



ERA CoBioTech (ERA-Net Cofund on Biotechnologies)

ACHEMA2018

Kick-off session: "Biotechnology
for a sustainable bioeconomy"

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

Project acronym: HOMBIOCAT

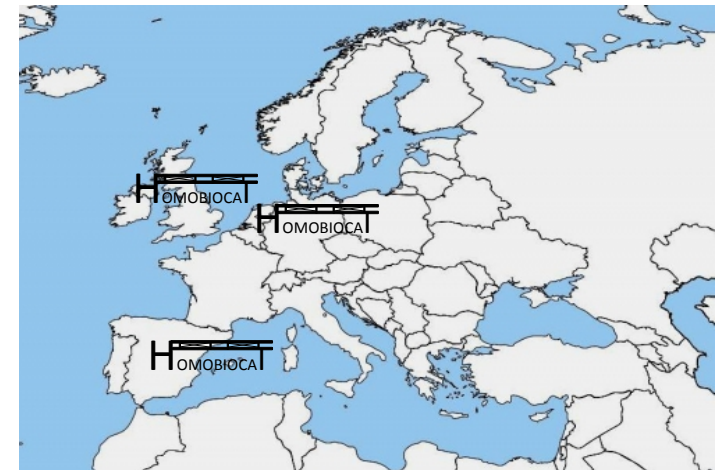
Name: Aitziber L. Cortajarena, Fernando López-Gallego



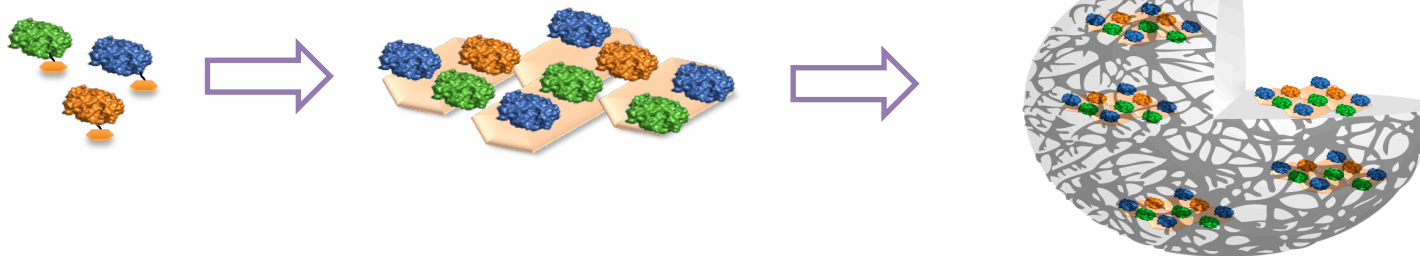
This project has received funding from the European Union's Horizon
2020 research and innovation programme under grant 722361

Frankfurt am Main, 13.06.2018

- 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**



- CIC biomaGUNE (research institute), Spain (CIC BMG)



- Bioassays (company), Spain (BIOA)



- University of Nottingham (university), UK (RUB)



- Ruhr-Universität Bochum (university), Germany (UNO)



- Third party: U. Zaragoza (university), Spain



- Total project budget: **1.130.000**

- Project start: 01/05/2018

Project Objectives:

- **Main:** Developing a **tool-box** for the fabrication of **hierarchically organized heterogeneous multi-functional biocatalysts**
 - **Problems to be solved :**
 - ✓ Need for a transition from fuel economy to bioeconomy
 - ✓ Need more efficient and sustainable processes in chemical manufacturing
 - ✓ Low efficiency of multi-enzyme systems for step-wise reactions in one pot
 - ✓ Isolated enzymes: loss spatial compartmentalization, low catalytic efficiency
 - ✓ Limited tools to control the spatial arrangement of multi-enzyme systems ex-vivo
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Project Objectives:

