The economics of periodic enzyme profiles
Supplementary information

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Contents

S1 Mathematical notation

Most formulae are written in matrix notation, with vectors, matrices, and tensors written in bold font. Mathematical symbols are listed in Table S1. For tensor products, I use the following notation: products $Y^i_{mn} = \sum_l A^i_l B^l_{mn}$ are written $\mathbf{Y} = \mathbf{A} \cdot \mathbf{B}$; product $X^i_{mn} = \sum_{pq} A^i_{pq} B^{p*}_m C^q_n$ are written as $\mathbf{X} = \mathbf{A}\,[\mathbf{B} \otimes \mathbf{C}]$. The symbol $\otimes$ denotes the Kronecker product, the star $*$ denotes the complex conjugate, and the dagger $\dagger$ denotes the adjoint, i.e. the conjugate transpose. Oscillations are described as the real parts of complex exponentials, for instance, $a(t) = \mathrm{Re}[\tilde{a}\ \mathrm{e}^{i\omega t}] = |\tilde{a}|\cos(\omega t) + \varphi(\tilde{a})$, where $\varphi(z)$ is the angle of a complex number z. Elasticities and response coefficients are used in their unscaled form throughout the text.

Symbol	Unit	Name
c_i	mM	Concentration
v_l	mM/s	Reaction rate
$v_l(\mathbf{c})$	mM/s	Reaction rate law
u_l	mM	Enzyme level
x_j		External parameter
p_m		System parameter (enzyme level or external)
y		System variable (concentration, reaction rate, or benefit function)
s_i	mM	Steady state concentration
j_l	mM/s	Steady state flux (reaction rate)
$f(\mathbf{u})$	D	Fitness function
$g(\mathbf{u}) = z(\mathbf{y})$	D	Benefit function
$h(\mathbf{u})$	D	Cost function
z_v	D/(mM/s)	Flux demand
$\tilde{y}_l$	D	Periodic enzyme gain
ω	s^{-1}	Circular frequency
$\mathbf{I}$		Identity matrix

Table S1: Mathematical symbols used. The physical units of some quantities depend on the case. The unit D (Darwin) is the hypothetical unit of the fitness function. Note that all MCA coefficients (elasticities, response and control coefficients) are used in their unscaled form.

S2 Dynamics of oscillations in metabolism: propagating perturbations

Static parameter changes can shift the stable reference state of a kinetic model. Periodic parameter changes will drive forced oscillations *and*, as a second-order effect, can shift the average state. In this section, I show how to compute these forced oscillations in biochemical systems in a second-order approximation. In section S5, I will demonstrate the approach step by step with simple example models. Here I recall general formulae from [1, 2, 3],

Steady states	
$\mathbf{R}^{\mathrm{y}}_{\mathrm{p}} = (R^{\mathrm{y_l}}_{\mathrm{p_m}})$	1st order response coefficients (for state variable y)
$\mathbf{R}^{\mathrm{y}}_{\mathrm{pp}} = (R^{\mathrm{y_l}}_{p_m p_n})$	2nd order response tensor ("synergy tensor")
$\mathbf{C}^{\mathrm{s}}$, $\mathbf{C}^{\mathrm{j}}$	Control coefficients (for concentrations and fluxes)
$\mathbf{E}_{\mathrm{c}} = (E_{li})$	Unscaled elasticity matrix
$\mathbf{E}_{\mathrm{cc}}$, $\mathbf{E}_{\mathrm{cp}}$, $\mathbf{E}_{\mathrm{pc}}$, $\mathbf{E}_{\mathrm{pp}}$	2nd order elasticity tensors

Periodic states	
$\mathbf{C}^{\mathrm{s}}(\omega)$, $\mathbf{C}^{\mathrm{j}}(\omega)$	Spectral control coefficients (for concentrations and fluxes)
$\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}(\omega)$	1st order spectral response matrix
$\tilde{\mathbf{R}}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)$, $\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)$	2nd order spectral response tensor (frequencies 0 and $2\,\omega$) (periodic synergy tensor)
$\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)$, $\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)$	2nd order periodic response tensor (frequencies 0 and $2\,\omega$) (periodic synergy tensor)

Table S2: Symbols for metabolic control analysis.

which equally apply to simple and complicated models. The paragraphs describe how rate perturbations in a single reaction can be described by spectral elasticities; how biochemical systems respond to small perturbations - which may be stationary or periodic - and how the response can be computed by response coefficients; how static, spectral, and periodic response coefficients are computed; and how periodic changes in transcript and enzyme levels are related to each other.

S2.1 Expansion of peridiodic rates by spectral elasticities

The unscaled reaction elasticities are defined as the derivatives of the rate laws $v_k(\mathbf{c}, \mathbf{p})$:

$$\begin{aligned} E^{v_k}_{\mathrm{c_m}} &= \frac{\partial r_k}{\partial c_m} \qquad E^{v_k}_{\mathrm{p_m}} = \frac{\partial r_k}{\partial p_m} \\ E^{v_k}_{\mathrm{c_m c_n}} &= \frac{\partial^2 r_k}{\partial c_m \partial c_n} \qquad E^{v_k}_{\mathrm{c_m p_n}} = \frac{\partial^2 r_k}{\partial c_m \partial p_n} \qquad E^{v_k}_{\mathrm{p_m p_n}} = \frac{\partial^2 r_k}{\partial p_m \partial p_n}. \end{aligned} \tag{S1}$$

They can be used to Taylor-expanded a rate law around a given reference state. In periodic metabolic states with cosine oscillations (frequency ω) in the reactant and enzyme levels, the rate will oscillate as well, and in a second-order approximation, the shift and amplitude can be determined from the periodic elasticities (see [3] and Figure S1).

I will illustrate this with a simple example. In model M1 in the article, a cosine-wave profile, our ansatz for enzyme profiles, provides an exact optimal solution. However, this only holds because the mass-action rate law is linear in both u and x. Typical enzymatic rate laws are nonlinear in x, so a harmonic substrate profiles will not induce harmonic optimal enzyme profiles. However, for small perturbations, they can be approximated by harmonic profiles. To do so, we expand the rate laws around the reference state: a rate change Δv, caused by deviations Δu and Δx, is approximated by

$$\Delta v \approx E_x\,\Delta x + E_u\,\Delta u + \frac{1}{2} E_{xx}\,\Delta x^2 + E_{ux}\,\Delta u \Delta x \tag{S2}$$

with the unscaled elasticities $E_x = \frac{\partial r}{\partial x}$, $E_u = \frac{\partial r}{\partial u} = \frac{v}{u}$, $E_{xx} = \frac{\partial^2 r}{\partial x^2}$, $E_{ux} = \frac{\partial^2 r}{\partial u \partial x} = \frac{1}{u}\,E_x$. The second-order elasticity E_{uu} vanishes because the enzyme level is a linear prefactor in the rate law. We consider only harmonic

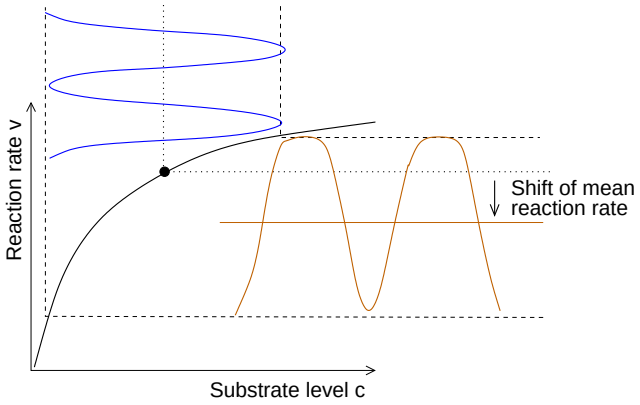

Figure S1: Periodic elasticities. A reaction rate (y-axis) depends on the substrate level (x-axis), as described by the rate law (black curve). Harmonic oscillation of the substrate level (blue curve, with y-axis representing time) lead to an oscillating rate (brown curve, time on x-axis). The minimal and maximal concentrations translate into minimal and maximal rates (dashed lines), while the average concentration translates into the median rate (dotted line), which differs from the average rate (brown straight line). This shows that oscillations around a constant substrate level can shift the average flux. The flux shift and amplitude, in a second-order aproximation, can be determined from the metabolic amplitude with the help of periodic elasticities.

enzyme profiles. With harmonic ryhthms of u and x, only the last term in Eq. (S2) will matter for periodic enzyme adaptions. The term resembles the mass-action rate law in model M1 in the article, with the mixed elasticity $E_{ux} = \frac{1}{u} E_x$ replacing the rate constant k, and the average rate shift due to enzyme adaption reads approximately

$$\Delta\langle v\rangle_t \approx \mathrm{Re}(\frac{1}{2} E_{ux}\, \tilde{u}\, \tilde{x}) = \frac{1}{2} E_{ux} |\tilde{u}|\, |\tilde{x}| \cos(\phi). \tag{S3}$$

The prefactor $E_{\tilde{u}\tilde{x}} = \frac{1}{2} E_{ux} = \frac{E_x}{2\,u}$, called the periodic second-order elasticity, relates the flux shift to the amplitudes. If we consider reactions with multiple substrates and products, the optimal enzyme rhythm will depend on all reactant rhythms together. Instead of Eq. (S3), we obtain

$$\Delta\langle v\rangle_t \approx \frac{1}{2} \mathrm{Re}(\tilde{u}\ \mathbf{E}_{ux}\ \tilde{\mathbf{x}}) \tag{S4}$$

where $\tilde{\mathbf{x}}$ is the vector of reactant amplitudes and $\mathbf{E}_{\tilde{u}\tilde{x}} = \frac{1}{2}\mathbf{E}_{ux} = \frac{1}{2u}\mathbf{E}_x$ is the row vector of periodic enzyme-reactant elasticities. As an example, consider a reaction $X \leftrightharpoons X$ with elasticities $E_X > 0$ and $E_Y \leq 0$. With given amplitudes $\tilde{a}$ and $\tilde{b}$, the average flux shift caused by enzyme adaption reads $\Delta\langle v\rangle_t = \mathrm{Re}[\frac{\tilde{u}}{2\,u}\,(E_X\,\tilde{x} + E_X\,\tilde{y})]$. If the enzyme amplitude is fixed, the enzyme must be in phase with $E_X\,\tilde{x} + E_Y\,\tilde{y}$ to achieve a maximal flux. If the reaction is irreversible ($E_Y = 0$), the enzyme must be in phase with X; if it is reversible, Y will peak after X (phase shift $0 < \phi < \pi$) and the enzyme will peak before X (see Figure 2 (d)).

S2.2 How biochemical systems respond to parameter perturbations

The metabolite levels in a kinetic model follow the balance equations

$$\frac{\mathrm{d}\mathbf{c}(t)}{\mathrm{d}t} = \mathbf{N}\ \mathbf{v}\,(\mathbf{c}(t), \mathbf{p}) \tag{S5}$$

with the stoichiometric matrix $\mathbf{N}$ (for internal metabolites), rate laws $\mathbf{v}(\mathbf{c}, \mathbf{p})$, and parameter vector $\mathbf{p}$. To account for metabolite dilution in growing cells, we can subtract a term $\kappa\,\mathbf{c}$ on the right hand side, where κ is the cell growth rate in s^{-1}, The parameters can comprise enzyme activities, enzyme kinetic parameters, and external metabolite levels. I assume that all vectors $\mathbf{p}$ in a region Ω_p in parameter space lead to stable steady states, with concentrations $\mathbf{s}(\mathbf{p})$ satisfying the steady state equation $0 = \mathbf{N}\mathbf{r}(\mathbf{s}(\mathbf{p}), \mathbf{p})$. We combine the stationary concentration vector $\mathbf{c} = \mathbf{s}(\mathbf{p})$ with the stationary flux vector $\mathbf{v}(\mathbf{p}) = \mathbf{r}(\mathbf{s}(\mathbf{p}), \mathbf{p})$ in a state vector $\mathbf{y}(\mathbf{p}) = \binom{\mathbf{s}(\mathbf{p})}{\mathbf{j}(\mathbf{p})}$. Next, we choose a parameter set $\bar{\mathbf{p}}$ inside Ω_p, which defines our reference state. Static parameter changes $\Delta\mathbf{p}$ will shift the state, and the new state vector $\mathbf{y}$ can be Taylor-expanded around $\bar{\mathbf{p}}$ into a series

$$\mathbf{y}(\bar{\mathbf{p}} + \Delta\mathbf{p}) \quad = \quad \mathbf{y}(\bar{\mathbf{p}}) + \mathbf{R}^\mathrm{y}_\mathrm{p}\Delta\mathbf{p} + \frac{1}{2}\,\mathbf{R}^\mathrm{y}_\mathrm{pp}(\Delta\mathbf{p} \otimes \Delta\mathbf{p}) + ... \tag{S6}$$

The expansion coefficients ("metabolic response coefficients") are collected in a first-order response matrix $\mathbf{R}^\mathrm{y}_\mathrm{p}$ and a second-order response tensor (or "synergy tensor") $\mathbf{R}^\mathrm{y}_\mathrm{pp}$. Both can be computed directly from the stoichiometric matrix and from the reaction elasticities (see section S2.3).

