The economic basis of periodic enzyme dynamics

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Abstract

Periodic enzyme activities can improve the metabolic performance of cells. As an adaptation to periodic environments or by driving metabolic cycles that can shift fluxes and rearrange metabolic processes in time to increase their efficiency. To study what benefits can ensue from rhythmic gene expression or posttranslational modification of enzymes, I propose a theory of optimal enzyme rhythms in periodic or static environments. The theory is based on kinetic metabolic models with predefined metabolic objectives, scores the effects of harmonic enzyme oscillations, and determines amplitudes and phase shifts that maximise cell fitness. In an expansion around optimal steady states, the optimal enzyme profiles can be computed by solving a quadratic optimality problem. The formulae show how enzymes can increase their efficiency by oscillating in phase with their substrates and how cells can benefit from adapting to external rhythms and from spontaneous, intrinsic enzyme rhythms. Both types of behaviour may occur different parameter regions of the same model. Optimal enzyme profiles are not passively adapted to existing substrate rhythms, but shape them actively to create opportunities for further fitness advantage: in doing so, they reflect the dynamic effects that enzymes can exert in the network. The proposed theory combines the dynamics and economics of metabolic systems and shows how optimal enzyme profiles are shaped by network structure, dynamics, external rhythms, and metabolic objectives. It covers static enzyme adaptation as a special case, reveals the conditions for beneficial metabolic cycles, and predicts optimally combinations of gene expression and posttranslational modification for creating enzyme rhythms.

Keywords: Metabolic oscillation, Metabolic control analysis, Optimal control, Enzyme oscillation, Allosynchrony, Pattern formation

1 Introduction

Many cell functions (e.g., cell cycle, photosynthesis [1], or metabolic oscillations [2 3]) and the life of organisms (e.g., sleep rhythms or seasonal flowering) are controlled by biological rhythms. Biological behaviour may be adapted to daily or yearly external rhythms, enabling organisms to perform their functions under the best possible conditions. Storage compounds may be produced as a preparation for night or winter times, a strategy known as anticipation. Other rhythms emerge spontaneously, rather than being induced from the outside, such as the menstrual cycle or the cell division cycle in microbes. Such rhythms also occur in cell metabolism. Yeast populations, for example, show spontaneous metabolic oscillations involving periodic, global gene expression changes [4 5] and a periodic production, storage,
and consumption of compounds [2, 6]. Experiments suggest that, while spontaneous oscillations exist in single cells, synchronisation between cells is needed to make them macroscopically visible [7]. In the experiments, each cell is subject to the periodic external conditions generated by the cell population. The sequence of physiological phases has been studied in detail [8], but it is unclear whether these oscillations have a biological function. Metabolic oscillations may be a simple side effect of dynamics, for example, of an overshooting regulation. May such oscillations, however, also provide a benefit? For example, cells might arrange metabolic processes in time to optimise their usage of resources [3]: scheduled in this way, biochemical processes may run in concerted actions or in a favourable temporal order, and mutually incompatible processes may be executed at different times to avoid deleterious side effects. It has also been claimed that “pumping”, i.e., driven by varying external conditions, can increase the efficiency of biochemical pathways [9].

Thus, while general mechanisms behind biochemical oscillations have been thoroughly studied [10], their possible functions are still debated. In this paper, I focus on cell metabolism as a special case. Since metabolic adaptation and metabolic rhythms are controlled by enzyme activities, I study the optimal choice of periodic enzyme profiles. Using models, I evaluate their effects on cellular fitness and study the shapes of optimal enzyme rhythms. The adaptation to external rhythms and the emergence of spontaneous metabolic cycles may look like unrelated phenomena, but as optimality problems they are closely related. First, in spontaneous cycles too, each pathway is adapted to a given periodic environment: the surrounding cell. Thus, behaviour within spontaneous cycles should obey the same rules as an adaptation to external perturbation. By converse, if a scheduling of processes in spontaneous metabolic rhythms is advantageous, similar scheduling patterns may also be beneficial when adapting to external rhythms. Second, if rhythmic environments can provide opportunities for beneficial adaptation, cells may actively create such environments (e.g., by synchronising their oscillations in a cell population). Therefore, I will describe both types of rhythms by a unified theory. To see whether rhythms can improve metabolic performance, I investigate dynamic metabolic models and search for periodic enzyme profiles that optimise the performance of some biological function. The rules for optimal enzyme rhythms should hold both in small, isolated pathways and in the entire metabolic network, comprising thousands of enzymes. To obtain tractable formulae, I will restrict the enzyme profiles to sine waves of small amplitude and determine their optimal amplitudes and phases. Using this framework, I will study how enzyme rhythms provide benefits, under what conditions spontaneous rhythms may emerge, and what principles underly the optimal amplitudes and phase shifts. As a side result, known formulae for quasi-static adaptation in such networks will be recovered [11].

