The economics of periodic enzyme profiles

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Abstract

Enzyme levels in cells can be an adaptions to periodic environments, but can also drive self-induced metabolic cycles. In both cases, rearranging metabolic processes in time can make them run more efficiently. If enzyme levels change periodically, how should they be coordinated within and between pathways? To predict optimal periodic enzyme profiles, I derive them from an optimality principle, which I am applying here to periodic metabolic states. I consider kinetic models controlled by periodic enzyme levels, possibly in a periodic environment. The enzyme profiles are chosen such that the metabolic performance is maximized. Assuming small perturbations, optimal static or periodic enzyme adaptions can be computed using metabolic control theory. To compute optimal enzyme rhythms, I compute pairwise fitness synergies between all periodic parameters and solve for optimal periodic enzyme profiles. They are applicable to large metabolic networks and show how factors like model structure, fitness functions, and external rhythm shape the periodic enzyme profiles. Rhythms can either be induced by the environment, or in some cases self-induced, suggesting a possible selection advantage of spontaneous metabolic cycles. Phase shifts between reactants and enzymes can affect enzyme efficiency, and orchestrated enzyme rhythms can increase and redirect fluxes and arrange metabolic processes optimally in time. The enzymes are not only passively adapted to existing periodic metabolite levels, but shape them actively to realise fitness advantages.

Keywords: Metabolic oscillation, Metabolic Control Analysis, Optimal control, Enzyme oscillation, Allosynchrony

1 Introduction

Rhythms are common in biology, both in cells (cell cycle, circadian photosynthesis [1], and metabolic oscillations [2, 3]) and in organisms (sleep rhythms, menstrual cycle, seasonal flowering). Some are adapted to natural daily and yearly rhythms, in order to perform certain tasks when conditions are best. Adaptive rhythms can entail anticipation, for instance, a production and storage of nutrients for night or winter times. Metabolic rhythms in yeast have been studied in detail: they entail global periodic gene expression [4, 5], entailing a periodic production, storage, and consumption of compounds [2, 6, 7]. Moreover, enzyme rhythms, in certain metabolic pathways, could increase the average flux. If this is true, self-induced rhythms, without any external trigger, might also bring an advantage. The mechanisms behind biochemical oscillations have been studied extensively. Here I shall concentrate on a complementary question: whether metabolic rhythms, involving enzyme variation, can bring functional advantages.

If rhythmic enzyme profiles can provide benefits, how would the optimal adaptive profile look like? If an organism adapts itself quasi-statically to a slowly varying environment, rhythms in the environment will induce enzyme rhythms. This behaviour should be a possible outcome of a theory optimal enzyme rhythms. If the environment changes faster, oscillations caused by the environment (and possibly, by enzyme rhythms) spread dynamically in

the network and reach different regions at different times, thus making a quasi-static adaption impossible. If each enzyme is adapted to its momentary substrate level ("just-in-time production"), a sudden external substrate supply would lead to a sequence of enzyme activations, propagating along a pathway. Accordingly, a periodic substrate supply would induce wave-like enzyme rhythms. However, such enzyme profiles would affect the metabolite dynamics and create an incentive for additional enzyme adaptions. These, again, affect the metabolic dynamics, and so on. The optimal enzyme profile have to be self-consistent, that is, optimally adapted to the dynamics creates by itself and by the environment. How would such an orchestration work in a metabolic networks comprising thousands of enzymes? How should they be mutually adpated to create utile metabolic cycles or to respond appropriately to periodic environments?

One may consider all biochemical processes a cell needs to run during an external cycle (e.g., one day), and see how they could be optimally arranged in time. Instead of running them all continuously, the cell can take advantage of existing oscillations and allocate processes to time windows where the conditions (availability of substrates, cofactors, or thermodynamic driving forces) are best. For instance, energy-consuming processes in plants may run during day time, where energy is easily available. Once the enzyme levels have been adjusted to rearrange the cellular processes in the metabolic cycle, the conditions (e.g., energy availability) during the cycle will change, which may make further adaptions necessary, provoking further changes, and so on. Altogether, the phases of the cycle may become more and more specialised in their tasks. For instance, one process may accumulate compounds which are then consumed by processes in the following phase. If enzymes have delayed, indirect effects in distant parts of metabolism, or if they produce metabolites for later use, the enzyme profiles have to anticipate the future conditions under which their actions will take effect. Such a scheduling of effects may shape their optimal time profiles.

To understand the tight interplay of metabolic dynamics and enzyme adaption in metabolic rhythms, we need mathematical models that combine two perspectives: the dynamics of metabolic pathways and the economics of enzyme usage. In practice, we use a kinetic metabolic model to describe fluxes and metabolite levels dynamically and dependent on enzyme levels. On top of this model, we solve an economic problem in which fluxes, metabolite levels and enzyme levels are scored by a fitness function, and enzyme profiles are chosen by optimality principles. Both layers – dynamic model and economic problem – are closely entangled.

Enzyme profiles for static or dynamic cost-benefit problems have been predicted by numerical optimisation. For instance, optimal temporal enzyme profiles in linear pathways, after a sudden addition of substrate, have been computed numerically [12, 13]: in the optimal strategy found in [12], enzymes are activated sequentially in time, and repressed again. Thus, the cell does only invest into enzymes when enough substrate has accumulated (see Figure 1 (a)). Such "just-in-time production" has also been observed experimentally in amino acid synthesis in *E. coli* [13]. Despite its apparent simplicity, the sequential activation of enzymes represents a subtle interplay between metabolic dynamics and enzyme adaption. If all enzymes were switched on immediately, downstream metabolites could appear much faster. However, the optimal solution delays this process and creates large concentration drops along the pathway, which enable the enzymes to work more efficiently. Thus, optimal enzyme levels are not just passively adapted to their substrate levels (as implied by the notion of "just-in-time production"), but they actively manage the metabolite profiles for the benefit of the cell.

Thus, enzyme rhythms are coupled both mechanistically (by physical causation) and functionally (by economic demands): once a biochemical process oscillates, others may need to be adapted and their enzymes will show periodic expression. This may cause further adaptions, involving even more enzymes. Since perturbations can propagate in biochemical networks like waves, the adapted enzyme profiles may show a complex pattern of amplitudes and phase shifts. A metabolic cycle emerges, in which periodic enzyme levels shape the global dynamic processes and are adapted to them at the same time.

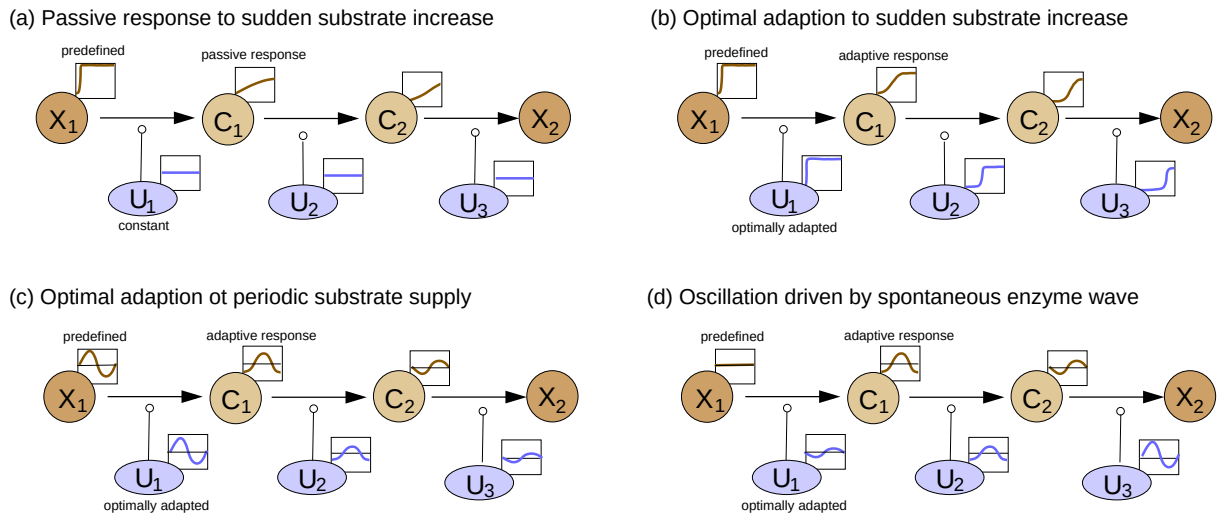

Figure 1: **Metabolic dynamics and enzyme adaption.** (a) Linear example pathway. After a sudden increase of the substrate level, metabolite pools increase with time delays. Circles represent metabolites (dark brown: external metabolites, with predefined level, light brown: internal metabolites), ellipses represent enzymes. (b) Just-in-time activation of enzyme levels speeds up the process. Enzyme levels increase when their substrate becomes available. In the strategy shown, enzymes are activated with time delays, each producing the substrate of the following enzyme. If this is an optimal strategy, we call it "induced" by the external change. (c) A periodic nutrient supply induces an enzyme rhythm "just in phase". Rhythmis levels of intermediates, caused by a periodic substrate level, are modulated by enzyme rhythms which exploit the varying substrate availability and increase the enzymes' efficiency. (d) Self-induced enzyme rhythms, causing oscillations of metabolite levels and fluxes in a constant environment. Under which circumstances can such rhythms be beneficial?

I will present a general approach to predicting optimal enzyme rhythms in metabolic systems. Since this article is about general solutions, potentially applicable to larger models, I shall not pursue a numerical optimisation of enzyme profiles in specific models, but develop a general theory. In the case of small static changes, the effects of environment and enzyme changes can be handled by metabolic control analysis (MCA), and optimal enzyme adaption profiles can be computed from metabolic response coefficients and fitness curvatures in the initial optimal state [10]. In reality, metabolic systems may not face abrupt discrete changes between steady states, but continuous fluctuations of supply and demand. To predict enzyme adaption in this case, I extend the MCA approach to periodic perturbations. If fluxes, metabolite levels, and enzyme levels vary rhythmically around given reference values (see Figure 1 (b)), the rhythms will interact and affect the metabolic performance. If this improves the metabolic performance, there will be an incentive for such rhythmic enzyme levels.

For small perturbations, oscillations of enzyme levels, metabolite levels, and fluxes can be approximated by cosine waves. Then, the entire dynamics can be captured by the amplitudes and phases, which can be treated by a variant of metabolic control theory [14]. In contrast to a full numerical optimisation, and following linear control theory used in engineering, this perturbation theory holds only for small oscillation amplitudes. At the same time, the theory yields general formulae for optimal enzyme profiles, is applicable to large metabolic networks, and can be used to prove fitness advantages of self-induced oscillations over steady states. Here I shall introduce the theory and illustrate it with small schematic examples. I first outline the problem of periodic enzyme levels in complex metabolic networks. Then I study enzyme rhythms in a single reaction, where they can provide benefits by exploiting existing rhythms of a reactant. Next, I study how enzyme rhythms can create and exploit metabolite rhythms in complex networks, and present a way to predict optimal periodic enzyme profiles for this case. Finally, I describe how enzyme rhythms may become utile, and discuss how mechanistic and economic aspects of the models,

e.g. causation and induction, are entangled and linked by metabolic control theory. Mathematical formulae are given in the appendix, and animated graphics can be found on www.metabolic-economics.de/enzyme-rhythms/.