Next, we consider small harmonic parameter variations $\mathbf{p}(t) = \bar{\mathbf{p}} + \mathrm{Re}[\tilde{\mathbf{p}}\,\mathrm{e}^{i\omega t}]$. The complex amplitudes $\tilde{p}_m$ describe the amplitudes and phases of individual parameters. The oscillations lead to forced oscillations of concentrations and fluxes: asymptotically (i.e., if initial conditions are defined for times $t \to -\infty$), the state vector $\mathbf{y}(t)$ changes periodically and can be expanded [3] as

$$\mathbf{y}(t; \bar{\mathbf{p}}, \tilde{\mathbf{p}}, \omega) \quad \approx \quad \mathbf{y}(\bar{\mathbf{p}}) + \mathrm{Re}\Big[\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}\;\tilde{\mathbf{p}}\;\mathrm{e}^{i\omega t}\Big] + \frac{1}{2}\,\mathbf{R}^\mathrm{y}_{\tilde{\mathrm{p}}\bar{\mathrm{p}}}(\tilde{\mathbf{p}} \otimes \tilde{\mathbf{p}}^*) + \frac{1}{2}\,\mathrm{Re}\Big[\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}\Big(\tilde{\mathbf{p}} \otimes \tilde{\mathbf{p}}\Big)\;\mathrm{e}^{i2\omega t}\Big]. \tag{S7}$$

In this second-order approximation, the parameter oscillations at frequency ω evoke state variable oscillations at frequencies ω (1^st order), 0 (2^nd order), and 2ω (2^nd order). The periodic response coefficients, in the matrix $\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}$ and in the tensors $\mathbf{R}^\mathrm{y}_{\tilde{\mathrm{p}}\bar{\mathrm{p}}}$, and $\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}$, are complex-valued and depend on the driving frequency ω. Formulae for their calculation are given in section S2.3. With Eq. (S7), the time average of the state vector reads

$$\langle\mathbf{y}\rangle_t \quad \approx \quad \mathbf{y}(\bar{\mathbf{p}}) + \frac{1}{2}\,\mathbf{R}^\mathrm{y}_{\tilde{\mathrm{p}}\bar{\mathrm{p}}}(\tilde{\mathbf{p}} \otimes \tilde{\mathbf{p}}^*). \tag{S8}$$

Equation (S8) shows that parameter oscillations can shift the time-averaged metabolic state and that this shift is a second-order effect. Since the system equations (S5) are time-shift invariant, the first-order response coefficients $\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}$ can only couple perturbations and effects of the same frequency. Therefore, oscillating parameters (with frequency $\omega \neq 0$) have no first-order effect on the time-average metabolic behaviour (at frequency $\omega = 0$). If we consider a single variable y (e.g., a metabolite level c, a reaction rate v, or the metabolic benefit function $g = z(\mathbf{y})$), we can write Eqs (S6) and (S8) as

$$\begin{aligned} y(\bar{\mathbf{p}} + \Delta\mathbf{p}) \quad &\approx \quad y(\bar{\mathbf{p}}) + \mathbf{R}^\mathrm{y}_\mathrm{p}\Delta\mathbf{p} + \frac{1}{2}\,\Delta\tilde{\mathbf{p}}^\mathrm{T}\mathbf{R}^\mathrm{y}_\mathrm{pp}\Delta\tilde{\mathbf{p}} \\ \langle y\rangle_t \quad &\approx \quad y(\bar{\mathbf{p}}) + \frac{1}{2}\,\tilde{\mathbf{p}}^\dagger\mathbf{R}^\mathrm{y}_{\tilde{\mathrm{p}}\bar{\mathrm{p}}}(\omega)\tilde{\mathbf{p}} \end{aligned} \tag{S9}$$

with a row vector $\mathbf{R}^\mathrm{y}_\mathrm{p}$, a symmetric matrix $\mathbf{R}^\mathrm{y}_\mathrm{pp}$, and a Hermitian matrix $\mathbf{R}^\mathrm{y}_{\tilde{\mathrm{p}}\bar{\mathrm{p}}}(\omega)$. These can be directly computed from network structure and elasticities in steady state.

S2.3 Formulae for static, spectral, and periodic response coefficients

How can we compute the static and periodic response coefficients for a given model? To account for potential conservation relations between metabolite concentrations [4], we split the stoichiometric matrix $\mathbf{N}$ into a product $\mathbf{N} = \mathbf{L}\mathbf{N}_\mathrm{R}$, where $\mathbf{N}_\mathrm{R}$ consists of linearly independent rows of $\mathbf{N}$.

Static response coefficients With the steady state variables given as functions $y_i(\mathbf{p}) = \binom{\mathbf{s}(\mathbf{p})}{\mathbf{j}(\mathbf{p})}$ of the system parameters, the static response coefficients [1, 2] are defined as

$$R^{\mathrm{y_l}}_{\mathrm{p_m}} = \frac{\partial y_l}{\partial p_m}, \qquad R^{\mathrm{y_l}}_{p_m p_n} = \frac{\partial^2 y_l}{\partial p_m \partial p_n}. \tag{S10}$$

With the control coefficient matrices [4]

$$\mathbf{C}^\mathrm{s} = -\mathbf{L}\,(\mathbf{N}_\mathrm{R}\mathbf{E}_\mathrm{c}\mathbf{L})^{-1}\mathbf{N}_\mathrm{R}, \qquad \mathbf{C}^\mathrm{j} = \mathbf{E}_\mathrm{c}\ \mathbf{C}^\mathrm{s} + \mathbf{I}, \tag{S11}$$

they can be written as [2]

$$\begin{aligned} \mathbf{R}^\mathrm{y}_\mathrm{p} &= \mathbf{C}^\mathrm{y}\ \mathbf{E}_\mathrm{p} \\ \mathbf{R}^\mathrm{y}_\mathrm{pp} &= \mathbf{C}^\mathrm{y} \cdot \mathbf{\Gamma} \\ \text{where}\quad \mathbf{\Gamma} &= \mathbf{E}_\mathrm{cc}(\mathbf{R}^\mathrm{s} \otimes \mathbf{R}^\mathrm{s}) + \mathbf{E}_\mathrm{cp}[\mathbf{R}^\mathrm{s} \otimes \mathbf{I}] + \mathbf{E}_\mathrm{pc}[\mathbf{I} \otimes \mathbf{R}^\mathrm{s}] + \mathbf{E}_\mathrm{pp}. \end{aligned} \tag{S12}$$

Spectral response coefficients The spectral response coefficients, introduced in [3], assume harmonic parameter perturbations and relate their Fourier components to the Fourier components of the responing state variables. With the spectral control coefficient matrices

$$\begin{aligned} \mathbf{C}^\mathrm{s}(\omega) &= -\mathbf{L}(\mathbf{N}_\mathrm{R}\mathbf{E}_\mathrm{c}\mathbf{L} - i\omega\mathbf{I})^{-1}\mathbf{N}_\mathrm{R} \\ \mathbf{C}^\mathrm{j}(\omega) &= \mathbf{E}_\mathrm{c}\ \mathbf{C}^\mathrm{s}(\omega) + \mathbf{I}, \end{aligned} \tag{S13}$$

they can be written as

$$\begin{aligned} \tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}(\omega) &= \mathbf{C}^\mathrm{y}(\omega)\ \mathbf{E}_\mathrm{p} \\ \tilde{\mathbf{R}}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega) &= \frac{1}{\sqrt{2\pi}}\mathbf{C}^\mathrm{y}(0) \cdot \mathbf{\Gamma}(\omega, -\omega) \\ \tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega) &= \frac{1}{\sqrt{2\pi}}\mathbf{C}^\mathrm{y}(2\omega) \cdot \mathbf{\Gamma}(\omega, \omega) \\ \text{where}\quad \mathbf{\Gamma}(\alpha, \beta) &= \mathbf{E}_\mathrm{cc}[\tilde{\mathbf{R}}^{\tilde{\mathrm{s}}}_{\tilde{\mathrm{p}}}(\alpha) \otimes \tilde{\mathbf{R}}^{\tilde{\mathrm{s}}}_{\tilde{\mathrm{p}}}(\beta)] + \mathbf{E}_\mathrm{cp}[\tilde{\mathbf{R}}^{\tilde{\mathrm{s}}}_{\tilde{\mathrm{p}}}(\alpha) \otimes \mathbf{I}] + \mathbf{E}_\mathrm{pc}[\mathbf{I} \otimes \tilde{\mathbf{R}}^{\tilde{\mathrm{s}}}_{\tilde{\mathrm{p}}}(\beta)] + \mathbf{E}_\mathrm{pp}. \end{aligned} \tag{S14}$$

The first-order spectral response coefficients (in the matrix $\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}(\omega)$) connect perturbations and effects at the same frequency ω. The second-order spectral response coefficients (in the tensors $\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)$ and $\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)$) correspond to effects at frequencies 0 and 2ω, respectively. For more details, see [3].

Periodic response coefficients In this article, oscillations are not described by complex exponentials, but by their real parts $p(t) = \bar{p} + \mathrm{Re}(\tilde{p}\,\mathrm{e}^{i\omega t})$. To expand these in Eqs (S8) and (S9), we need a rescaled version the spectral response coefficients, called periodic response coefficients. The first-order coefficients are identical, but

for the second order, we obtain a prefactor of $\frac{\sqrt{2\pi}}{2}$ (proof in section S6.2), and therefore

$$\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega) = \frac{\sqrt{2\pi}}{2}\,\tilde{\mathbf{R}}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega), \qquad \mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega) = \frac{\sqrt{2\pi}}{2}\,\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega). \tag{S15}$$

These are the coefficients needed in Eqs (S8) and (S9). For slow oscillations ($\omega \approx 0$), the second-order periodic response coefficients converge to half of the second-order static response coefficients, that is,

$$\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}}(\omega = 0) = 0 \tag{S16}$$

$$\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega = 0) = \frac{1}{2}\mathbf{R}^{\mathrm{y}}_{\mathrm{pp}} \tag{S17}$$

$$\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega = 0) = \frac{1}{2}\mathbf{R}^{\mathrm{y}}_{\mathrm{pp}} \tag{S18}$$

For fast oscillations ($\omega \to \infty$), they become

$$\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega) \approx \frac{1}{2}\,\mathbf{C}^{\mathrm{y}}\,\mathbf{E}_{\mathrm{pp}}. \tag{S19}$$

S2.4 Phase shifts between mRNA and enzyme levels

Periodic enzyme levels can be realised by periodic gene expression. However, when comparing predicted optimal enzyme rhythms to expression data, we need to consider the time lag between mRNA and protein peaks. Moreover, mRNA profiles with high relative amplitudes may lead to moderate or negligible enzyme oscillations. To model this, we assume that protein production is proportional to the current mRNA level and that proteins are degraded or diluted linearly. The protein level $p(t)$ will follow the differential equation

$$\frac{\mathrm{d}p(t)}{\mathrm{d}t} = \alpha\, m(t) - \kappa\, m(t) \tag{S20}$$

with mRNA concentration $m(t)$, a rate constant α for protein production, and an effective rate constant κ for protein degradation, which may entail enzyme dilution in growing cells; the rate constants may differ between proteins. According to the model, an harmonic mRNA profile $m(t) = \bar{m} + \mathrm{Re}(\tilde{m}\mathrm{e}^{i\omega t})$ leads to a protein profile $p(t) = \bar{p} + \mathrm{Re}(\tilde{p}\,\mathrm{e}^{i\omega t})$ with curve parameters

$$\bar{p} = \frac{\alpha}{\kappa}\bar{m}, \qquad \tilde{p} = \frac{\alpha}{\kappa + i\omega}\,\tilde{m}. \tag{S21}$$

In turn, to obtain a predefined protein profile $p(t) = \bar{p} + \mathrm{Re}(\tilde{p}\,\mathrm{e}^{i\omega t})$, we need a periodic mRNA profile with

$$\bar{m} = \frac{\kappa}{\alpha}\bar{p}, \qquad \tilde{m} = \frac{\kappa + i\omega}{\alpha}\,\tilde{p}. \tag{S22}$$

To avoid negative mRNA levels, these values need to satify $\tilde{m} \le \bar{m}$, and accordingly, protein profiles must satify the inequality $|\tilde{p}| \le (1 + \omega/\kappa)^{-1/2}\,|\bar{p}|$. The phase shift between mRNA peak and protein peak is $\tan(\omega/\kappa)$; for $\omega \approx 0$, mRNA and protein are in phase; for high frequencies, the protein peaks after the mRNA with a phase shift of $\pi/2$. Proteins with a slow turnover (small κ) show large phase shifts, whereas if oscillations are slow compared to protein turnover ($\omega \ll \kappa$), the phase shifts are negligible.

