A METABOLIC-LIKE CYCLE FOR SYNTHETIC APPLICATIONS

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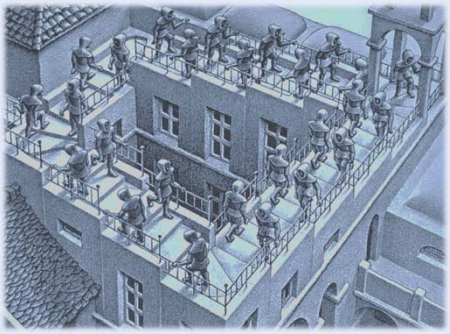
The concept:

Systems Biocatalysis (SysBiocat)[1] is a new approach consisting of organizing enzymes *in vitro* in order to generate an *artificial metabolism*[2] for synthetic purposes. In order to furnish an example of the concept, an in vitro artificial cycle interconverting chemical functional groups through a series of six enzyme catalyzed reactions was setup.[3] The addition of any of the substrates established a steady state in which concentrations of all the four components remained unchanged, and transformation of any component into another is possible by breaking the cycle omitting one (or more) of the enzymatic activities. We believe that this example of an artificial metabolism may constitute a novel approach towards the synthesis of useful products by modern applied biocatalysis. Such an in vitro strategy circumvents the presence of deviating/competing metabolic pathways as well as the issues related to enzymes inhibition or regulation as observed at the cellular level

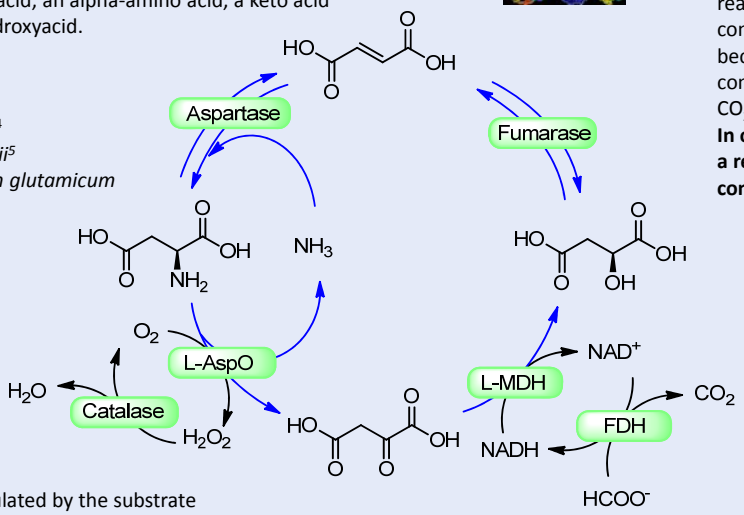


The *futile* cycle:

In this view, we managed to design and build up, employing six enzymes, the following biochemical-like cycle (***fumarate cycle***) connecting among them an unsaturated carboxylic acid, an alpha-amino acid, a keto acid and the corresponding alpha-hydroxyacid.

The enzymes:

- AspB from *Bacillus* sp. YM55-1[4]
- L-AspO from *Sulfolobus tokodaii*[5]
- Catalase from *Corynebacterium glutamicum*
- L-MDH from bovine heart
- FDH from *Pseudomonas* sp.
- Fumarase from porcine heart



The multienzymatic cycle is regulated by the substrate availability, enzymes activity and the (ir)reversibility of the single reactions. The stability of the biocatalysts in the chosen conditions is clearly a key feature.

After adding all the enzymes into the system, the cycle is closed: a stationary state is eventually reached, where the system undergoes a continuous counterclockwise rotation, and becomes in practical terms a multienzymatic complex carrying out the oxidation of formate to CO_2 by means of atmospheric dioxygen.
In other words, this new, artificial system mimicks a respiratory cycle which is fueled by the combustion of formic acid.

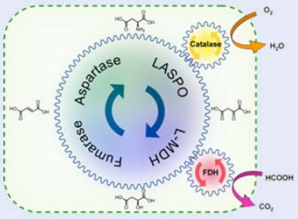
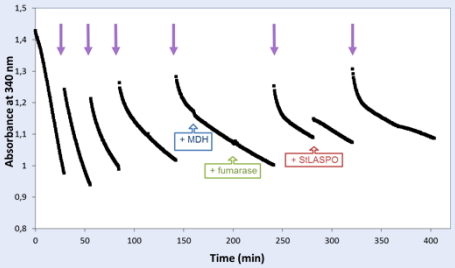


Figure 1: UV @ 340 nm

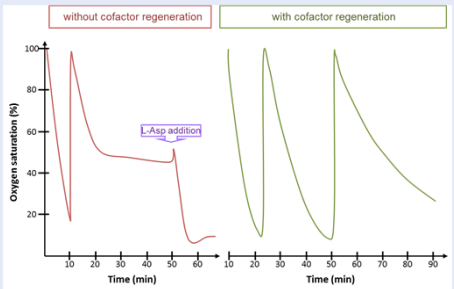
Cycle from L-malate to L-malate (0.5 µmol), without cofactor regeneration (no FDH). Every purple arrow indicates the addition of 0.12 µmol of NADH.



The cycle is limited and regulated by the addition of NADH, whereas the "internal" species are continuously interconverted.

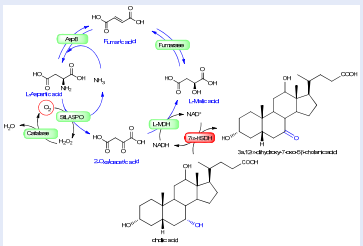
Figure 2: Oxygen consumption

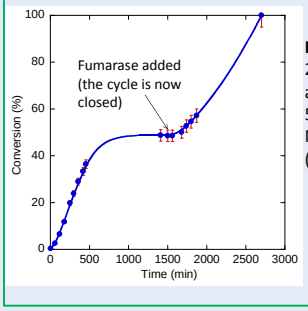
Cycle from L-Asp to L-Asp, molecular oxygen as limiting reagent



Work in progress:

The cycle can be seen as a multienzymatic system acting as a NADH oxidase, so it can be coupled to synthetically useful biooxidations, for example on cholic acid.[6]





Initial conditions:
20 mM fumaric acid
50 mM cholic acid
No fumarase
(open cycle)

References:
1) Systems Biocatalysis (*SysBiocat*) is the COST Action CM-1303
2) Fessner, W.-D.; Walter, C., (1992), *Angew. Chem. Int. Ed. Engl.*, 31: 614–616
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