- **Specific Aims:**

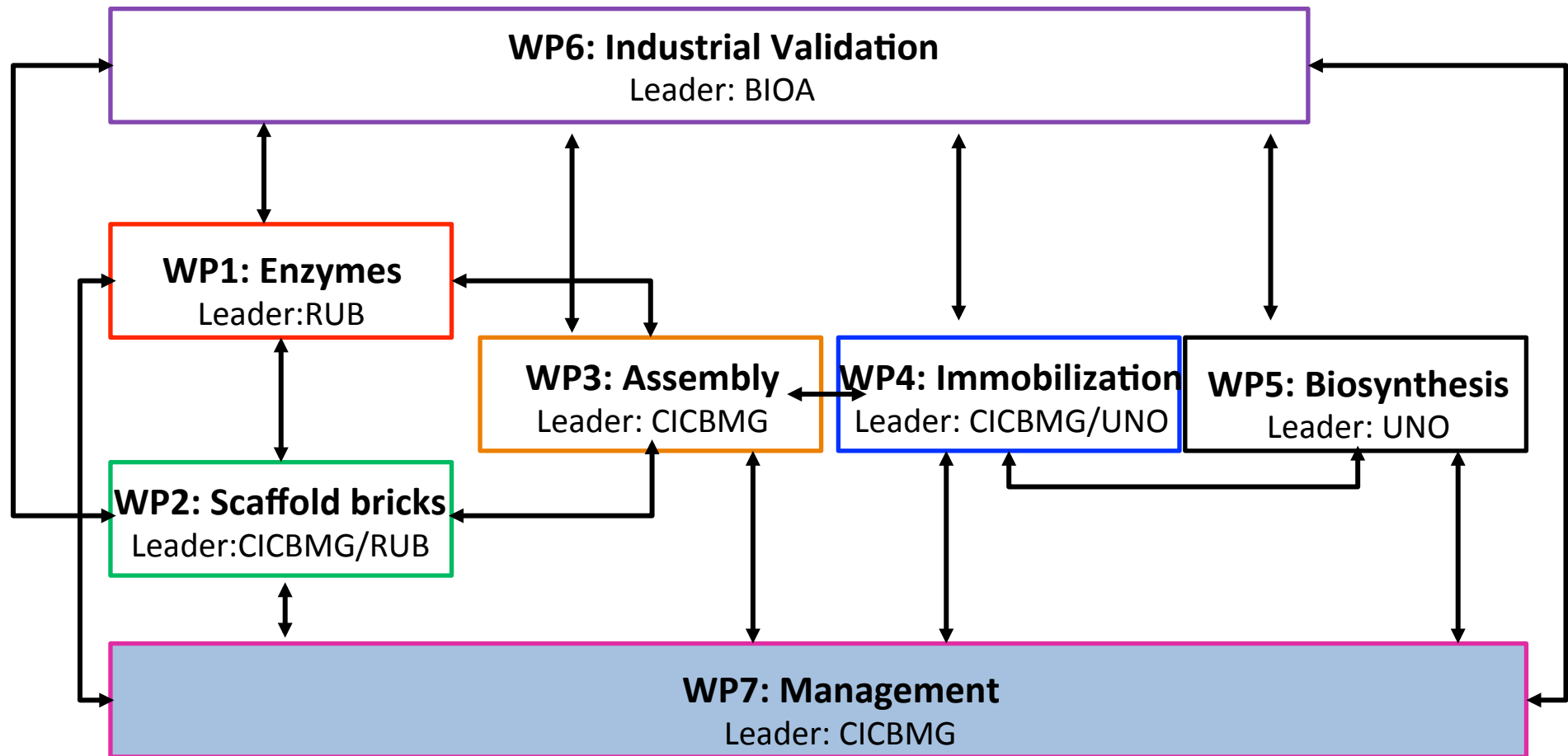
Case of study: *Synthesis of ω -amino acids from renewable feedstocks*

- ✓ Design and expression of 4 enzymatic modules
 - ✓ Design and expression of scaffolding protein units
 - ✓ Genetic toolbox: different enzymes fused with distinct scaffolding units
 - ✓ Solid-phase assembly of multi-enzyme systems into scaffolds *in vitro*
 - ✓ Co-immobilization of enzymatic assemblies on solid materials
 - ✓ Synthesis of ω -amino acids from renewable sources (diols or bio-oils) catalyzed by multi-functional heterogeneous biocatalysts
 - ✓ 10 L scale-up production under industrially relevant conditions and use real biorenewable feedstocks
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Scientific Approach and Project Topic Area

