3.3. Solving r-deFBA problems

We have a linear **m**ixed **i**nteger **o**ptimal **c**ontrol problem of the form

$$\min_{\boldsymbol{u}(\cdot),\boldsymbol{y}(\cdot),\bar{\boldsymbol{x}}(\cdot)} \int_{t_0}^{t_{\mathrm{end}}} \mathrm{e}^{-\varphi \cdot t} \cdot \boldsymbol{\Phi}_1^\top \cdot \boldsymbol{y}(t)\,\mathrm{d}t + \boldsymbol{\Phi}_2^\top \cdot \boldsymbol{y}(t_0) + \boldsymbol{\Phi}_3^\top \cdot \boldsymbol{y}(t_{\mathrm{end}}) \quad (3.1\text{a})$$

$$\text{s.t.} \quad \dot{\boldsymbol{y}}(t) = \mathbf{S}_2 \cdot \boldsymbol{u}(t) \quad (3.1\text{b})$$

$$\mathbf{0} = \mathbf{S}_1 \cdot \boldsymbol{u}(t) \quad (3.1\text{c})$$

$$\boldsymbol{lb} \leq \boldsymbol{u}(t) \leq \boldsymbol{ub} \quad (3.1\text{d})$$

$$\mathbf{H}_{\boldsymbol{y}} \cdot \boldsymbol{y}(t) + \mathbf{H}_{\boldsymbol{u}} \cdot \boldsymbol{u}(t) \leq \boldsymbol{h} \quad (3.1\text{e})$$

$$\mathbf{0} \leq \boldsymbol{y}(t) \quad (3.1\text{f})$$

$$\mathbf{H}_{\mathbb{B},\boldsymbol{y}} \cdot \boldsymbol{y}(t) + \mathbf{H}_{\mathbb{B},\boldsymbol{u}} \cdot \boldsymbol{u}(t) + \mathbf{H}_{\mathbb{B},\bar{\boldsymbol{x}}} \cdot \bar{\boldsymbol{x}}(t) \leq \boldsymbol{h}_{\mathbb{B}} \quad (3.1\text{g})$$

$$\mathbf{B}_{\boldsymbol{y}_0} \cdot \boldsymbol{y}(t_0) + \mathbf{B}_{\boldsymbol{y}_{\mathrm{end}}} \cdot \boldsymbol{y}(t_{\mathrm{end}}) = \boldsymbol{b}_{\mathrm{bndry}} \quad (3.1\text{h})$$

$$\boldsymbol{y}(t) \in \mathbb{R}^{n_y}, \, \boldsymbol{u}(t) \in \mathbb{R}^{n_u} \quad (3.1\text{i})$$

$$\bar{\boldsymbol{x}}(t) \in \mathbb{B}^{n_{\bar{x}}} \quad (3.1\text{j})$$

3.3.1. Example: Self-replicator

The translation of the r-deFBA problem of a regulatory self-replicator introduced in Liu and Bockmayr (2020) into the MILP given above will be shown in the following.
The flux vector is given by

$$\boldsymbol{u}(t) = \begin{pmatrix} v_{C_1}(t) & v_{C_2}(t) & v_Q(t) & v_R(t) & v_{T_1}(t) & v_{T_2}(t) & v_{RP}(t) \end{pmatrix}^\top, \quad (3.2)$$

and the species vector reads

$$\boldsymbol{y}(t) = \begin{pmatrix} C_1(t) & C_2(t) & Q(t) & R(t) & T_1(t) & T_2(t) & RP(t) \end{pmatrix}^\top, \quad (3.3)$$

and thus

$$\dot{\boldsymbol{y}}(t) = \begin{pmatrix} \dot{C_1}(t) & \dot{C_2}(t) & \dot{Q}(t) & \dot{R}(t) & \dot{T_1}(t) & \dot{T_2}(t) & \dot{RP}(t) \end{pmatrix}^\top. \quad (3.4)$$

Note that the species vector does not contain species for which a quasi-steady state is assumed (QSSA) since these are by definition not dynamic ($\dot{M} = 0$).

