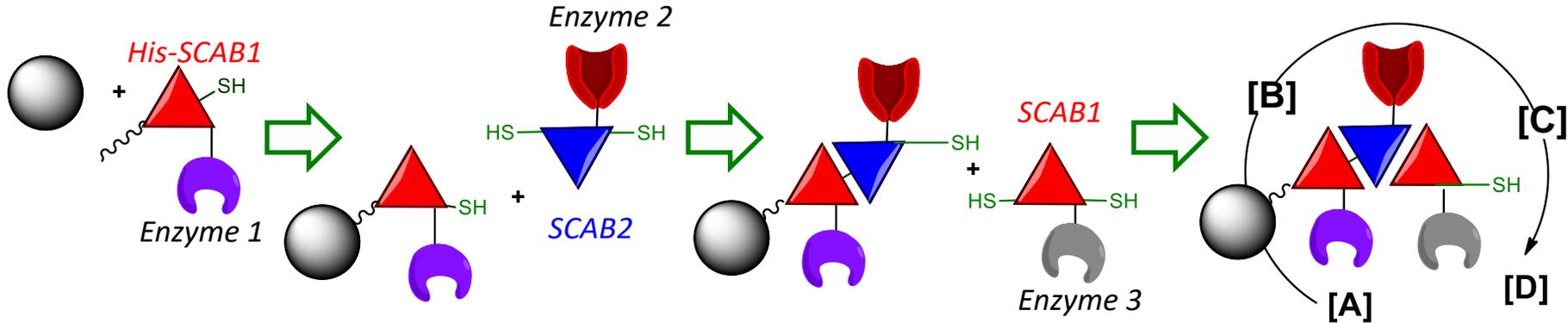
Orthogonal biobricks: intra-repeat interactions stabilized with disulfide bonds

