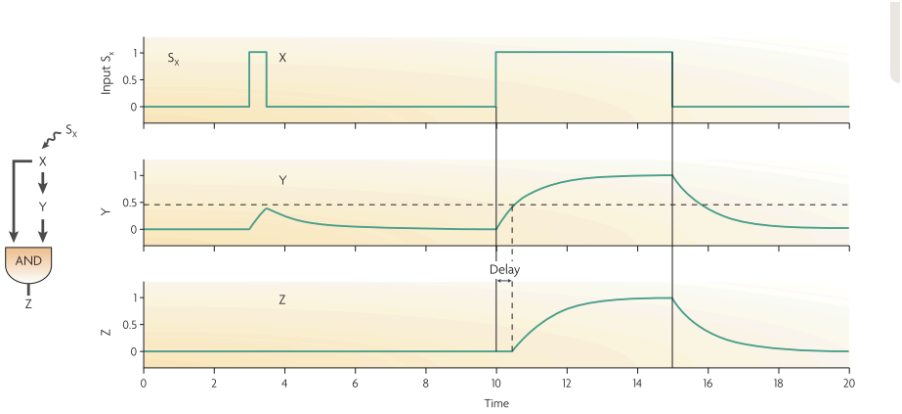
BB1110 HMWK 2

PART 1 Synthetic biology.

1. Consider the arabinose feed-forward motif from Alon and Lecture 08

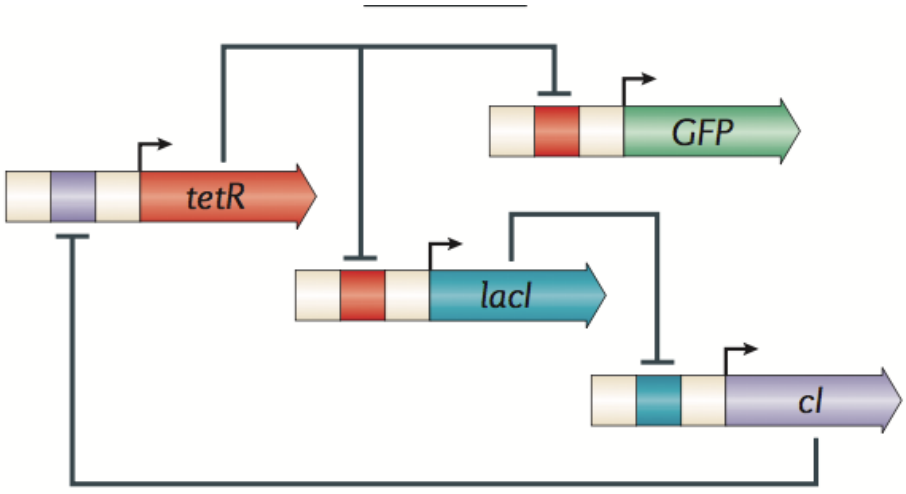
A) Now lets pretend that Z is under an OR gate instead. Draw how Y and Z would respond to a "step change" increase in Sx, and a "step change" decrease in Sx.

b) What is the molecular mechanism for the AND gate? Can you think of how an OR gate could look? Consult slides from L08 if you want.



The feed-forward motif containing an AND gate.

2. Consider the following gene circuit, which consists of three repressor proteins (see LacI) and GFP. In this circuit, each protein represses a protein downstream, and each protein is in turn repressed by the protein upstream.



A) Draw what you think the concentration TetR would look like over time (consider a starting concentration of 1). Do you think GFP expression would follow similar pattern?

B) What could such a genetic circuit be used for? Are there analogous circuits to this in biology?

PART 2 Cell factory.
The Calvin cycle enzymes capture CO2 in plants. They are not present in yeast. You have a great idea: by putting in some of these enzymes into a yeast, you can capture CO2 *and* produce ethanol! Let´s explore the CO2-to-ethanol cell factory with a metabolic model.

3. You clone the Calvin cycle enzymes from a cyanobacteria into yeast. You try to grow the yeast with CO2 but they do not grow. Why not? *(Hint: think about how plants do this)*

4. You now give the yeast some glucose to go with the CO2. You see that the yeast grow faster than the wild type before. In fact, your new yeast produces more ethanol than the original. Look at the metabolic model on p2. If ethanol increased, what product decreased? Can you think of an explanation why (Hint: cofactors)?