The prediction of optimal enzyme profiles, in metabolic networks and in time, is a difficult problem. In slowly changing environments, enzyme levels could be quasi-statically adapted, thus assuming a constant environment in every moment. Then, slow environmental rhythms would promote slow, synchronous enzyme rhythms. Such a strategy, however, would miss the opportunity to produce storage compounds in times of plenty and to use them when conditions are worse. It also ignores that metabolism itself is dynamic: when external or enzyme levels oscillate fast, their effects percolate the network like waves, reaching different pathways with different phase shifts. The idea of a quasi-static adaptation does not even apply in this case. Instead, cells could exploit existing (e.g., day-night) rhythms to their advantage: they could arrange biochemical processes in time and allocate each process to a phase that provides the best biochemical conditions (availability of substrates, cofactors, or thermodynamic driving force). Plants, for
Objective: fast production of product

(a) Passive response to a sudden substrate increase
(b) Optimal adaption to a sudden substrate increase
Objective: fast production of product

(c) Optimal adaption to a periodic substrate supply
Objective: Increase of average flux
(d) Autonomous enzyme rhythms
Can waves of enzyme activity increase the average flux?

Figure 1: Metabolic dynamics and enzyme adaptation. Circles represent metabolites (dark brown: external substrate with predefined level; light brown: internal metabolites), ellipses represent enzymes. (a) Linear pathway. After a sudden increase in substrate level, metabolite levels rise with time delays. (b) “Just-in-time” activation of enzyme activities. When substrate becomes available, enzymes are activated one by one, each producing the substrate of the following enzyme. This sequential activation can speed up the process (i.e., the time until the half-maximal product concentration is reached) at a fixed total enzyme cost [12, 13]. (c) “Just-in-phase” enzyme rhythm adapted to a periodic substrate supply. A periodic substrate level causes metabolite rhythms along the chain. These rhythms can be modulated by enzyme rhythms that increase the average flux and, therefore, enzyme efficiency. (d) Spontaneous enzyme rhythms can enforce metabolite and flux rhythms in a constant environment. In this article, I show that such spontaneous rhythms can be beneficial, and how their optimal phases and amplitudes can be computed.

example, may produce and store energy during daytime, when light is available, some energy-consuming processes could be shifted to night hours. This scheduling, implemented by rhythmic enzyme levels, would affect metabolic dynamics and create incentives for further enzyme adaptation. This would again affect metabolic dynamics, and so on. In the resulting metabolic cycle, the cell “specialises” on different processes in different temporal phases, not only adapted to the current external conditions, but also using products from previous phases and producing compounds for phases to come. Ideally, the metabolic cycle and the corresponding enzyme profile are self-consistent, i.e., optimally adapted to the dynamics created by the environment and by the enzyme profile itself.

How can we predict such enzyme rhythms mathematically? Enzyme allocation can be framed as an optimal control problem in which metabolite concentrations and rates are dynamically described by a kinetic model, and optimal enzyme profiles, as control variables, are determined by optimality. This approach has been used to predict to static enzyme levels [14], long-term enzyme adaptation [11] and temporal enzyme profiles [12, 13]. For example, consider a metabolic pathway whose substrate becomes suddenly available. In what temporal order should the pathway enzymes be activated? If the pathway is described by a kinetic model with constant enzyme activities, the levels of pathway intermediates increase sequentially (Figure 1 (a)). However, this process can be improved, at a constant total enzyme investment, if the enzyme levels can vary in time. A numerical optimisation of the enzyme profiles [12] or of the regulation mechanisms behind them [13] predicts a sequential induction of enzymes (Figure 1 (b)), a behaviour experimentally observed
in amino acid biosynthesis [13]. In the optimal strategy found in [12], each enzyme remains inactive until enough of its substrate has accumulated (through the action of previous enzymes); then, it is activated. The sequential expression of protein was dubbed “just-in-time production” because enzymes are made when needed, i.e., when substrate becomes available. Here I ask the same question – what enzyme profiles will maximise metabolic performance? – for periodic metabolic behaviour. For example, in a pathway with a periodic substrate supply, could the flux be increased by usage of periodic enzyme levels? Would there be a sequential, i.e. wave-like, activity pattern (Figure 1 (c)), and could cells benefit from “spontaneous” enzyme rhythms in constant environments (Figure 1 (d))? 

