**Metabolic Regulation Assignment**

**Work in teams so you can share out the workload (3 people max per team).**

**Write a report on your studies. In your write up indicate how each member in your team contributed to the study. Provide all Python scripts with your report.**

**Introduce your report with a one-page description of flux balance analysis and a one page description of flux control (i.e flux control coefficients) in kinetic models.**

**1. Flux Balance Analysis**

You are given an SBML model of the central part of E coli metabolism. The model has no kinetic laws. The model includes a reaction step that represents growth.

Build a flux balance model of E coli metabolism. Use the growth reaction as the objective function for the linear programming. We will provide a python script that will convert an SBML model into a list of strings representing the ODEs. Use lpSolve IDE implement the flux balance model. lpSolveIDE requires you to enter the ODE explicitly hence the need for the python script to convert the SBML model into a list ODEs.

Once you have put together the flux balance model you will need to add some additional constraints:

Set

GLC\_feed = 0.23 mM/s  
CYTB0 = 0.702 mM/s  
XCH\_ACE2 = 0 mM/s

Maximize the growth rate (expressed as mM/s) according to:

116 G6P + 204 E4P + 845 PGA3 + 1010 OAA + 610 AKG + 1601 PYR + 507 R5P + 293 PEP + 73 GAP + 40F6P + 10169 NADPH + 2118 ACCOA + 2004 NAD + 30508 ATP => 10169 NADP + 2004 NADH + 30508 ADP + 30508 P

**Specific Tasks:**

a) Use the model to predict the fluxes through all the reactions.

b) List the reactions that are essential for growth by knocking out each reaction in turn and computing the growth rate using flux balance analysis.

c) Add a new reaction that bleeds a fixed amount of mass from pyruvate. Investigate how this bleed affects the grown rate. Plot growth rate as a function of the bleed rate.

**Information on the Model:**

The current model simulates the metabolic operation of *E. coli* K-12 MG1655 during exponential growth phase, under aerobic condition and glucose limitation (μ = 0.1 h-1).

The model comprises three compartments: the environment and the cell which is divided in two compartments (cytoplasm and periplasm). The periplasmic volume represents 20% of the cell volume [1]. The model contains 77 species and 68 reactions constitutive of the central carbon and energy pathways of *E. coli.*

transport reactions between the environment and the periplasm   
glucose phosphotransferase system (PTS)   
glycolytic and gluconeogenic pathways (EMP)   
pentose phosphate pathway (PP)   
Entner-Doudoroff pathway (ED)   
anaplerotic reactions (AR)   
tricarboxylic acids cycle (TCA)   
glyoxylate shunt (GS)   
oxidative phosphorylation (OP)   
synthesis of biomass

**2. Comparison with Kinetic Model**

You are now provided with the equivalent, but full kinetic model of the E coli model from assignment 1. Run a simulation of this model to determine the fluxes.

a) Compare the fluxes you computed using flux balance analysis with the kinetic model.

b) Do the same bleed experiment you do in 1.c. How does it compare with 1.c? Use a simple reaction step k\*Pyruvate for the bleed step.

c) Remove the bleed step. Compute all the flux control coefficients for the growth rate with respect to each enzyme step. Write python scripts to automate this! Form a table that ranks the control.

d) Do the steps with the highest flux control in c) correspond to the regulated steps (yellow in diagram)? Explain your observation.

e) Compute the flux control coefficients for the glycolytic flux with the respect to each enzyme step. Form a table that ranks the control from high to low

f) Add a bleed pathway from Pyruvate and compute the flux control coefficients for the glycolytic flux with the respect to each enzyme step. Investigate how the control distribution is affected by the rate of the bleed.

g) Given your studies, what steps would you recommend a metabolic engineer change in order to maximize the amount of flux coming off the bleed step?