5. When solving a metabolic model, the set of equations is often *underdetermined.* Why is this? How does an "objective" function help?

6. You would like to increase the ethanol production more. You know how to knock out genes in the lab. How would you use the model to help you decide which genes to knock out?

7. Unfortunately, model on p2 is not always accurate in predicting the amount of ethanol. You want to make a genome-scale metabolic model. List at least 3 steps (as best you can) needed to construct an accurate genome-scale model. What data (or databases) could you use?

PART 3 Screening and selection.
Your miljövänlig ethanol is a huge hit. One day you get a call from Naturvårdsverket. They say that during summer, motorboats leak a lot of oil into the waterways. They wonder, can you make a yeast that eats oil?

8. You have recently read a Richard Dawkins book and have an idea: You will *evolve* your yeast strain to eat oil. Evolution starts with random mutation. What are two ways to cause random mutations to the genome of your yeast?

9. Describe how you would *screen* your yeast library to find cells that grow fast on oil.

10. Can you think of a *selection* procedure for fast growth on oil?

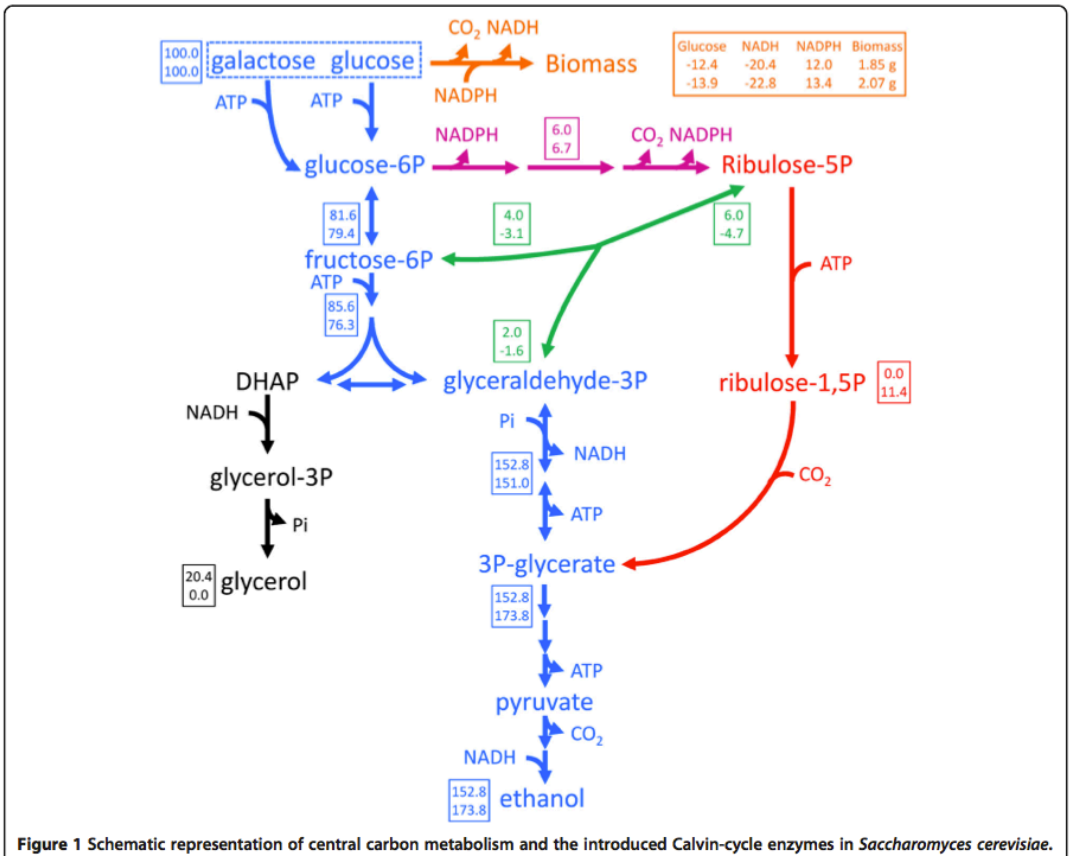


Figure 1 Schematic representation of central carbon metabolism and the introduced Calvin-cycle enzymes in *Saccharomyces cerevisiae*.

The numbers in boxes are the relative fluxes of each metabolite upon solving the model (note that glucose and galactose fluxes are set to 100). Top number: no Calvin cycle enzymes. Bottom number. with Calvin cycle enzymes.