Sequential Metabolic Phases as a Means to Optimize Cellular Output in a Constant Environment

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Supplementary Information

Analysis of the minimal example

The simplicity of the minimal example allows us to analyze formally how the parameters determine the performance of the different strategies.

**Strategy A: Single flux mode**  For strategy A, the reference case, the demanded output $\Gamma > 0$ is produced by a single flux mode $v$. Without loss of generality, we assume $\tau v_1 = \Gamma_1$, $\tau v_2 = \Gamma_2$ and set $r := \Gamma_2/\Gamma_1 = v_2/v_1$. Using the steady-state assumption $v_0 = v_1 + v_2$, we obtain $v^\top = (v_0, v_1, v_2)^\top = v_1 \cdot (1 + r, 1, r)^\top$ (again $\cdot^\top$ denotes transposition). Thus, there is only the unknown $v_1$, which has to be maximized in order to minimize $\tau(1) = \Gamma_1/v_1$. From $v > 0$, we get $g = (1, 1, 1)^\top$. Setting the kinetic parameters to $\eta_j = 1$, $j = 1, 2, 3$, the upper bounds on the fluxes (cf. 6) are given by

$$ub_j := A_{tot} k c_j \frac{1}{\gamma_A + g \cdot \gamma} = A_{tot} k c_j \frac{1}{\gamma_A + \gamma_0 + \gamma_1 + \gamma_2}, \text{ for } j = 0, 1, 2.$$ 

Maximizing $v_1$ under the constraint $v \leq ub$, we obtain $v_1 = \min(ub_0/(1 + r), ub_1, ub_2/r)$ or equivalently

$$\tau(1) = \frac{\Gamma_1}{v_1} = \max \left( \frac{\Gamma_1 + \Gamma_2}{ub_0}, \frac{\Gamma_1}{ub_1}, \frac{\Gamma_2}{ub_2} \right). \quad (S1)$$

**Strategy B: Switching between two MinModes**  Next we consider the case where the two minimal gene sets $\chi_1, \chi_2$ are separately activated in two time intervals with flux vectors $w^1, w^2$. Here $w^1$ is only producing the target metabolite $P_1$ and $w^2$ only $P_2$. Applying the steady-state condition, we get $w^1 = (w^1_0, w^1_1, 0)^\top$, $w^2 = (w^2_0, 0, w^2_2)^\top$. For $w^1$, we have the upper bounds

$$ub^{1}_0 := A_{tot} k c_0 \frac{1}{\gamma_A + \gamma_0 + \gamma_1}, \quad ub^{1}_1 := A_{tot} k c_1 \frac{1}{\gamma_A + \gamma_0 + \gamma_1}, \quad ub^{1}_2 = 0,$$

whereas as for $w^2$ we get

$$ub^{2}_0 := A_{tot} k c_0 \frac{1}{\gamma_A + \gamma_0 + \gamma_2}, \quad ub^{2}_1 = 0, \quad ub^{2}_2 := A_{tot} k c_2 \frac{1}{\gamma_A + \gamma_0 + \gamma_2}.$$

Maximizing $w^1_0$ resp. $w^2_0$ under the constraint $w^1 \leq ub^1$ resp. $w^2 \leq ub^2$ yields

$$w^1 = \min(ub^1_0, ub^1_1) \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix} \text{ and } w^2 = \min(ub^2_0, ub^2_2) \begin{pmatrix} 1 \\ 0 \\ 1 \end{pmatrix}. $$
For the durations, we get
\[ \tau_1 = \frac{\Gamma_1}{\min(ub_0^0, ub_1^1)} = \max \left( \frac{\Gamma_1}{ub_0^0}, \frac{\Gamma_1}{ub_1^1} \right) \quad \text{and} \quad \tau_2 = \frac{\Gamma_2}{\min(ub_0^2, ub_2^2)} = \max \left( \frac{\Gamma_2}{ub_0^2}, \frac{\Gamma_2}{ub_2^2} \right). \]

Whether or not the solution \( w^1 \) and \( w^2 \) outperforms the single flux vector \( v \), i.e., whether or not \( \tau_1 + \tau_2 < \tau(1) \) depends on the demand \( \Gamma \) and the upper bounds \( ub, ub^1, ub^2 \). We discuss two cases in more detail.

First suppose \( ub_0 \) is small, such that \( \tau(1) = (\Gamma_1 + \Gamma_2)/ub_0 \) and \( ub_0 < ub_1^1, ub_2^2 \). It follows \( \tau_1 < \Gamma_1/ub_0, \tau_2 < \Gamma_2/ub_0 \) and so \( \tau_1 + \tau_2 < \tau(1) \). In other words, switching from \( w^1 \) to \( w^2 \) is more efficient than the single flux mode \( v \).

Second, assume \( ub_0 \) is large, such that \( \tau(1) = \Gamma_1/ub_1 \geq \Gamma_2/ub_2 \). Using Eqn. ???, we get \( (\Gamma_1 + \Gamma_2)/ub_0 \leq \Gamma_i/ub_i \), which implies \( \Gamma_i/ub_0 \leq \Gamma_i/ub_i \), for \( i = 1, 2 \). Since \( ub_0 \geq ub_i \Leftrightarrow ub_0^0 \geq ub_i^i \) and using Eqn. ???, we get \( \tau_i = \Gamma_i/ub_i^i \), for \( i = 1, 2 \). The switching solution thus has the duration \( \tau_1 + \tau_2 = \Gamma_1/ub_0^1 + \Gamma_2/ub_0^2 \). As long as \( \Gamma_2/ub_0^2 \) is not too small, this will be larger than \( \tau(1) = \Gamma_1/ub_0^1 \), the duration of the single mode solution. Taking a closer look at the ratio \( \Gamma_1/\Gamma_2 \), we observe that a smaller value of \( \Gamma_1 \) and a larger value of \( \Gamma_2 \) in this situation are favorable for the single mode solution. On the one hand, increasing \( \Gamma_1 \) by a factor \( c > 1 \) increases also \( \tau(1) \) by \( c \), whereas \( \tau_1 + \tau_2 \) increases by a strictly smaller factor (as long as \( \Gamma_2 > 0 \)). On the other hand, decreasing \( \Gamma_2 \) has no effect on \( \tau(1) \), while the duration of the switching solution is decreased. We conclude that the single mode solution performs best compared to the switching solution, i.e., \( \tau(1)/(\tau_1 + \tau_2) \) is minimal, if we have equality in our assumption, i.e., \( \Gamma_1/ub_1 = \Gamma_2/ub_2 \), or equivalently \( \Gamma_1/\Gamma_2 = ub_1^1/ub_2^2 \).

Strategies C or D Between the two extreme strategies A and B, there are the intermediate strategies C and D. Strategy A can be seen as the limit case of strategy C or D, when \( \tau_2 \) goes to zero. Strategy B is a limit case of C resp. D, when \( v_2^1 \) resp. \( v_1^2 \) vanishes.