S3 Economics of oscillations in metabolism: optimal enzyme profiles

To derive formulae for optimal enzyme profiles in the presence of external perturbations, we write the fitness as a function of the external parameters x_j and enzyme levels u_l and compute its first and second derivatives.

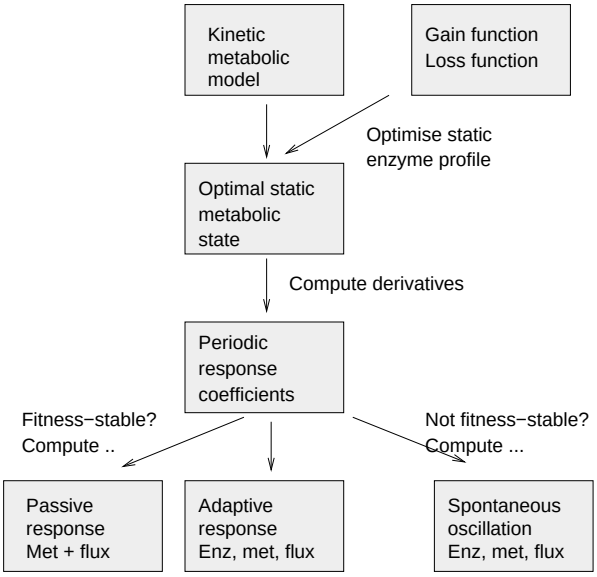

Figure S2:

S3.1 Fitness under static perturbations

With fixed values for the external parameters x_j and enzyme levels u_l, and solving for a steady state, we obtain a reference state with state vector $\mathbf{y}(\mathbf{x},\mathbf{u}) = \binom{\mathbf{s}(\mathbf{x},\mathbf{u})}{\mathbf{j}(\mathbf{x},\mathbf{u})}$. Let $(\bar{\mathbf{x}},\bar{\mathbf{u}})$ denote a reference parameter set. For parameter sets $\binom{\mathbf{x}}{\mathbf{u}} = \binom{\bar{\mathbf{x}}}{\bar{\mathbf{u}}} + \binom{\Delta\mathbf{x}}{\Delta\mathbf{u}}$, the expansion (S6) for changes $\Delta\mathbf{x}$, $\Delta\mathbf{u}$, and $\Delta\mathbf{y}$ reads

$$\Delta\mathbf{y} \approx (\mathbf{R}^{\mathrm{y}}_{\mathrm{x}}\ \mathbf{R}^{\mathrm{y}}_{\mathrm{u}})\begin{pmatrix}\Delta\mathbf{x}\\ \Delta\mathbf{u}\end{pmatrix} + \frac{1}{2}\begin{pmatrix}\mathbf{R}^{\mathrm{y}}_{\mathrm{xx}} & \mathbf{R}^{\mathrm{y}}_{\mathrm{xu}}\\ \mathbf{R}^{\mathrm{y}}_{\mathrm{ux}} & \mathbf{R}^{\mathrm{y}}_{\mathrm{uu}}\end{pmatrix}\left(\begin{pmatrix}\Delta\mathbf{x}\\ \Delta\mathbf{u}\end{pmatrix}\otimes\begin{pmatrix}\Delta\mathbf{x}\\ \Delta\mathbf{u}\end{pmatrix}\right). \tag{S23}$$

In a second-order expansion, the benefit and cost changes can be approximated as

$$\begin{aligned} g &\approx g(\bar{\mathbf{u}}) + \mathbf{z}_{\mathrm{y}}\cdot\Delta\mathbf{y} + \frac{1}{2}\Delta\mathbf{y}^{\mathrm{T}}\mathbf{Z}_{\mathrm{yy}}\Delta\mathbf{y}\\ h &\approx h(\bar{\mathbf{u}}) + \mathbf{h}_{\mathrm{u}}\cdot\Delta\mathbf{u} + \frac{1}{2}\Delta\mathbf{u}^{\mathrm{T}}\mathbf{H}_{\mathrm{uu}}\Delta\mathbf{u}. \end{aligned} \tag{S24}$$

The expansions (S23) and (S24) can be inserted into the fitness formula $f = z(\mathbf{y}(\mathbf{x},\mathbf{u})) + h(\mathbf{u})$. Differentiating the fitness function $f(\bar{\mathbf{x}}+\Delta\mathbf{x},\bar{\mathbf{u}}+\Delta\mathbf{u})$ by the external changes $\Delta\mathbf{x}$ and enzymes $\Delta\mathbf{u}$ yields the gradients and

Hessian matrices

$$\begin{aligned}
\mathbf{f}_{\mathrm{x}}^{\mathrm{T}} &= \mathbf{z}_{\mathrm{y}}^{\mathrm{T}}\, \mathbf{R}_{\mathrm{x}}^{\mathrm{y}} \\
\mathbf{f}_{\mathrm{u}}^{\mathrm{T}} &= \mathbf{z}_{\mathrm{y}}^{\mathrm{T}}\, \mathbf{R}_{\mathrm{u}}^{\mathrm{y}} - \mathbf{h}_{\mathrm{u}}^{\mathrm{T}} \\
\mathbf{F}_{\mathrm{ux}} &= \mathbf{z}_{\mathrm{y}}^{\mathrm{T}} \cdot \mathbf{R}_{\mathbf{ux}}^{\mathrm{y}} + \mathbf{R}_{\mathrm{u}}^{\mathrm{y}^{\mathrm{T}}}\, \mathbf{Z}_{\mathrm{yy}} \mathbf{R}_{\mathrm{x}}^{\mathrm{y}} \\
\mathbf{F}_{\mathrm{uu}} &= \mathbf{z}_{\mathrm{y}}^{\mathrm{T}} \cdot \mathbf{R}_{\mathbf{uu}}^{\mathrm{y}} + \mathbf{R}_{\mathrm{u}}^{\mathrm{y}^{\mathrm{T}}}\, \mathbf{Z}_{\mathrm{yy}} \mathbf{R}_{\mathrm{u}}^{\mathrm{y}} - \mathbf{H}_{\mathrm{uu}}.
\end{aligned} \tag{S25}$$

S3.2 Fitness under periodic perturbations

Shifts of average state variables, caused by periodic perturbations Now we assume that the external parameters oscillate around the reference state (no average shift):

$$\begin{aligned}
\mathbf{x}(t) &= \bar{\mathbf{x}} + \mathrm{Re}[\tilde{\mathbf{x}}\, \mathrm{e}^{i\omega t}] \\
\mathbf{u}(t) &= \bar{\mathbf{u}} + \mathrm{Re}[\tilde{\mathbf{u}}\, \mathrm{e}^{i\omega t}].
\end{aligned} \tag{S26}$$

The time average of $\mathbf{u}$ is simply $\bar{\mathbf{u}}$. The time average $\bar{\mathbf{y}}$ is expanded by Eq. (S8) using the second-order periodic response coefficients:

$$\Delta\bar{\mathbf{y}} \approx \frac{1}{2} \begin{pmatrix} \mathbf{R}_{\tilde{\mathrm{x}}\tilde{\mathrm{u}}}^{\mathrm{y}} & \mathbf{R}_{\tilde{\mathrm{x}}\tilde{\mathrm{u}}}^{\mathrm{y}} \\ \mathbf{R}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}^{\mathrm{y}} & \mathbf{R}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}^{\mathrm{y}} \end{pmatrix} \left(\begin{pmatrix} \tilde{\mathbf{x}} \\ \tilde{\mathbf{u}} \end{pmatrix} \otimes \begin{pmatrix} \tilde{\mathbf{x}} \\ \tilde{\mathbf{u}} \end{pmatrix} \right). \tag{S27}$$

There is no first-order term because $\mathbf{R}_{\tilde{\mathrm{x}}}^{\mathrm{y}}$ and $\mathbf{R}_{\tilde{\mathrm{u}}}^{\mathrm{y}}$ vanish.

Fitness functional applied to time-averaged state variables ("fluctuation-insensitive fitness") We define a fitness by applying the fitness function $f = z(\mathbf{y}(\mathbf{x}, \mathbf{u})) + h(\mathbf{u})$ to time-averaged state variables. Since the time averages of the enzyme levels remain constant ($\Delta\mathbf{u} = 0$), benefit and cost can be expanded as

$$\begin{aligned}
g(\langle\mathbf{y}\rangle_t) &\approx g(\bar{\mathbf{u}}) + \mathbf{z}_{\mathrm{y}} \cdot \Delta\bar{\mathbf{y}} + \frac{1}{2}\, \Delta\bar{\mathbf{y}}^{\mathrm{T}} \mathbf{Z}_{\mathrm{yy}} \Delta\bar{\mathbf{y}} \\
h(\langle\mathbf{u}\rangle_t) &= h(\bar{\mathbf{u}}).
\end{aligned} \tag{S28}$$

and with Eq. (S27), the derivatives of $f = g(\langle\mathbf{y}\rangle_t) - h(\langle\mathbf{u}\rangle_t)$ read

$$\begin{aligned}
\mathbf{f}_{\tilde{\mathrm{x}}}^{\mathrm{T}} &= 0 \\
\mathbf{f}_{\tilde{\mathrm{u}}}^{\mathrm{T}} &= 0 \\
\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}} &= \mathbf{z}_{\mathrm{y}}^{\mathrm{T}} \cdot \mathbf{R}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}^{\mathrm{y}} \\
\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}} &= \mathbf{z}_{\mathrm{y}}^{\mathrm{T}} \cdot \mathbf{R}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}^{\mathrm{y}}.
\end{aligned} \tag{S29}$$

Fitness functional based on time-averaged fitness values ("fluctuation-sensitive fitness") Instead of averaging the state variables over time and applying the fitness function afterwards, we can also evaluate the fitness in each time point and then average over time. This yields additional terms in the fitness Hessians. To derive them, we expand the time courses $y(t)$ to second order (obtaining a sum of harmonic oscillations), expand the fitness function in each time point, integrate over one oscillation period (duration T) and collect all terms up

to second order. For the cost term, we obtain:

$$\begin{aligned}\langle h(\mathbf{u})\rangle_t &= \frac{1}{T}\int_0^T h(\bar{\mathbf{u}} + \mathrm{Re}[\tilde{\mathbf{u}}\mathrm{e}^{i\omega t})]\mathrm{d}t \\ &\approx \frac{1}{T}\int_0^T h(\bar{\mathbf{u}}) + \mathbf{h}_\mathrm{u}\cdot\mathrm{Re}[\tilde{\mathbf{u}}\mathrm{e}^{i\omega t}] + \frac{1}{2}(\tilde{\mathbf{u}}\mathrm{e}^{i\omega t})^\dagger \mathbf{H}_\mathrm{uu}(\tilde{\mathbf{u}}\mathrm{e}^{i\omega t})\mathrm{d}t \\ &= h(\bar{\mathbf{u}}) + \frac{1}{4}\tilde{\mathbf{u}}^\dagger\mathbf{H}_\mathrm{uu}\tilde{\mathbf{u}}. \end{aligned} \tag{S30}$$

The first-order term cancels out in the integration . The benefit term is computed similarly, yielding

$$\langle g(\mathbf{y})\rangle_t = g(\bar{\mathbf{y}}) + \frac{1}{4}\tilde{\mathbf{y}}^\dagger\mathbf{Z}_\mathrm{yy}\tilde{\mathbf{y}} \qquad \text{where} \quad \tilde{\mathbf{y}} = \mathbf{R}^\mathrm{y}_\mathrm{x}\tilde{\mathbf{x}} + \mathbf{R}^\mathrm{y}_\mathrm{u}\tilde{\mathbf{u}}.$$

