**Exercise for Constraint-based Modeling of Cellular Networks**
**5 January 2023**

**Homework should be sent to Anika (ankueken@uni-potsdam.de)**

**Hand in your commented code / answers for all exercise tasks as homework.**

## Simplified OptReg—extension to simplified OptKnock

Use the *E. coli* core model to determine the optimal reaction eliminations/ up- or down-regulations for increasing flux through "Fumarase". The problem is to find the minimum number of modifications such that while having at least 90% of the maximum biomass in the *E. coli* model, flux through fumarase reaction should be increased by 70%.

Copy the code from last week where you:

- Set the lower bound of 'Fumarase' (FUM) and 'ATP maintenance requirement' reaction to zero.
- Calculate the optimum biomass flux $z^*$, under the steady state constraints.
- Calculate the optimum flux through fumarase reaction $w_{FUM}$, under steady state constraints at the optimum biomass.
- Split reversible reactions in the model into two irreversible reactions.

1. Use *flux variability analysis (FVA)* to find the minimum and maximum values for all reaction fluxes, while (1) fixing the biomass reaction flux to the maximum biomass ($v^L$ and $v^U$) and (2) having no constraint on biomass production ($v^{min}$ and $v^{max}$).

2. In addition to the steady-state constraint, implement the following constraints for the simplified OptReg to model modifications knock-out, up- and down-regulation. Also, determine the objective function and the lower and upper bounds for all needed variables.

$$\varepsilon y_i^k \leq v_i$$
$$v_i \leq v_i^{max} y_i^k + (1 - y_i^k)\varepsilon$$

$$v_i^{min} y_i^u + \left(v_i^U (1 - C) + v_i^{max} C\right)(1 - y_i^u) \leq v_i$$
$$v_i \leq v_i^{max}(1 - y_i^u) + v_i^U y_i^u$$

$$v_i^{min}\left(1 - y_i^d\right) + v_i^L y_i^d \leq v_i$$
$$v_i \leq \left(v_i^L (1 - C) + v_i^{min} C\right)(1 - y_i^d) + v_i^{max} y_i^d$$

$$y^d, y^u, y^k \in \{0,1\}$$

3. Add a constraint that guarantees that a reaction can be a target of at most a single type of genetic manipulation ($y_i^u$ up-regulation, $y_i^d$ down-regulation, $y_i^k$ knock-out)

$$(1 - y_i^u) + (1 - y_i^d) + (1 - y_i^k) \leq 1$$

4. For reversible reactions $j$ add the following constraints:

$$\begin{aligned} y_j^k &= y_{j+1}^k \\ y_j^u + y_{j+1}^u &\geq 1 \\ y_j^d + y_{j+1}^d &\geq 1 \end{aligned}$$

   Where $y_j^u$ denotes the integer variable associated with the forward reaction and $y_{j+1}^u$ is the integer associated with the respective backward reaction.

Use the *intlinprog* function to solve your designed optimization problem. The final output should be a 2-column array in which the first column contains the name of reactions needed to be manipulated for increasing "fumarase" flux and the second column shows the type of manipulation ($C = 0.001, \varepsilon = 1e - 6$).

Example output:

| | |
|---|---|
| Reaction A | upregulated |
| Reaction B | downregulated |
| Reaction C | upregulated |
| Reaction D | knocked-out |

**Find alternative solution using integer cut**

Having one solution from the previous task, augment the same MILP with an additional constraint to check if there is an alternative solution for this problem. This integer cut constraint makes the previously found solution infeasible.