Production of biofuels such as ethanol induces a potent stress on the microorganisms that produce them. Thus, engineering product tolerance is essential to improve yields and process efficiency. We demonstrate the discovery of gene overexpression targets to significantly improve ethanol tolerance in *Saccharomyces cerevisiae*. Using the Cytostat continuous culture technique we screened for a sustainable ethanol tolerance mechanism in a pool of *S. cerevisiae* cells transformed with a genome-wide overexpression library. We discovered that an overlapping sequence containing genes HAP4 and SLD2 represented 90% of selected clones from a starting pool of 1,588, and that co-overexpressing these genes is sufficient to confer an increase of nearly 50% in both the growth rate and ethanol productivity under ethanol stress. In addition, overexpression of these genes prevents the drastic effects of ethanol stress on cell size and the cell cycle. Co-overexpression of HAP4 and SLD2 leads to significantly improved kinetics of glucose consumption and ethanol productivity during fed-batch growth under ethanol stress. As demonstrated for the case of ethanol production the approach presents a general strategy for the improvement of a wide range of biotechnology processes.

177. Microbial Production of Renewable Monoethylene Glycol
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Monoethylene glycol (MEG) is an important commodity chemical with such applications as antifreeze and as a raw material for poly(ethylene terephthalate) which is utilized for plastic packaging and polyester fabric. Currently, MEG is produced in large volumes (approximately 19 million metric tons in 2010), primarily from fossil fuels. As a sustainable alternative, we propose a single-step bioprocess in which plant-derived carbohydrates are converted by engineered microorganisms into renewable MEG. Toward this goal, we have engineered novel metabolic pathways for the biological production of MEG into strains of *E. coli*. The general metabolic engineering strategy for the conversion of sugars into MEG is that pentoses are split into 2-carbon and 3-carbon compounds, hexoses are split into two 3-carbon compounds, and the respective 2-carbon and 3-carbon intermediates are independently converted into MEG. Here, we validate these pathways and demonstrate the microbial production of MEG from various sugars, including one engineered strain that produces 40 g/L MEG from xylose at a yield of 0.35 g-MEG/g-xylose.

178. Improving the Internal Flux Distributions from Genome Scale Metabolic Models of *S. cerevisiae*
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Genome Scale Metabolic Models can be used to simulate the intracellular flux distributions under steady state conditions using different simulation tools such as Flux Balance Analysis (FBA)1. Lately, these models proved to be useful for predicting gene knock-outs that optimize the production of important industrial targets2–4. To obtain a good correlation between simulations and in vivo results, it is important to validate beforehand how well the model allows to predict the metabolic fluxes for the wild-type organism.

In this work, the accuracy of the simulated intracellular flux distributions in *Saccharomyces cerevisiae* was evaluated. The results revealed that steady-state simulations performed with FBA and the available genome-scale models [5–8] under fully aerobic conditions contained relevant mismatches in important areas of central metabolism, when compared with in vivo data9, 10 and physiological knowledge, namely the absence of flux in the Pentose Phosphate Pathway. Since many of these mismatches are associated with reactions involving the cofactors NADP+/NADPH and NAD+/NADH, all the enzymatic reactions that included these cofactors were manually curated.

Because under fully aerobic conditions the ratios of NADPH/NADP+ and NAD+/NADH are high, it was assumed that the concentration of these cofactors would drive reactions near equilibrium in one direction. Therefore, if a reaction was found to be near equilibrium, its reversibility was constrained in the direction of NADPH consumption or NADH production.

To verify if the modifications applied had any effect on the predicted fluxes for the central carbon metabolism, the
models5–8 were used for FBA simulations and the results were compared with experimental fluxes. The simulations performed with the curated models revealed several improvements in the Pentose Phosphate Pathway and other parts of NADPH metabolism, resulting in a flux distribution much closer to experimental values9, 10. The new flux distribution was then used as a reference for the MOMA11 methodology in an in silico optimization of the production of two organic acids to evaluate its impact on quality the results. It was observed that the knock-out mutants obtained were consistent with experimental evidences in the literature and were only valid when the curated model was used.

In sum, it was shown that a careful curation of the wild-type network can improve the simulation accuracy, resulting in a better correlation with experimental data. Since in vivo strain design is very time consuming, these results can prove important to boost the reliability of in silico optimizations. However, since the proposed changes are only valid under specific conditions (full aerobiosis) it can also be concluded that the accuracy of flux distribution prediction in large-scale models might be dependent on condition-specific modifications.

References:
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179. The Yeast Pathway Kit: A Method for Rational or Combinatorial Metabolic Pathways Design in Saccharomyces cerevisiae

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Metabolic engineering often requires multiple genetic modifications in order to alter the properties of a target organism, especially if an entirely new metabolic pathway has to be established. Pathway elements can be rationally or randomly assembled, the latter facilitates selection of the best performing pathway from a set of randomly generated pathways if a suitable screening or selection procedure is available. Methods described so far allow either rational or random assembly of metabolic pathways in in vivo or in vitro, but not both and usually providing few reusable genetic elements such as promoters and genes. We present here the Yeast Pathway Kit (YPK) that aids rational or random metabolic pathway assembly using the same genetic parts. The system is based on efficient and rapid cloning using positive selection vector in combination with hierarchical in-vivo gap repair. As a proof of principle, we assembled xylose metabolic pathways with up to eight genes producing a recombinant S. cerevisiae strain able to grow on xylose with a specific growth rate of 0.181 h⁻¹. YPK relies on PCR reactions with short primers resulting in a relatively low cost of construction compared to other protocols such as Gibson assembly or DNA assembler.