BIOCON-CO2: from carbon dioxide to valuable chemicals

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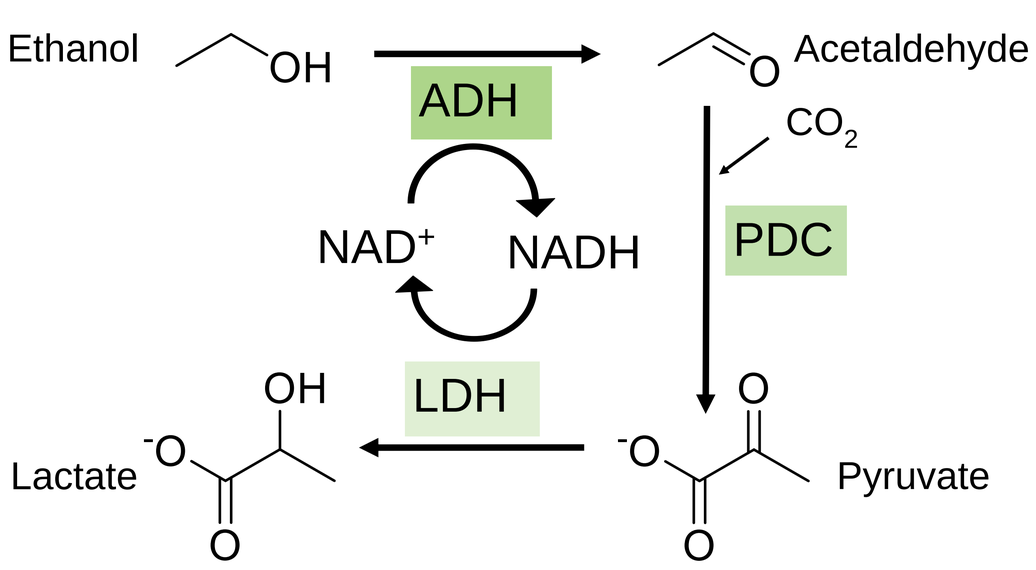
The aims of BIOCON-CO2:

- reduce the emissions of CO_2 coming from industrial production
- exploit the carbon source to produce high value compounds



RUG contribution to BIOCON-CO2

Enzymatic CO_2 solubilisation

Biocatalysts stabilisation

Enzymatic CO_2 solubilisation

Tackle inhibition from contaminants

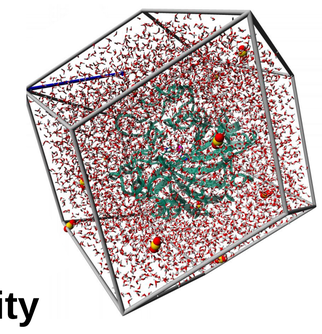
To further increase the concentration of CO_2 in solution, we convert it to bicarbonate using carbonic anhydrase.

Target enzyme:
human carbonic anhydrase II

$$CO_2 + H_2O \xrightarrow{\text{hCAII}} HCO_3^- + H^+$$

Contaminants are present in heavy industry off-gases, which behave as inhibitors of the enzyme. To decrease the affinity of these small molecules for carbonic anhydrase, we perform molecular dynamics and design mutants.

Simulation of wild type with inhibitors → Rational mutagenesis based on trajectories → Simulation of mutants with inhibitors



Increase protein stability

As further described in this poster, we are applying the FRESCO protocol to carbonic anhydrase, in order to make the enzyme more resistant to the conditions in which its reaction will be required. Such measure is required to make the enzyme last longer, in this way making the process cheaper and efficient.

Biocatalysts stabilisation

FRESCO pipeline

High resolution structure

FoldX | Rosetta ddg | Molecular dynamics

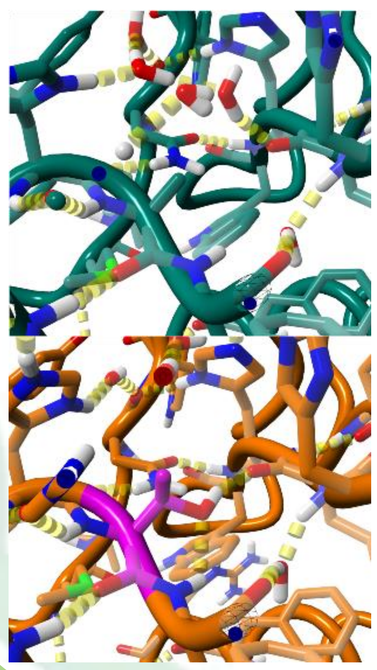
Visual inspection of predicted mutants

Experimental verification of mutants

The FRESCO protocol is composed of in silico and in vitro evaluation steps. In this way the number of variants to be tested at the bench is reduced while remaining significant.

Here we present the different advancement stages of the process for the enzymes that are part of our project.

PDC: mutants selection *in silico*



In the first FRESCO step, the crystallographic structure is checked and adapted for the following energy calculations, disulphide bonds discovery and molecular dynamics simulations. After this, a reasonable pool of mutants is proposed to the operator, who checks them on the screen and selects the promising ones according to criteria such as: loss or gain of H bonds, hydrophobicity and flexibility of the side chains. In PDC case the mutants to be screened were 1200.

ADH: recombinant expression of mutants

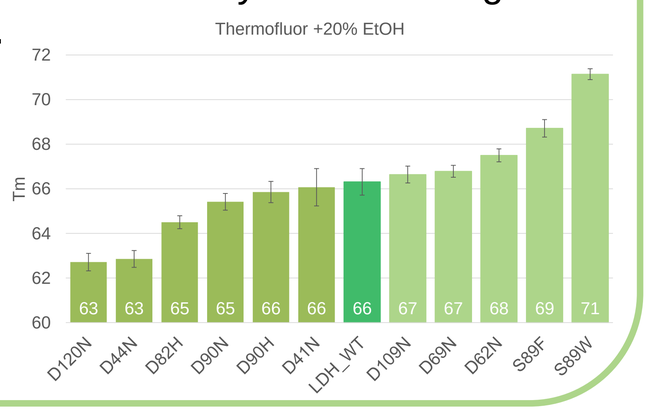
Once the number of selected mutants has been reduced, mutagenesis primers are ordered and the mutants are expressed in small scale, typically in 96-wells plates. For ADH, visual inspection suggested 93 mutants to be expressed.

Selected pool of stabilising mutants → Primers design and mutagenesis → Expression in small scale → Stabilisation screening by Thermofluor → Combination of positive mutants

Expressed proteins are purified and their melting temperature determined by Thermofluor or ThermoFAD assay. By comparing with wild type enzyme, a number of variants is selected and mutations can be combined to create a super-resistant version of the enzyme.

LDH: testing mutants thermoresistance

In the LDH case, 71 variants were selected, expressed in *E.coli* and purified. Because of the high Tm of the wild type enzyme (95°C), 20% ethanol was added to the mixture for the Thermofluor assay, in this way destabilising the samples in the same way. Also, since ethanol will be present in the final reaction, this values reflect the actual stability of the enzyme in the solvent-rich environment.



Our partners

Our targets

- Industry CO_2 Suppliers
- Regulators/Policy Makers
- Scientific Community
- Biotech Industry
- Public and Private Investors
- Related H2020 Initiatives
- Chemical / Food / Plastic Industries
- Local Authorities/Decision Makers
- General Public

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