If fluxes, metabolite levels, and enzyme activities oscillate around a static reference state, these rhythms can affect metabolic performance. To study this, I describe the metabolic system by a kinetic model and score the fluxes, metabolite levels, and enzyme activities by a fitness function. In the model, fitness results from a compromise between metabolic performance and enzyme cost (e.g., of enzyme production). Given external conditions (e.g., a periodic external substrate level), I search for periodic enzyme profiles of maximal fitness. Since large-amplitude profiles are difficult to treat, I use a perturbation theory: I assume small-amplitude rhythms and approximate them by sine-wave functions; enzyme activities, metabolite levels, and fluxes can then be represented by amplitudes and phases and the entire optimality problem can be formulated in terms of these variables (see Figure 11 in appendix). To describe how amplitudes and phases of metabolites, enzyme, and fluxes affect each other, methods from Metabolic Control Analysis (MCA) can be used [15]. By expanding the fitness function to second order around a (previously optimised) static reference state, the search for optimal enzyme profiles can be formulated as a quadratic optimality problem with linear constraints. I have previously studied enzyme adaptation to static external changes with a similar approach [11]. In that case, I computed optimal long-term changes in enzyme activity, after external perturbation, from metabolic response coefficients and from curvatures of the fitness function. Now, to predict enzyme rhythms, the static response coefficients will be replaced by their periodic counterparts.

In this article, I first study how substrate rhythms in a single reaction can provide an incentive for rhythmic enzyme levels. Then I consider metabolic pathways and networks and derive optimal enzyme rhythms from pairwise fitness synergies and amplitude constraints. I further discuss how optimal enzyme rhythms can be realised by gene expression and enzyme modification and which factors determine the shape of an optimal enzyme rhythm. Whether and how such rhythms are mechanistically realised in cells is a separate question and is not addressed here. The focus on optimal control, rather than concrete regulation mechanisms, is also reflected in my terminology: for example, if an external rhythm promotes an enzyme rhythm, this means that it provides a fitness incentive for showing this enzyme rhythm; and if I call enzyme rhythms spontaneous, this simply means that they provide a fitness advantage without any external rhythms. How such rhythms may emerge mechanistically is beyond the scope of this paper. The example models below are simple, but the method equally applies to complex metabolic models as well. A number of such models are shown at www.metabolic-economics.de/enzyme-rhythms/, and general formulae are explained in the appendix and in the supplementary information.
Box 1: Beneficial enzyme oscillation in a single reaction

(a) Reaction with adaptive enzyme rhythm

(b) Enzyme efficiency depends on phase difference

Figure 2: Enzyme profile optimally adapted to a given substrate rhythm. (a) If an enzyme activity $u$ oscillates in phase with the substrate level $x$, this can increases the average flux even if the average enzyme activity remains constant. (b) Enzyme rhythm described by a complex amplitude.

We consider a reaction $X \rightarrow Y$ with an irreversible mass-action rate law $\nu(u, x) = v u k x$, where the enzyme activity $u(t)$ is the concentration of enzyme $U$ in its active form (units: reaction rate $v$ in mM/s, enzym activity $u$ in mM, substrate level $x$ in mM, rate constant $k$ in mM$^{-1}$ s$^{-1}$). Given a substrate rhythm $x(t)$, which enzyme rhythm $u(t)$ (i.e., which relative phase and amplitude) will maximise the flux? We limit ourselves to harmonic profiles

$$x(t) = \bar{x} + \text{Re}(\tilde{x} e^{i\omega t}), \quad u(t) = \bar{u} + \text{Re}(\tilde{u} e^{i\omega t})$$

