Keynote speaker / **S8: 5**

New tools for the simulation and optimization of microbes in metabolic engineering problems

Isabel Rocha

IBB – Centre of Biological Engineering, University of Minho, Braga, Portugal

Industrial Biotechnology is increasingly replacing chemical processes in numerous industrial sectors since it allows the use of renewable raw-materials and provides a more sustainable manufacturing base. The field of Metabolic Engineering (ME) has thus gained a major importance since it allows the design of improved microorganisms for industrial applications, starting with wild-type strains that usually have low production capabilities in terms of the target compounds. The ultimate aim of ME is to identify genetic manipulations *in silico* leading to improved microbial strains, that can be implemented using novel molecular biology techniques. This task, however, is a complex one, requiring the existence of reliable (genome-scale-) metabolic models for strain simulation and robust optimization algorithms for target identification. Strain simulation is usually performed by using Linear or Quadratic Programing methods that assume a steady state over the intracellular metabolites. However, there is no guarantee that the engineered cells actually function according to the optimal pathway predicted by these methods. In this scope, we have been working towards the use of a modification of the concept of Control Effective Fluxes to be able to find Metabolic Engineering solutions that couple growth with product formation while considering optimal, as well as sub-optimal routes and their efficiency. Regarding strain optimization, the most common task is to solve a bi-level optimization problem, where the strain that maximizes the production of a given compound is sought, while trying to keep the organism viable. Several different algorithms have been proposed to address this problem, namely mixed integer linear programming. More recently, we have proposed the use of stochastic meta-heuristics, such as Evolutionary Algorithms (EAs) and Simulated Annealing (SA). These approaches allow to solve the Metabolic Engineering problem in a considerable shorter time, originating a family of (sub)optimal solutions. Moreover, they are quite flexible regarding the use of non-linear objective functions. However, so far optimization approaches have been limited to the tasks of selecting the best set of genes to knockout from an organism. To extend the manipulation possibilities, we have been using both dynamic and steady-state models in modified formulations to account for gene over and under expression. In this way, it is possible to indicate the set of genes that should be modified, the type of modification that should be performed and the degree of over and underexpression. These algorithms have been validated with different case-studies, namely the production of lactic and succinic acid with *E. coli* and *S. cerevisiae* and some are already available in the open source and user friendly software tool Optflux.

1 - Burgard AP, et al.. Biotechnol Bioeng, 84:647–657, 2003, 2 – Patil K, et al. BMC Bioinformatics, 6(308), 2005., 3 - Rocha M. et al. BMC Bioinformatics 9:499, 2008., 4 - Rocha I. et al. BMC Systems Biology, 4(45), 1-12, 2010.