Together, the time-averaged fitness terms

$$\begin{aligned}\langle g(\mathbf{y})\rangle_t &\approx g(\bar{\mathbf{u}}) + \mathbf{z}_\mathrm{y}\cdot\Delta\bar{\mathbf{y}} + \frac{1}{2}\Delta\bar{\mathbf{y}}^\mathrm{T}\mathbf{Z}_\mathrm{yy}\Delta\bar{\mathbf{y}} + \frac{1}{4}\tilde{\mathbf{y}}^\dagger\mathbf{Z}_\mathrm{yy}\tilde{\mathbf{y}} \\ \langle h(\mathbf{u})\rangle_t &= h(\bar{\mathbf{u}}) + \frac{1}{4}\tilde{\mathbf{u}}^\dagger\mathbf{H}_\mathrm{uu}\tilde{\mathbf{u}} \end{aligned} \tag{S31}$$

yield the first and second order derivatives of $f = \langle g(\mathbf{y}) - h(\mathbf{u})\rangle_t$

$$\begin{aligned}\mathbf{f}_{\tilde{\mathrm{x}}} &= 0 \\ \mathbf{f}_{\tilde{\mathrm{u}}} &= 0 \\ \mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}} &= \mathbf{z}_\mathrm{y}^\mathrm{T}\,\mathbf{R}^\mathrm{y}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}} + \frac{1}{2}\mathbf{R}^{\tilde{\mathrm{y}}\,\dagger}_{\tilde{\mathrm{u}}}\mathbf{Z}_\mathrm{yy}\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{x}}} \\ \mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}} &= \mathbf{z}_\mathrm{y}^\mathrm{T}\,\mathbf{R}^\mathrm{y}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}} + \frac{1}{2}\mathbf{R}^{\tilde{\mathrm{y}}\,\dagger}_{\tilde{\mathrm{u}}}\mathbf{Z}_\mathrm{yy}\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{u}}} - \frac{1}{2}\mathbf{H}_\mathrm{uu}. \end{aligned} \tag{S32}$$

Since $\mathbf{R}^\mathrm{y}_{\tilde{\mathrm{p}}\bar{\mathrm{p}}}(\omega = 0) = \frac{1}{2}\mathbf{R}^\mathrm{y}_\mathrm{pp}$, the Hessians become half of the static Hessians $\mathbf{F}_\mathrm{ux}$ and $\mathbf{F}_\mathrm{uu}$. Finally, we can apply stationary and periodic perturbations at the same time. Between stationary and periodic perturbations, there are no second-order synergy terms (mixed second derivatives) that could shift the metabolic state on average, so the derivatives of our fitness function $F(\bar{\mathbf{x}}, \bar{\mathbf{u}}; \Delta\bar{\mathbf{x}}, \Delta\bar{\mathbf{u}}; \tilde{\mathbf{x}}, \tilde{\mathbf{u}})$ with respect to $\Delta\bar{\mathbf{x}}, \Delta\bar{\mathbf{u}}, \tilde{\mathbf{x}}$, and $\tilde{\mathbf{u}}$ are given by Eqs. (S29) and (S32).

S3.3 Bounds for enzyme amplitudes

The complex amplitude $\tilde{u}$ of an enzyme profile $\bar{u} + \mathrm{Re}[\tilde{u}\mathrm{e}^{i\omega t}]$ is limited by a number of constraints:

1. The amplitude cannot be larger than the average enzyme level: $|\tilde{u}| \le \bar{u}$; otherwise, the enzyme level would become negative.

2. If the enzyme itself is linearly degraded with rate constant κ then, as shown in section S2.4, the enzyme amplitude needs to satisfy $\tilde{u} \le \sqrt{\frac{\kappa}{\kappa+\omega}}\,\bar{u}$. Otherwise the corresponding mRNA level would become negative. This constraint is tighter than the previous one; both constraints become equal at frequency $\omega = 0$.

3. The time derivative of the enzyme level, $\mathrm{d}u/\mathrm{d}t = \tilde{u}\,\omega\,-\sin(\omega t)$, shows that the maximal rate of decrease,

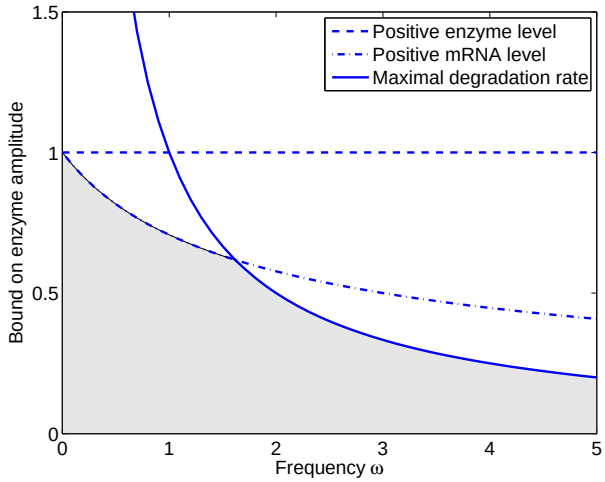

Figure S3: Enzyme amplitude constraints, assuming an average enzyme level $\bar{u}$ and a degradation constant $\kappa = 1$. The shaded region contains the allowed enzyme amplitudes.

$\tilde{u}\,\omega$, is reached in a moment when the enzyme level has its average value of $\bar{u}$. The degradation rate in this moment reads $\kappa\,\bar{u}$. Since the maximal rate of decrease cannot be larger than the degradation rate in the same moment, we obtain another constraint $\tilde{u} \leq \frac{\kappa}{\omega}\bar{u}$.

Thus, the upper bound of an enzyme amplitude depends on the frequency. All constraints together are shown in Figure S3.

S3.4 Enzyme profiles that realise a predefined metabolic behaviour

Given external parameter rhythms $\tilde{\mathbf{x}}$ and some rhythms $\tilde{\mathbf{c}}$ and $\tilde{\mathbf{v}}$ to be realised, what enzyme profiles $\tilde{\mathbf{u}}$ will realise these rhythms as closely as possible and at a minimal cost? Guessing such enzyme profiles is difficult, not only because of the complex system dynamics, but also because they need to be self-consistent, i.e. all profiles must be adapted to the dynamic state they create. To compute such profiles, we need to invert the propagation of perturbations in the network. Such inverse problems can be numerically hard, but approximations using MCA, as used in [5] for optimal control of steady states, make them tractable.

For harmonic perturbations of a single frequency, the forward propagation – in a first-order approximation – is described by periodic response coefficients, so the enzyme profile for this frequency could be computed by inverting the response matrix. For instance, to realise a predefined complex amplitude profile $\tilde{\mathbf{c}} \stackrel{!}{=} \tilde{R}^{\tilde{c}}_{\tilde{u}}\tilde{\mathbf{u}} + \tilde{R}^{\tilde{c}}_{\tilde{x}}\tilde{\mathbf{x}}$, we may set $\tilde{\mathbf{u}} = \tilde{R}^{\tilde{c}}_{\tilde{u}}{}^{-1}[\tilde{\mathbf{c}} - \tilde{R}^{\tilde{c}}_{\tilde{x}}\tilde{\mathbf{x}}]$. If no solution exists, we may employ a least-squares optimisation, and if the solution is underdetermined, a regularisation term. Altogether, the criterion for the enzyme amplitude vector $\mathbf{u}$ would read $||(\tilde{\mathbf{c}} - \tilde{R}^c_x\,\tilde{\mathbf{x}}) - \tilde{R}^{\tilde{c}}_{\tilde{u}}\tilde{\mathbf{u}}|| + \tilde{\mathbf{u}}^\dagger\,\tilde{\mathbf{u}} \stackrel{!}{=} \min$. To realise a non-harmonic time course $\mathbf{c}(t)$, we may synthesise it by adding enzyme rhythms of different frequencies.

S3.5 Enzyme levels and enzyme activities

S4 Periodic economic potentials

S4.1 Periodic economic potentials

Flux value balance. Given the periodic economic potentials, we can compute the value of an enzyme rhythm.

Case 1. Values in a static reference state. Assume that we are in a non-oscillatory state. To derive the balance equation, we consider a compensated variation of the enzyme rhythm. Consider a variation $\Delta\tilde{u}_l$ of the enzyme rhythm. To first order, it can be compensated by harmonic supply fluxes with amplitude $\tilde{\varphi}_i = -n_{il}\,\frac{\partial \tilde{v}_l}{\partial \tilde{u}_l}\Delta\tilde{u}_l$. Since the reaction rate (at unchanged reactant profiles) changes linearly with the enzyme level, we can set $\frac{\partial \tilde{v}_l}{\partial \tilde{u}_l} = \bar{v}_l/\bar{u}_l$ and obtain the (negative) compensation value

$$0 = \sum_i \tilde{w}_i\,\tilde{\varphi}_i = \sum_i -\tilde{w}_i\,n_{il}\,\frac{\bar{v}_l}{\bar{u}_l}\,\Delta\tilde{u}_l \tag{S33}$$

which compensates the additional enzyme investment $h_{\tilde{u}_l}\,\Delta\tilde{u}_l$, so

$$\sum_i \tilde{w}_i\,n_{il}\,\frac{\bar{v}_l}{\bar{u}_l}\,\Delta\tilde{u}_l = h_{\tilde{u}_l}\,\Delta\tilde{u}_l. \tag{S34}$$

We obtain the balance equation

$$\sum_i \tilde{w}_i\,n_{il}\,\bar{v}_l = h_{\tilde{u}_l}\,\bar{u}_l. \tag{S35}$$

In a periodic state with enzyme rhythms $\tilde{\mathbf{u}}$, the periodic enzyme loss $h_{\tilde{\mathbf{u}}}$ can be approximated by $h_{\tilde{\mathbf{u}}} \approx H_{\tilde{u}\tilde{u}}\,\tilde{\mathbf{u}}$

Case 2. Values in an oscillating state. Now we consider a state that is already harmonically driven at a frequency ω. We assume a static reference state and perturb it by deviations $\Delta\bar{\mathbf{u}}$ and $\tilde{\mathbf{u}}$ (for enzyme levels) and $\Delta\bar{\mathbf{x}}$ and $\tilde{\mathbf{x}}$ (for external parameters). Accordingly, the metabolic state will be changed by $\Delta\bar{\mathbf{c}}$ and $\tilde{\mathbf{c}}$ (for internal metabolite levels) and $\Delta\bar{\mathbf{v}}$ and $\tilde{\mathbf{v}}$ (for fluxes). The amplitudes refer to oscillations at the driving frequency ω. Higher harmonics will in general exist, but we do not consider them here. Now we consider the effects of small changes in $\Delta\bar{\mathbf{u}}$ and $\tilde{\mathbf{u}}$ on $\Delta\bar{\mathbf{v}}$ and $\tilde{\mathbf{v}}$, *assuming* that the metabolite profile remains unchanged (e.g., by keeping it unchanged with the help of virtual supply fluxes). We obtain the economic balance equations (proof section S6.3)

$$\begin{aligned}
\mathrm{Dg}(\mathbf{u})\,\mathbf{h}^{\mathbf{u}} &= \mathrm{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})[[\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} + \mathbf{z}^{\mathrm{v}}] + \mathbf{A}\,[\mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} + \tilde{\mathbf{z}^{\mathrm{v}}}]] \\
\mathrm{Dg}(\mathbf{u})\,\tilde{\mathbf{h}}^{\mathbf{u}} &= \mathrm{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})[\mathbf{A}\,[\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} + \mathbf{z}^{\mathrm{v}}] + [\mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} + \tilde{\mathbf{z}^{\mathrm{v}}}]]
\end{aligned} \tag{S36}$$

where $\mathbf{h}^{\mathbf{u}}$ and $\tilde{\mathbf{h}}^{\mathbf{u}}$ are the losses for additional enzyme variations $\delta\mathbf{u}$ and $\delta\tilde{\mathbf{u}}$.