Scaffold-Bricks

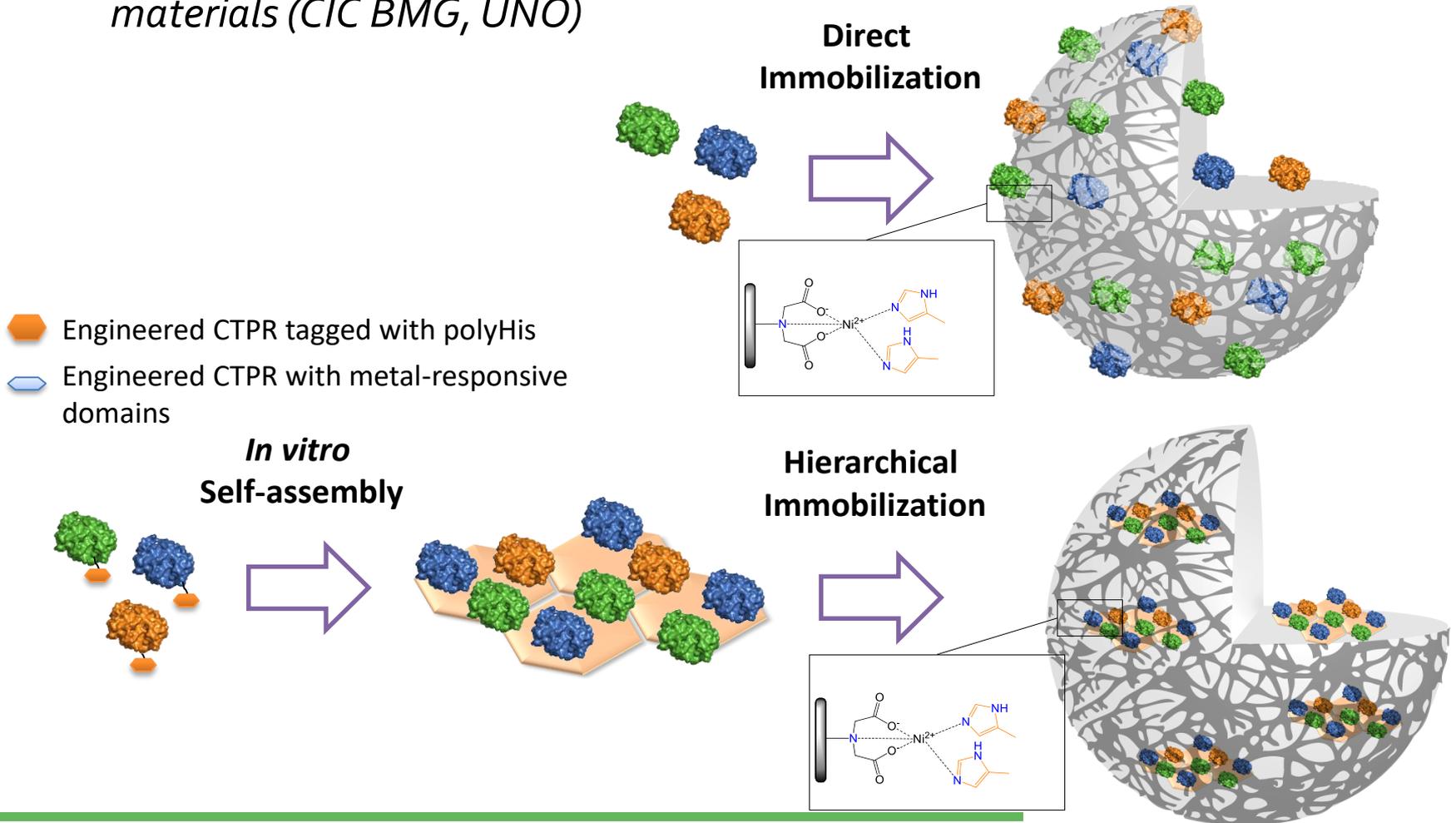


- WP3 *In vitro* assembly of enzymes tagged for ordered scaffolding (CIC BMG)

Solid-phase assembly of enzymes: His-SCAB1:SCAB2:SCAB1

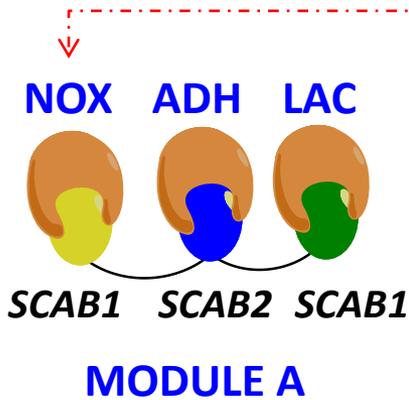


- WP4 Immobilization of the single enzymes and enzymatic assemblies on solid materials (CIC BMG, UNO)



- WP5 ω -Amino acids synthesis by heterogeneous biocatalysts (M18-M36)

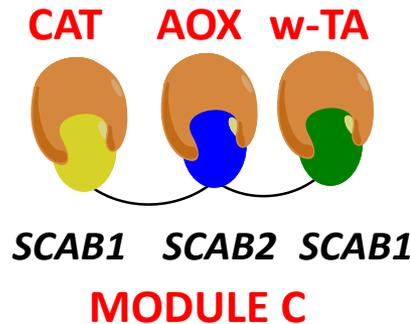
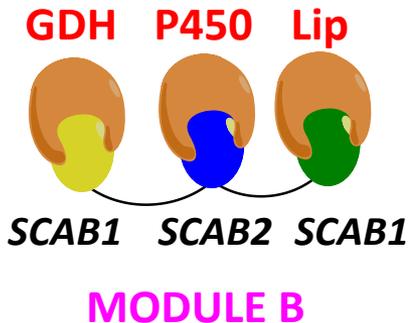
PATHWAY 1 (from diols) (Module A +C + D (optional))



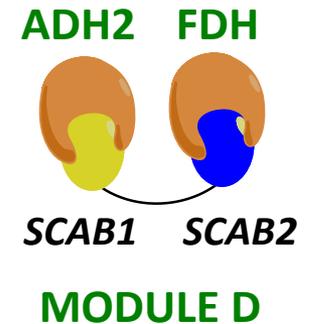
Shifting the transamination equilibrium

- **System 1:** Acetone removal by Stripping/Evaporation
- **System 2:** Acetone removal by enzymatic elimination (Module D)
- **System 3:** Unexpensive diamines