Dynamics of External Species and Macromolecules

$$\dot{C}_1(t) = -v_{C1}(t)$$
$$\dot{C}_2(t) = -v_{C2}(t)$$
$$\dot{\boldsymbol{P}}(t) = \boldsymbol{v}_P(t) - \boldsymbol{kd}_p \cdot \boldsymbol{P}(t)$$

TO DO: Degradation Rates. Ich habe mir überlegt, dass man das so im SBML implementieren könnte: man definiert weiterhin den Abbau als Reaktionen, setzt aber `fbc:fluxLowerBound` und `fbc:fluxUpperBound` gleich Parameter `kd_<protein_id>` und definiert dann noch eine `AssignmentRule`, die den Parameter in jedem Schritt neu berechnet: $kd\_{<}protein\_id{>} = p(t) \cdot kd_p$, wobei kd_p einen festen Wert hat.

Using Equation 3.1b, $\mathbf{S}_2$ contains a row for each dynamical species and a column for each reaction:

$$\mathbf{S}_2 = \begin{array}{c} \\ C_1 \\ C_2 \\ Q \\ R \\ T_1 \\ T_2 \\ RP \end{array} \begin{array}{c} \begin{matrix} v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \end{matrix} \\ \left(\begin{matrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{matrix}\right) \end{array} \tag{3.5}$$

Steady-state assumption for internal metabolites

$$\dot{M}(t) = v_{C_1}(t) + v_{C_2}(t) - \sum n_p \cdot v_p(t) = 0$$

Using Equation 3.1c, $\mathbf{S}_1$ contains a row for each QSSA species and a column for each reaction:

$$\mathbf{S}_1 = M \begin{array}{c} \begin{matrix} v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \end{matrix} \\ \left(\begin{matrix} 1 & 1 & -300 & -7459 & -400 & -1500 & -300 \end{matrix}\right) \end{array} \tag{3.6}$$

Lower and Upper Flux Bounds

Lower and Upper Flux Bounds are not used in this example, and in general are optional in (r-)deFBA models Reimers et al. (2017b); Liu and Bockmayr (2020). Therefore, in Equation 3.1d all lower bounds are set to 0 (since all reactions in the regulatory self-replicator are irreversible), and upper bounds for fluxes are not given (achieved by a constant $u = $ big M).

$$\boldsymbol{lb}(t) = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}^\top \tag{3.7}$$

$$\boldsymbol{ub}(t) = \begin{pmatrix} u & u & u & u & u & u & u \end{pmatrix}^\top \tag{3.8}$$

Quota Constraint

$$n_Q \cdot Q(t) \geq \Phi_Q \sum_{p \in \mathcal{P}} n_p \cdot P(t) \iff \Phi_Q \sum_{p \in \mathcal{P}} n_p \cdot P(t) - n_Q \cdot Q(t) \leq 0$$

The quota constraint can be implemented using Equation 3.1e. Since $Q \in \mathcal{P}$, its entry reads $n_Q Q(t) - \Phi_Q n_Q Q(t) = (\Phi_Q - 1)n_Q$. Since no fluxes are involved here, $\mathbf{H}_{u,1}$ contains only zeros.

$$\mathbf{H}_{y,1} = Q \begin{array}{c} \begin{matrix} C_1 & C_2 & M & Q & R & T_1 & T_2 & RP \end{matrix} \\ \left(\begin{matrix} 0 & 0 & 0 & (\Phi_Q - 1)300 & \Phi_Q \cdot 7459 & \Phi_Q \cdot 400 & \Phi_Q \cdot 1500 & \Phi_Q \cdot 300 \end{matrix}\right) \end{array} \tag{3.9}$$

$$\mathbf{H_{u,1}} = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \tag{3.10}$$

$$\mathbf{h_1} = \begin{pmatrix} 0 \end{pmatrix} \tag{3.11}$$

Enzyme Capacity Constraint

The inequalities

$$v_{C_1}(t) \leq k_{cat1} \cdot T_1(t)$$
$$v_{C_2}(t) \leq k_{cat2} \cdot T_2(t)$$
$$\sum \frac{v_p(t)}{k_{cat}^{p,R}} \leq R(t)$$

can be expressed in the form $H_C \cdot V \leq H_E \cdot P \iff -H_E \cdot P + H_C \cdot V \leq 0$ Waldherr et al. (2015) and thus be implemented by using Equation 3.1e. $\mathbf{H_{y,2}}$ and $\mathbf{H_{u,2}}$ both contain a row for each catalytic macromolecule $p \in \mathcal{E}$.

