

Supporting Information Text S1

Nomenclature

i – metabolites; j – reactions; l – non - native reactions; n – enzymes; g – genes

$J_{LB} = \{j \in J \mid \text{the flux through reaction } j \text{ has a lower bound}\}$

$L_{LB} = \{l \in L \mid \text{the flux through non - native reaction } l \text{ has a lower bound}\}$

$J_{GPR} = \{j \in J \mid \text{reaction } j \text{ has known GPR associations}\}$

$N(j) = \{n \in N \mid \text{enzyme } n \text{ is associated with reaction } j\}$

$G(n) = \{g \in G \mid \text{gene } g \text{ is associated with enzyme } n\}$

c_j – outer objective coefficient; p_j – inner objective coefficient; S_{ij} – stoichiometric coefficient

v_j – flux through reaction j ; w_l – flux through reaction l ; $u_i, \lambda_j, h_j, \eta_l, g_l$ – dual variables

v_j^{lb} – lower bound on v_j ; w_l^{lb} – lower bound on w_l ; h_j^{lb} / h_j^{ub} – lower / upper bounds on h_j

$y_g / b_n / d_j$ – binary variables for gene / enzyme / reaction status

α – penalty for gene deletion; β – penalty for reaction addition

γ – penalty for dual variables h_j ; v_j^{wt} – wildtype flux through reaction j

k – number of allowed gene deletions; k' – number of allowed reaction additions

A. OptORF without transcriptional regulatory constraints

$$\max \quad \sum_j c_j v_j - \alpha \sum_g (1 - y_g) \quad (\text{A.1})$$

$$\text{s.t.} \quad \sum_j S_{ij} v_j = 0 \quad \forall i \in I : (u_i) \quad (\text{A.2})$$

$$v_j \geq v_j^{lb} \quad \text{if } d_j = 1 \quad \forall j \in J_{LB} : (\lambda_j) \quad (\text{A.3})$$

$$v_j = 0 \quad \text{if } d_j = 0 \quad \forall j \in J : (h_j) \quad (\text{A.4})$$

$$\sum_i S_{ji} u_i = p_j \quad \text{if } d_j = 1 \quad \forall j \in J \setminus J_{LB} \quad (\text{A.5})$$

$$\sum_i S_{ji} u_i - \lambda_j = p_j \quad \text{if } d_j = 1 \quad \forall j \in J_{LB} \quad (\text{A.6})$$

$$\sum_i S_{ji} u_i + h_j = p_j \quad \text{if } d_j = 0 \quad \forall j \in J \quad (\text{A.7})$$

$$\lambda_j \geq 0 \quad \forall j \in J_{LB} \quad (\text{A.8})$$

$$\sum_j p_j v_j = \sum_{j \in J_{LB}} (-v_j^{lb}) \lambda_j \quad (\text{A.9})$$

$$d_j \geq b_n \quad \forall j \in J_{GPR}, n \in N(j) \quad (\text{A.10})$$

$$d_j \leq \sum_{n \in N(j)} b_n \quad \forall j \in J_{GPR} \quad (\text{A.11})$$

$$(b_n - 1) \geq \sum_{g \in G(n)} (y_g - 1) \quad \forall n \in N \quad (\text{A.12})$$

$$b_n \leq y_g \quad \forall n \in N, g \in G(n) \quad (\text{A.13})$$

$$\sum_g (1 - y_g) \leq k \quad (\text{A.14})$$

$$h_j^{lb} \leq h_j \leq h_j^{ub} \quad \forall j \in J \quad (\text{A.15})$$

$$d_j, b_n, y_g \in \{0, 1\} \quad (\text{A.16})$$

B. SimOptStrain

$$\max \quad \sum_j c_j v_j - \alpha \sum_g (1 - y_g) - \beta \sum_l z_l \quad (\text{B.1})$$

$$\text{s.t.} \quad \sum_j S_{ij} v_j + \sum_l T_{il} w_l = 0 \quad \forall i \in I : (u_i) \quad (\text{B.2})$$

$$v_j \geq v_j^{lb} \quad \text{if } d_j = 1 \quad \forall j \in J_{LB} : (\lambda_j) \quad (\text{B.3})$$

$$v_j = 0 \quad \text{if } d_j = 0 \quad \forall j \in J : (h_j) \quad (\text{B.4})$$

$$w_l \geq w_l^{lb} \quad \text{if } z_l = 1 \quad \forall l \in L_{LB} : (\eta_l) \quad (\text{B.5})$$

$$w_l = 0 \quad \text{if } z_l = 0 \quad \forall l \in L : (g_l) \quad (\text{B.6})$$