+

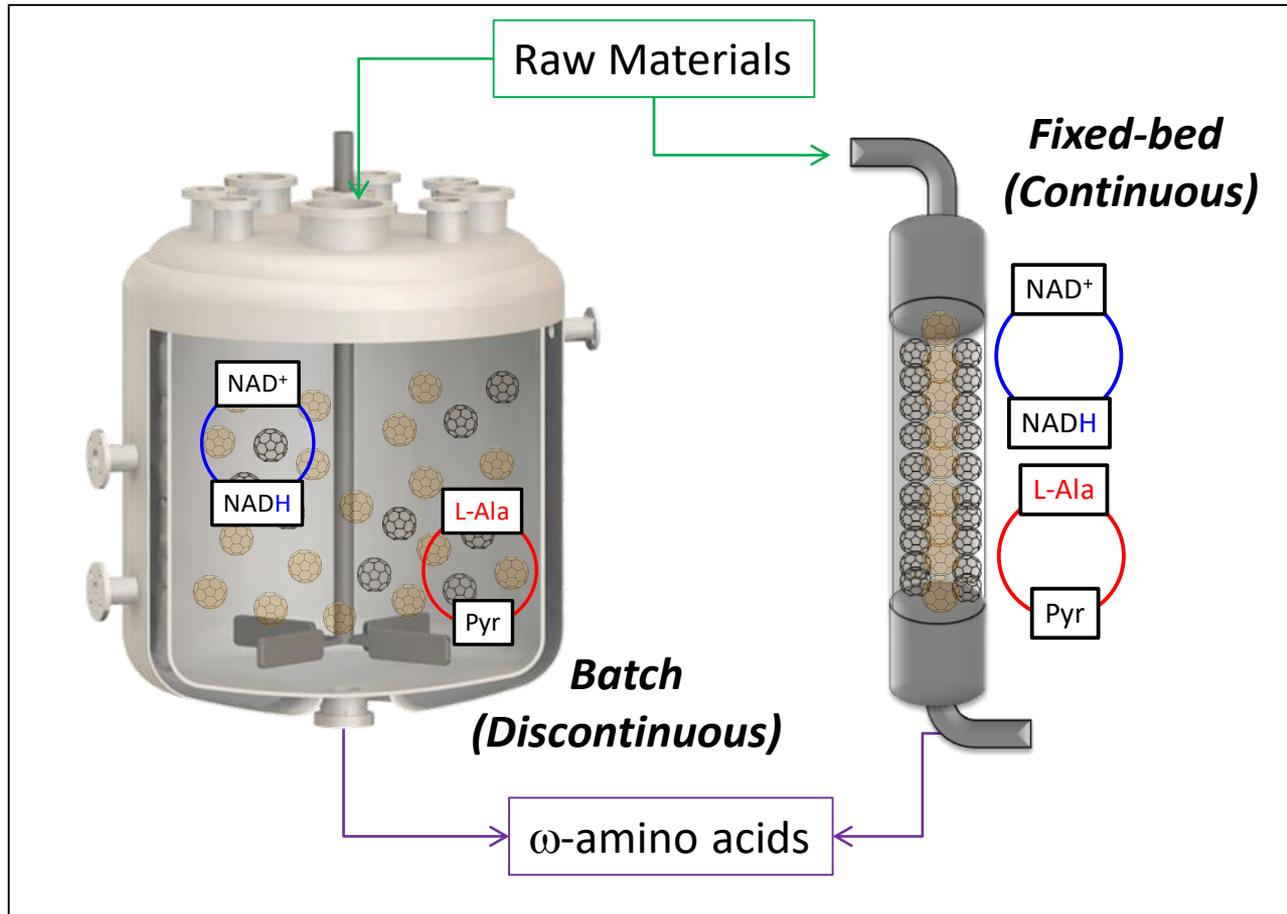


OPTIONAL



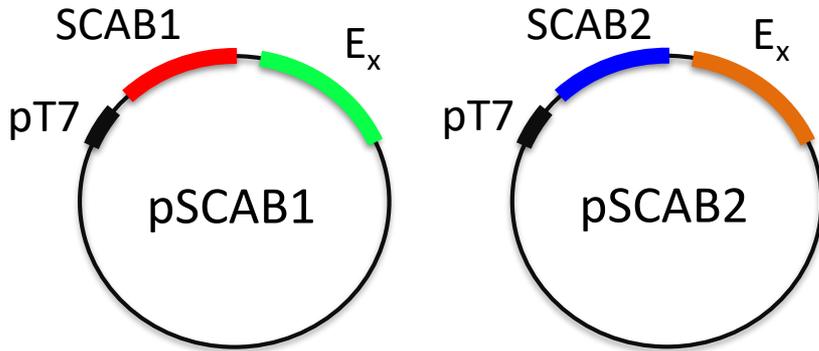
PATHWAY 2 (from oils) (Module B +C + D (optional))

- WP5 ω -Amino acids synthesis by heterogeneous biocatalysts (M18-M36)



WP6 Fabrication of multi-functional heterogeneous biocatalysts hierarchically organized in an industrial environment: Validation and demonstration (BIOA) (M12-M36)

1. Plasmid kit



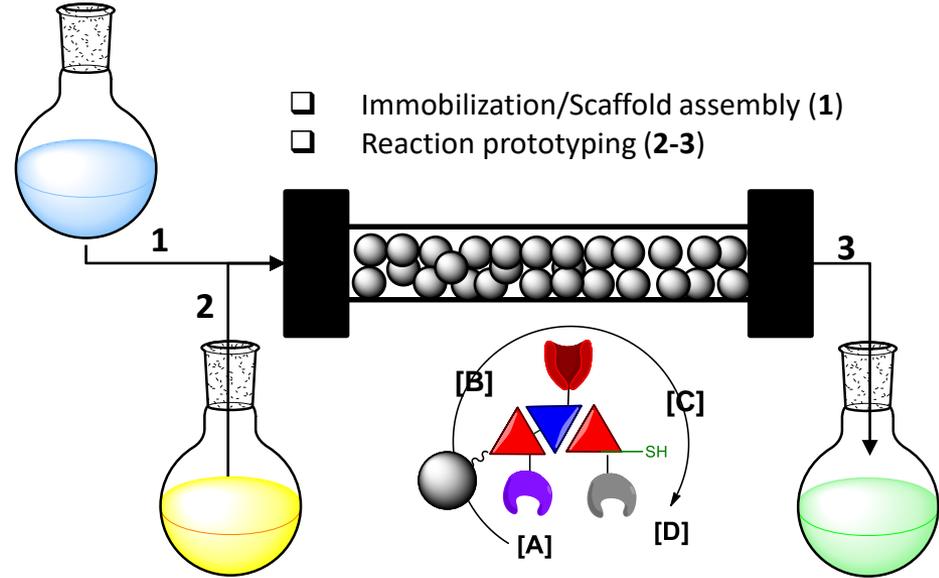
Enzyme pool



Progress and Outcomes

2. Solid phase kit

Scaffold Units



Raw substrates (diols and acids)

ω-amino acids

Engineered HOMBIOCAT plasmids with different resistances: Enable commercialization

● *Major outcomes to date*

Scientific:

Three complete multienzymatic modules, 1 partial multi-enzymatic modules

Two successful scaffolding strategies : SCABs and TRAPs

Proof of concept scaffolding of module D

Multiple solid-phase immobilization materials and strategies

Engineered set of plasmids – patentable

3 ongoing PhD thesis

Dissemination:

11 Scientific papers, 1 book chapter, 3 in progress

5 contributions to conferences

Outreach : 2 project outreach talks , 1 showcase

● *Major problems and delays*

Different starting dates – different countries

Delay funding- Spain – 2019 ; UNO- delay in hiring

COVID-19 lab closure

Contact

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Consortium