2 Dynamics and economics of a single rhythmic enzyme

To see how periodic enzyme rhythms may bring benefits, we first consider a single reaction. If the substrate level varies periodically, synchronous enzyme rhythms (at a fixed average enzyme level) can increase the average flux. Thus, the effective catalytic activity of the enzyme increases. Our example model is shown in Figure 2: a reaction X $\rightarrow$ Y with irreversible mass-action rate law[1] $v(u,x) = u\,k\,x$. The variable u is the enzyme level (or, possibly, the enzyme activity, i.e., the concentration of enzyme in its active state). We study the reaction with a predefined substrate time course[2] $x(t)$ and choose an appropriate enzyme profile $u(t)$. Specifically, we consider substrate and enzyme profiles $x(t) = \bar{x} + \mathrm{Re}(\tilde{x}\,\mathrm{e}^{i\omega t})$ and $u(t) = \bar{u} + \mathrm{Re}(\tilde{u}\,\mathrm{e}^{i\omega t})$ with average levels $\bar{x}$ and $\bar{u}$ and complex amplitudes $\tilde{x}$ and $\tilde{u}$. Complex exponentials are convenient for describing oscillations: their real part yields a cosine wave with amplitude $|\tilde{u}|$ and a phase shift given by the phase angle of $\tilde{u}$ (a calculation, with cosine functions instead of complex exponentials, is shown in SI S5.2). Given a substrate profile $x(t)$, which enzyme rhythm (i.e., which phase and amplitude) will maximise the flux? By inserting $x(t)$ and $u(t)$ into the rate law, we obtain the time-dependent rate

$$v(t) = \quad = \underbrace{k\,\bar{u}\,\bar{x}}_{\bar{v}} + \underbrace{k\,\mathrm{Re}(\tilde{u}\,\mathrm{e}^{i\omega t})\bar{x}}_{\langle\cdot\rangle=0} + \underbrace{k\,\bar{u}\,\mathrm{Re}(\tilde{x}\,\mathrm{e}^{i\omega t})}_{\langle\cdot\rangle=0} + \underbrace{k\,\mathrm{Re}(\tilde{u}\,\mathrm{e}^{i\omega t})\mathrm{Re}(\tilde{x}\,\mathrm{e}^{i\omega t})}_{\frac{k}{2}\mathrm{Re}(\tilde{u}\,\tilde{x}\,\mathrm{e}^{i2\omega t})+\frac{k}{2}\mathrm{Re}(\tilde{u}^*\,\tilde{x})} \,. \tag{1}$$

It consists of four terms: first, the unperturbed reference flux. Then, two first-order terms describing oscillations caused by the periodic parameters, whose time-averages vanish. The last term describes synergies between the rhythms and can be split into $\frac{k}{2}\mathrm{Re}(\tilde{u}\,\tilde{x}\,\mathrm{e}^{i2\omega t}) + \frac{k}{2}\mathrm{Re}(\tilde{u}^*\,\tilde{x})$ where the star $*$ denotes the complex conjugate (proof in SI S6.1). The two terms describe a harmonic oscillation of frequency 2ω and a shift of the average flux [14]. We can write this shift as

$$\Delta\bar{v} = \langle\Delta v\rangle_t = \frac{k}{2}\,|\tilde{x}|\,|\tilde{u}|\,\cos(\varphi) \tag{2}$$

where φ is the phase shift between $x(t)$ and $u(t)$. This is the effect we are interested in. If the rate law is non-linear, Eq. (3) has to be modified: in a small-amplitude approximation, we obtain

$$\Delta\bar{v} = \langle\Delta v\rangle_t = \frac{E_\mathrm{x}}{2\bar{u}}\,|\tilde{x}|\,|\tilde{u}|\,\cos(\varphi) \tag{3}$$

where $E_\mathrm{x} = \partial r/\partial x$ is the enzyme's substrate elasticity (details in SI S2.1). The average flux can be tuned by the phase shift between substrate and enzyme: if both are in phase ($\varphi = 0$), the flux increases by $\frac{E_\mathrm{x}\,|\tilde{u}|\,|\tilde{x}|}{2\bar{u}}$ even though the average enzyme level remains unchanged. While the average ratio $\langle v/u\rangle_t = k\langle x\rangle_t = k\,\bar{x}$ remains fixed, the efficiency $\langle v\rangle/\langle u\rangle$ – the average rate, divided by the average enzyme level – increases, i.e. the enzyme molecules are used more efficiently. If $x(t)$ and $u(t)$ have opposite phases, the average flux decreases. I call this synergism between substrate and enzyme rhythms an allosynchrony. Allosynchrony resembles allosteric regulation, but the signal is not the metabolite level itself, but a metabolite oscillation with defined amplitude and phase. That enzyme rhythms can increase the average flux is a dynamical fact – but a fact with economical consequences.

[1] Units: rate v in mM/s, enzyme level u in mM, and substrate level x in mM, and a rate constant k in $\mathrm{mM}^{-1}\ \mathrm{s}^{-1}$.

[2] In general, the varying parameter x need not be a substrate level, but could be any parameter affecting the rate. For instance, ATP production by ATP synthase in plants depends on a proton gradient, which effectively sets the equilibrium constant of the ADP $\rightarrow$ ATP reaction. During the night, the is too low and ATP would be destroyed, so the ATP synthase should be shut down (light reaction).

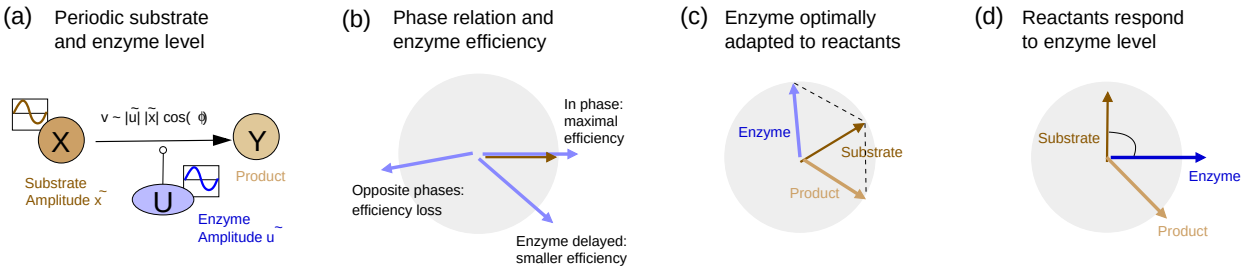

Figure 2: **Adaption of a single enzyme to periodic substrate levels.** (a) If enzyme and substrate levels oscillate in phase (at a fixed average enzyme level), the average flux increases, so the enzyme becomes more efficient. (b) Cosine waves can be described by complex amplitudes and phases (arrows rotating counter-clockwise; length: amplitude; angle: phase). The real parts (projections to x-axis) describe the time-dependent concentration changes $\Delta x(t)$ and $\Delta u(t)$. The enzyme efficiency in (a) depends on the phase shift between substrate and enzyme. Example phase shifts with different efficiencies are shown. (c) Enzyme rhythm $\tilde{u}$, optimally adapted to substrate and product rhythms $\tilde{x}$ and $\tilde{y}$ in a reversible reaction. The optimal enzyme rhythm $\tilde{u}^{\mathrm{opt}}$ is in phase with the term $E_X\,\tilde{x} + E_Y\,\tilde{y}$, where $E_X > 0$ and $E_Y < 0$ are the reactant elasticities in the reference state. Figure (c) shows the construction for $E_X = 1, E_Y = -1$, i.e., $\tilde{u}^{\mathrm{opt}} = \tilde{x} - \tilde{y}$. (d) In a simple pathway $\xrightarrow{v_0} X \xrightarrow{u\,k_x\,x} Y \xrightarrow{k_Y\,y}$ (model see SI S5.1), an enzyme rhythm evokes substrate and product rhythms. The substrate X peaks before the enzyme, the product Y afterwards.

Due to allosynchrony, the same enzyme investment yields a higher average flux, or equivalently, the same average flux can be achieved with a lower enzyme investment. Therefore, substrate rhythm may render enzyme rhythms profitable or, in my terminology, *induce* them.

We can phrase the result in two ways: one the one hand, we can say that the enzyme is adapted in every moment to the available substrate; on the other, we can assume that there is a constant total enzyme investment, which can be rearranged in time. This can be seen as two types of strategies, tactical or strategic ones. Tactically, we optimise the enzyme level in every moment given the current reactant levels. This will work for slow external oscillations: the enzyme level is adapted quasi-statically, following the optimal-response curve $u^{\mathrm{opt}}(x)$ for steady states. For small perturbations, this curve can be linearly approximated with a slope[3] $R_x^{u,opt} = \mathrm{d}u^{\mathrm{opt}}(\bar{x})/\mathrm{d}\bar{x} = -f_{\mathrm{uu}}^{-1}\,f_{\mathrm{ux}}$. Thus, a tactical adaption yields an enzyme rhythm in phase with the substrate and with an amplitude $\tilde{u}^{\mathrm{opt}} = R_x^{u,opt}\,\tilde{x}$.

If all metabolite profiles were fixed and given, such a tactical adaption would be optimal[5]. In reality, enzyme rhythms influence their reactant levels and, therefore, create metabolic oscillations. This possibility will affect their optimal choice. If the oscillations are fast, they will propagate through the network as waves, a quasi-stationary adaption will be impossible, and an adaption in each time-point cannot be optimal. Now, periodic behaviour must be devised *strategically*, i.e. the entire time profile must be optimised together, anticipating the future impacts of each momentary behaviour. Like in the previous calculation of flux changes, we could first consider constant

[3]The optimal-response curve depends on the interplay of flux benefits and enzyme costs and can be obtained from an optimisation problem: consider an objective function $g(u,x) = z(v(u,x))$, an investment function $h(u)$, and the fitness function $f(u,x) = g(u,x) - h(u)$ given by their difference[4]. We search for enzyme profiles that maximise the fitness under given external conditions. If the investment function is not too steep, the optimal static enzyme level $\bar{u}^{\mathrm{opt}}(x) = \mathrm{argmax}_u f(u,\bar{x})$ in our example reaction increases with the external substrate concentration $\bar{x}$ (see model M2 in SI S5.2, with a reversible rate law). If the substrate level moves from its reference value $\bar{x}$ to a value $\bar{x} + \Delta x$, the enzyme level must change in order to remain optimal. With the fitness curvatures $f_{\mathrm{uu}} = \frac{\partial^2 f}{\partial u^2} < 0$ and $f_{\mathrm{ux}} = \frac{\partial^2 f}{\partial u\,\partial x} > 0$ of the fitness function $f(u,x)$ in an initial optimal state, the optimal adaption to a small increase Δx reads (see Methods C)

$$\Delta u^{\mathrm{opt}} \quad \approx \quad -f_{\mathrm{uu}}^{-1}\,f_{\mathrm{ux}}\,\Delta x. \tag{4}$$

The adaption Δu^{opt} has the same sign as Δx and increases the fitness by $\Delta f = f_{\mathrm{ux}}\,\Delta\bar{u}_{\mathrm{opt}}\,\Delta x + \frac{1}{2} f_{\mathrm{uu}}\,\Delta\bar{u}_{\mathrm{opt}}^2 = -\frac{1}{2} f_{\mathrm{uu}}^{-1}\,f_{\mathrm{ux}}^2 \Delta x^2 > 0$. Since the fitness function is negatively curved, any enzyme variation would decrease the fitness.

[5]Since we allow only harmonic enzyme profiles for our strategic solutions, both strategies lead to the same results. For freely chosen enzyme profiles, the optimum profile would be a delta peek at the moment of highest substrate level, but such a profile would be unrealistic since real enzyme levels arise from production and degradation/dilution and are therefore smooth.