with central values $\bar{x}$ and $\bar{u}$ and complex amplitudes $\tilde{x}$ and $\tilde{u}$. Circular frequency $\omega$, frequency $f$, and period length $T$ are related as $\omega = 2 \pi f = 2 \pi / T$. By inserting $x(t)$ and $u(t)$ into the rate law, we obtain the time-dependent rate

$$v(t) = \frac{k \bar{u} \tilde{x} + k \text{Re}(\tilde{u} e^{i\omega t})\tilde{x} + k \bar{u} \text{Re}(\tilde{\bar{x})} e^{i\omega t}) + \frac{k \text{Re}(\tilde{\bar{u}} e^{i\omega t})\text{Re}(\tilde{x} e^{i\omega t})}{\frac{k}{2} \text{Re}(\tilde{\bar{u}} e^{i\omega t}) + \frac{k}{2} \text{Re}(\tilde{\bar{x}} e^{i\omega t})}.$$  

This formula contains five terms: the static reference flux $\bar{v}$, two periodic terms of frequency $\omega$, caused by linear effects of the periodic parameters, and two synergy terms (see SI S1.1). The synergy term $\frac{k}{2} \text{Re}(\tilde{\bar{u}} e^{i\omega t})$ describes an oscillation of frequency $2 \omega$, while the synergy term $\Delta v = \frac{k}{2} \text{Re}(\tilde{\bar{u}} e^{i\omega t}) \bar{u} e^{i\omega t}$ describes a shift of the average flux [15] (the star denotes the complex conjugate). The flux shift is the effect we are interested in. It can be written as

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

with a phase difference $\Delta \varphi = \varphi(\tilde{x}) - \varphi(\tilde{u})$. If a nonlinear rate law $\nu = u r(x)$ is considered, we can assume that the oscillation amplitudes are small, and use the linear approximation

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

with the unscaled substrate elasticity $E_x = \partial v / \partial x$ (see SI S1.1). In both cases, the average flux $\langle v \rangle = \bar{v} + \Delta \bar{v}$ depends on the phase shift between substrate and enzyme. If substrate and enzyme are in phase ($\Delta \varphi = 0$), the flux increases by $\frac{E_x |\tilde{u}| |\tilde{x}|}{2 \bar{u}}$. Thus, the efficiency $\langle v \rangle / \langle u \rangle$ – the average rate, divided by the average enzyme level – increases, while average enzyme activity and average ratio $\langle v/u \rangle_t = k \langle \tilde{x} \rangle_t = k \bar{x}$ remain fixed. In contrast, if $x(t)$ and $u(t)$ oscillate with opposite phases, average flux and enzyme efficiency will decrease.
2 Results

2.1 Rhythmic enzyme levels can increase enzyme efficiency

In reactions with oscillating substrate levels, synchronous enzyme rhythms can increase the average flux at a fixed average enzyme activity. An example is shown in Box 1. To denote such synergisms between substrate and enzyme rhythms, I introduce the term “allosynchrony”. Unlike allosteric regulation, which depends on momentary metabolite levels, allosynchrony depends on orchestrated oscillations with a common frequency and defined amplitudes and phases. Metabolic efficiency (e.g., an average flux, divided by the average enzyme activity) is closely related to cost efficiency (i.e., benefit per enzyme investment). When we compare enzyme profiles of identical cost (e.g., profiles with a fixed average enzyme level and a linear cost function), a high fitness directly depends on high cost-efficiency. Thus, allosynchrony is a dynamic effect, but it also has consequences for enzyme economy. If a substrate rhythm allows cells to increase its fluxes at a given enzyme cost or to reach the same flux at a lower cost by allosynchrony, it creates an incentive for enzyme rhythms or, in the terms I’m introducing, it promotes these enzyme rhythms.