S4.2 Periodic economic potentials

Can enzyme-economic analysis also be applied to periodic states and periodic enzyme profiles? As a basic ideas of enzyme-economic analysis, the investment into enzymes has to be justified by their metabolic benefit, which

can thus be seen as an incentive for enzyme expression. This incentive can be quantified by the extra benefit that would be generated by small virtual changes of enzyme levels in steady states. If the stationary flux v of a single reaction (with mass-action rate law $v = k\,u\,x$) is the objective function f, the derivative $\bar{f}_u = \frac{\partial v}{\partial u} = k\,x$ is the enzyme gain. Since the enzyme level is a prefactor in the rate law, the enzyme benefit, in this case, can be written as a product $f = f^{\mathrm{u}} \cdot u$. In an optimal state with positive enzyme levels, it must match the marginal cost $h_u = \frac{\partial h}{\partial u}$.

Let us now apply the same thought to time-dependent behaviour. If a reaction flux is directly scored by the benefit function, we can define a momentary demand for the enzyme: in an isolated reaction with varying substrate levels, the enzyme will produce a different flux per enzyme molecule in every moment. This can be quantified by a momentary enzyme gain $f^{\mathrm{u}}(t) = \frac{\partial v(t)}{\partial u(t)} = k\,x(t)$. If a certain total enzyme cost can be distributed over one oscillation period, the cell should concentrate enzyme expression in the moments when the enzyme is most valuable. If the substrate level is harmonic $x(t) = \bar{x} + \mathrm{Re}[\tilde{x}\exp(i\omega t)]$, then also the momentary benefit $\frac{\partial v(t)}{\partial u(t)} = k\,x(t)$ will also change periodically and can be described by a complex amplitude: $f^{\mathrm{u}}(t) = k\,\bar{x} + \mathrm{Re}[k, \tilde{x}\exp(i\omega t)]) = \bar{f}_u + \mathrm{Re}[\tilde{f}_u \exp(i\omega t)])$. Actually, the ideal enzyme profile in this case would be a short, delta-like peak in the moment of highest substrate level; but in reality, enzyme profiles have to be smooth and, in our approach, they are assumed to be harmonic $u(t) = \bar{u} + \mathrm{Re}[\tilde{u}\exp(i\omega t)]$. In this case, the change of the benefit functional (assuming fixed $\bar{u}$ and omitting constant terms) reads:

$$\Delta f \quad \approx \quad \langle k\,\Delta x\,\Delta u\rangle_t = \frac{1}{2}\mathrm{Re}(k\,\tilde{x}^*\,\tilde{u}) = \mathrm{Re}(\tilde{f}_u^*\,\tilde{u}). \tag{S37}$$

Written like this, the benefit change resembles the enzyme benefit $\bar{f}^{\mathrm{u}} \cdot u$ in steady states, but the benefit amplitude $\tilde{f}_u^* = \frac{\delta \Delta f}{\delta \tilde{u}}$ and the enzyme amplitude $\tilde{u}$ are complex numbers and the real part of the product is taken. If the enzyme amplitude $|\tilde{u}|$ is limited, a that this limit is reached and that enzyme rhythm and enzyme gain are in phase. A larger absolute value of $|\tilde{f}_u^*|$ means a larger demand. Therefore, we can regard the complex number $\tilde{f}_u^*$ as the incentive for enzyme rhythms, with the phase angle denoting the optimal phase. Eq. (S37) is still based on a mass-action rate law. For other rate laws, we apply a second-order approximation with the second-order unscaled elasticity $\bar{E}_{xu} = \frac{\partial^2 v}{\partial x\,\partial u}$ and obtain a similar equation with a periodic enzyme value $\tilde{f}_u = \frac{\delta \langle f\rangle_t}{\delta \tilde{u}} \approx \frac{\bar{E}_{xu}}{2}\,\tilde{x}$. The periodic enzyme gain shows how strongly the periodic enzyme modulation improves the overall benefit, and in which phase it is most effective.

In larger networks, periodic substrate levels will not be predefined, but emerge from the metabolic dynamics. Therefore, enzymes do not only realise benefits *given* their substrate levels, but they can also *actively change* the substrate levels, influence the dynamics of the entire system, and realise benefits in distant parts of the network. The enzyme gain has to be redefined to include global influences, phase delays, and anticipation; in particular, instead of the reaction elasticities (which isolated reactions, in which reactant levels controlled and the flux depends directly on reactant and enzyme levels), we need to employ response coefficients (which hold for extended systems in which fluxes and concentrations follow the system dynamics). Given periodic external perturbations (with amplitude $\tilde{x}$), we consider the overall fitness shift as a function of the enzyme amplitudes $\tilde{u}$. A first-order expansion with respect to $\tilde{u}$ (regarding synergies between $\tilde{\mathbf{x}}$ and $\tilde{\mathbf{u}}$ as first-order effects) reads (proof see SI S4.3)

$$\Delta f \approx \tilde{\mathbf{x}}^\dagger R^f_{\tilde{x}\tilde{u}}\tilde{\mathbf{u}} = [\Delta\tilde{\mathbf{w}} + \mathbf{z}^{\mathrm{v}}]^\dagger\,\mathrm{Dg}(\frac{\mathbf{v}}{\mathbf{u}})\,\tilde{\mathbf{u}} = \tilde{\mathbf{f}}_u^\dagger\,\tilde{\mathbf{u}}. \tag{S38}$$

In the first equation, the synergies between the amplitudes $\tilde{x}_j$ and $\tilde{u}_l$ are expressed using the second-order, spectral

response matrix $R^f_{\tilde{x}\tilde{u}}$. Then, the unscaled enzyme elasticities $\bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}} = v_l/u_l$ are split off and the remaining spectral control matrix is expressed in terms of the gradient $\partial f/\partial v_l$ of the metabolic benefit as a function f of the reaction rates v_l and the vector $\Delta\tilde{\mathbf{w}} = \mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}$, containing the differences of the periodic economic potentials $\tilde{w}_i$ along the reactions. By their definition (see SI), these potentials describe how a small, additional, periodic influx of a certain metabolite would influence the benefit function, taking into account all its dynamic effects on the periodic metabolic state. Equation (S38) shows that the complex-valued enzyme values $\tilde{f}_{u_l}$ – including their optimal phase shifts – can be directly computed from the periodic economic potentials. For reactions without direct influence on the benefit ($f_{v_l} = 0$), the periodic enzyme gains are simply determined by the periodic economic potential differences $\Delta\tilde{w}_l$. Otherwise, the marginal benefits f_{v_l} have to be added. Importantly, and in contrast to the calculations in the previous sections, Eq. (S38) describes synergies between external oscillations and enzyme levels as first-order effects of the enzyme levels, whereas all self-synergies and synergies between the enzyme rhythms are ignored.

To maximise the benefit in Eq. (S38), the enzyme amplitudes $\tilde{u}_l$ would have to increase to infinity unless they are restricted by upper bounds. In fact, the computed enzyme values $\tilde{f}_{u_l}$ describe the benefits only for small amplitudes at the onset of enzyme rhythms. We may use them to estimate the phases and relative amplitudes in the optimal amplitude vector $\tilde{\mathbf{u}}$ very roughly, but not their proper scaling since the enzyme benefit given by Eq. (S38) increase linearly with the amplitude. To obtain a unique solution, we need to employ regularisation, e.g., require that the fitness increase Δf in Eq. (S38) be maximised, but at limited amplitudes $|\tilde{u}_l| \leq u_l^{\max}$.

Periodic enzyme gains and periodic economic potentials would be hard to measure in experiments. However, Eq. (S38) shows that self-consistent, optimal enzyme rhythms can be understood in terms of a local importance of the reactants, which changes periodically. Like in normal enzyme-economic analysis, their importance is quantified by economic potentials, which subsume all relevant details of the optimisation in the larger network.

S4.3 Periodic economic potentials and flux gain balance

To prove Eq. (S38), we consider a thought experiment: a metabolic system is periodically perturbed by a single parameter with amplitude $\tilde{x}$. That is, fluxes and metabolite levels are already oscillating. Now we add a small oscillation of one enzyme (amplitude $\tilde{u}_l$) and, for compensation, periodic supply fluxes of the reactants i with amplitudes $\tilde{\varphi} = -n_{il}\bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\,\tilde{u}$. To first order in $\tilde{u}$, the reaction flux will be periodically change ($\tilde{v}_l = \bar{E}^{\mathrm{v_l}}_{\mathrm{u_l}}\tilde{u}_l$), but the reactant concentrations behave as before, and also the (periodic) behaviour of surrounding system will remain as before. The only fitness change due to the compensated perturbation arises in the reaction itself. It reads, to first order in $\tilde{u}$

$$\Delta f^{\mathrm{comp}} = \frac{\partial f}{\partial v}\tilde{v} = \frac{\partial f}{\partial v}\bar{E}_u\tilde{u} \tag{S39}$$

However, this has to be equal to the overall fitness change given in terms of the perturbations $\tilde{u}$ and $\tilde{\varphi}$ (again, to first order in these variables)

$$\tilde{x}^\dagger R^f_{\tilde{x}\tilde{u}}\tilde{u} + \tilde{x}^\dagger R^f_{\tilde{x}\tilde{\varphi}}\tilde{\varphi} \;\; = \;\; \tilde{x}^\dagger R^f_{\tilde{x}\tilde{u}}\tilde{u} - \tilde{x}^\dagger R^f_{\tilde{x}\tilde{\varphi}} N^{\mathrm{T}}\,\bar{E}_u\,\tilde{u} \tag{S40}$$

and thus

$$\left[\tilde{x}^\dagger R^f_{\tilde{x}\tilde{\varphi}} N^{\mathrm{T}} + \frac{\partial f}{\partial v_l}\right] \mathrm{Dg}(\bar{\mathbf{E}}_{\mathrm{u}})\tilde{u} \;\; = \;\; \tilde{x}^\dagger R^f_{\tilde{x}\tilde{u}}\tilde{u}. \tag{S41}$$

which can also be cast as

$$\left[\tilde{\mathbf{w}}^\dagger \mathbf{N}^{\mathrm{T}} + \mathbf{z}^{\mathrm{v}}\right] \frac{\mathbf{v}}{\mathbf{u}} \tilde{\mathbf{u}} \quad = \quad f_{\tilde{u}}\, \tilde{u}. \tag{S42}$$

S5 Example models

S5.1 Rate oscillations cause reactants to oscillate

Model 1 In kinetic models, periodic perturbations of reaction rates will make the substrate and product levels oscillate, and the rhythms will propagate from one reaction to the other. As an example, consider two reactions $X \xrightarrow{k_x\, x} Y \xrightarrow{k_Y\, y}$ with irreversible mass-action kinetics $v_1 = k_x\, x$ and $v_2 = k_Y\, y$. We can perturb reaction 1 through the enzyme or through the substrate. Let us consider the substrate: if the substrate level $x(t)$ varies harmonically with complex amplitude $\tilde{x}$ and frequency ω, and the enzyme level is constant, Y will show a complex amplitude $\tilde{y} = \frac{k_X}{k_Y + i\omega}\tilde{x}$. This means that Y will peak after X with a phase shift $\phi = \arctan(\omega/k_Y)$ while the amplitude decreases from X to Y by a factor $|\frac{\tilde{y}}{\tilde{x}}| = \frac{k_X}{\sqrt{k_Y^2+\omega^2}}$. In a linear pathway, the rhythmic perturbation will propagate with a local wavelength $2\,\pi/\phi$ and a speed $V = \phi/\omega$ (in units of reactions per second).

Model 2 In general, rate perturbations affect both subtrates and products: consider three reactions $\xrightarrow{v_0} X \xrightarrow{u\, k_x\, x} Y \xrightarrow{k_Y\, y}$ with irreversible mass-action kinetics and a constant production of X. In a first-order approximation of the rate law, an enzyme rhythm with amplitude $\tilde{u}$ will evoke reactant amplitudes $\tilde{x} = \frac{i\, k_X}{\omega}\tilde{u}$ and $\tilde{y} = \frac{k_X}{k_Y + i\,\omega}\,\tilde{u}$. As shown in Figure 2 (c), U will peak after X (phase shift $\pi/2$) and before B (phase shift between 0 and $\pi/2$, depending on the rate constants).

S5.2 Single enzyme

In this section, I analyse oscillations in a single enzymatic reaction. In contrast to the example in the article, I assume a reversible rate law, and for illustration purposes, cosine functions are used instead of complex exponentials. I first describe the dynamics, deriving synergies between enzyme and reactant rhythms, and then the economics, computing an optimal enzyme rhythm for a quadratic cost function.