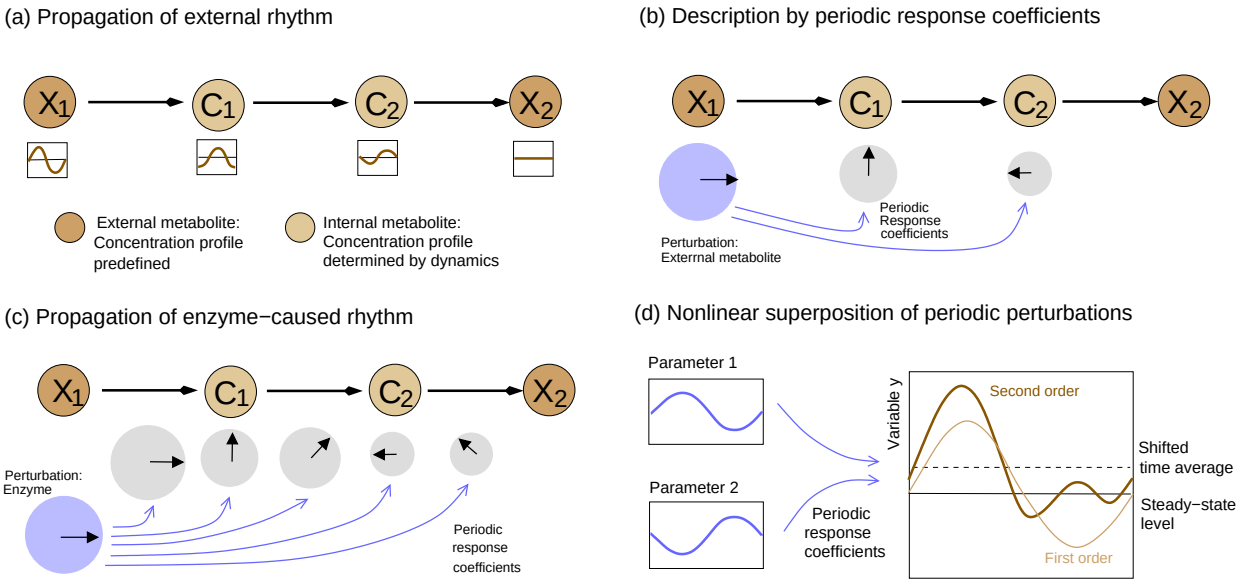

Figure 3: **Dynamics of forced oscillations.** (a) Metabolic pathway with profiles of external substrate X_1 and product X_2 determined by the environment. A harmonic substrate level x_1 (brown curve) evokes wave-like concentration and flux changes. Phase shifts between metabolites reflect their order along the chain. (b) In a linearised model (approximation $\Delta\mathbf{v} \approx \mathbf{E}_u\Delta\mathbf{u}+\mathbf{E}_c\Delta\mathbf{c}+\mathbf{E}_x\Delta\mathbf{x}$), the forced oscillations are given by phase-shifted cosine waves. Amplitudes and phases (shown by arrow lengths and angles) are described by complex-valued amplitudes. In a linear approximation, a metabolite's complex amplitude (index i) is obtained by multiplying the complex amplitude of a perturbation parameter (index m) with the spectral response coefficient $\tilde{R}^{c_i}_{p_m}(\omega)$ between the parameter and the concentration c_i (complex value shown by arrow). (c) The effects of enzyme rhythms can be described by spectral response coefficients as well. Forced metabolite and flux rhythms are shown.(d) A pair of oscillating parameters (frequency ω) causes oscillations in a state variable y; in a second-order approximation, the forced oscillations consist of an average shift (second-order effect), harmonic oscillations of frequency ω (first-order effect), and harmonic oscillations of frequency 2ω (second-order effect).

enzyme levels as a starting point (optimized to the average external conditions), and then add sine waves whose amplitudes and phases must be optimised. The sine waves would change the enzyme levels at different times, but keep the average values unchanged. Thus, enzyme investments would be shifted to where they are most profitable, but at a constant total investment $\langle u(t)\rangle_t$.

3 Dynamics of periodic enzyme profiles

Now we move on to metabolic networks. State variables (internal concentrations c_i and reaction rates v_l) and enzyme levels u_l are described by vectors and reaction rates depend on reactant levels and external parameters: enzyme levels u_l and external metabolite levels x_j. Fluctuations caused by perturbed reaction rates reach different parts of the network with different delays and can feed back on the initial reaction. To predict which of those enzyme fluctuations may be profitable, all dynamic effects need to be taken into account: thus, we first need to capture how periodic perturbations propagate dynamically in kinetic models.

If enzyme or external metabolite levels oscillate, the metabolite levels and fluxes will show forced oscillations, whose amplitudes and phases depend on network structure and rate laws. If the perturbations are small, their amplitudes and phases can be described by spectral reponse coefficients [16, 14] (see Figure 3). In practice, we start from a stable steady state with fixed external parameters and non-zero enzyme levels, our reference

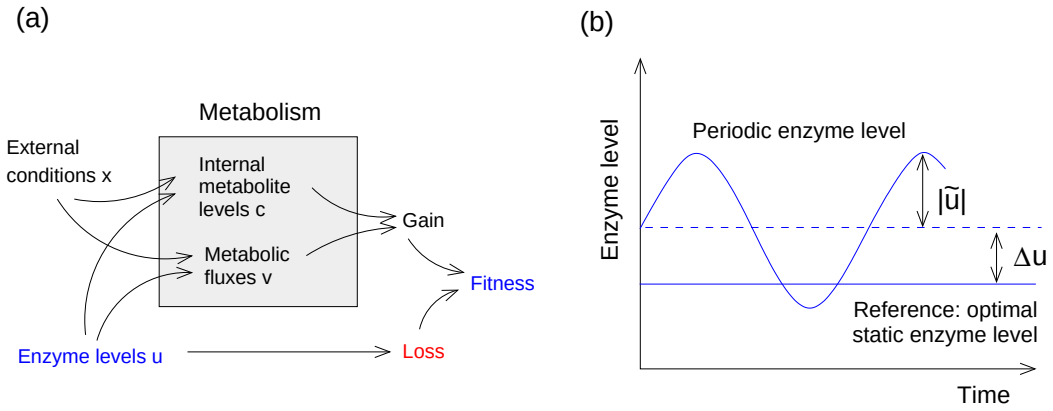

Figure 4: **Optimal enzyme profiles as an economic benefit-cost problems.** (a) Enzyme levels control the metabolic state, and thus the metabolic objective function. The fitness (difference of metabolic objective and enzyme investment) needs to be maximised. (b) Harmonic enzyme profile. Starting from a steady reference state (straight solid line), the profile is changed by a time-average shift Δu and a complex amplitude $\tilde{u}$, which represents the amplitude and phase of a harmonic oscillation.

state. Then, we assume that external parameters $x_j(t)$ and enzyme levels u_l oscillate harmonically with circular frequency ω, shifts $\Delta\bar{x}_j$ and Δu_l or their mean values, and complex amplitudes $\tilde{x}_j$ and $\tilde{u}_l$. The perturbations are assumed to be small, allowing for a Taylor expansion. In a first-order approximation, a harmonic parameter rhythm evokes harmonic metabolite and flux oscillations of the same frequency. The amplitude vectors $\tilde{\mathbf{c}}$ and $\tilde{\mathbf{v}}$ are linear functions of the amplitude vectors $\tilde{\mathbf{x}}$ and $\tilde{\mathbf{u}}$, with coefficients given by the spectral response coefficients [14]. The linearised model acts as a low-pass filter: the system shows quasi-stationary responses at low frequencies, high-frequency perturbations are damped, and there may be resonances at intermediate frequencies [14]. For mixed perturbations (multiple parameters and frequencies), the forced oscillations follow from a linear superposition. As the perturbation amplitudes become larger, the first-order approximation becomes inaccurate. In a second-order expansion, external and enzyme rhythms act synergistically, evoke higher harmonics, and can shift average concentrations, fluxes, and the metabolic utility (see appendix A and SI S2).

4 Economics of periodic enzyme profiles

Once we can simulate the dynamic effects of enzyme rhythms, we can turn this around and study optimal control problems: for instance, which enzyme rhythms can evoke some intended metabolic behaviour at low enzyme costs, and in the presence of external rhythms $\tilde{x}$? Such inverse problems can be numerically hard, but our approximation by MCA makes them tractable (see SI S3.4). In [10], static response coefficients were used to predict enzyme adaptions between steady states. Here, in order to predict periodic enzyme profiles, I use the same method but with periodic response coefficients.

To formulate general benefit-cost problems for metabolic networks, we define a metabolic objective as a function of internal metabolite levels c_i and fluxes v_l. We write the objective as $z(\mathbf{y})$, where the state vector $\mathbf{y}$ contains metabolite levels and fluxes[6]. Next, we define a fitness function $f(\mathbf{u},\mathbf{x}) = g(\mathbf{u},\mathbf{x}) - h(\mathbf{u})$ with an enzyme utility $g(\mathbf{u}.\mathbf{x}) = z(\mathbf{y}(\mathbf{u},\mathbf{x}))$ and an enzyme investment function $h(\mathbf{u})$. There are two types of model parameters: external parameters x_j, which represent given conditions (here: external metabolite levels) and control parameters

[6]The vector $\mathbf{y}$ can also include any other state variables appearing in the metabolic model, e.g., pH values, compartment sizes, and so on.

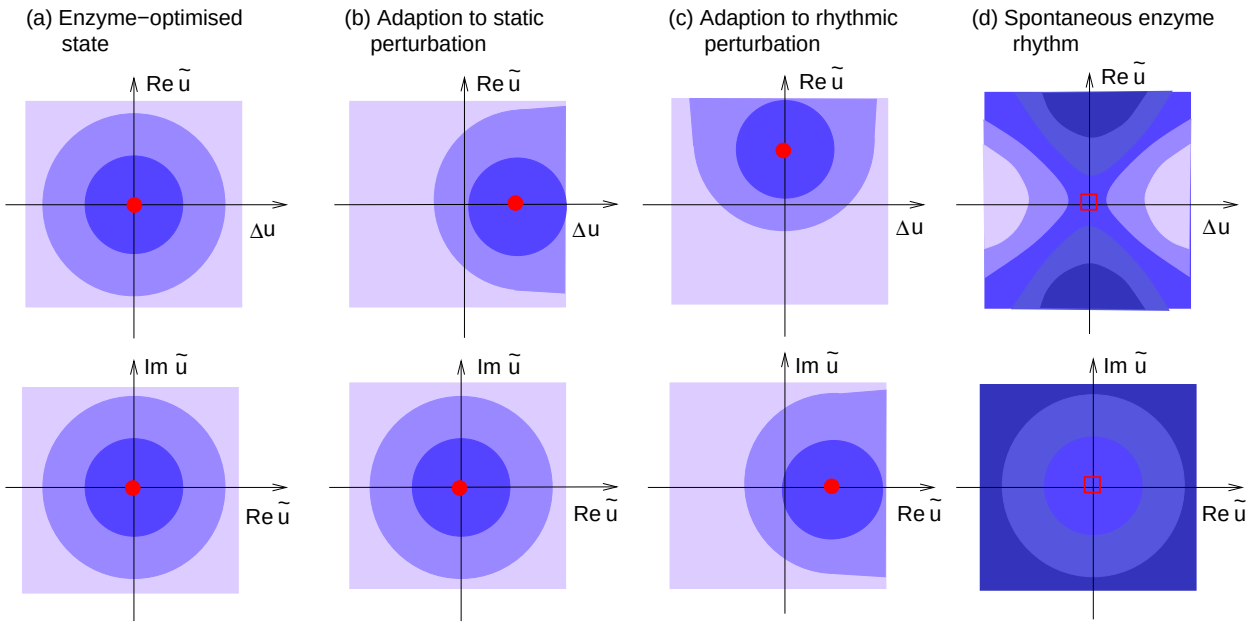

Figure 5: **Fitness landscape and prediction of optimal enzyme adaptions.** To study enzyme adaptions, we consider the fitness as a function of enzyme profiles, represented by static shifts Δu and complex amplitudes $\tilde{u}$. The panels show the fitness as a function of static and rhythmic enzyme deviations $\mathrm{Re}(\tilde{u})$ and Δu (top) or $\mathrm{Re}(\tilde{u})$ and $\mathrm{Im}(\tilde{u})$ (bottom) in the presence of external perturbations Δx or $\tilde{x}$. (a) Unperturbed fitness landscape ($\Delta x = \tilde{x} = 0$; fitness contours shown in blue; dark colours represent high fitness). The fitness maximum (dot) is the state of optimal static enzyme levels. The curvature matrices $\mathbf{F}_{\mathrm{uu}}$ and $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}(\omega)$ (fitness curvatures for Δu and $\tilde{u}$) are negative definite, so any change Δu or $\tilde{u}$ would decrease the fitness. (b) An external parameter shift Δx displaces the optimum, creating an incentive for a static enzyme adaption Δu. (c) An external rhythm with amplitude $\tilde{x}$ displaces the optimum, creating an incentive for an enzyme rhythm with amplitude $\tilde{u}$. In the example shown, the optimal enzyme profile is in phase with the perturbation. (d) Economic instability against enzyme rhythms: if $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$ has a positive eigenvalue, self-induced oscillations ($\tilde{u} \neq 0$) can increase the fitness even in the absence of external oscillations, but not static enzyme changes $\Delta\bar{u}$, could do so.