2.2 Dynamics of enzyme rhythms in pathways and networks

Enzyme rhythms in a metabolic network resemble the rhythms in single reactions, but with a number of complications. First, only some metabolite profiles (the “external” ones) are predefined, while all others emerge from the system dynamics and depend on the enzyme profiles. Second, perturbations propagate through biochemical networks as dampened waves and the emerging enzyme profiles may show complex patterns of amplitudes and phase shifts. Third, all enzymes must be optimised simultaneously; in the resulting metabolic cycle, the solution must be self-consistent, i.e., each enzyme must be adapted to all other enzyme rhythms, which are also optimally adapted. To model how enzyme rhythms shape the metabolic states dynamically, and are shaped by them economically, we consider a kinetic model in which state variables (internal concentrations $c_i$ and reaction rates $v_l$) and enzyme activities $u_l$ are described by vectors. The rate laws depend on reactant levels $c_i$, enzyme activities $u_l$, and external metabolite levels $x_j$. When a reaction rate is perturbed, the resulting fluctuations reach different parts of the network with different delays. To predict beneficial enzyme rhythms, we need to model how periodic perturbations propagate in the kinetic model.

Oscillating enzyme activities will cause oscillations in internal metabolite levels and fluxes, with amplitudes and phases depending on network structure, rate laws, and oscillation frequencies. If the amplitudes are small, as per (3), the forced amplitudes and phases can be predicted with the help of spectral response coefficients [15] (see Figure 3). To describe these oscillations, we first consider a stable steady state with

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2 The distinction between enzyme activity $u(t)$ (concentration of enzyme molecules in the right modification state) and the enzyme concentration $p(t)$ (concentration of enzyme molecules) will become important below, when desired enzyme rhythms would be too fast to be realised by expression changes.

3 External parameters $x$ can describe metabolite levels or other quantities that affect the reaction rates. For example, ATP production by ATP synthase in plants depends on a proton gradient, which affects the equilibrium constant of the ADP $\rightarrow$ ATP conversion. At night, the gradient is too low and ATP synthase would start degrading ATP, so it should be shut down. Here, the proton gradient could be treated as an external parameter $x$, and the ATP synthase activity as a control parameter $u$ to be optimised.
fixed external parameters and enzyme activities. This is our reference state. We then assume that external parameters \(x_j(t)\) and enzyme activities \(u_l(t)\) vary periodically with circular frequency \(\omega\), complex amplitudes \(\hat{x}_j\) and \(\hat{u}_l\), and shifts \(\Delta \hat{x}_j\) and \(\Delta \hat{u}_l\) of the central values. To describe the resulting forced oscillations, we use a Taylor approximation. In a first-order approximation, harmonic perturbations lead to harmonic metabolite and flux oscillations of the same frequency. The amplitude vectors \(\hat{\mathbf{c}}\) and \(\hat{\mathbf{v}}\) depend linearly on the amplitude vectors \(\hat{\mathbf{x}}\) and \(\hat{\mathbf{u}}\) (e.g., \(\hat{\mathbf{c}} = \hat{R}_S^{c} \hat{\mathbf{x}} + \hat{R}_S^{c} \hat{\mathbf{u}}\)), with the spectral response coefficients as expansion coefficients. Similarly, average parameter shifts \(\Delta \hat{\mathbf{x}}\) and \(\Delta \hat{\mathbf{u}}\) lead to average shifts \(\Delta \hat{\mathbf{c}} = \hat{R}_S^{c} \Delta \hat{\mathbf{x}} + \hat{R}_S^{c} \Delta \hat{\mathbf{u}}\) with static response coefficients as prefactors. A linearised metabolic model acts as a low-pass filter: at low frequencies, it shows a quasi-stationary response, while high-frequency perturbations are strongly dampened. At intermediate frequencies, resonance can occur. To describe mixed perturbations, with multiple perturbation parameters and frequencies, we first consider single parameters and frequencies, compute the resulting forced oscillations, and add them all up. At larger perturbation amplitudes, the