- Combination of **synthetic biology** and **biotechnological approaches**
 - **Topic Area 1: Sustainable production and conversion of different types of feedstocks and bioresources into added value products**
 - ✓ Developing a new, sustainable, eco-efficient and competitive technology
 - ✓ Obtaining high added value products from renewable raw materials
 - **Topic Area 2: Development of new products, value-added products and supply services**
 - ✓ New technological products: Protein scaffolds
 - ✓ Versatile technology to engineer multi-enzyme systems
 - ✓ Technology applicable for precise control over spatial arrangement
 - ✓ Commercial kits for scaffolding and immobilization of multienzyme systems
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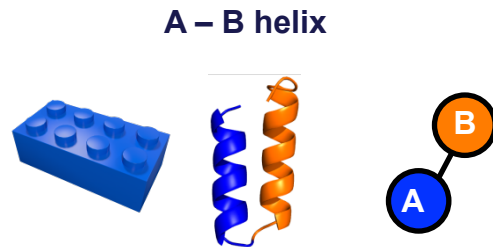
Workflow overview



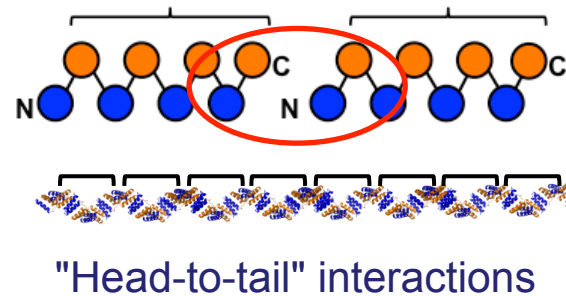
- **WP1** Design and Fabrication of multi-enzymes systems (RUB)