(here: enzyme levels) to be optimised. For a reference state, we fix the external concentrations x_j and determine a static enzyme profile $\mathbf{u}^{\mathrm{opt}}$ that yields a stable steady state of maximal fitness. If enzyme levels vanish in this state, we omit their reaction from the model. This is our reference state. If we perturb it by parameter changes $\Delta\mathbf{x}$, an optimal enzyme adaption $\Delta\bar{\mathbf{u}}$ can be computed with the help of second-order metabolic response coefficients [10]. Here, we are concerned with periodic changes, which require periodic response coefficients instead [14] (for a short description, see SI S2). To define a fitness for metabolic time courses, we need fitness functionals, and we consider two possibilities. In the *fluctuation-sensitive fitness* $F = z(\langle\mathbf{y}\rangle_t) - h(\langle\mathbf{u}\rangle_t)$, the fitness function is evaluated in each moment and then averaged over time. In the *fluctuation-insensitive fitness* $F = \langle z(\mathbf{y}) - h(\mathbf{y})\rangle$, the fitness function is applied to the average state values. In both cases, the total fitness depends on time-averaged values (either of state variables, or of metabolic objective, enzyme investment, and fitness).

If our reference state has parameter profiles $\bar{\mathbf{u}}$ and $\bar{\mathbf{x}}$, we consider enzyme profiles $\mathbf{u}(t) = \bar{\mathbf{u}} + \Delta\bar{\mathbf{u}} + \mathrm{Re}(\tilde{\mathbf{u}}\mathrm{e}^{i\,\omega\,t})$ and external profiles $\mathbf{x}(t) = \bar{\mathbf{x}} + \Delta\bar{\mathbf{x}} + \mathrm{Re}(\tilde{\mathbf{x}}\mathrm{e}^{i\,\omega\,t})$ in the perturbed, periodic state. The resulting fitness $F(\Delta\mathbf{u}, \Delta\tilde{\mathbf{x}}, \tilde{\mathbf{u}}, \tilde{\mathbf{x}})$ is a function of the shift vectors $\Delta\bar{\mathbf{u}}$ and $\Delta\bar{\mathbf{x}}$ and of the complex amplitude vectors $\tilde{\mathbf{u}}$ and $\tilde{\mathbf{x}}$ (see Figure 5). Starting from the reference state, we expand this function to second order and obtain the perturbation-dependent fitness

change[7]

$$\Delta F \approx \left[\Delta \mathbf{x}^{\mathrm{T}}\, \mathbf{F}_{\mathrm{xu}} \Delta \mathbf{u} + \frac{1}{2} \Delta \mathbf{u}^{\mathrm{T}}\, \mathbf{F}_{\mathrm{uu}} \Delta \mathbf{u}\right] + \left[\mathrm{Re}[\tilde{\mathbf{x}}^{\dagger}\, \mathbf{F}_{\tilde{\mathrm{x}}\tilde{\mathrm{u}}} \tilde{\mathbf{u}}] + \frac{1}{2} \tilde{\mathbf{u}}^{\dagger}\, \mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}} \tilde{\mathbf{u}}\right]. \tag{5}$$

The first term in brackets describes the effect of static perturbations, the second term the effect of rhythmic perturbations at frequency ω. The curvature matrices ($\mathbf{F}_{\mathrm{uu}}$ and $\mathbf{F}_{\mathrm{ux}}$ for static changes, $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$ and $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}$ for rhythms) describe the local curvatures of the fitness F with respect to $\Delta\mathbf{u}$, $\Delta\mathbf{x}$, $\tilde{\mathbf{u}}$, and $\tilde{\mathbf{x}}$, i.e. if the local shape of the fitness landscape around the unperturbed state. The curvatures depend on the kinetic model, enzyme investment and metabolic objective function, and on the type of fitness functional (fluctuation-sensitive or fluctuation-insensitive). If the optimal reference state is given, they can be easily computed (see appendix B). $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$ and $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}$ depend on the frequency ω; if the flux-sensitive fitness is used, we obtain the limiting cases $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}(\omega = 0) = \frac{1}{2}\mathbf{F}_{\mathrm{uu}}$ and $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}(\omega = 0) = \frac{1}{2}\mathbf{F}_{\mathrm{ux}}$ for infinitely slow perturbations.

With Eq. (5), we can compute the fitness effects of agiven enzyme rhythm. All necessary information is contained in the curvature matrices $\mathbf{F}_{\mathrm{uu}}$ and $\mathbf{F}_{\mathrm{ux}}$ (for static perturbations) or $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$ and $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}$ (for periodic perturbations). The matrix elements represent different types of fitness synergies: between enzymes and external parameters (elements of $\mathbf{F}_{\mathrm{ux}}$ and $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}$), between enzymes only (off-diagonal elements of $\mathbf{F}_{\mathrm{uu}}$ and $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$), and enzyme self-synergies (diagonal elements of $\mathbf{F}_{\mathrm{uu}}$ and $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$). Self-synergies tend to be negative for different reasons: due to dynamic self-inhibition; due to non-linear investment functions and fluctuation-sensitive fitness functionals; and possibly because of frequency-dependent enzyme investments, which could be considered (see below). In contrast, synergies between enzymes and external parameters can be beneficial. In total, the fitness effect of an enzyme rhythm comprises all these effects. If the net effect is positive, enzyme rhythms may bring a selection advantage in evolution.

Given an external perturbation profile (which can be static or periodic), what is the optimal enzyme profile? In Eq. (5), each matrix element in $\mathbf{F}_{\tilde{\mathrm{x}}\tilde{\mathrm{u}}}$ describes a fitness synergy between one external parameter and one enzyme: if the external parameter oscillates, it creates *incentives* for enzymes to oscillate, with phase angles defined by the fitness synergy. The enzyme oscillations, however, will then show fitness synergies between each other, as described by the elements of $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$. Each enzyme rhythm, once it exists, creates new incentives for other enzyme rhythms, and so on. What is the resulting optimal solution? To compute it, we go back to Eq. (5) and maximise F as a function of the enzyme profiles. The unpertubed reference state shows by definition an optimal static enzyme profile. To avoid complications, we require that all enzymes are active in the reference state. Cases in which enzymes are activated under oscillations are discussed in the appendix. Next, we can ask if the reference state, without external perturbations, is economically stable against periodic enzyme rhythms. If it is, the cell should avoid any fluctuations in the enzyme levels, and enzyme rhythms should only appear as adaptions to external parameter oscillations. If it is not, there is an incentive for some enzyme oscillations, which are then called *self-induced*.

How can optimal periodic adaptions be computed? A static perturbation $\Delta\mathbf{x}$ will move the optimum point $\bar{\mathbf{u}}$ by $\Delta\mathbf{u}^{\mathrm{opt}}$. This is exactly the adaption the enzyme profile needs to follow (Figure 5 (b)). Periodic perturbations can be treated similarly: an external rhythm with amplitude vector $\tilde{\mathbf{x}}$ would shift the fitness optimum by $\tilde{\mathbf{u}}^{\mathrm{opt}}$ in the subspace of amplitude vectors $\tilde{\mathbf{u}}$; this shift describes the enzyme amplitudes and phases required for adaption. If there are no restrictions on the shift vectors, they can be directly computed from maximising Eq. (5) for given $\Delta\mathbf{x}$ and $\tilde{\mathbf{x}}$. The two bracket terms, related to static and periodic shifts, can be treated separately: from $\Delta\mathbf{x}$ follows

[7] Terms containing only $\Delta\mathbf{x}$ and $\tilde{\mathbf{x}}$ have been omitted because they do not matter for choosing the enzyme profiles. Some other terms vanish: terms linear in $\Delta\mathbf{u}$ vanish because of the optimality condition $\mathbf{f}_{\mathrm{u}} = 0$ in the reference state. Linear terms in $\tilde{\mathbf{u}}$ cannot cause time-average shifts. In the second-order approximation, there are no interactions between pairs of perturbations at different frequencies, in particular not between static and periodic perturbations.

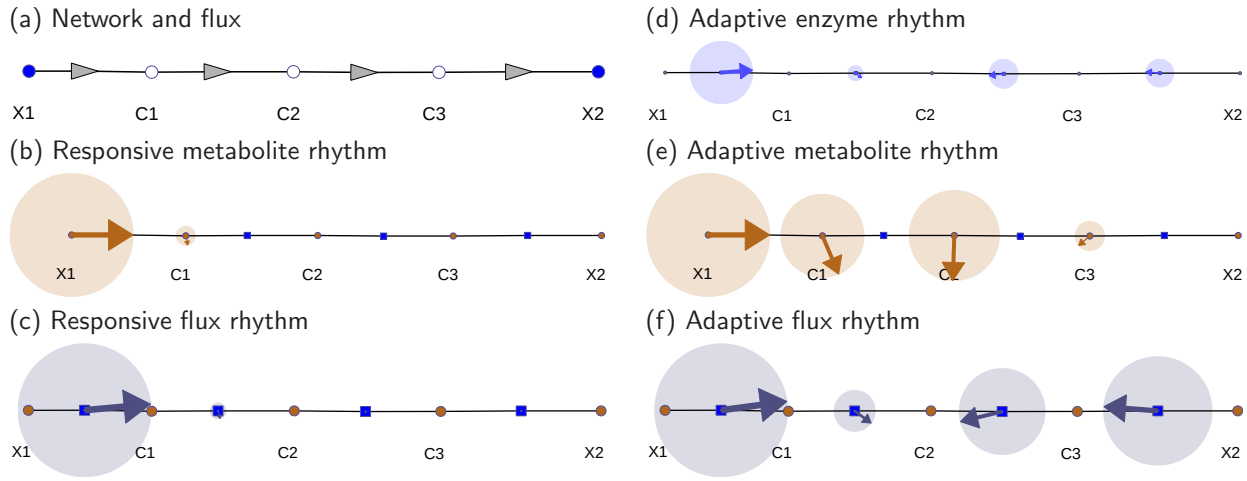

Figure 6: **Enzyme rhythms in a linear pathway.** (a) Network structure (metabolites shown as circles, external metabolites X1 and X2 in blue, flux by arrows). For model details, see SI S5.3. (b) An external substrate rhythm evokes metabolite and flux oscillations, which propagate as damped waves. The panels show different types of variables: (i) external substrate (periodic) and enzyme levels (constant, optimised for static conditions); (ii) internal metabolite levels; (iii) reaction fluxes; (iv) fitness, utility, and investment. (c) Harmonic fluxes, resulting from the external perturbation. (d) Enzyme rhythms optimally adapted to the external perturbation. The first enzyme is approximately in phase with the pathway substrate, the others are phase-shifted according to their position in the pathway. (e) and (f) show the corresponding metabolite and flux rhythms.