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\(\hat{R}_S^{c}(\omega)\) resemble reaction elasticities, but refer to an entire network. In an isolated reaction, periodic reactant levels and enzyme activities lead to a periodic reaction rate whose amplitude can be obtained from the periodic elasticities. In a network, external metabolite and enzyme rhythms provoke forced oscillations of the metabolic state (i.e., internal metabolite levels and fluxes). Since metabolite levels are not predefined, but dynamic, the output rhythms are described by periodic response coefficients. There can be synergies between enzymes (while in single reactions, due to vanishing second-order elasticities \(E_{uu} = 0\), enzyme rhythms alone have no second-order effects).
Figure 4: Prediction of optimal enzyme profiles. A rhythmic enzyme profile is characterised by a static shift $\Delta \bar{u}$ and a complex amplitude $\tilde{u}$ (see Figures 11 (a) and 12). The fitness of an enzyme-controlled metabolic system can be seen as a function of the static and rhythmic deviations $\Delta \bar{u}$ and $\Re(\tilde{u})$. The panels show the fitness landscape under different perturbations $\Delta \bar{x}$ or $\tilde{x}$. (a) Unperturbed fitness landscape ($\Delta \bar{x} = \tilde{x} = 0$; fitness contours shown in blue; dark blue represents high fitness). The fitness maximum (dot) is our reference state. The fitness synergies $F_{uu}$ and $F_{\tilde{u}\tilde{u}}(\omega)$ in this point (fitness curvatures for $\Delta \bar{u}$ and $\tilde{u}$) are negative, so any change $\Delta \bar{u}$ or $\tilde{u}$ would decrease the fitness. (b) An external parameter shift $\Delta \bar{x}$ changes the landscape, displaces the optimum point, and promotes a static enzyme adaptation $\Delta \bar{u}$ (red arrow). (c) An external rhythm with amplitude $\tilde{x}$ displaces the optimum and promotes an enzyme rhythm with amplitude $\tilde{u}$. (d) If the reference state is a saddle point of the fitness landscape (red square), it is fitness-unstable against enzyme rhythms. Static enzyme changes $\Delta \bar{u}$ would be costly, but spontaneous oscillations ($\tilde{u} \neq 0$) around the reference state can increase the fitness even in the absence of external oscillations.

first-order approximation becomes unreliable and a second-order expansion is needed. It shows new effects like the synergistic interactions between enzymes, leading to higher harmonics at frequency $2 \omega$, as well as shifts in the average concentrations and fluxes (see appendix A and SI S1).

2.3 Economics of enzyme rhythms in pathways and networks

Once we can simulate the effects of enzyme rhythms, e.g., forced oscillations and shifts of metabolic fluxes, we can turn the question around and study how metabolic systems can be optimally controlled by enzymes. For example, which enzyme profile yields a desired metabolic behaviour at a minimal cost in the presence of an external rhythm $\tilde{x}$? Such inverse problems can be difficult to solve, but an approximation for small amplitudes makes them tractable (see SI S2.3). To formulate cost-benefit problems for metabolic networks (see Figure 12), we collect the internal metabolite levels $c_i$ and fluxes $v_l$ in a vector $s$ and score them by a metabolic benefit function $b(s)$. The vector may also contain other state variables such as pH values or compartment sizes. Among the model parameters, we distinguish two types: external parameters $x_j$ representing the environment (here: external metabolite levels), and control parameters $u_l$ to be optimised (here: enzyme activities). The fitness $f(u, x) = g(u, x) - h(u)$ is the difference of metabolic benefit $g(u, x) = b(s(u, x))$ and enzyme cost $h(u)$. The metabolic benefit $b(c, v)$ may, e.g., increase with production fluxes and decreases for high intermediate levels; the cost $h$ increases with the enzyme activities $u_l$.

To define a static reference state with parameter profiles $x^{ref}$ and $u^{ref}$, we fix the external concentrations
$x_j$ and optimise the static enzyme levels $u_j^{opt}$ for maximal fitness. As an optimality condition, $f(u, x)$ must have negative curvatures with respect to $u$. Enzymes whose levels vanish in the reference state are removed from the model. Now we can study enzyme adaptation to external perturbations. For example, we perturb the reference state by a static parameter change $\Delta x$ and solve for the optimal static enzyme change $\Delta \bar{u}$ using second-order metabolic response coefficients. Optimal adaptations to periodic perturbations can be computed similarly, using periodic response coefficients [15] (see SI S1). We start from the optimal reference state, consider periodic external profiles $x(t) = x^{ref} + \Delta x + \text{Re}(\bar{x}e^{i\omega t})$ and enzyme profiles $u(t) = u^{ref} + \Delta \bar{u} + \text{Re}(\bar{u}e^{i\omega t})$, and evaluate the resulting periodic state (Figure S4(b)). To score metabolic time courses by fitness values, we define a fitness functional. We consider two possibilities. For a state-averaged fitness $F = \langle b(s) - b(\bar{s}) \rangle$, we apply our static fitness function $f$ to the average metabolic state (fluxes and concentrations). For a fitness-averaged fitness $F = b(\langle s \rangle_t) - h(\langle u \rangle_t)$, the fitness function is evaluated in every moment and then averaged over time. In both cases, the overall fitness depends on time averages; in the first case, it is insensitive to temporal fluctuations; in the second case, temporal fluctuations can affect the fitness. Given our periodic parameter profiles, the fitness can be written as a function $F(\Delta \bar{u}, \Delta \bar{x}, \bar{u}, \bar{x})$ of shift vectors $\Delta \bar{u}$ and $\Delta \bar{x}$ and complex amplitude vectors $\bar{u}$ and $\bar{x}$ (see Figure 4). Near the reference state, this function can be quadratically expanded as