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

Building block: CTPR Unit



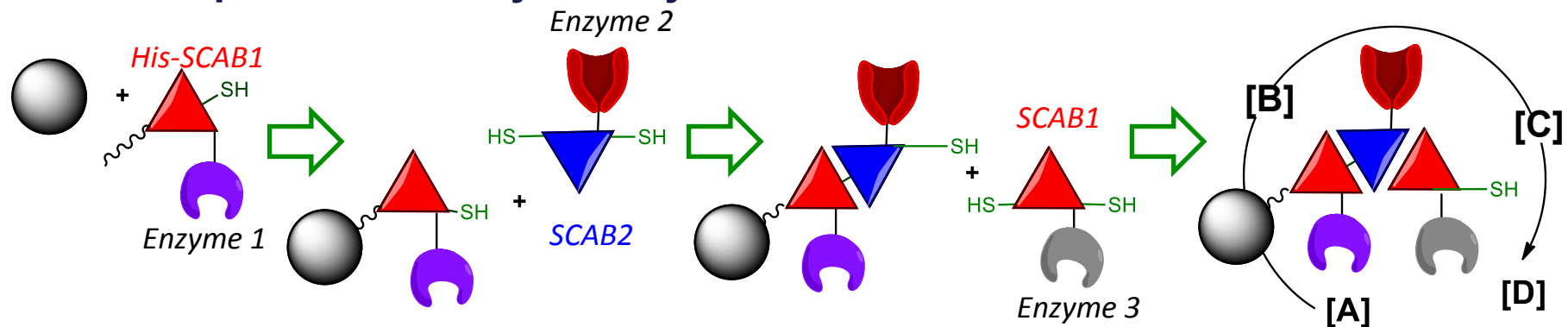
Linear assemblies



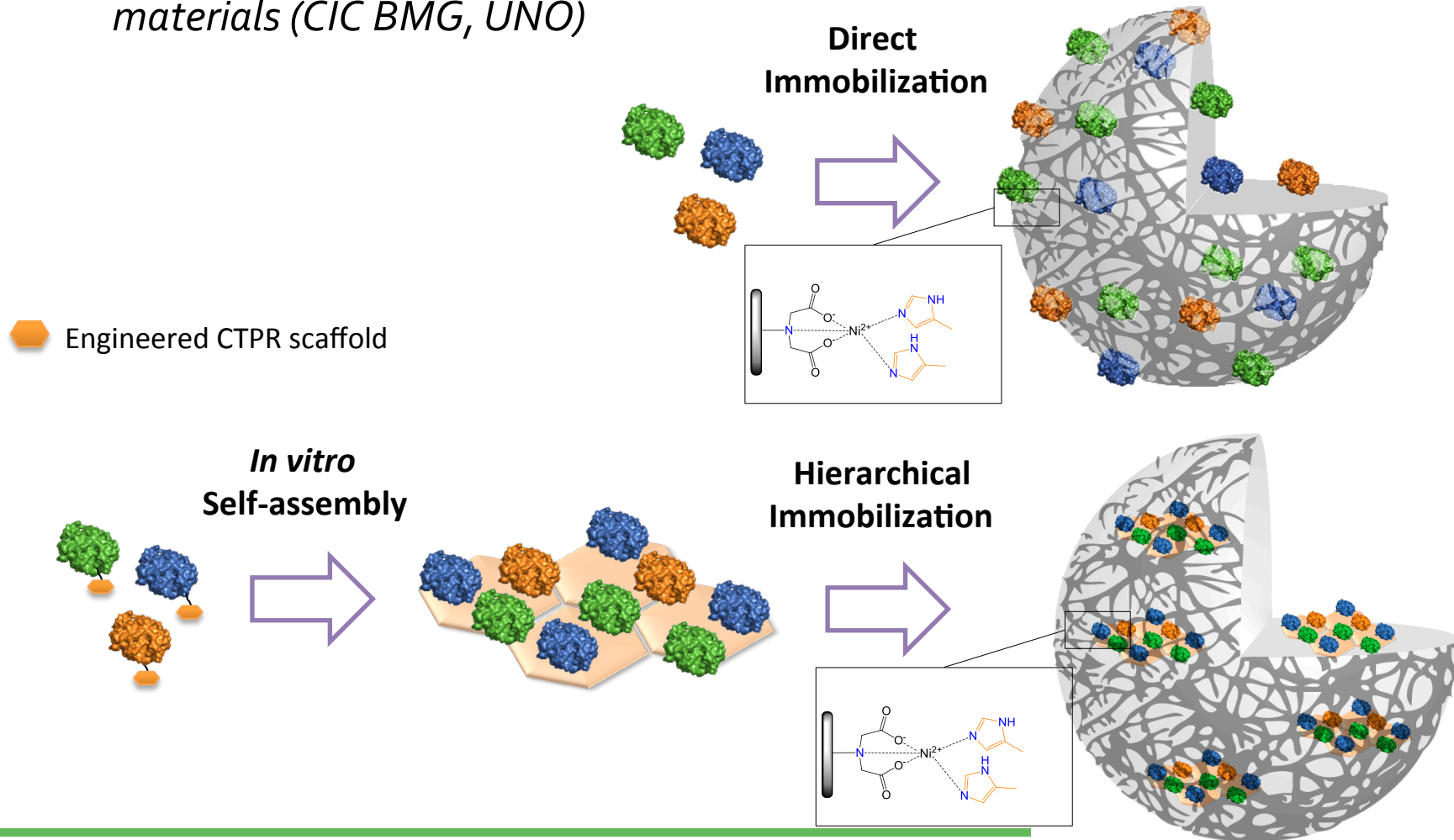
Design Orthogonal biobricks

- **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)

