



Regulatory-Metabolic Network Meta Integration and Modelling in *Saccharomyces cerevisiae*: Ethanol as a Case-Study

PHD in Biotechnology and Biosciences

Diogo Alexandre Carracedo Couceiro (diogo.c.couceiro@tecnico.ulisboa.pt)

Metabolism is much more than a set of connected reactant-catalyst-product combinations, an assumption too often made by standard genome-scale metabolic models. Metabolism is complex and dynamic, and in close liaison with environmental conditions that control metabolism through signaling/regulatory pathways. Altogether, it's vital to consider all these players when wishing to represent cell behavior using mathematical models, in a way that they can be useful to increase our ability to optimize cell factories through transcription factor manipulation.

Fusing Qualitative (Boolean) Regulatory Models into Dynamic Quantitative Mixed Regulatory Metabolic Models

Method 1 TF-TMG : Estimating TF-Target Metabolic Genes Expression Changes

YeastRACT as of this point in the work contained solely qualitative associations. The question is then how qualitative regulatory models can be fused with quantitative metabolic models resulting in a dynamic **quantitative** mixed regulatory-metabolic model. Well, why not make educated guesses?

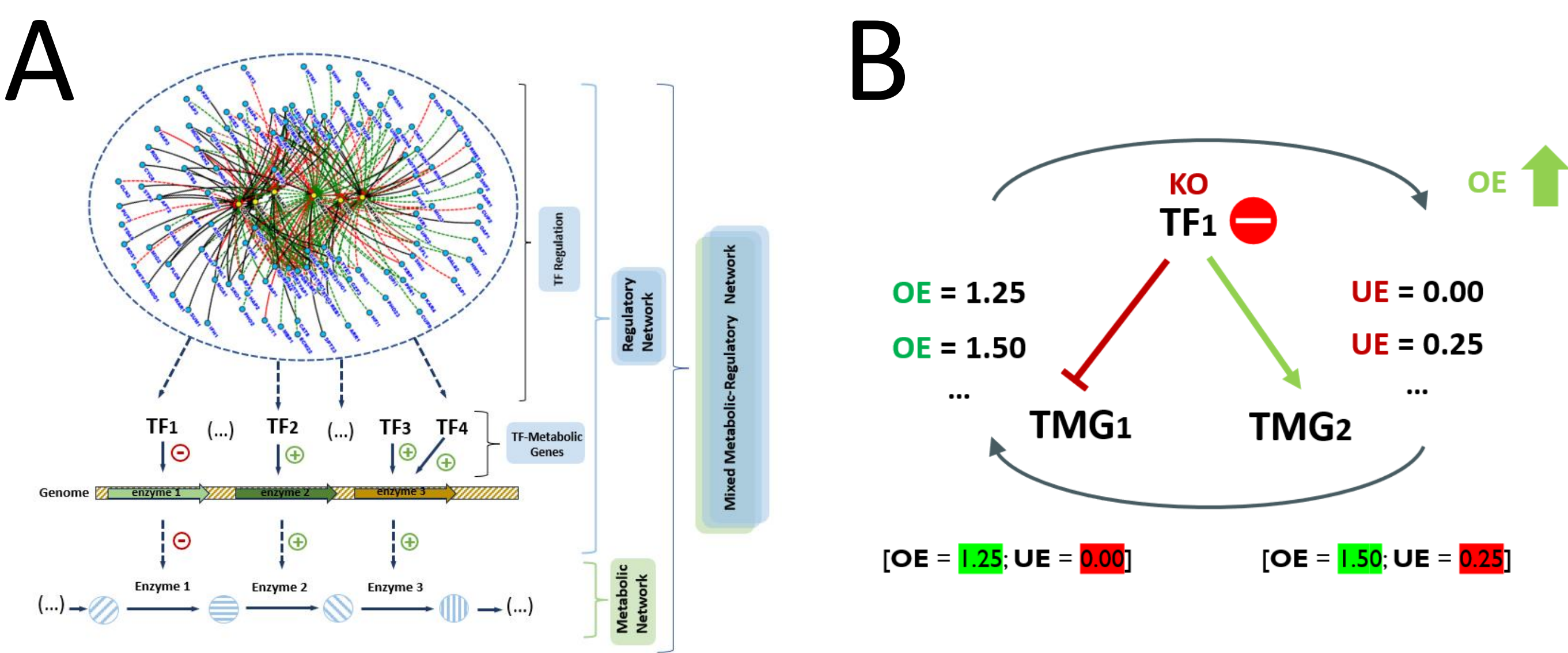


Figure 1: A) Defining architecture is essential. There will be a foundation corresponding to the metabolic model (only metabolic reactions) with an upstream multilayered and very complex level (regulation). How do changes at this level affect this foundation quantitatively? **B)** When lacking quantitative information, we can make a series of estimates wide enough in their range that the true effect will be contained somewhere in the solution field.