Dynamics We consider a single reaction with substrate X_1 and product X_2 and respective concentrations x_1 and x_2. The reaction is driven by an enzyme with activity u and reversible mass-action kinetics. For simplicity, we set both rate constants to 1, so the reaction rate reads

$$v = u(x_1 - x_2). \tag{S43}$$

Now we consider reactant levels set by the environment

$$\begin{aligned} x_1(t) &= \bar{x}_1 + \tilde{x}_1 \cos(\omega t) \\ x_2(t) &= \bar{x}_2. \end{aligned} \tag{S44}$$

and possible periodic enzyme levels

$$u(t) \quad = \quad \bar{u} + \tilde{u}\cos(\omega t + \phi). \tag{S45}$$

To ensure that u remains positive, we require that $\bar{u} \geq 0$ and $|\tilde{u}| \leq \bar{u}$; the phase shift ϕ can vary between 0 and 2π and the amplitudes $\tilde{x}_1$ and $\tilde{u}$ are real numbers. The time-dependent reaction rate reads

$$\begin{aligned} v(t) &= (\bar{u} + \tilde{u}\cos(\omega t + \phi))(\bar{x}_1 + \tilde{x}_1\cos(\omega t) - \bar{x}_2) \\ &= \bar{u}\,(\bar{x}_1 - \bar{x}_2) + \tilde{u}\,\cos(\omega t + \phi)(\bar{x}_1 - \bar{x}_2) + \bar{u}\,\tilde{x}_1\cos(\omega t) + \\ &\quad \tilde{u}\,\cos(\omega t + phi)\tilde{x}_1\cos(\omega t). \end{aligned} \tag{S46}$$

The last term follows from multiplying the two cosine functions: it describes a synergy between the periodic substrate and enzyme levels, leading to an average flux shift and to flux oscillations with frequency $2\,\omega$. Averaged over an oscillation period, the flux reads

$$\bar{v} = \frac{1}{T}\int_0^T v(t)\,\mathrm{d}t = \bar{u}\,(\bar{x}_1 - \bar{x}_2) + \frac{1}{2}\tilde{u}\,\tilde{x}_1\cos(\phi). \tag{S47}$$

All oscillatory terms from Eq. (S46) average out; the first term in Eq. (S47) is the flux that would result from the steady reference state; the second term describes a shift, caused by the synergy between the periodic reactant and enzyme levels. The $\cos^2$ term, averaged over an oscillation period, yields a factor of $\frac{1}{2}$.

Economics The flux in this model could be increased to arbitrarily high values by increasing the enzyme level, and be further increased by oscillations (where the average value needs to be as large as the amplitude, to avoid negative values). For a more realistic economic description, I assume that an enzyme level u comes at a cost

$$\begin{aligned} h(u(t)) &= \alpha u(t) + \frac{\beta}{2}u^2(t) \\ &= \alpha\Big(\bar{u} + \tilde{u}\cos(\omega t + \phi)\Big) + \frac{\beta}{2}\Big(\bar{u}^2 + 2\,\bar{u}\,\tilde{u}\cos(\omega t + \phi) + \tilde{u}^2\cos^2(\omega t + \phi)\Big) \end{aligned} \tag{S48}$$

with positive coefficients α and β. Averaged over time, the cost reads

$$\langle\bar{h}\rangle_t = \frac{1}{T}\int_0^T h(t)\,\mathrm{d}t = \alpha\,\bar{u} + \frac{\beta}{2}\bar{u}^2 + \frac{\beta}{4}\tilde{u}^2 = h(\bar{\mathbf{u}}) + \frac{\beta}{4}\tilde{u}^2. \tag{S49}$$

Again, the cosine terms cancel out and the $\cos^2$ term, averaged over an oscillation period, yields a factor of $\frac{1}{2}$. Compared to the reference state, the cost is increased, and the additional cost depends on oscillation amplitude and cost curvature. The fitness function F, given by the difference of the flux (benefit) and the enzyme cost, both averaged over time, reads

$$F = \langle y\rangle_t - \langle h\rangle_t = \left(\bar{u}(\bar{x}_1 - \bar{x}_2) + \frac{1}{2}\tilde{u}\,\tilde{x}_1\cos(\phi)\right) - \left(\alpha\bar{u} + \frac{\beta}{2}\bar{u}^2 + \frac{\beta}{4}\tilde{u}^2\right). \tag{S50}$$

To maximise the fitness for a given substrate amplitude $\tilde{x}_1$, the cell needs to choose the optimal enzyme profile, that is, the pair $(\bar{u}, \tilde{u})$ that maximizes F given $\tilde{x}$ under the constraints $\bar{u} \geq 0$, $|\tilde{u}| \leq \bar{u}$. The average reactant levels $\bar{x}_1 > \bar{x}_2$ are fixed and given. To determine the optimal values $\bar{u}$ and $\tilde{u}$, we collect all terms that depend

either on the time averages ($\bar{u}$) or on the oscillations ($\tilde{u}$ and ϕ):

$$F = \left((\bar{x}_1 - \bar{x}_2 - \alpha)\bar{u} - \frac{\beta}{2}\bar{u}^2\right) + \left(\frac{1}{2}\tilde{u}\tilde{x}_1\cos(\phi) - \frac{\beta}{4}\tilde{u}^2\right). \quad \text{(S51)}$$

Without inequality constraints for $\bar{u}$ and $\tilde{u}$, we can maximise both terms separately and obtain

$$\begin{aligned} \bar{u}^{\mathrm{opt}} &= (\bar{x}_1 - \bar{x}_2 - \alpha)/\beta \\ \cos(\phi^{\mathrm{opt}}) &= 1 \\ \tilde{u}^{\mathrm{opt}} &= \tilde{x}_1/\beta. \end{aligned} \quad \text{(S52)}$$

For $\tilde{x} = 0$, we obtain the enzyme-optimal reference state ($\tilde{u}^{\mathrm{opt}} = 0$). We still need to check if the constraints are satisfied: the solution for $\bar{u}$ only holds if $\alpha < \bar{x}_1 - \bar{x}_2$: the benefit of an enzyme molecule produced (if no enzyme is present yet) must exceed its own cost; otherwise, we obtain a boundary optimum at $\bar{u} = 0$ and the enzyme is not expressed. The optimum for $\tilde{u}$ holds whenever $\tilde{u}^{\mathrm{opt}} \leq \bar{u}^{\mathrm{opt}}$, otherwise, we obtain a boundary optimum at $\tilde{u} = \bar{u}^{\mathrm{opt}}$.

S5.3 Linear metabolic pathway

S5.4 Yeast central metabolism

Example: periodic adaption in central metabolism to varying ATP/ADP ratio Optimal enzyme rhythms in larger networks become more complex. Figure S4 shows the optimal adaption to periodic ATP and ADP levels in yeast central metabolism. The model is described in more detail in SI S5.4. As an external perturbation, opposing changes of the ATP and ADP levels were applied. The perturbation, by itself, evokes wide-spread driven oscillations in fluxes and metabolite levels. As an optimal adaption, the model predicts a relatively homogeneous up- and downregulation of all enzymes in glycolysis, while the enzymes in the TCA cycle are almost inactive.

S5.5 Glucose storage model

S6 Proofs

S6.1 Periodic parameter synergies evoke static shifts and second harmonics

In the model M1 in the article, a synergy between substrate and enzyme rhythm (quadratic term in $\tilde{u}$ and $\tilde{x}$) leads to a static shift (frequency 0) and a second harmonic (frequency $2\,\omega$):

$$\begin{aligned} \mathrm{Re}(\tilde{u}\,\mathrm{e}^{i\omega t})\mathrm{Re}(\tilde{x}\,\mathrm{e}^{i\omega t}) &= \frac{1}{2}(\tilde{u}\,\mathrm{e}^{i\omega t} + \tilde{u}^*\,\mathrm{e}^{-i\omega t})\frac{1}{2}(\tilde{x}\,\mathrm{e}^{i\omega t} + \tilde{x}^*\,\mathrm{e}^{-i\omega t}) \\ &= \frac{1}{4}\left[\tilde{u}\,\tilde{x}\mathrm{e}^{i2\omega t} + \tilde{u}^*\,\tilde{x} + \tilde{u}\,\tilde{x}^* + \tilde{u}^*\,\tilde{x}^*\,\mathrm{e}^{-i2\omega t}\right] \\ &= \frac{1}{2}\mathrm{Re}(\tilde{u}\,\tilde{x}\,\mathrm{e}^{i2\omega t}) + \frac{1}{2}\mathrm{Re}(\tilde{u}^*\,\tilde{x}). \end{aligned} \quad \text{(S53)}$$

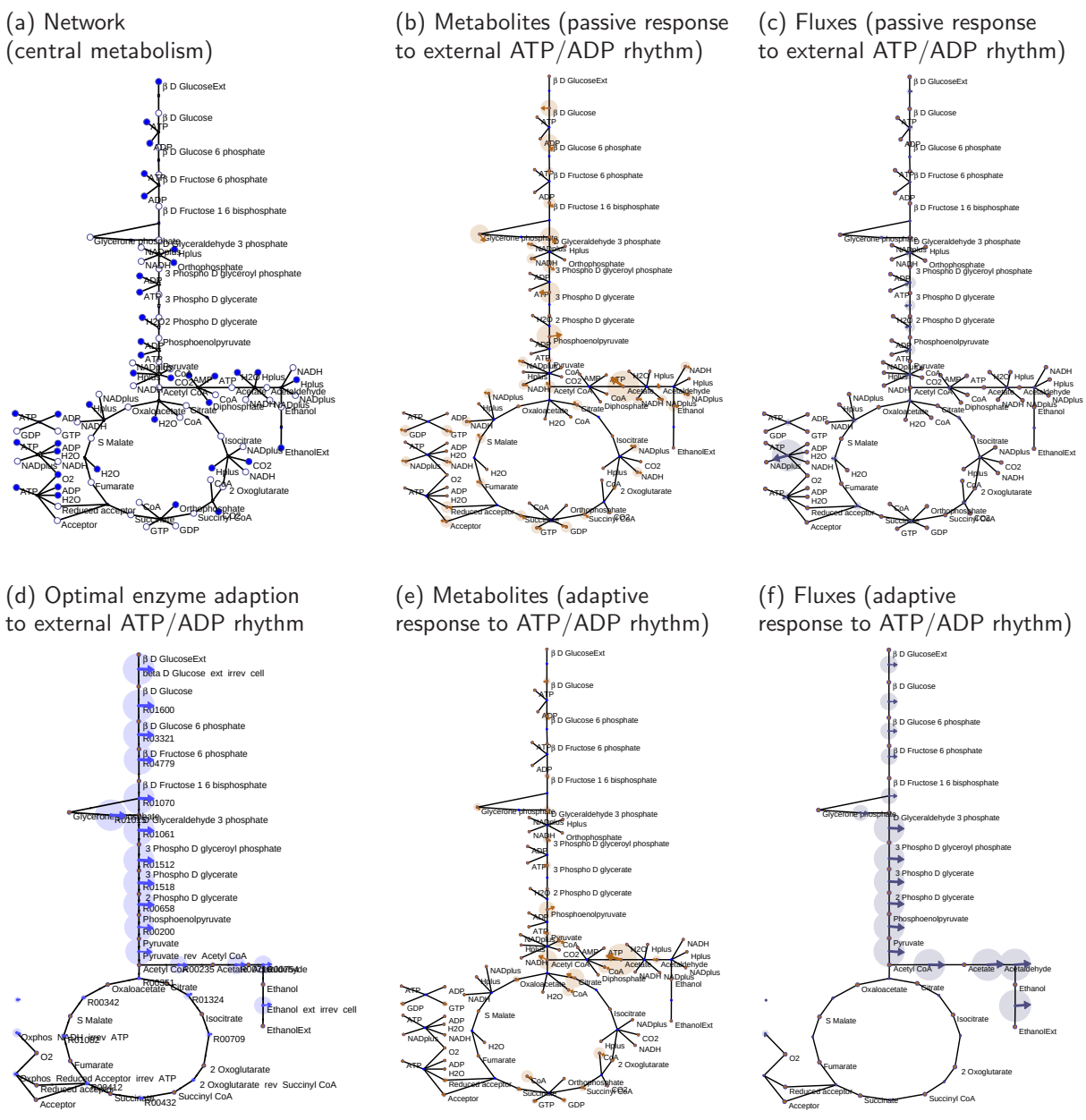

Figure S4: **Enzyme adaption to periodic ATP and ADP levels in central metabolism of the yeast S. cerevisiae.** (a) Network structure (external metabolites in blue, allosteric activation and inhibition shown by blue and red arcs). (b) Concentration rhythms evoked by a predefined ATP/ADP oscillation (amplitudes and phases shown by arrows. (c) Flux rhythms evoked by ATP/ADP oscillation. (d) Adaptive enzyme rhythm (complex amplitudes shown by arrows). (e) Concentration rhythm caused by adaptive enzyme rhythm. (f) Flux rhythm caused by adaptive enzyme rhythm. Driven oscillations and optimal enzyme rhythm were computed using the MCA-based approximation described in the text.