$\Delta\mathbf{u}$, and from $\tilde{\mathbf{x}}$ follows $\tilde{\mathbf{u}}$. For a static perturbation $\Delta\mathbf{x}$, the optimal static adaption reads

$$\Delta\mathbf{u}^{\mathrm{opt}} \approx -\mathbf{F}_{\mathrm{uu}}^{-1}\mathbf{F}_{\mathrm{ux}}\,\Delta\mathbf{x} \tag{6}$$

similar to Eq. (4). For a periodic perturbation $\tilde{\mathbf{x}} \neq 0$, the optimal enzyme amplitudes read

$$\tilde{\mathbf{u}}^{\mathrm{opt}} \approx -\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}^{-1}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}\,\tilde{\mathbf{x}}. \tag{7}$$

By inserting $\Delta\mathbf{u}^{\mathrm{opt}}$ or $\tilde{\mathbf{u}}^{\mathrm{opt}}$ into Eq. (5), we obtain the fitness change, for instance $\Delta F = \tilde{\mathbf{u}}^{\mathrm{opt}\,\dagger}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}\,\tilde{\mathbf{x}} = -\tilde{\mathbf{x}}^{\dagger}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}^{\dagger}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}^{-1}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}\tilde{\mathbf{x}}$ for adaptions to a periodic perturbation. However, equations (6) and (7) only apply if the shifts $\bar{u}_l$ and amplitudes $\tilde{u}_l$ are unconstrained. In reality, enzyme shifts and amplitudes are limited in their magnitudes: to prevent enzyme levels from becoming negative, static shifts Δu_l must stay above $-\bar{u}_l$, and amplitudes $|\tilde{u}_l|$ must stay below the mean level $\bar{u}_l + \Delta u_l$. These and other constraints are discussed in SI S3.3. If a solution of Eq. (6) or (7) violates these constraints, the equations are not applicable and have to optimise Eq. (5) numerically under constraints. This problem can be avoided by choosing small perturbation amplitudes $\tilde{\mathbf{x}}$ – unless the reference state contains inactive enzymes (which could become active in the oscillating state). This case and other extensions of the theory are discussed in appendix D.

5 Understanding the shapes of optimal enzyme rhythms

We can now compute optimal enzyme rhythms for given kinetic models, possibly with external rhythms to induce the enzyme rhythms. The formulae are summarised in the appendix, and the resulting workflow for calculation is shown in Figure S2 in SI. We shall now consider the enzyme rhythms arising in some simple example models, and then discuss, again, the logic behind them, and general conclusions that cen be drawn.

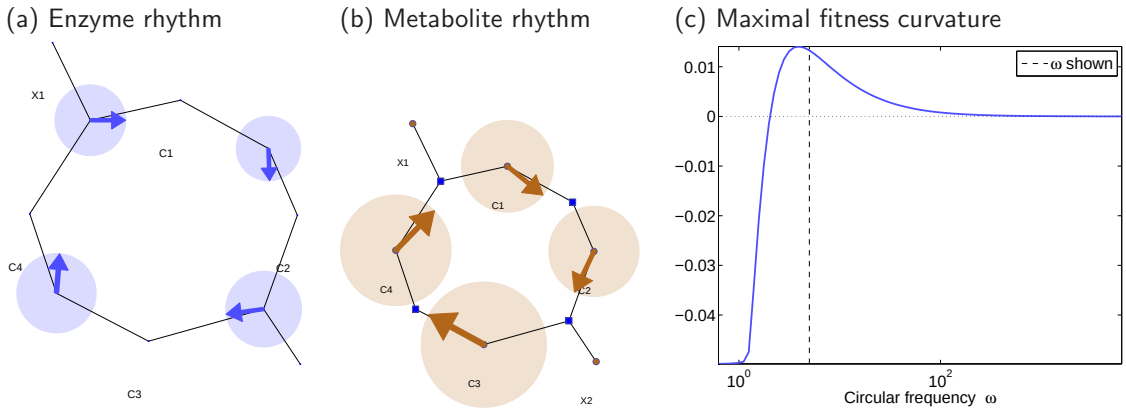

Figure 7: **Self-induced enzyme rhythms in a metabolic loop** (a) Network structure. The reference fluxes follow the loop, with in inflow from metabolite X1 and and outflow to metabolite X2. An optimal self-induced enzyme rhythm is shown by arrows. The enzyme amplitudes are phase-shifted along the circular flux. (b) Corresponding metabolite rhythm. (c) Global fitness synergy (maximal fitness curvature) as a function of the frequency. The synergy is positive for a wide range of frequencies, indicating an incentive for self-induced oscillations, with a maximum at a finite frequency.

First, we study the flux in a simple metabolic pathway in the presence of given external substrate rhythms, similar to Figure 1. To make the enzymes more efficient, they need to be synchronised with their respective reactants, so we can expect waves of activation propagating along the chain. Figure 6 (a) shows the result (for model details, see SI S5.3). In its structure, the pathway resembles the pathways studied in [12, 13], but here we consider harmonic substrate levels instead of a sudden increase. Starting from the optimised reference state, a static substrate increase would induce an increase of all enzyme levels. Harmonic rhythms, without adaption, would evoke a wave of phase-shifted oscillations along the chain (Figure 6 (b)). The fitness can be further increased by adaptive enzyme rhythms with amplitudes and phase shifts determined by Eq. (7)

In the linear pathway, external oscillations induce a wave of enzyme activation. If we close the pathway to form a loop, the enzyme waves can form a closed loop and, thereby, provide benefits without external induction. Figure 7 shows an example. The maximal fitness curvature (also called global fitness synergy), shows a maximum at a positive frequency. This is the frequency at which enzyme rhythms increase the flux in the most cost-efficient way. Much faster or slower oscillations will not be beneficial (although they could still be beneficial as adaptions to external rhythms).

Thus, we can study different networks and predict optimal enzyme rhythms. But what is the general logic behind these patterns? A general theme is the transition from local interactions to global patterns. On the level of metabolic dynamic, the local interplay between metabolite levels and reaction rates leads to propagating waves or steady state changes, which are global. On the level of enzyme allocation, pairwise fitness effects between enzyme rhythms (as expressed by the fitness curvature matrix) lead to a global optimal enzyme pattern. In both cases, it is a matrix inversion that brings us from local to global descriptions (see Figure 8). In the kinetic model, the enzymes, metabolites, and reactions are directly linked by reaction elasticities, which lead to a sparse Jacobian matrix. Their global dependence, via propagating waves, is captured by global periodic response coefficients, which we obtain from the Jacobian by matrix inversion. In the economic calculation, we first determine the pairwise fitness synergies; being based on the response coefficients, they reflect the global metabolic dynamics, but they are still "direct", economically local effects between enzymes. From this, the global economic behaviour can be obtained by Eqs (6) and (7). Again, the step from local to global properties happens by a matrix inversion.

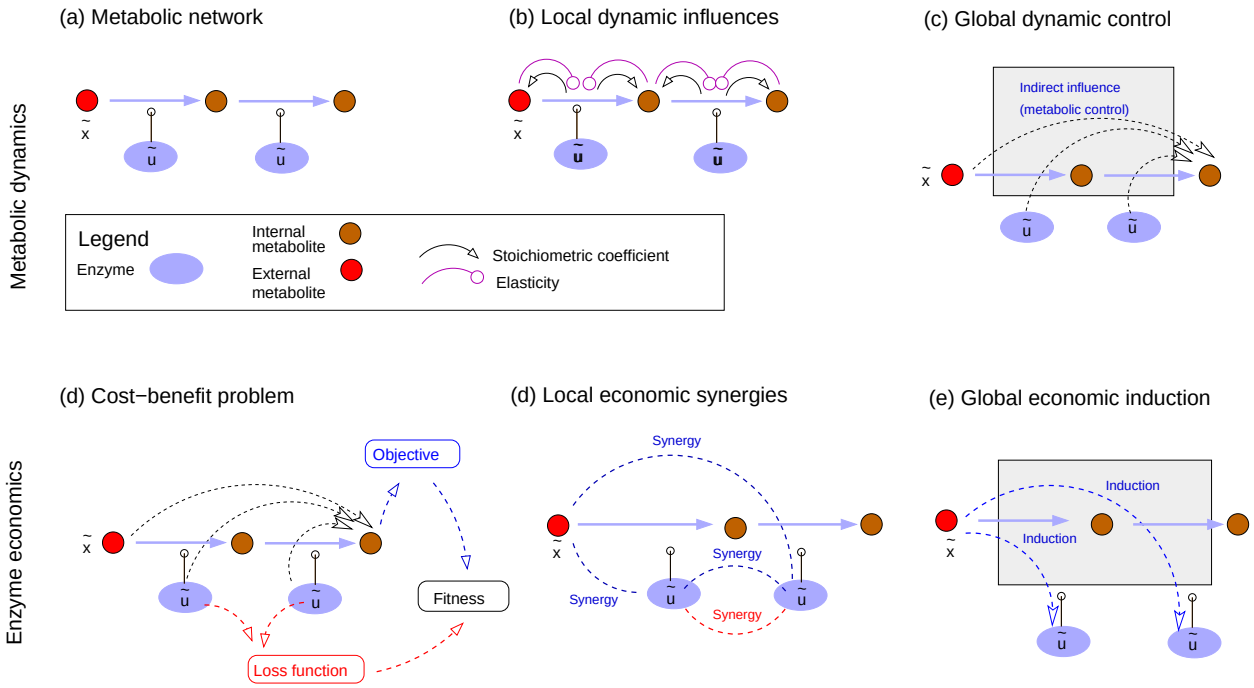

Figure 8: **Dynamics and economics of enzyme rhythms are closely connected.** The pictures show the dynamic and the economic level of the calculations for a schematic example. On both levels, local and global interactions are tightly linked. (a) Metabolic network structure. (b) In metabolic control analysis, elements of the kinetic model (reactions, metabolites, enzymes) are locally connected through elasticities and stoichiometric coefficients. (c) The resulting response coefficients (static or periodic) relate perturbations in external or enzyme levels to the global responses of state variables (metabolite levels and fluxes). (d) In the economic model, enzyme profiles are scored by utility (via state variables) and investment functions. (e) Quadratic expansion around the reference enzyme profile. The fitness function depends on pairwise fitness synergies between all oscillating parameters. (f) Given the optimality principle, external rhythms can *induce* enzyme rhythms, which will be globally orchestrated and reflect model structure, perturbation, and objective function.

To understand orchestrated enzyme rhythms in complex networks, a first step is to consider pairwise synergies between enzymes. Thus, we simply ask: if an enzyme (or an external parameter) oscillates, how will this render other enzyme oscillation more or less profitable? Such synergies can have many possible reasons (most of them arise from how enzymes, together, affect metabolic dynamics). The following three are particularly intuitive: (i) *Supply coupling*. A rhythm in one enzyme creates time windows in which other processes (to be driven by other enzymes) are favourable. For instance, these other enzymes could become more efficient in these phases because their substrates are abundant or because the reactions show large thermodynamic driving forces. (ii) *Avoidance coupling*. An enzyme rhythm creates time windows in which certain other processes should be avoided (for instance, phases in which reactive oxygen species are abundant and make DNA replication problematic in this moment). (iii) *Cost coupling*. If the investment function penalises the sum of all enzyme levels more than linearly, the upregulation of one enzyme creates an incentive to downregulate other enzymes in the same moment. Thus, an existing enzyme rhythm induces other rhythms with opposite phases.

If a reaction is embedded in a metabolic pathway, an enzyme rhythm in this reaction will affect the reactant levels and change their phase relations. On the contrary, an enzyme rhythm can also exploit existing reactant rhythms by allosynchrony. Now we may ask: in a static environment, could a spontaneous enzyme rhythm cause reactant oscillations and exploit the very same oscillations to work more efficiently? A counterexample is shown in Figure 2 and explained in appendix S5.1: for an oscillating enzyme, we obtain two phase relations, one between the enzyme and the evoked reactant rhythms, and another one between reactant rhythms and the optimally adapted

enzyme rhythm that would follow from them. If both phase relations match, a spontaneous rhythms would be profitable. In the example in Figure 2, this is not the case: quite the contrary, when the enzyme is most active, the substrate is depleted and the product accumulates, which slows down the reaction exactly when the enzyme is abundant. Thus, the enzyme rhythm effectively inhibits the enzyme by allosynchrony.

