# Bridging Systems and Synthetic Biology for the development of Improved Microbial Cell Factories

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**PI short CV:**
Author of 80 papers published in international journals, 6 books. Received PhD in Chemical Engineering in 1995, at the University of Porto. Main research interests: BioProcess Systems Engineering, Systems Biology.

## Objectives

The primary goal of this project is to develop and apply systems and synthetic biology tools for improving *E. coli* microbial cell factories for the production of amino acids.

The overall project is organized into six tasks:

- To construct a more reliable mathematical model and better simulation tools for an accurate prediction of *E. coli* phenotypes under different environmental and genetic conditions.

- To collect appropriate high-throughput experimental data measuring fluxomics, metabolomics and transcriptomics for the purpose of model improvement and validation.

- To apply advanced algorithms based on the concept of EFMs in order to elucidate major metabolic routes used under different environmental conditions

- To develop and apply mathematical and computational tools for identifying metabolic engineering targets

- To design Synthetic Biology strategies for programming gene expression in response to intracellular metabolic states

- To implement selected metabolic engineering and synthetic biology approaches in order to obtain in vivo improved microbial cell factories.

## Work plan

This project aims to develop and apply systems and synthetic biology tools for improving microbial cell factories for the production of amino acids.

These compounds represent interesting case-studies for metabolic engineering, because they have been increasingly used as supplements for human food and animal feed with a special emphasis on L-glutamic acid and L-lysine. Moreover, they are good representatives of the success of Industrial Biotechnology: a few years ago only a small number were produced by bioprocesses, while nowadays almost all 20 natural L-amino acids are produced by fermentation or enzyme technologies.

The microorganism to be used is the bacterium *Escherichia coli*, for which sufficient knowledge has been accumulated in recent years to perform these tasks and also because this organism is able to produce naturally all the 20 amino acids from inorganic nitrogen sources.

The main tasks of the project encompass the entire cycle of metabolic engineering and are guided by advanced approaches from Systems and Synthetic Biology approaches.

The cycle of the project starts with the construction of improved mathematical models representing both metabolic and regulatory processes from different data sources and using state-of-the-art bioinformatics tools. Experiments on wild-type *E. coli* strains will be performed to adjust and validate the developed models and to help in the understanding the governing objectives that determine a particular physiological state. In this context, an innovative methodology that integrates the concept of Elementary Flux Modes (EFMs) with Projection to Latent Structures will be applied for elucidating major metabolic routes.

The refined model will then be used to predict *in silico* molecular targets for knockouts, gene addition and under/overexpression using advanced optimization algorithms developed in-house. These strategies will then be directly implemented in *E. coli* or further analyzed and advanced using Synthetic Biology approaches to program or enhance gene expression before implementation.

**Results**

So far, efforts have been focusing on the development of the metabolic-regulatory model for *E. coli*. The data from different databases have been integrated and several models have been generated in SBML format that are being debugged and validated. The information that these models aim to integrate and the most relevant datasources is shown in the next Figure.