S6.2 Derivation of Eq. (S19) for periodic response coefficients

The prefactor of the periodic response coefficients can be derived as follows. We consider a Fourier transformation with the same prefactor convention as in [3]:

$$x(t) = \frac{1}{\sqrt{2\pi}} \int \tilde{x}\, \mathrm{e}^{i\,\omega\,t} \mathrm{d}t, \qquad \tilde{x}(\omega) = \frac{1}{\sqrt{2\pi}} \int x\, \mathrm{e}^{-i\,\omega\,t} \mathrm{d}t. \tag{S54}$$

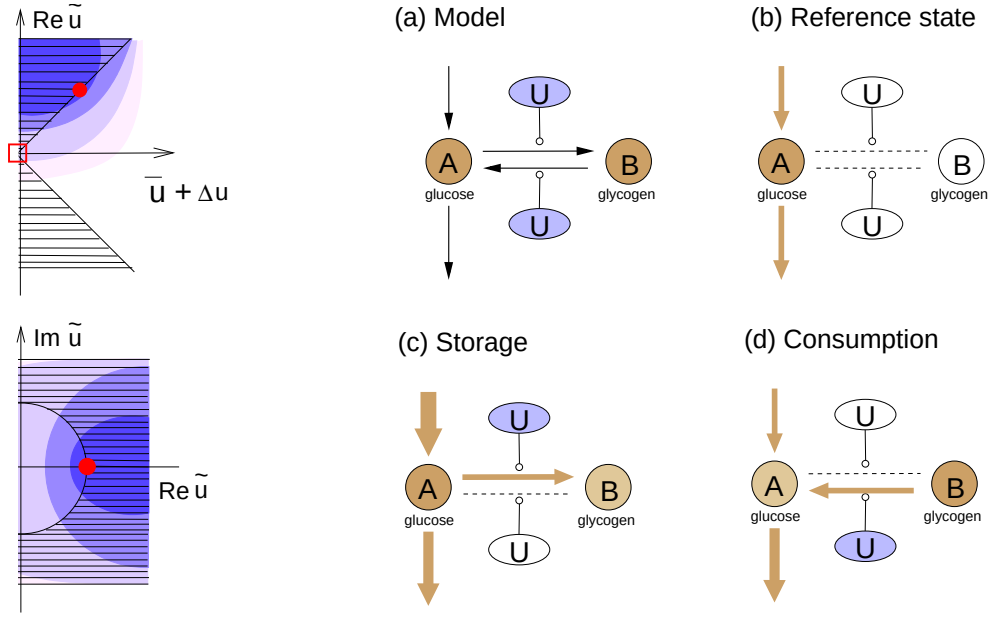

Figure S5: **Enzyme levels that become active only when induced by external oscillation.** Left: Due to constraints in the fitness landscape, the optimal static state may contain inactive enzymes. Scheme like in Figure 5; the x-axis shows the total level $\bar{u} + \Delta u$. Enzyme amplitudes and average levels are constrained (forbidden region in profile space shaded), which allows for boundary optima. With the fitness function shown, the enzyme is inactive when confined to the optimal steady state (square), but becomes active if oscillations are allowed (dot). Right: Model of glycogen storage and consumption. (a) Model structure. Glucose and glycogen are assumed to be slowly diluted (not shown). (b) Fluxes in the reference state. In steady state, storage enzymes would lead to a drain of glucose and to enzyme investments, so they remain inactive. (c) Fluxes during storage phase; overly high glucose levels are decreased. (d) Consumption phase; the flux is stabilised by reconverting glycogen into glucose.

A harmonic parameter perturbation with complex amplitude vector $\tilde{\mathbf{p}}$,

$$\Delta\mathbf{p}(t) \quad = \quad \mathrm{Re}[\tilde{\mathbf{p}}\,\mathrm{e}^{i\omega t}] = \frac{1}{2}\left[\tilde{\mathbf{p}}\,\mathrm{e}^{i\omega t} + \tilde{\mathbf{p}}^*\,\mathrm{e}^{-i\omega t}\right] \tag{S55}$$

has the Fourier transform

$$\hat{\mathbf{p}}(\alpha) = \frac{\sqrt{2\pi}}{2}\,\delta_\alpha(\omega)\;\tilde{\mathbf{p}} + \frac{\sqrt{2\pi}}{2}\,\delta_\alpha(-\omega)\;\tilde{\mathbf{p}}^*. \tag{S56}$$

The Fourier components of the state variables can be approximated using the spectral response cofficients $\tilde{\mathbf{R}}^{\tilde{\mathbf{y}}}_{\tilde{\mathbf{p}}}$, i.e. the functional derivatives between the respective Fourier components [3]. In a first-order expansion, the Fourier component at frequency α reads

$$\hat{\mathbf{y}}(\alpha) \quad \approx \quad \tilde{\mathbf{R}}^{\tilde{\mathbf{y}}}_{\tilde{\mathbf{p}}}(\alpha)\;\hat{\mathbf{p}}(\alpha) = \frac{\sqrt{2\pi}}{2}\;\delta_\alpha(\omega)\;\tilde{\mathbf{R}}^{\tilde{\mathbf{y}}}_{\tilde{\mathbf{p}}}(\omega)\;\tilde{\mathbf{p}} + \frac{\sqrt{2\pi}}{2}\,\delta_\alpha(-\omega)\;\tilde{\mathbf{R}}^{\tilde{\mathbf{y}}}_{\tilde{\mathbf{p}}}(-\omega)\;\tilde{\mathbf{p}}^*. \tag{S57}$$

By applying the inverse Fourier transformation, we obtain the behaviour in the time domain

$$\Delta\mathbf{y}(t) \quad \approx \quad \frac{1}{2}\left(\tilde{\mathbf{R}}^{\tilde{\mathbf{y}}}_{\tilde{\mathbf{p}}}(\omega)\;\tilde{\mathbf{p}}\;\mathrm{e}^{i\omega t} + \tilde{\mathbf{R}}^{\tilde{\mathbf{y}}}_{\tilde{\mathbf{p}}}(-\omega)\,\tilde{\mathbf{p}}^*\mathrm{e}^{-i\omega t}\right) = \mathrm{Re}[\tilde{\mathbf{R}}^{\tilde{\mathbf{y}}}_{\tilde{\mathbf{p}}}(\omega)\,\tilde{\mathbf{p}}\,\mathrm{e}^{i\omega t}]. \tag{S58}$$

because $\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}(-\omega)$ and $\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}(\omega)$ are complex conjugates. Therefore, our first-order expansion cofficient $\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}(\omega)$, the periodic response coefficient, is identical with the spectral response cofficient $\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}}(\omega)$. Now consider the second order. An expansion with spectral response cofficients yields additional terms in the Fourier transform, to be added to Eq. (S57):

$$\begin{aligned}
&\delta_\alpha(0)\,\tilde{\mathbf{R}}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)\left(\frac{\sqrt{2\pi}}{2}\tilde{\mathbf{p}}\otimes\frac{\sqrt{2\pi}}{2}\tilde{\mathbf{p}}^*\right)\\
&+\frac{1}{2}\,\delta_\alpha(2\omega)\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)\left(\frac{\sqrt{2\pi}}{2}\tilde{\mathbf{p}}\otimes\frac{\sqrt{2\pi}}{2}\tilde{\mathbf{p}}\right)+\frac{1}{2}\,\delta_\alpha(-2\omega)\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}*}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)\left(\frac{\sqrt{2\pi}}{2}\tilde{\mathbf{p}}^*\otimes\frac{\sqrt{2\pi}}{2}\tilde{\mathbf{p}}^*\right)\\
=\;&\frac{2\pi}{4}\left(\delta_\alpha(0)\,\tilde{\mathbf{R}}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)\,[\tilde{\mathbf{p}}\otimes\tilde{\mathbf{p}}^*]+\frac{1}{2}\,\delta_\alpha(2\omega)\,\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)\,[\tilde{\mathbf{p}}\otimes\tilde{\mathbf{p}}]+\frac{1}{2}\,\delta_\alpha(-2\omega)\,\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}*}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)\,[\tilde{\mathbf{p}}^*\otimes\tilde{\mathbf{p}}^*]\right)
\end{aligned}\tag{S59}$$

In the time domain (Eq. (S28)), we obtain the additional terms

$$\begin{aligned}
&\ldots+\frac{\sqrt{2\pi}}{4}\left(\tilde{\mathbf{R}}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)[\tilde{\mathbf{p}}\otimes\tilde{\mathbf{p}}^*]+\frac{1}{2}\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)[\tilde{\mathbf{p}}\otimes\tilde{\mathbf{p}}]\,\mathrm{e}^{i2\omega t}+\frac{1}{2}\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}*}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)[\tilde{\mathbf{p}}^*\otimes\tilde{\mathbf{p}}^*]\mathrm{e}^{-2i\omega t}\right)\\
=\;&\ldots+\frac{1}{2}\mathsf{Re}\left(\frac{\sqrt{2\pi}}{2}\tilde{\mathbf{R}}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)[\tilde{\mathbf{p}}\otimes\tilde{\mathbf{p}}^*]\right)+\frac{1}{2}\mathsf{Re}\left(\frac{\sqrt{2\pi}}{2}\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)[\tilde{\mathbf{p}}\otimes\tilde{\mathbf{p}}]\mathrm{e}^{2i\omega t}\right).
\end{aligned}\tag{S60}$$

The periodic response coefficients, needed for th expansion in Eqs (S7) and (S8), therefore read

$$\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)=\frac{\sqrt{2\pi}}{2}\,\tilde{\mathbf{R}}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega),\qquad \mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)=\frac{\sqrt{2\pi}}{2}\,\tilde{\mathbf{R}}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega).\tag{S61}$$

S6.3 Economic balance equations for periodically perturbed system Eq. (S36)

We consider the effects of small changes in $\Delta\bar{\mathbf{u}}$ and $\tilde{\mathbf{u}}$ on $\Delta\bar{\mathbf{v}}$ and $\tilde{\mathbf{v}}$, *assuming* that the metabolite profile remains unchanged (e.g., by keeping it unchanged with the help of virtual supply fluxes). To this aim, we write down the expansion of $\Delta\bar{\mathbf{v}}$ and $\tilde{\mathbf{v}}$, keeping only relevant terms containing $\Delta\bar{\mathbf{u}}$ and $\tilde{\mathbf{u}}$:

$$\begin{aligned}
\Delta\bar{\mathbf{v}}&\approx E^v_u\,\Delta\bar{\mathbf{u}}+\Delta\bar{\mathbf{c}}^{\mathrm{T}}\,E^v_{cu}\,\Delta\bar{\mathbf{u}}+\mathsf{Re}(\tilde{\mathbf{c}}^\dagger\,E^v_{\tilde{c}\tilde{u}}\,\Delta\tilde{\mathbf{u}})\\
\Delta\tilde{\mathbf{v}}&\approx E^{\tilde{v}}_{\tilde{u}}\,\tilde{\mathbf{u}}+\Delta\bar{\mathbf{c}}^{\mathrm{T}}\,E^{\tilde{v}}_{c\tilde{u}}\,\tilde{\mathbf{u}}+\tilde{\mathbf{c}}^\dagger\,E^{\tilde{v}}_{\tilde{c}u}\,\Delta\bar{\mathbf{u}}
\end{aligned}\tag{S62}$$