6 Discussion

In contrast to purely mechanistic model, they do not describe how processes take place in reality, but how they *should* take place to reach some objective. Here I applied this economic (or functional, or teleological) approach to periodic behaviour, asking how biochemical processes should vary during the cell cycle, between day and night, or during a year. Should organisms in periodic environments maintain homoeostasis, or rather use external changes as an opportunity to run different biochemical processes at different times? How should biochemical processes be synchronised or separated in time, and how should they follow each other in metabolic cycles? To pose such questions mathematically, I formulated them as optimal control problem for kinetic metabolic models. One could either optimise the regulation systems that create oscillations or the enzyme profiles as such: in the optimisation, enzyme profiles are treated as control variables, external influences are treated as perturbation parameters, and both together shape a dynamic metabolic state, which then determines the fitness.

Natural rhythms are often portrayed as an alternation between discrete states, like sleeping and being awake, discrete phases of the cell cycle, or the temporal compartments proposed in [2, 6]). Some real metabolic changes may be smooth variations rather than abrupt jumps, in particular if the perturbations have been dampened on their way through the metabolic network. To describe this situation, rhythms were here constructed by adding smooth small-amplitude oscillations to a stationary reference state. Cosine waves are well suited because they are eigenfunctions of the time-shift operator and basis functions of the Fourier transformation, allowing us to approximate other dynamic changes by Fourier synthesis [14].

The formalism does not assume a specific objective function, but works for a wide range of possible fitness functions to be chosen by the modeller. What would be suitable choices when describing real cells[8]?

When a fitness function for single metabolic states has been chosen, one needs to apply it to time courses. I considered two types of fitness functionals, which represent contrary assumptions about time scales: in the *fluctuation-insensitive fitness*, the original fitness function is applied to the time-averaged metabolic state, so enzymes can control the fitness by shifting the mean values. In the *fluctuation-sensitive fitness*, the fitness is evaluated in every moment in time and then averaged; this makes it sensitive to temporal variation (unless the fitness function is linear). If linear enzyme investment functions or fluctuation-insensitive costs are used, enzyme rhythms are cost-neutral and fitness changes are solely determined by the metabolic performance.

Generally, it seems that temporal enzyme profiles, in their expression patterns across the metabolic network or in their phase shifts, "portray" the metabolic network structure (and the sequences of biochemical processes) . If such portrayal exists, we may ask if it can be explained by optimality principles. As an analogy, we may think of our visual system. Visual perception is particularly well suited in processing visual patterns like moving edges – exactly those that allow us to recognise objects in visual scenes. This may be a result of evolution or of adaptions during development. Thus, our capacities for recognition reflect the statistics, and possibly the relevance, of natural visual stimuli [9].

[8]

[9] A similar notion of reflection is expressed in J.W. Goethe's poem: "Wär nicht das Auge sonnenhaft, die Sonne könnt es nie erblicken; Läg nicht in uns des Gottes eigne Kraft, wie könnt uns Göttliches entzücken?". (Were not the eye sunlike, it could never see the sun; were not within us God's own force, how could we delight in anything divine?).

In metabolism, the expression profiles of enzymes show specific patterns both in time and across the network. In [13, 19], metabolic enzymes and complex-forming proteins were found to be expressed "on demand" and reflecting their order of usage. Similar cases of reflection are predicted for metabolic models: specifically, optimal enzyme profiles are related to metabolic control and response coefficients, which describe the enzyme's effects on the metabolic objective. For an optimisation of static enzyme levels, Klipp and Heinrich [20] showed that the optimal levels are proportional to their scaled control coefficients, that is, to their influence on the metabolic fluxes. Likewise, static enzyme patterns, again predicted by optimality, reflect static fitness synergies [10]: enzymes that strongly control the fitness and enzymes for which differential expression is cheap should show large adaptive changes. Here, we observed the same thing for periodic adaptions: optimal enzyme rhythms show coordinated patterns and, like in the computer experiments in [12] and [13], their order matches the way perturbations propagate along the pathway.

The optimality-based prediction of periodic behavior could apply to other biological phenomena, possibly with medical applications. On the one hand, one may study physiological rhythms like the day-night rhythm in the human body. Studies in terms of biological function, not just of mechanisms, could show how rhythmic adaptive processes, like glucose management and other tasks in the liver, should be ideally organised in the day-night cycle and what constrains them.

On the other hand, one may use the same optimisation approach to devise schemes for administration of drugs. In the models, drug dosages, instead of enzyme levels, would be the control variables. The mechanistic part of the model may cover pharmacokinetics and pharmacodynamics, i.e., the distribution and effects of drugs in the body. Bringing together physiological rhythms and the effects of drugs, one could determine optimal phases of dosing within existing rhythms, or combination therapies with phase-shifted dosages, which may exploit allosynchronic synergisms or antagonisms – for instance, using one drug to make cells more susceptible to the other, with a phase delay.

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A Dynamics of forced oscillations in biochemical networks

To see the possible benefits of enzyme rhythms, we need to study how enzymes affect the metabolic state, that is, how periodic perturbations propagate in metabolic networks. I recall some formulae from [10] and [14]. A

metabolic system is described by a system of rate equations $\mathrm{d}\mathbf{c}/\mathrm{d}t = \mathbf{N}\,\mathbf{r}(\mathbf{c},\mathbf{p})$ with a concentration vector $\mathbf{c}$, the stoichiometric matrix $\mathbf{N}$ for internal metabolites, and rate laws $v_l(\mathbf{c},\mathbf{p})$ forming the flux vector $\mathbf{v} = \mathbf{v}(\mathbf{c},\mathbf{p})$. To describe metabolite dilution in growing cells, we can subtract $\mathbf{v}^{\mathrm{dil}} = \kappa\,\mathbf{c}$ on the right, where κ is the cell growth rate. The vector $\mathbf{p}$ contains model parameters like external concentrations (subvector $\mathbf{x}$) and enzyme levels (subvector $\mathbf{u}$). For a reference state, we choose a stable steady state, with a concentration vector $\mathbf{s}$ satisfying the stationarity condition $0 = \mathbf{N}\,\mathbf{v}(\mathbf{s},\mathbf{p})$. A solution $\mathbf{s}(\mathbf{p})$ must exist for all parameter sets $\mathbf{p}$ in a region around our reference parameter set $\mathbf{p}_0$. Now we consider a periodically changing parameter vector

$$\mathbf{p}(t) \quad = \quad \mathbf{p}_0 + \mathrm{Re}(\tilde{\mathbf{p}}\ \mathrm{e}^{i\omega t}) \tag{8}$$

with a complex amplitude vector $\tilde{\mathbf{p}}$ and circular frequency ω. The perturbations cause waves of concentration and flux changes, which propagate through the network. In linear systems, the concentration and flux oscillations will by harmonic with frequency ω. In nonlinear systems, they contain higher harmonics and their time averages can deviate from the reference state. Such nonlinear effects can be studied by second-order perturbation theory [14] (for an overview, see Figure 3). Here, we only consider the time-average shifts (of metabolite concentrations, fluxes, and metabolic fitness) because only these are scored by the fitness functionals.

The direct effects of reactant and enzyme rhythms on the reaction rates are described by spectral elasticities [14]. Since the enzyme levels appear as prefactors in the rate laws $v_l = u_l\,r_l(\mathbf{c})$, enzyme rhythms alone, at constant reactant levels, cannot shift the average flux, nor evoke higher harmonics. However, reactant rhythms, possibly in combination with enzyme rhyth,s, can have such effects (see Figure S1). Consider two varying parameters

$$a(t) = \bar{a} + \mathrm{Re}(\tilde{a}\ \mathrm{e}^{i\omega t}), \qquad b(t) = \bar{b} + \mathrm{Re}(\tilde{b}\ \mathrm{e}^{i\omega t}) \tag{9}$$

with complex amplitudes $\tilde{a}$ and $\tilde{b}$ and equal frequency ω. If the phase shift $\varphi(\tilde{a}) - \varphi(\tilde{b})$ is positive, a peaks before b. For small-amplitude perturbations, the perturbed reaction rate reads

$$v(t) \approx \bar{v} + E_a\,\Delta a(t) + E_b\,\Delta b(t) + \frac{1}{2}E_{aa}\,\Delta a(t)^2 + E_{ab}\,\Delta a(t)\,\Delta b(t) + \frac{1}{2}E_{bb}\,\Delta b(t)^2 \tag{10}$$

with the reference rate $\bar{v}$ and reaction elasticities E_a, E_b, E_{aa}, E_{ab}, and E_{bb}. The average shift caused by harmonic perturbations reads

$$\Delta\langle v\rangle_t \approx \frac{1}{2}E_{\tilde{a}\tilde{a}}\,|\tilde{a}|^2 + E_{\tilde{a}\tilde{b}}\,|\tilde{a}|\,|\tilde{b}|\cos(\Delta\varphi) + \frac{1}{2}E_{\tilde{b}\tilde{b}}\,|\tilde{b}|^2 \tag{11}$$

with periodic second-order elasticities $E_{\tilde{a}\tilde{a}} = \frac{1}{2}E_{aa}$, $E_{\tilde{a}\tilde{b}} = \frac{1}{2}E_{ab}$, and $E_{\tilde{b}\tilde{b}} = \frac{1}{2}E_{bb}$. If parameter a represents an enzyme level u and parameters b a reactant level x, we can set $E_{\tilde{a}\tilde{a}} = E_{\tilde{u}\tilde{u}} = 0$, $E_{\tilde{a}\tilde{b}} = E_{\tilde{u}\tilde{x}} = \frac{1}{2\,u}E_x$, and $E_{\tilde{b}\tilde{b}} = \frac{1}{2}E_{xx}$. Thus, the shift term depending on the enzyme level reads

$$\Delta\langle v\rangle_t \approx \frac{E_{\tilde{u}\tilde{x}}}{2}\,|\tilde{u}|\,|\tilde{x}|\cos(\Delta\varphi) = \frac{E_x}{2\,u}\,|\tilde{u}|\,|\tilde{x}|\cos(\Delta\varphi) \tag{12}$$

similar to Eq. (3) (with $E_{\tilde{u}\tilde{x}}$ replacing the prefactor $k/2$).