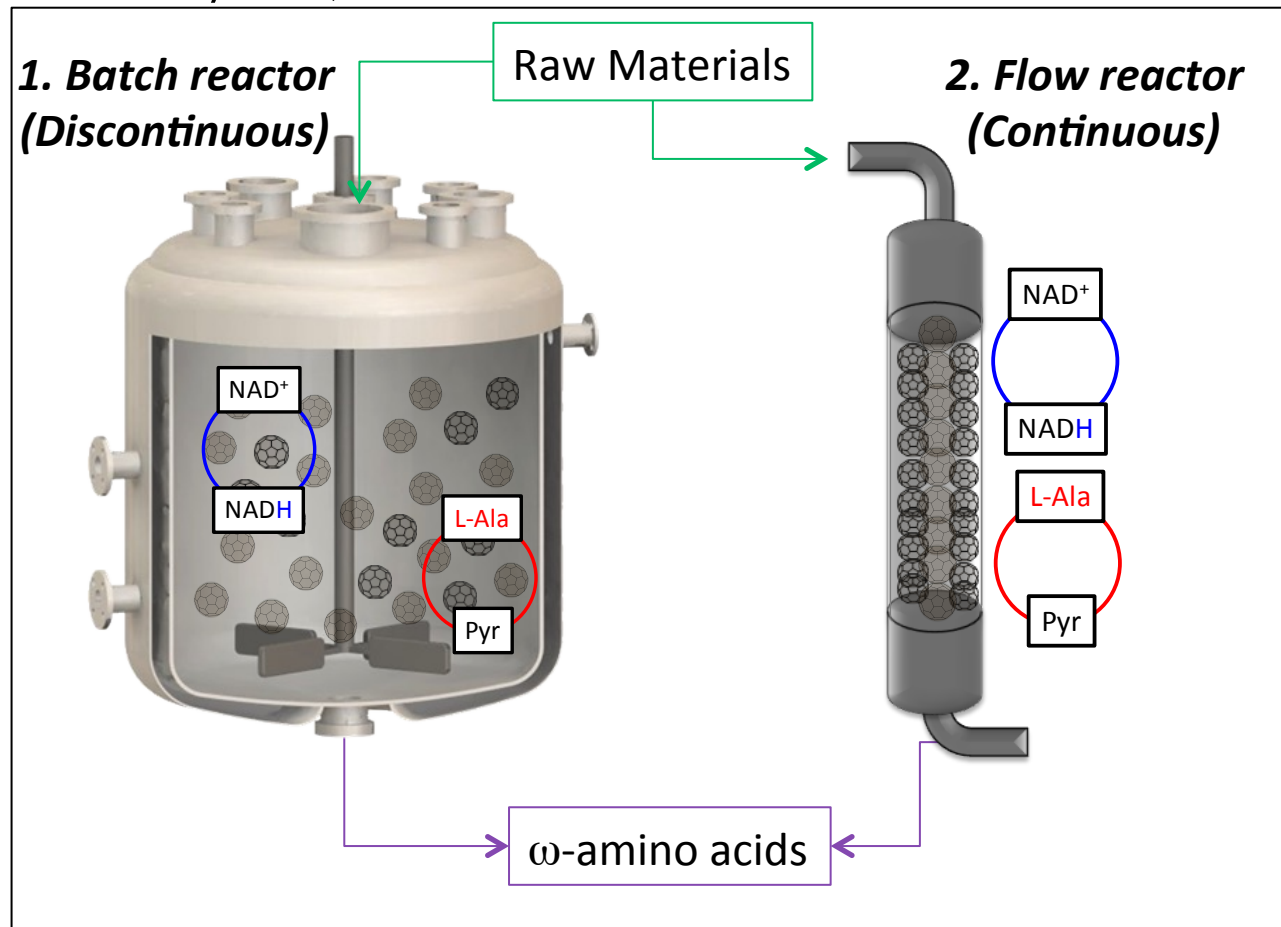


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- **WP5** *ω -Amino acids synthesis by heterogeneous biocatalysts (UNO, CIC BMG, RUB)*

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

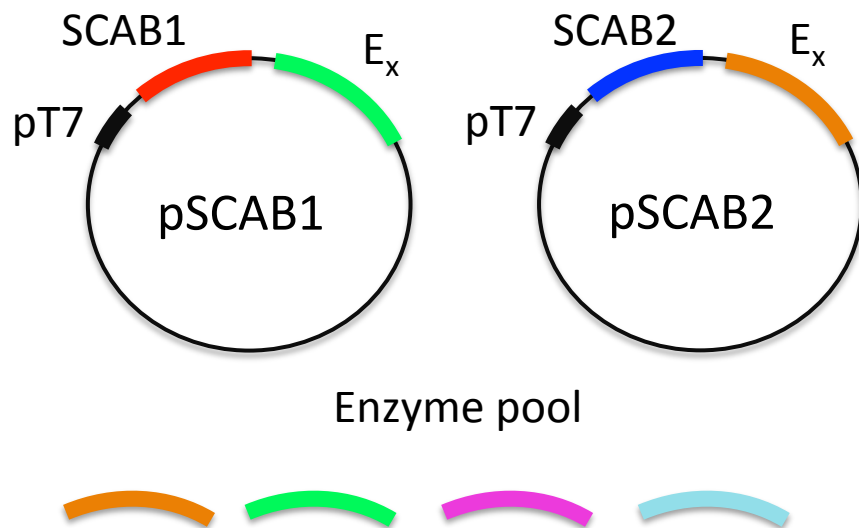
PATHWAY 2 (from bio-oils) Module B +C + D (optional)