$$\mathbf{H_{y,2}} = \begin{array}{c} \\ T_1 \\ T_2 \\ R \end{array} \begin{array}{cccccccc} C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \begin{pmatrix} 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 0 \end{pmatrix} \end{array} \tag{3.12}$$

$$\mathbf{H_{u,2}} = \begin{array}{c} \\ T_1 \\ T_2 \\ R \end{array} \begin{array}{ccccccc} v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \\ \begin{pmatrix} 3000^{-1} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 2000^{-1} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 4.2^{-1} & 0.1689^{-1} & 3.15^{-1} & 0.84^{-1} & 4.2^{-1} \end{pmatrix} \end{array} \tag{3.13}$$

$$\mathbf{h_2} = \begin{pmatrix} 0 & 0 & 0 \end{pmatrix}^{\top} \tag{3.14}$$

Control of discrete jumps

$$\overline{RP}(t) = 1 \iff C_1(t) \geq \gamma \Rightarrow l(1 - \overline{RP}) \leq C_1 - \gamma \leq (u + \epsilon)\overline{RP} - \epsilon$$
$$\overline{T_2}(t) = 0 \iff RP(t) \geq \alpha \Rightarrow l(1 - \overline{T_2}) \leq RP - \alpha \leq (u + \epsilon)\overline{T_2} - \epsilon$$

where $l = -\text{big M}$, $u = \text{big M}$, $\epsilon > 0$.
These four inequalities can be reformulated as:

$$l(1 - \overline{RP}) \leq C_1 - \gamma \iff -l \cdot \overline{RP} - C_1 \leq \gamma - l \qquad\qquad I$$
$$C_1 - \gamma \leq (u + \epsilon)\overline{RP} - \epsilon \iff -(u + \epsilon)\overline{RP} + C_1 \leq \gamma - \epsilon \qquad\qquad II$$
$$l(1 - \overline{T_2}) \leq RP - \alpha \iff -l \cdot \overline{T_2} - RP \leq \alpha - l \qquad\qquad III$$
$$RP - \alpha \leq (u + \epsilon)\overline{T_2} - \epsilon \iff -(u + \epsilon)\overline{T_2} + RP \leq \alpha - \epsilon \qquad\qquad IV$$

and enforced using Equation 3.1g such that $\mathbf{H}_{\mathbb{B},y,1}$ denotes which species amounts govern the state changes of the boolean variables ($\mathbf{H}_{\mathbb{B},y,1}$ analogous, if the boolean variables depend on fluxes).

$$\mathbf{H}_{\mathbb{B},y,1} = \begin{array}{c} \\ I \\ II \\ III \\ IV \end{array} \begin{array}{c} \begin{array}{ccccccc} C_1 & C_2 & Q & R & T_1 & T_2 & RP \end{array} \\ \left(\begin{array}{ccccccc} -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{array} \right) \end{array} \tag{3.15}$$

$$\mathbf{H}_{\mathbb{B},u,1} = \begin{array}{c} \\ I \\ II \\ III \\ IV \end{array} \begin{array}{c} \begin{array}{ccccccc} v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \end{array} \\ \left(\begin{array}{ccccccc} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{array} \right) \end{array} \tag{3.16}$$

$$\mathbf{H}_{\mathbb{B},\bar{x},1} = \begin{array}{c} \\ I \\ II \\ III \\ IV \end{array} \begin{array}{c} \begin{array}{cc} \overline{RP} & \overline{T_2} \end{array} \\ \left(\begin{array}{cc} -l & 0 \\ \epsilon - u & 0 \\ 0 & -l \\ 0 & \epsilon - u \end{array} \right) \end{array} \tag{3.17}$$

$$h_{\mathbb{B},1} = \begin{pmatrix} \gamma - l & \gamma - \epsilon & \alpha - l & \alpha - \epsilon \end{pmatrix}^{\top} \tag{3.18}$$

Control of continuous dynamics by discrete states

$$\overline{RP}(t) = 1 \Rightarrow v_{RP}(t) \geq \epsilon_{RP} \land \overline{RP}(t) = 0 \Rightarrow v_{RP}(t) = 0 \iff \epsilon_{RP} \cdot \overline{RP} \leq v_{RP} \leq u_{RP} \cdot \overline{RP}$$
$$\overline{T_2}(t) = 1 \Rightarrow v_{T_2}(t) \geq \epsilon_{T_2} \land \overline{T_2}(t) = 0 \Rightarrow v_{T_2}(t) = 0 \iff \epsilon_{T_2} \cdot \overline{T_2} \leq v_{T_2} \leq u_{T_2} \cdot \overline{T_2}$$