Other terms vanish because (i) the second-order enzyme elasticities E^v_{uu} vanish; or (ii) because frequencies do not match (e.g., the coupling of two static changes cannot yield a periodic change). If we now apply changes to the enzyme profiles, the flux changes read:

$$\begin{aligned}
\delta\mathbf{v}&=\frac{\partial\bar{\mathbf{v}}}{\partial\Delta\bar{\mathbf{u}}}\delta\bar{\mathbf{u}}+\frac{\partial\bar{\mathbf{v}}}{\partial\tilde{\mathbf{u}}}\delta\tilde{\mathbf{u}}=\left[E^v_u+\Delta\bar{\mathbf{c}}^{\mathrm{T}}\,E^v_{cu}\right]\,\delta\bar{\mathbf{u}}+\left[\tilde{\mathbf{c}}^\dagger\,E^v_{\tilde{c}\tilde{u}}\right]\,\delta\tilde{\mathbf{u}}\\
\delta\tilde{\mathbf{v}}&=\frac{\partial\tilde{\mathbf{v}}}{\partial\Delta\bar{\mathbf{u}}}\delta\bar{\mathbf{u}}+\frac{\partial\tilde{\mathbf{v}}}{\partial\tilde{\mathbf{u}}}\delta\tilde{\mathbf{u}}=\left[\tilde{\mathbf{c}}^\dagger\,E^{\tilde{v}}_{\tilde{c}u}\right]\,\delta\bar{\mathbf{u}}+\left[E^{\tilde{v}}_{\tilde{u}}+\Delta\bar{\mathbf{c}}^{\mathrm{T}}\,E^{\tilde{v}}_{c\tilde{u}}\right]\,\delta\tilde{\mathbf{u}}
\end{aligned}\tag{S63}$$

With $E^v_u=E^{\tilde{v}}_{\tilde{u}}=\mathsf{Dg}(\mathbf{v})\mathsf{Dg}(\mathbf{u})^{-1}$ and $E^v_{cu}=E^{\tilde{v}}_{\tilde{c}u}=E^{\tilde{v}}_{c\tilde{u}}=E^{v\,\mathrm{T}}_c\,\mathsf{Dg}(\mathbf{u})^{-1}$, this yields

$$\begin{aligned}
\delta\mathbf{v}&=\left[\mathsf{Dg}(\mathbf{v}+E^v_c\,\Delta\bar{\mathbf{c}})\mathsf{Dg}(\mathbf{u})^{-1}\right]\,\delta\bar{\mathbf{u}}+\left[\mathsf{Dg}(E^v_c\,\tilde{\mathbf{c}}^\dagger)\,\mathsf{Dg}(\mathbf{u})^{-1}\right]\,\delta\tilde{\mathbf{u}}\\
\delta\tilde{\mathbf{v}}&=\left[\mathsf{Dg}(E^v_c\,\tilde{\mathbf{c}})\mathsf{Dg}(\mathbf{u})^{-1}\right]\,\delta\bar{\mathbf{u}}+\left[\mathsf{Dg}(\mathbf{v}+E^v_c\,\Delta\bar{\mathbf{c}})\mathsf{Dg}(\mathbf{u})^{-1}\right]\,\delta\tilde{\mathbf{u}}
\end{aligned}\tag{S64}$$

After introducing the mean flux vector $\bar{\mathbf{v}}^{\mathrm{per}} = \mathbf{v} + E_c^v\,\Delta\bar{\mathbf{c}}$ (the average in the periodic state) and the matrix $\mathcal{E} = \begin{pmatrix} \mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})\,\mathsf{Dg}(\mathbf{u})^{-1} & \mathsf{Dg}(E_c^v\,\tilde{\mathbf{c}})\,\mathsf{Dg}(\mathbf{u})^{-1} \\ \mathsf{Dg}(E_c^v\,\tilde{\mathbf{c}})\,\mathsf{Dg}(\mathbf{u})^{-1} & \mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})\,\mathsf{Dg}(\mathbf{u})^{-1} \end{pmatrix}$ we can write this as

$$\begin{pmatrix} \delta\mathbf{v} \\ \delta\tilde{\mathbf{v}} \end{pmatrix} = \mathcal{E}\begin{pmatrix} \delta\mathbf{u} \\ \delta\tilde{\mathbf{u}} \end{pmatrix} \tag{S65}$$

The virtual fluxes needed to compensate thus read

$$\begin{pmatrix} \delta\varphi \\ \delta\tilde{\varphi} \end{pmatrix} = -\begin{pmatrix} \mathbf{N} & 0 \\ 0 & \mathbf{N} \end{pmatrix}\begin{pmatrix} \delta\mathbf{v} \\ \delta\tilde{\mathbf{v}} \end{pmatrix} = -\begin{pmatrix} \mathbf{N} & 0 \\ 0 & \mathbf{N} \end{pmatrix}\mathcal{E}\begin{pmatrix} \delta\mathbf{u} \\ \delta\tilde{\mathbf{u}} \end{pmatrix} \tag{S66}$$

The internal enzyme gains (static or periodic) are defined as the total derivatives of the utility function with respect to enzyme changes; the economic potentials (static or periodic) are defined as the total derivatives of the utility function with respect to virtual fluxes:

$$\begin{aligned} \mathbf{w}^{\mathbf{u}} &= \frac{\partial g}{\partial \delta\mathbf{u}}, \qquad \tilde{\mathbf{w}}^{\mathbf{u}} = \frac{\partial \mathbf{g}}{\partial \delta\tilde{\mathbf{u}}} \\ \mathbf{w}^{\mathbf{c}} &= \frac{\partial g}{\partial \delta\varphi}, \qquad \tilde{\mathbf{w}}^{\mathbf{c}} = \frac{\partial \mathbf{g}}{\partial \delta\tilde{\varphi}} \end{aligned} \tag{S67}$$

Considering a static enzyme variation $\delta\mathbf{u}, \tilde{\delta}\mathbf{u}$ and assuming a full compensation (and a utility function that is not explicitly dependent on fluxes), we can require

$$\begin{aligned} 0 &= \mathbf{w}^{\mathbf{u}\mathrm{T}}\,\delta\mathbf{u} + \tilde{\mathbf{w}}^{\mathbf{u}\dagger}\,\delta\tilde{\mathbf{u}} + \mathbf{w}^{\varphi}\,\delta\varphi + \tilde{\mathbf{w}}^{\varphi}\,\delta\tilde{\varphi} \\ &= \begin{pmatrix} \mathbf{w}^{\mathbf{u}} \\ \tilde{\mathbf{w}}^{\mathbf{u}} \end{pmatrix}^{\dagger}\begin{pmatrix} \delta\mathbf{u} \\ \delta\tilde{\mathbf{u}} \end{pmatrix} + \begin{pmatrix} \mathbf{w}^{\mathbf{c}} \\ \tilde{\mathbf{w}}^{\mathbf{c}} \end{pmatrix}^{\dagger}\begin{pmatrix} \delta\varphi \\ \delta\tilde{\varphi} \end{pmatrix} \\ &= \begin{pmatrix} \mathbf{w}^{\mathbf{u}} \\ \tilde{\mathbf{w}}^{\mathbf{u}} \end{pmatrix}^{\dagger}\begin{pmatrix} \delta\mathbf{u} \\ \delta\tilde{\mathbf{u}} \end{pmatrix} - \begin{pmatrix} \mathbf{w}^{\mathbf{c}} \\ \tilde{\mathbf{w}}^{\mathbf{c}} \end{pmatrix}^{\dagger}\begin{pmatrix} \mathbf{N} & 0 \\ 0 & \mathbf{N} \end{pmatrix}\mathcal{E}\begin{pmatrix} \delta\mathbf{u} \\ \delta\tilde{\mathbf{u}} \end{pmatrix} \end{aligned} \tag{S68}$$

After omitting the variation vector $\binom{\delta\mathbf{u}}{\delta\tilde{\mathbf{u}}}$ and transposing the equation, we obtain (noting that $\mathcal{E}$ is symmetric)

$$\begin{aligned} \begin{pmatrix} \mathbf{w}^{\mathbf{u}} \\ \tilde{\mathbf{w}}^{\mathbf{u}} \end{pmatrix} &= \mathcal{E}\begin{pmatrix} \mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} \\ \mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} \end{pmatrix} \\ &= \begin{pmatrix} \mathsf{Dg}(\mathbf{u})^{-1}\,\mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}}) & \mathsf{Dg}(\mathbf{u})^{-1}\,\mathsf{Dg}(E_c^v\,\tilde{\mathbf{c}}) \\ \mathsf{Dg}(\mathbf{u})^{-1}\,\mathsf{Dg}(E_c^v\,\tilde{\mathbf{c}}) & \mathsf{Dg}(\mathbf{u})^{-1}\,\mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}}) \end{pmatrix}\begin{pmatrix} \mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} \\ \mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} \end{pmatrix} \\ &= \begin{pmatrix} \mathsf{Dg}(\mathbf{u})^{-1}\,\mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}}) & 0 \\ 0 & \mathsf{Dg}(\mathbf{u})^{-1}\,\mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}}) \end{pmatrix}\begin{pmatrix} \mathbf{I} & \mathbf{A} \\ \mathbf{A} & \mathbf{I} \end{pmatrix}\begin{pmatrix} \mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} \\ \mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} \end{pmatrix} \end{aligned} \tag{S69}$$

where $\mathbf{A} = \mathsf{Dg}(E_c^v\,\tilde{\mathbf{c}})\,\mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})^{-1}$. If we consider the values in the unperturbed reference state, $\mathbf{A}$ vanishes and the two equations decouple:

$$\begin{aligned} \mathsf{Dg}(\mathbf{u})\,\mathbf{w}^{\mathbf{u}} &= \mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})\,\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} \\ \mathsf{Dg}(\mathbf{u})\,\tilde{\mathbf{w}}^{\mathbf{u}} &= \mathsf{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})\,\mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} \end{aligned} \tag{S70}$$

Otherwise, we obtain the coupled equations

$$\begin{aligned} \mathrm{Dg}(\mathbf{u})\,\mathbf{w}^{\mathbf{u}} &= \mathrm{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})[\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} + \mathbf{A}\,\mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}}] \\ \mathrm{Dg}(\mathbf{u})\,\tilde{\mathbf{w}}^{\mathbf{u}} &= \mathrm{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})[\mathbf{A}\,\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} + \mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}}] \end{aligned} \tag{S71}$$

This is only the indirect value. For the direct value, we need to add the effects of a possible direct flux gain,

$$\delta g^{\mathrm{direct}} = \mathbf{z}^{\mathrm{v}\mathrm{T}}\,\delta\mathbf{v} + \tilde{\mathbf{z}^{\mathrm{v}}}^{\dagger}\delta\tilde{\mathbf{v}} = \begin{pmatrix}\mathbf{z}^{\mathrm{v}} \\ \tilde{\mathbf{z}^{\mathrm{v}}}\end{pmatrix}^{\dagger} \mathcal{E} \begin{pmatrix}\delta\mathbf{u} \\ \delta\tilde{\mathbf{u}}\end{pmatrix} \tag{S72}$$

The formulae for the entire value thus read

$$\begin{aligned} \mathrm{Dg}(\mathbf{u})\,\mathbf{w}^{\mathbf{u}} &= \mathrm{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})[[\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} + \mathbf{z}^{\mathrm{v}}] + \mathbf{A}\,[\mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} + \tilde{\mathbf{z}^{\mathrm{v}}}]] \\ \mathrm{Dg}(\mathbf{u})\,\tilde{\mathbf{w}}^{\mathbf{u}} &= \mathrm{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})[\mathbf{A}\,[\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} + \mathbf{z}^{\mathrm{v}}] + [\mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} + \tilde{\mathbf{z}^{\mathrm{v}}}]] \end{aligned} \tag{S73}$$

Assuming optimality, that is a balance between enzyme cost and benefit, we obtain the equations

$$\begin{aligned} \mathrm{Dg}(\mathbf{u})\,\mathbf{h}^{\mathbf{u}} &= \mathrm{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})[[\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} + \mathbf{z}^{\mathrm{v}}] + \mathbf{A}\,[\mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} + \tilde{\mathbf{z}^{\mathrm{v}}}]] \\ \mathrm{Dg}(\mathbf{u})\,\tilde{\mathbf{h}}^{\mathbf{u}} &= \mathrm{Dg}(\bar{\mathbf{v}}^{\mathrm{per}})[\mathbf{A}\,[\mathbf{N}^{\mathrm{T}}\mathbf{w}^{\mathbf{c}} + \mathbf{z}^{\mathrm{v}}] + [\mathbf{N}^{\mathrm{T}}\tilde{\mathbf{w}}^{\mathbf{c}} + \tilde{\mathbf{z}^{\mathrm{v}}}]] \end{aligned} \tag{S74}$$

where $\mathbf{h}^{\mathbf{u}}$ and $\tilde{\mathbf{h}}^{\mathbf{u}}$ are the losses for additional enzyme variations $\delta\mathbf{u}$ and $\delta\tilde{\mathbf{u}}$.

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