- **WP5** ω -Amino acids synthesis by heterogeneous biocatalysts (UNO, CIC BMG, RUB)

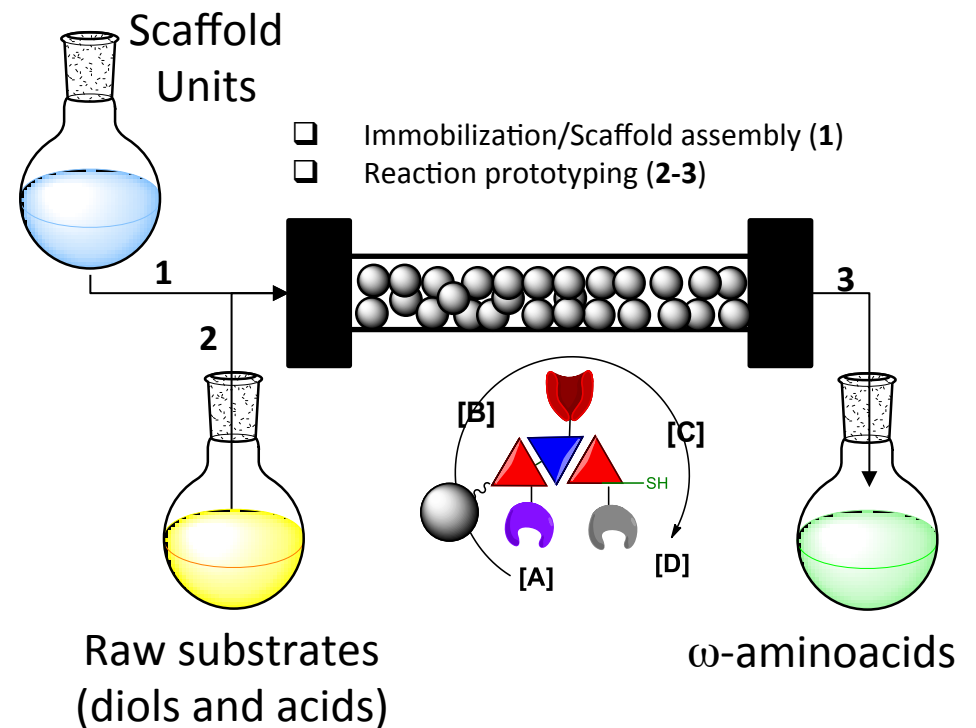


- **WP6** *Fabrication of multi-functional heterogeneous biocatalysts hierarchically organized in an industrial environment: Validation and demonstration of versatile technology (BIOA)*