$$\Delta F(\Delta \bar{u}, \bar{u}, \omega) \approx \Delta \bar{x}^T F_{x\bar{x}} \Delta \bar{u} + \frac{1}{2} \Delta \bar{u}^T F_{uu} \Delta \bar{u} + \text{Re}[\bar{x}^T F_{\bar{x}\bar{x}}(\omega) \bar{u}] + \frac{1}{2} \bar{u}^T F_{\bar{u}\bar{u}}(\omega) \bar{u} \tag{5}$$

The fitness synergy matrices ($F_{uu}$ and $F_{ux}$ for static changes, $F_{u\bar{u}}$ and $F_{u\bar{x}}$ for rhythmic changes) contain the local curvatures of $F$ with respect to $\Delta \bar{u}$, $\Delta \bar{x}$, $\bar{u}$, and $\bar{x}$ and describe the shape of the fitness landscape around the reference state. They depend on the kinetic model, on cost and benefit function, and on the fitness functional used (fitness-averaged or state-averaged). If the reference state is known, the matrices can be easily computed with the help of MCA (see appendix B). In Eq. (5), the first term in brackets describes the effect of static perturbations, while the second term describes the effect of rhythms at frequency $\omega$. Irrelevant terms have been omitted from the formula: terms that contain only $\Delta \bar{x}$ and $\bar{x}$ do not matter for the choice of enzyme profiles; terms that are linear in $\Delta \bar{u}$ vanish because of the optimality condition $f_s = 0$ in the reference state; terms that are linear in $\bar{u}$ cannot lead to time-average shifts. The expansion Eq. (5) holds for harmonic perturbations of a single frequency. In the second-order approximation used (and assuming a time-independent fitness function), there are no synergies between perturbations of different frequencies, and no synergies between static and periodic perturbations. For mixed perturbations with multiple frequencies, we can sum or integrate over contributions from different frequencies.

Our formula (5) yields the fitness advantage of enzyme rhythms based only on synergy matrices $F_{uu}$ and $F_{ux}$ (for static perturbations) or $F_{u\bar{u}}$ and $F_{u\bar{x}}$ (for periodic perturbations). The matrix elements represent three sorts of synergies: synergies between external parameters and enzymes (elements of $F_{ux}$ and $F_{u\bar{x}}$), synergies between enzymes (off-diagonal elements of $F_{uu}$ and $F_{u\bar{u}}$), and enzyme self-synergies (diagonal elements of $F_{uu}$ and $F_{u\bar{u}}$). The fitness effect of an enzyme profile results from all these effects: if their

\[ In the optimisation, we consider enzyme profiles that lead to stable steady states.

\[ The synergy matrices $F_{u\bar{u}}$ and $F_{u\bar{x}}$ depend on the frequency. In the limiting case of slow oscillations ($\omega \to 0$), and assuming a fitness-averaged fitness functional, the matrices can be obtained from the static synergy matrices by $F_{u\bar{u}}(\omega = 0) = \frac{1}{2} F_{uu}$ and $F_{u\bar{x}}(\omega = 0) = \frac{1}{2} F_{ux}$.

\[ This linear superposition is only possible if the higher-order terms (beyond the quadratic expansion) are neglected, if our fitness function is not explicitly time-dependent, and if our combined solution does not violate any constraints (see SI S4.4).

\[ 9]
sum is positive, the enzyme rhythm will be beneficial. Self-synergies will either be negative, because of dynamic self-inhibition or because of fitness-averaged fitness functionals with non-linear cost functions. To make a global enzyme rhythm beneficial, these negative effects must be compensated by large beneficial synergy effects between external parameters and enzymes or among enzymes.