$$\sum_i S_{ji} u_i = p_j \quad \text{if } d_j = 1 \quad \forall j \in J \setminus J_{LB} \quad (\text{B.7})$$

$$\sum_i S_{ji} u_i - \lambda_j = p_j \quad \text{if } d_j = 1 \quad \forall j \in J_{LB} \quad (\text{B.8})$$

$$\sum_i S_{ji} u_i + h_j = p_j \quad \text{if } d_j = 0 \quad \forall j \in J \quad (\text{B.9})$$

$$\sum_i T_{li} u_i = 0 \quad \text{if } z_l = 1 \quad \forall l \in L \setminus L_{LB} \quad (\text{B.10})$$

$$\sum_i T_{li} u_i - \eta_l = 0 \quad \text{if } z_l = 1 \quad \forall l \in L_{LB} \quad (\text{B.11})$$

$$\sum_i T_{li} u_i + g_l = 0 \quad \text{if } z_l = 0 \quad \forall l \in L \quad (\text{B.12})$$

$$\lambda_j \geq 0 \quad \forall j \in J_{LB} \quad (\text{B.13})$$

$$\eta_l \geq 0 \quad \forall l \in L_{LB} \quad (\text{B.14})$$

$$\sum_j p_j v_j = \sum_{j \in J_{LB}} (-v_j^{lb}) \lambda_j + \sum_{l \in L_{LB}} (-w_l^{lb}) \eta_l \quad (\text{B.15})$$

$$d_j \geq b_n \quad \forall j \in J_{GPR}, n \in N(j) \quad (\text{B.16})$$

$$d_j \leq \sum_{n \in N(j)} b_n \quad \forall j \in J_{GPR} \quad (\text{B.17})$$

$$(b_n - 1) \geq \sum_{g \in G(n)} (y_g - 1) \quad \forall n \in N \quad (\text{B.18})$$

$$b_n \leq y_g \quad \forall n \in N, g \in G(n) \quad (\text{B.19})$$

$$\sum_g (1 - y_g) \leq k \quad (\text{B.20})$$

$$\sum_l z_l \leq k' \quad (\text{B.21})$$

$$h_j^{lb} \leq h_j \leq h_j^{ub} \quad \forall j \in J \quad (\text{B.22})$$

$$d_j, b_n, y_g, z_l \in \{0,1\} \quad (\text{B.23})$$

C. BiMOMA

$$\max \quad \sum_j c_j v_j - \alpha \sum_g (1 - y_g) - \gamma \sum_j h_j^2 \quad (\text{C.1})$$

$$\text{s.t.} \quad \sum_j S_{ij} v_j = 0 \quad \forall i \in I : (u_i) \quad (\text{C.2})$$

$$v_j \geq v_j^{lb} \quad \text{if } d_j = 1 \quad \forall j \in J_{LB} : (\lambda_j) \quad (\text{C.3})$$

$$v_j = 0 \quad \text{if } d_j = 0 \quad \forall j \in J : (h_j) \quad (\text{C.4})$$

$$\sum_i S_{ji} u_i = v_j - v_j^{wt} \quad \text{if } d_j = 1 \quad \forall j \in J \setminus J_{LB} \quad (\text{C.5})$$

$$\sum_i S_{ji} u_i + \lambda_j = v_j - v_j^{wt} \quad \text{if } d_j = 1 \quad \forall j \in J_{LB} \quad (\text{C.6})$$

$$\sum_i S_{ji} u_i + h_j = v_j - v_j^{wt} \quad \text{if } d_j = 0 \quad \forall j \in J \quad (\text{C.7})$$

$$\lambda_j \geq 0 \quad \forall j \in J_{LB} \quad (\text{C.8})$$

$$\sum_j \frac{1}{2} v_j^2 - v_j^{wt} v_j \leq \sum_j -\frac{1}{2} v_j^2 + \sum_{j \in J_{LB}} v_j^{lb} \lambda_j \quad (\text{C.9})$$

$$d_j \geq b_n \quad \forall j \in J_{GPR}, n \in N(j) \quad (\text{C.10})$$

$$d_j \leq \sum_{n \in N(j)} b_n \quad \forall j \in J_{GPR} \quad (\text{C.11})$$

$$(b_n - 1) \geq \sum_{g \in G(n)} (y_g - 1) \quad \forall n \in N \quad (\text{C.12})$$

$$b_n \leq y_g \quad \forall n \in N, g \in G(n) \quad (\text{C.13})$$

$$\sum_g (1 - y_g) \leq k \quad (\text{C.14})$$

$$h_j^{lb} \leq h_j \leq h_j^{ub} \quad \forall j \in J \quad (\text{C.15})$$

$$d_j, b_n, y_g \in \{0,1\} \quad (\text{C.16})$$