We saw that reactant and enzyme rhythms in a single reaction have synergistic effects on the average flux and that their coupling can be described by second-order spectral elasticities. . This observation can be extended to complex metabolic networks, in which internal rhythms are evoked by rhythms of enzymes and external metabolites. Predefined enzyme and external rhythms are linked to the resulting flux and concentration rhythms by spectral response coefficients. For two perturbation parameters $a(t)$ and $b(t)$, the second-order expansion of a state variable y (metabolic flux or internal concentration) yields a sum of three effects: forced oscillations with frequencies ω

and 2ω, and a shift of the average value (frequency 0). Accordingly, the shift (compare Eq. (14))

$$\Delta\langle y\rangle \;\approx\; \frac{1}{2}\, R^{\mathrm{y}}_{\tilde{\mathrm{a}}\tilde{\mathrm{a}}}(\omega)\, \tilde{a}^2 + \frac{1}{2} R^{\mathrm{y}}_{\tilde{\mathrm{b}}\tilde{\mathrm{b}}}(\omega)\, \tilde{b}^2 + \frac{1}{2}\mathrm{Re}(\mathrm{e}^{-i\Delta\varphi} R^{\mathrm{y}}_{\tilde{\mathrm{a}}\tilde{\mathrm{b}}}(\omega))\, |\tilde{a}||\tilde{b}| \tag{13}$$

contains three terms: the first two represent self-synergies of a and of b, for instance, a dynamic self-inhibition. The third term describes a synergy between both parameters, with magnitude and sign depending on their phase shift $\Delta\varphi$. Since Eq. (13) contains no first-order term, the reference state (where $\tilde{a} = \tilde{b} = 0$) is a local extremum of the shift $\Delta\langle y\rangle$; it can be a minimum, a maximum, or a saddle point. In the second-order approximation, average shifts can only arise from parameters oscillating at the same frequency. Let us now consider a system with many periodic parameters. Summing over their pairwise synergies – each described by Eq. (13) – yields the total time-average shift

$$\Delta\langle y\rangle \;\approx\; \frac{1}{2}\, \tilde{\mathbf{p}}^{\dagger}\, \mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega)\, \tilde{\mathbf{p}}. \tag{14}$$

The row vector $\tilde{\mathbf{p}}^{\dagger}$ is the adjoint (i.e., the complex conjugate transpose) of $\tilde{\mathbf{p}}$. The matrix $\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}$ is Hermitian, depends on ω, and can be computed from the stoichiometric matrix $\mathbf{N}$ and the elasticities (for details, see SI S2). Its elements, the second-order periodic response coefficients defined in SI S2.3, resemble the second-order spectral control coefficients from [14] except for a scaling factor. At high frequencies, the matrix approaches $\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{p}}\tilde{\mathrm{p}}}(\omega) \approx \frac{1}{2}\, \mathbf{C}^{\mathrm{y}}\, \mathbf{E}_{\mathrm{pp}}$ where $\mathbf{C}^{\mathrm{y}}$ is the static control matrix for y [14] and $\mathbf{E}_{\mathrm{pp}}$ the second-order elasticity tensor (Eq. (S19) in SI). Since there are no second-order elasticities between enzyme levels ($\mathbf{E}_{\mathrm{uu}} = \mathbf{0}$), the second-order response matrix $\mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}(\omega)$ (called synergy matrix), for frequencies $\omega \to \infty$, will vanish as well. This means that high-frequency enzyme rhythms, in the absence of other periodic parameters, have little effect on the average concentrations and fluxes.

B Metabolic fitness function

Assuming that the enzyme profile $\mathbf{u}$ is scored by a fitness function

$$f(\mathbf{u}) = z(\mathbf{y}(\mathbf{u})) - h(\mathbf{u}) \tag{15}$$

with a metabolic objective $z(\mathbf{y})$ scoring the fluxes and concentration (collected in a vector $\mathbf{y}$) and a investment term $h(\mathbf{u})$ scoring the enzyme levels [10]. The control variably vector $\mathbf{u}$ can also contain other parameters like the dilution rate. If the model describes a metabolic pathway, the functions $z(\mathbf{y})$ and $h(\mathbf{u})$ can capture the pathway's effective fitness contributions within the surrounding network; their choice depends on the modeller's ideas and the purpose of the model. The cost of protein expression has been studied experimentally in [11] and [21], and the observed cost functions – defined by growth rate deficits caused by artificial expression of idle proteins – show positive slopes and curvatures. In models, the curvatures may also be allowed to vanish. Metabolic performance and enzyme investment can be expanded to second order

$$\begin{aligned} z(\mathbf{y} + \Delta\mathbf{y}) &\approx z(\mathbf{y}) + \mathbf{z}_{\mathrm{y}} \cdot \Delta\mathbf{y} + \frac{1}{2}\Delta\mathbf{y}^{\mathrm{T}}\mathbf{Z}_{\mathrm{yy}}\Delta\mathbf{y} \\ h(\mathbf{u} + \Delta\mathbf{u}) &\approx h(\mathbf{u}) + \mathbf{h}_{\mathrm{x}} \cdot \Delta\mathbf{u} + \frac{1}{2}\Delta\mathbf{u}^{\mathrm{T}}\mathbf{H}_{\mathrm{uu}}\Delta\mathbf{u}, \end{aligned} \tag{16}$$

where $\mathbf{z}_\mathrm{y}$, $\mathbf{Z}_\mathrm{yy}$, $\mathbf{h}_\mathrm{x}$, and $\mathbf{H}_\mathrm{uu}$ are the gradients and curvature matrices of the functions z and h. For a metabolic system with external parameters x_j (e.g. external concentrations), the steady-state fitness reads

$$f(\mathbf{u},\mathbf{x}) = z(\mathbf{y}(\mathbf{u},\mathbf{x})) - h(\mathbf{u}). \tag{17}$$

With this function, we can formulate an optimal-control problem for $\mathbf{u}$: given external parameters x_j, the control parameters u_l must be chosen to locally maximise the fitness $f(\mathbf{u},\mathbf{x})$. The resulting state is called enzyme-optimal. If all inactive reactions are omitted from the network, the fitness gradient $f_\mathrm{u} = (\partial f/\partial u_l)$ will vanish and the fitness curvature matrix $\mathbf{F}_\mathrm{uu}$ must have negative eigenvalues. The fitness curvature matrices read

$$\begin{aligned} \mathbf{F}_\mathrm{ux} &= \mathbf{z}_\mathrm{y}^\mathrm{T}\ \mathbf{R}^\mathrm{y}_\mathrm{xu} + \mathbf{R}^{\mathrm{y}\,\mathrm{T}}_\mathrm{x}\mathbf{Z}_\mathrm{yy}\mathbf{R}^\mathrm{y}_\mathrm{u} \\ \mathbf{F}_\mathrm{uu} &= \mathbf{z}_\mathrm{y}^\mathrm{T}\ \mathbf{R}^\mathrm{y}_\mathrm{xx} + \mathbf{R}^{\mathrm{y}\,\mathrm{T}}_\mathrm{x}\mathbf{Z}_\mathrm{yy}\mathbf{R}^\mathrm{y}_\mathrm{x} - \mathbf{H}_\mathrm{uu}. \end{aligned} \tag{18}$$

Now we return to periodic perturbations. We allow all parameters to oscillate around their reference values at a circular frequency ω:

$$\begin{aligned} \mathbf{x}(t) &= \bar{\mathbf{x}} + \mathrm{Re}[\tilde{\mathbf{x}}\ \mathrm{e}^{i\omega t}] \\ \mathbf{u}(t) &= \bar{\mathbf{u}} + \mathrm{Re}[\tilde{\mathbf{u}}\ \mathrm{e}^{i\omega t}]. \end{aligned} \tag{19}$$

This may lead to average shifts of a state variable y, and its time average can be expanded, with Eq. (13), as

$$\langle y\rangle \approx y(\bar{\mathbf{x}},\bar{\mathbf{u}}) + \frac{1}{2}\begin{pmatrix}\tilde{\mathbf{x}}\\ \tilde{\mathbf{u}}\end{pmatrix}^\dagger \begin{pmatrix} \mathbf{R}^\mathrm{y}_{\tilde{\mathrm{x}}\tilde{\mathrm{x}}}(\omega) & \mathbf{R}^\mathrm{y}_{\tilde{\mathrm{x}}\tilde{\mathrm{u}}}(\omega) \\ \mathbf{R}^\mathrm{y}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}(\omega) & \mathbf{R}^\mathrm{y}_{\tilde{\mathrm{x}}\tilde{\mathrm{u}}}(\omega) \end{pmatrix} \begin{pmatrix}\tilde{\mathbf{x}}\\ \tilde{\mathbf{u}}\end{pmatrix}. \tag{20}$$

To score a dynamic enzyme profile $\mathbf{y}(t)$ by its fitness, we assess time averages over an oscillation period and consider two possibilities[10]: In the *fluctuation-insensitive fitness functional*, we apply the fitness function (15) to time-averaged state variables:

$$F^\mathrm{ins} = z(\langle\mathbf{y}(t)\rangle_t) - h(\langle\mathbf{u}(t)\rangle_t). \tag{21}$$

In the *fluctuation-sensitive fitness functional*, we evaluate the fitness function in each moment and take the average:

$$F^\mathrm{sen} = \langle g(\mathbf{y}(t)) - h(\mathbf{u}(t))\rangle_t. \tag{22}$$

The two fitness functionals lead to different fitness landscapes and different optimal enzyme profiles. In both cases, a fitness change caused by periodic perturbations can be expanded as a quadratic function of the amplitude vectors $\tilde{\mathbf{x}}$ and $\tilde{\mathbf{u}}$. From the periodic response coefficients and the fitness derivatives, we obtain the curvature matrices. For a fluctuation-insensitive fitness Eq. (21), they read

$$\begin{aligned} \mathbf{F}^\mathrm{ins}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}} &= \mathbf{z}_\mathrm{y}^\mathrm{T}\ \mathbf{R}^\mathrm{y}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}(\omega) \\ \mathbf{F}^\mathrm{ins}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}} &= \mathbf{z}_\mathrm{y}^\mathrm{T}\ \mathbf{R}^\mathrm{y}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}(\omega). \end{aligned} \tag{23}$$

[10] Both functionals can be seen as special cases of a functional in which the curves of metabolite state variables are convoluted with a kernel function and then the fitness function is applied and averaged over time. With such functionals, the fitness would not be realised immediately or after an infinite time, but on a time scale defined by the kernel function.

For the fluctuation-sensitive fitness Eq. (22), they contain additional terms:

$$\begin{aligned}\mathbf{F}^{\mathrm{sen}}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}} &= \mathbf{z}^{\mathrm{T}}_{\mathrm{y}}\ \mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}(\omega) + \frac{1}{2}\left(\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{u}}}(\omega)\right)^{\dagger}\mathbf{Z}_{\mathrm{yy}}\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{x}}}(\omega) \\ \mathbf{F}^{\mathrm{sen}}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}} &= \mathbf{z}^{\mathrm{T}}_{\mathrm{y}}\ \mathbf{R}^{\mathrm{y}}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}(\omega) + \frac{1}{2}\left(\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{u}}}(\omega)\right)^{\dagger}\mathbf{Z}_{\mathrm{yy}}\mathbf{R}^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{u}}}(\omega) - \frac{1}{2}\mathbf{H}_{\mathrm{uu}}. \end{aligned} \tag{24}$$

For slow oscillations ($\omega \approx 0$), we can approximate $R^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{x}}} \approx R^{\mathrm{y}}_{\mathrm{x}}$, $R^{\tilde{\mathrm{y}}}_{\tilde{\mathrm{u}}} \approx R^{\mathrm{y}}_{\mathrm{u}}$, $R^{\mathrm{y}}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}} \approx \frac{1}{2}R^{\mathrm{y}}_{\mathrm{uu}}$, and $R^{\mathrm{y}}_{\tilde{\mathrm{x}}\tilde{\mathrm{u}}} \approx \frac{1}{2}R^{\mathrm{y}}_{\mathrm{xu}}$, and Eq. (24) yields the fitness curvature matrices Eq. (18) for the static case, multiplied by $1/2$. The factor $1/2$ appears because the static curvature matrices refer to a constant perturbation, while the periodic curvature matrices in Eq. (24), even in the limit $\omega \to 0$, refer to a mixture of (positive and negative) perturbations, realising only $\sqrt{1/2}$ of their maximal amplitude on average.

C Optimal enzyme profiles

The expansion in Eq. (5) describes how the fitness is affected by small variations of enzyme levels and external parameters, which can be static or periodic. The central terms in this formula are the fitness curvature matrices given by Eqs (23) and (24), which describe self-synergies or pairwise synergies between enzymes and perturbation parameters. Knowing these matrices, we can predict enzyme profiles for different scenarios.

1. **Criterion for optimal steady state.** Given constant external parameters x_j, a constant enzyme profile with u_l will represent an *internal optimum* if they are strictly positive, the fitness gradient $\mathbf{f}_{\mathrm{u}}(\mathbf{x},\mathbf{u})$ vanishes, and the curvature matrix $\mathbf{F}_{\mathrm{uu}}(\mathbf{x},\mathbf{u})$ is negative definite (that is, all its eigenvalues are negative). Such a pair $(\mathbf{x},\mathbf{u})$ is called an enzyme-balanced parameter set. In contrast, the enzyme profile represents a *boundary optimum* (or an "incompletely enzyme-balanced state") if some of the enzyme levels vanish and the corresponding fitness slopes $\partial f/\partial u_l$ are negative, while all other reactions are enzyme-balanced.