- **1. Plasmid kit**



- **2. Solid phase kit**



- *WP7 Management and coordination (CIC BMG)*

- Scientific and Administrative coordination
- Communication and Dissemination

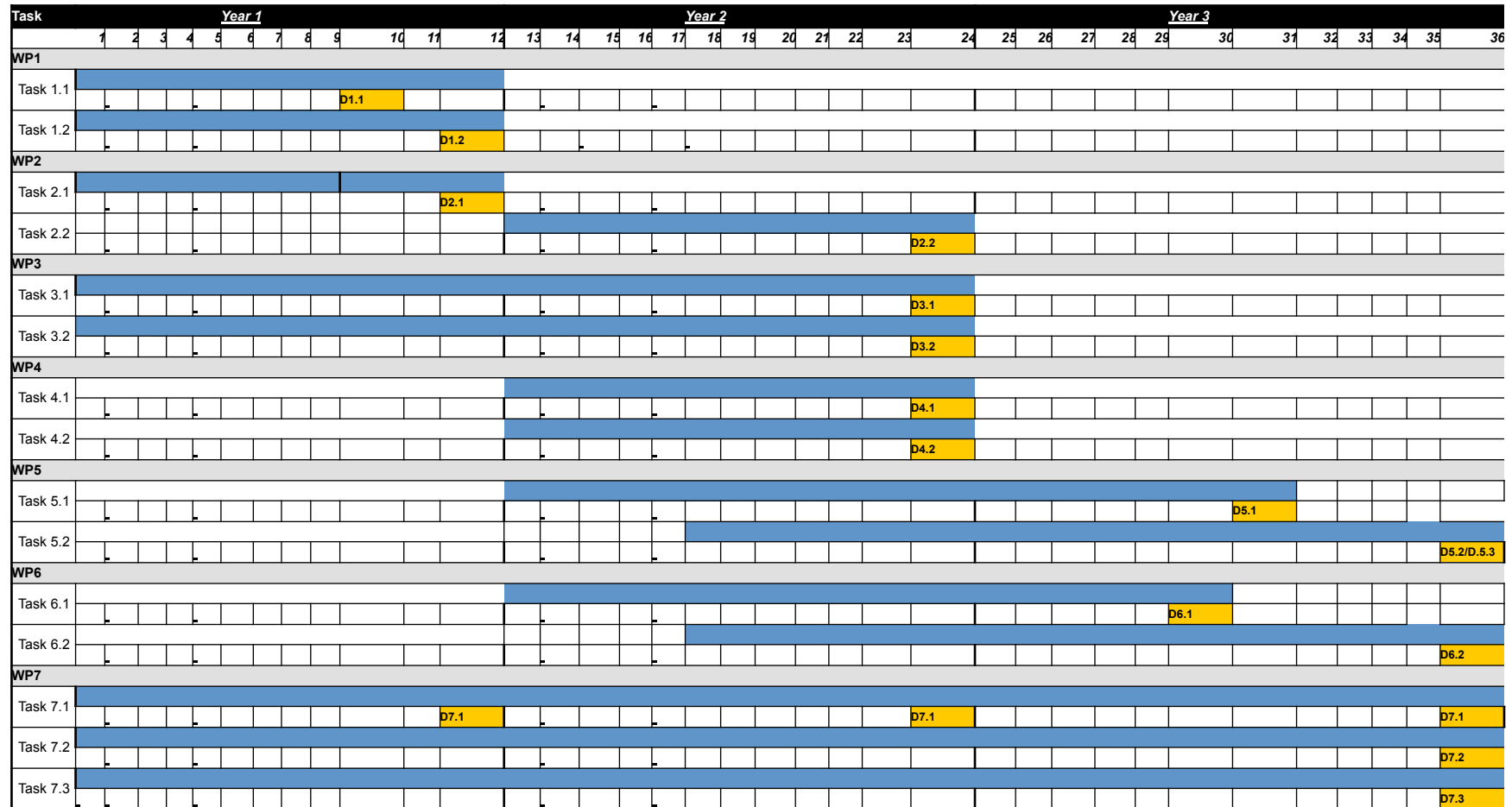
Identified stakeholders: scientific community, industry, students, and public/consumers

Responsible Research and Innovations (RRI) aspects: engagement of societal actors