These four inequalities can be reformulated as:

$$\begin{array}{lll}
\epsilon_{RP} \cdot \overline{RP} \leq v_{RP} & \iff -v_{RP} + \epsilon_{RP} \cdot \overline{RP} \leq 0 & V \\
v_{RP} \leq u_{RP} \cdot \overline{RP} & \iff v_{RP} - u_{RP} \cdot \overline{RP} \leq 0 & VI \\
\epsilon_{T_2} \cdot \overline{T_2} \leq v_{T_2} & \iff -v_{T_2} + \epsilon_{T_2} \cdot \overline{T_2} \leq 0 & VII \\
v_{T_2} \leq u_{T_2} \cdot \overline{T_2} & \iff v_{T_2} - u_{T_2} \cdot \overline{T_2} \leq 0 & VIII
\end{array}$$

Again, Equation 3.1g is used to implement these inequalities. $\mathbf{H}_{\mathbb{B},y,2}$ describes the fluxes that are regulated by the given boolean variables.

$$\mathbf{H}_{\mathbb{B},y,2} = \begin{array}{c} \\ V \\ VI \\ VII \\ VIII \end{array} \begin{array}{c} \begin{array}{ccccccc} C_1 & C_2 & Q & R & T_1 & T_2 & RP \end{array} \\ \left(\begin{array}{ccccccc} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{array} \right) \end{array} \tag{3.19}$$

$$
\mathbf{H}_{\mathbb{B},u,2} = \begin{array}{c} \\ V \\ VI \\ VII \\ VIII \end{array}
\begin{array}{ccccccc} v_{C_1} & v_{C_2} & v_Q & v_R & v_{T_1} & v_{T_2} & v_{RP} \\ \left(\begin{array}{ccccccc} 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \end{array}\right) \end{array}
\tag{3.20}
$$

$$
\mathbf{H}_{\mathbb{B},\bar{x},2} = \begin{array}{c} \\ V \\ VI \\ VII \\ VIII \end{array}
\begin{array}{cc} \overline{RP} & \overline{T_2} \\ \left(\begin{array}{cc} \epsilon_{RP} & 0 \\ -u & 0 \\ 0 & \epsilon_{T_2} \\ 0 & -u \end{array}\right) \end{array}
\tag{3.21}
$$

$$
h_{\mathbb{B},2} = \begin{pmatrix} 0 & 0 & 0 & 0 \end{pmatrix}^{\top}
\tag{3.22}
$$

Initial amounts for species

Equation 3.1h can be used to set initial and final amounts for species (the latter is necessary to enforce cyclic behaviour). $\mathbf{B}_{y_0}$ simply is an identity matrix, and b_{bndry} contains the respective initial amounts.

$$
\mathbf{B}_{y_0} = \begin{array}{c} \\ C_1 \\ C_2 \\ Q \\ R \\ T_1 \\ T_2 \\ RP \end{array}
\begin{array}{ccccccc} C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \left(\begin{array}{ccccccc} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{array}\right) \end{array}
\tag{3.23}
$$

$$
\mathbf{B}_{y_{\text{end}}} = \begin{array}{c} \\ C_1 \\ C_2 \\ Q \\ R \\ T_1 \\ T_2 \\ RP \end{array}
\begin{array}{ccccccc} C_1 & C_2 & Q & R & T_1 & T_2 & RP \\ \left(\begin{array}{ccccccc} 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{array}\right) \end{array}
\tag{3.24}
$$

$$
b_{\text{bndry}} = \begin{pmatrix} 1000 & 500 & 20 & 0.15 & 0.01 & 0.001 & 0.001 & 0 \end{pmatrix}^{\top}
\tag{3.25}
$$

Lin gibt tatsächlich einen Anfangswert für M an! Also müssen wie QSSA-Spezies doch in y(t) behalten und dann bei der Einheitsmatrix $\mathbf{B}_{y_0}$ in der Diagonalen 0 einsetzen, wenn kein Anfangswert gegeben wird?

Initial levels for qualitative species

Since the rules are enforced at any time, initial levels don't need to be stated explicitly in the MILP.