2. **Optimal adaption to static external perturbations.** Let $(\bar{\mathbf{x}},\bar{\mathbf{u}})$ be an enzyme-balanced parameter set and $\Delta\mathbf{x}$ some static perturbation of $\bar{\mathbf{x}}$. A static enzyme change $\Delta\mathbf{u}^{\mathrm{opt}}$ is called an optimal adaption to $\Delta\mathbf{x}$ if the new parameter set $(\bar{\mathbf{x}}+\Delta\mathbf{x}, \bar{\mathbf{u}}+\Delta\mathbf{u}^{\mathrm{opt}})$ is optimal again. For small changes $\Delta\mathbf{x}$, the optimal adaption can be approximated by [10]

$$\Delta\mathbf{u}^{\mathrm{opt}}(\Delta\mathbf{x}) \approx \mathbf{A}^{\mathrm{u}}_{\mathrm{x}}\ \Delta\mathbf{x} \tag{25}$$

   with the adaption matrix $\mathbf{A}^{\mathrm{u}}_{\mathrm{x}} = -\mathbf{F}^{-1}_{\mathrm{uu}}\mathbf{F}_{\mathrm{ux}}$ and the curvature matrices $\mathbf{F}_{\mathrm{uu}}$ and $\mathbf{F}_{\mathrm{ux}}$ given by Eq. (18). If the optimla state was enzyme optimal, the fitness matrix $\mathbf{F}_{\mathrm{uu}}$ is invertible. The formula can be derived easily: the fitness gradient must vanish before the perturbation ($\mathbf{f}_{\mathrm{u}}(\bar{\mathbf{x}},\bar{\mathbf{u}}) = 0$) and after the adaption ($\mathbf{f}_{\mathrm{u}}(\bar{\mathbf{x}}+\Delta\bar{\mathbf{x}}, \bar{\mathbf{u}}+\Delta\mathbf{u}) = 0$). Expanding these equations to first order and setting their difference to zero, we obtain $\Delta f_{\mathrm{u}} = \mathbf{F}_{\mathrm{uu}}\Delta\mathbf{u} + \mathbf{F}_{\mathrm{ux}}\Delta\mathbf{x} = 0$, and solving for $\Delta\mathbf{u}$ yields Eq. (25).

3. **Optimal adaption to external oscillations.** Given harmonic external perturbations, optimal periodic enzyme levels can be computed accordingly by

$$\tilde{\mathbf{u}}^{\mathrm{opt}}(\tilde{\mathbf{x}}) \approx \tilde{\mathbf{A}}^{\mathrm{u}}_{\mathrm{x}}\ \tilde{\mathbf{x}}. \tag{26}$$

   The periodic adaption matrix $\tilde{\mathbf{A}}^{\mathrm{u}}_{\mathrm{x}} = -\mathbf{F}^{-1}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{x}}}$ is derived from the fitness curvature matrices for periodic perturbations.

4. **Utile and self-induced oscillations.** In the absence of external perturbations, the fitness change caused by an enzyme rhythm $\tilde{\mathbf{u}}$ depends on the periodic fitness synergy matrix $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$:

$$\Delta F \approx \frac{1}{2}\,\tilde{\mathbf{u}}^{\dagger}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}\,\tilde{\mathbf{u}}. \tag{27}$$

Any vectors $\tilde{\mathbf{u}}$ with a positive fitness change ΔF, and in particular any eigenvector of $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$ with a positive eigenvalue, describes a profitable enzyme rhythm. Since $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$ is Hermitian, its eigenvalues are real-valued and its eigenvectors span the space of all enzyme amplitude vectors. If it has positive eigenvalues and if potential enzyme rhythms $\tilde{\mathbf{u}}$ are restricted to a fixed norm $||\tilde{\mathbf{u}}||$, a maximal fitness increase is provided by the eigenvector $\tilde{\mathbf{u}}^{\mathrm{opt}}$ with the largest eigenvalue (called global fitness synergy $\sigma(\omega)$). The relative amplitudes and phases of enzymes follow from the elements of $\tilde{\mathbf{u}}^{\mathrm{opt}}$. For enzyme rhythms to appear spontaneously, the reference state must be optimal with respect to static enzyme changes, but not with respect to certain enzyme rhythms. In other words, all eigenvalues of $\mathbf{F}_{\mathrm{uu}}$ must be negative, but at least one eigenvalue of $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}(\omega)$ for some frequency $\omega \neq 0$ must be positive. If utile enzyme rhythms exist at different frequencies, the most utile one has be the best chances to be selected for in evolution. To be selected for, an enzyme rhythms must provide a higher fitness gain than other similar rhythms, in particular those at frequency $\omega = 0$. its fitness gain must not only be positive, but also show a local maximum at a finite frequency. If the maximum is at $\omega = 0$, there would be no selection advantage to actual oscillations.

D Extending the theory

The cost-benefit analysis of enzyme rhythms can be extended in several ways:

1. **Constraints on other variables.** In the optimisation of $\Delta\mathbf{u}$ and $\tilde{\mathbf{u}}$ by Eq. (5), the concentrations, fluxes, the total enzyme level, or time averages of these quantities can be restricted by constraints. Positivity constraints on the enzyme levels are mandatory. Soft constraints could be implemented by additional terms in the fitness function.

2. **Loss by non-optimal enzyme profiles.** Even if cells do not realise optimal enzyme profiles, we may still use models to quantify their fitness losses. As shown in Figure 5 (c), wrong phases and amplitudes can lead to fitness losses, and phase shifts deviating by more than $\pi/2$ make a profitable enzyme rhythm costly. The fitness loss can be computed by Eq. (5): if the amplitude vector $\tilde{\mathbf{u}}$ deviates from the optimal vector $\tilde{\mathbf{u}}^{\mathrm{opt}}$ by $\tilde{\mathbf{u}}^{\mathrm{mis}} = \tilde{\mathbf{u}} - \tilde{\mathbf{u}}^{\mathrm{opt}}$, the fitness change can be approximated by

$$\Delta F^{\mathrm{mis}} \approx \mathrm{Re}[\tilde{\mathbf{x}}^{\dagger}\,\mathbf{F}_{\tilde{\mathrm{x}}\tilde{\mathrm{u}}}\,\tilde{\mathbf{u}}^{\mathrm{mis}} + \tilde{\mathbf{u}}_{\mathrm{opt}}^{\dagger}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}\tilde{\mathbf{u}}^{\mathrm{mis}} + \tilde{\mathbf{u}}^{*\dagger}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}\tilde{\mathbf{u}}^{\mathrm{mis}}] = \mathrm{Re}[\tilde{\mathbf{u}}^{*\dagger}\,\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}\tilde{\mathbf{u}}^{\mathrm{mis}}]. \tag{28}$$

If the reference state does not allow for self-induced enzyme rhythms (negative definite curvature matrix $\mathbf{F}_{\tilde{\mathrm{u}}\tilde{\mathrm{u}}}$), Eq. (28) will predict a fitness loss.

3. **Other control variables.** Describing the metabolite levels as state variables and enzyme levels as control variables is, of course, a bit arbitrary. One could also describe enzymes mechanistically, whereas mRNA species, transcription factors, or drug dosages affecting the system could be control variables. Another possible control variable is the cell growth rate (which enters the metabolic model in the form of dilution). After optimising stationary enzyme levels and dilution rate (for instance, with the biomass production rate as the objective), one could test how these variables should be adapted together to a periodic demand, e.g. during the cell cycles. Since growth relies on metabolism (e.g., on production of membrane components),

but also affects metabolism via dilution, a joint periodic variation in growth and enzyme levels (e.g. during the cell cycle) could shift metabolite levels, redirect fluxes, and change the average enzyme levels needed.

4. **Boundary optima: enzymes that only become active during oscillations** Finally, we consider a case that we have excluded so far: enzymes that are inactive in the reference state but active under oscillations – in both cases following the optimality principle. In order to start oscillating, the enzyme must move from a zero level to a positive average level. This means that amplitudes and average level are now coupled not only at large oscillation amplitudes, but as soon as the oscillations begin. Thus, it is not possible anymore to treat static and periodic adaptions separately, and Eqs (6) and (7) do not even apply under very small perturbations. Instead, Eq. (5) must be maximised numerically under constraints.

   In our second-order approximation of the fitness functional, enzyme rhythms of different frequencies are completely independent, that is, they show no synergies in relation on time-average fluxes, metabolite concentrations, or fitness terms. Therefore, it seems that they can be treated separately. However, enzyme rhythms can always become coupled by amplitude constraints. This happens, in particular, in cases where the reference state contains inactive enzymes. In such cases, the reference state may be economically unstable with a first-order benefit to oscillations. An example model (demonstrating the use of storage metabolites) is described in SI S5.5.

5. **Frequency-dependent costs.** Experiments show that the cost of a sudden articifical protein expression (measured by growth deficits) is first high, but decreases after some hours; the reason may be a slow adaption of ribosome numbers [21]. If such cost adaptions happen generally, the average cost of enzymes should not only depend on the momentary enzyme level, but additionally increase with the frequency of enzyme oscillations. In metabolic cycles, the changes could possibly be anticipated, so the frequency-dependent costs may be smaller than expected from th ecost after sudden perturbations.

6. **Periodic fitness functions** We assumed that model and fitness function are not explicitly time-dependent and that time-dependencies arise only via perturbation parameters. Instead, fitness functions could vary periodically. This would allow us to model different enzyme costs or metabolic demands at different times of the day. Time-dependent fitness functions would break the time-shift invariance of the model (which before was only broken by external perturbations) and would yield new terms in the fitness expansion, including a first-order fitness contribution by periodic enzyme levels and a mixed second-order term between enzyme shifts and enzyme amplitudes. In addition, the formulae for the other terms will change. Periodic fitness functions arise naturally if our pathways are embedded in larger systems. If our pathway supplies material to the surrounding system (a system scored by a constant fitness function, but subject to periodic perturbations), and if we describe this by an effective fitness function for our pathway, the new fitness function will have an explicit periodic time dependence. Thus, periodic fitness functions are even necessary to make our formalism able to handle a subdivision of models (invariance against model modularisation).

7. **Oscillations involving covalent modification and allosteric regulation**. Each enzyme rhythms has a maximal possible amplitude depending on the average enzyme levels and on the oscillation frequency. For high frequencies, only small amplitudes are possible. Enzyme activities could be regulated much faster (and thus, could show much larger relative amplitudes) by allosteric regulation and covalent modifications like phosphorylation. However, if oscillations are created by these mechanisms, a fraction of enzymes will remain unused; higher oscillation amplitudes (achieved by fast regulation mechanisms) are effectively paid by higher average enzyme levels: in models, we can describe this as follows: we allow for enzyme amplitudes to exceed the normal constraints, but we penalise this by a special enzyme investment function: in the formula, the investment function $h(\bar{\mathbf{u}}, |\tilde{\mathbf{u}}|)$ is replaced by $h(\bar{\mathbf{u}}^*, \tilde{\mathbf{u}}^*)$ where $u_l^* = u_l, \tilde{u}_l^* = \tilde{u}_l$ for all

enzymes l that do not violate the constraint (i.e. $|\tilde{u}_l| \leq |\tilde{u}_l^{\max}|$) (assuming no allosteric regulation), and $u_l^* = u_l + |\tilde{u}_l| - |\tilde{u}_l^{\max}|, \tilde{u}_l^* = \tilde{u}_l^{\max}$ for all enzymes l that violate the constraint (i.e. $|\tilde{u}_l| > |\tilde{u}_l^{\max}|$) (assuming that the level u_l describes only the active enzyme fraction, and that true enzyme level, which determines the cost, is higher).