Figure: C) YeastRACT already allows for metabolite optimization by manipulating TF expression, with integration of 160 TFs. **D)** Wet-lab quantification validates *in silico* predictions for the three best single deletion mutants for the TFs MIG1p, CST6p and CRZ1p, all producing more ethanol than the around 11g/L produced by the wt. A 30% increase!

Method 2 TF-TF-TMG : Integrating TF-TF interactions and Stable States as the Missing Link!

Inter-TF regulations cannot be omitted. They are rich, complex and multidirectional. The question of how to fuse qualitative and quantitative models remains. What if we fuse all TF-TF interactions into one single result we can quantitatively integrate just like we did before?

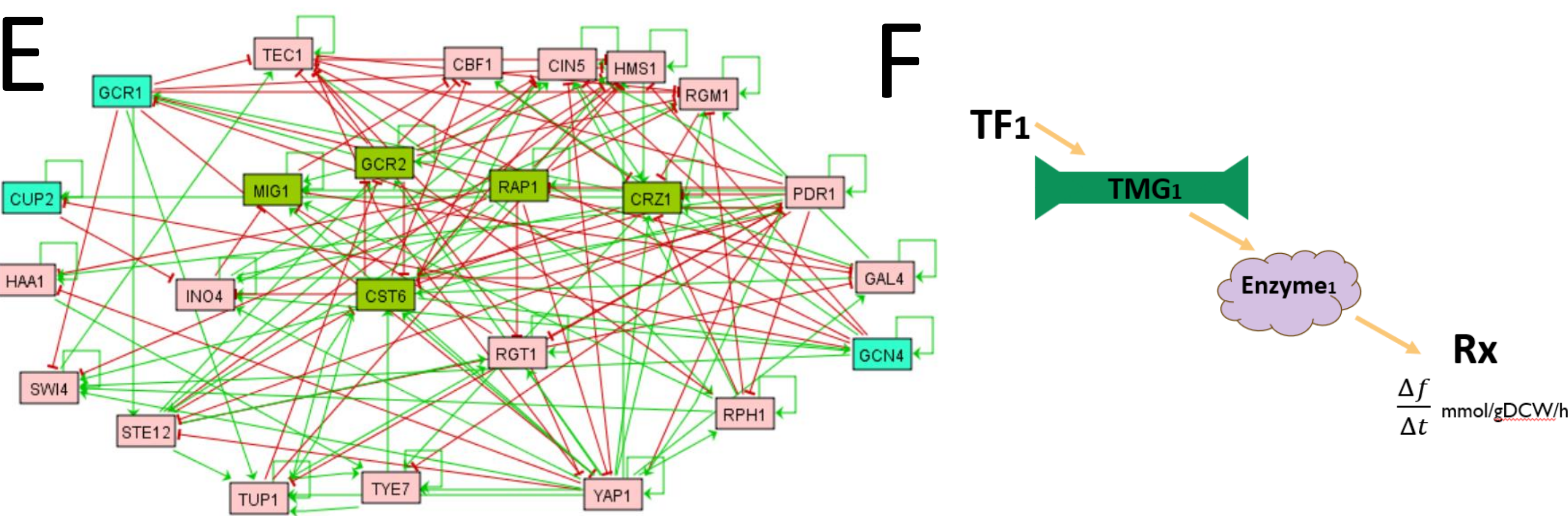


Figure: E) The first step is building a TF only Boolean regulatory model. This corresponds to the entirety of the model's upper layer. It provides us with Stable States which have biological significance as representations of biological energetic optimization and therefore indicate optimal TF differential expressions. **F)** There is a direct link between TF expression and metabolic reaction flux. Stable States are the missing link!

Reg. Type	SS Change	$\Delta f / \Delta t$ (wt)	Effect	Mutant	Run	Final Score		Notes
						Best Hit	Second Best	
Activator	0 to 1	≠0	↑	Δcst6	1	2.263	0.882	Correct!!
	1 to 0	≠0	↓		2	1.474	0.793	
	1 to 0	≠0	↓		3	1.316	0.759	
	0 to 1	≠0	↑	Δcrz1	1	2.579	1.517	
Inhibitor	0 to 1	≠0	↓		2	2.105	0.448	
	1 to 0	≠0	↑		3	1.263	0.931	
	0 to 1	≠0	↓	Δmig1	1	4.737	0.403	
	1 to 0	≠0	↑		2	2.895	0.522	
	1 to 0	≠0	↑		3	2.842	0.463	

Figure: G) Stable States are directly linked with flux across the metabolic network. The change in Boolean activation status thus indicates several UE or OE cases depending on the initial reaction's flux. **H)** Triplicate runs were performed and all three previously tested mutants were indicated once again as good candidates. The higher the score the most likely the prediction. All highest scores coincide with higher ethanol production. These predictions are reliable.

Updating YeastRACT: Integrating over 65K Quantitative Expression Entries in the Database and quantitatively manipulating TF Expression

YeastRACT has undergone a major update over the past year, integrating around 65K quantitative transcriptomic entries. A new tool for metabolite optimization by quantitatively manipulating TF expression is already available following much of the same rational as the previous two approaches. Try it out!!