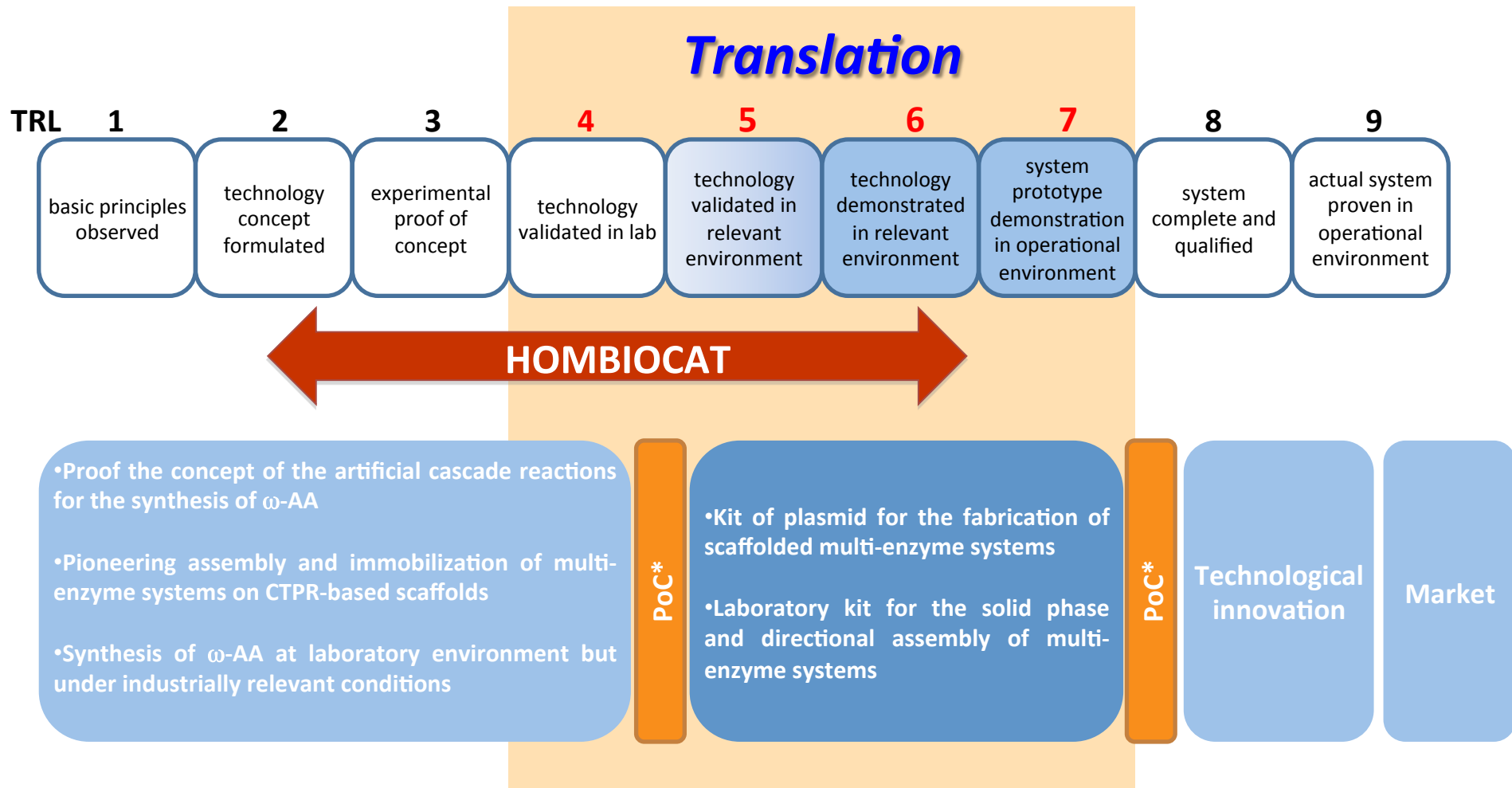
- IPR and Data Management

Data will be made publicly available ensuring its sustainability and accessibility (Biosharing standards, <https://biosharing.org/>; ZENODO and OpenAIRE repositories)

Project chronogram



● *TRL Plan*



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- ***Expected outcomes***
 - ***Technology for the production of ω -amino acids, currently generated by highly pollutant chemical synthesis, from renewable raw materials such as vegetable oils and fermented diols***
 - ***Novel multi-functional heterogeneous biocatalysts widely applicable to the available feedstocks***
 - **Platform of bio-based building blocks to arrange any multi-component system.**

 - ***Planned implementation and exploitation of results***
 - ***Patentability analysis***
 - ***Technology evaluation, marketing planning, and industrial engagement***
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GOALS

- ❖ *Developing a tool-box for the fabrication of hierarchically organized heterogeneous multi-functional biocatalysts*
- ❖ *Heterogeneous biocatalyst able to transform renewable raw materials into ω -amino acids*


CHALLENGES

- ❖ Development artificial biosynthetic pathways using cell-free systems
- ❖ Spatial organization of the multi-enzyme system to avoid accumulation of both intermediates and toxic by-products
- ❖ Assembly of scaffolds and enzyme on solid materials to operate different reactor designs (batch and fixed bed)

SOLUTIONS

- ❖ Protein engineering to pair the enzyme activities with the necessities of the artificial pathway
 - ❖ Nanometric organization using biomolecular scaffolds
 - ❖ Micrometric compartmentalization using porous beads where to heterogenize and confine the different modules in the microscale
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#Hombiocat

Consortium

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UK | CHINA | MALAYSIA

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