2.4 Optimal enzyme profiles

Our static reference state is optimal against static enzyme changes, but it may still be improvable by spontaneous enzyme rhythms. If it is, there will be an incentive for such rhythms. Otherwise, enzyme rhythms can only be promoted by external rhythms. Given an external perturbation – a static shift $\Delta \bar{x}$ or a rhythm $\tilde{x}$ – what will be the optimal enzyme profile look like? To compute this profile, we consider the fitness landscape $F(\Delta \bar{u}, \tilde{u})$ given by Eq. (5), assume an external perturbation $\bar{x}(t)$, and determine the enzyme profile (defined by parameters $\Delta \bar{u}$ and $\tilde{u}$) that maximises $F$. Without perturbation, the system remains in the reference state (with $\Delta \bar{u} = \tilde{u} = 0$) because this is the optimum point. A static perturbation $\Delta \bar{x}$ changes the fitness landscape and displaces the optimum point $\bar{u}$. If the new optimum is still within the constraints (e.g., no enzyme levels become negative), the displacement $\Delta \bar{u}^{opt}$ yields the optimal enzyme adaptation (Figure 4(b)). Adaptations to periodic perturbations can be found in a similar way: an external rhythm with amplitude vector $\tilde{x}$ displaces the optimum point by $\tilde{u}^{opt}$ (in the space of amplitude vectors $\tilde{u}$), and the vector $\tilde{u}^{opt}$ describes the amplitudes and phases of the optimal enzyme profile. In both cases, the optimal profile is computed by maximising Eq. (5) at given perturbations $\Delta \bar{x}$ or $\tilde{x}$. If there are no further constraints, the bracket terms for static and periodic shifts can be optimised separately. Then, a static perturbation $\Delta \bar{x}$ promotes a static adaptation

$$
\Delta \bar{u}^{opt} \approx - F^{-1}_{\bar{u} \bar{u}} F_{\bar{u} \bar{x}} \Delta \bar{x},
$$

and a periodic perturbation $\tilde{x}$ promotes a periodic adaptation with amplitude vector

$$
\tilde{u}^{opt} \approx - F^{-1}_{\tilde{u} \tilde{u}} F_{\tilde{u} \tilde{x}} \tilde{x}.
$$

Can enzyme rhythms $\tilde{u}$ be beneficial in the absence of external rhythms? This would require a a positive fitness change $\Delta F = \tilde{u} F_{\tilde{u} \tilde{u}} \tilde{u}$ by the enzyme rhythms alone, i.e., the matrix $F_{\tilde{u} \tilde{u}}$ must have positive eigenvalues (as in Figure 4(d)). We can test this by computing the maximal eigenvalue of $F_{\tilde{u} \tilde{u}}(\omega)$, called principal fitness synergy $\sigma(\omega)$. If the value is positive, the corresponding eigenvector represents a beneficial spontaneous rhythm; in fact, it represents the most beneficial rhythm under the amplitude constraint $||\tilde{u}|| = \beta$, where $\beta$ is a fixed small number. In contrast, if $\sigma(\omega)$ remains negative for all frequencies $\omega$, the reference state is fitness-stable against spontaneous enzyme rhythms.

As an example, we consider a simple metabolic pathway. We start from a static reference state with given substrate level and optimal static enzyme activities. A sudden increase in substrate level would promote (i.e., provide an incentive for) increasing the enzyme activities (sequentially in time, or statically as a long-term behaviour). What about a periodic substrate rhythm? At constant enzyme activities, the external

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8Negative self-synergies could also be caused by frequency-dependent enzyme costs. I briefly discuss this below.
rhythm would lead to dampened waves of concentrations and fluxes along the pathway (Figure 5(a)). By adding a wave-like enzyme rhythm that synchronizes the enzymes with their substrates, the average flux will be increased by allosynchrony, i.e., the substrate oscillation promotes wave-like enzyme rhythms (Figure 5(b)). In this model, however, the principal fitness synergy remains negative for all frequencies, so spontaneous enzyme waves without an external rhythm would not be beneficial. This changes if we modify the model, assuming that intermediate metabolites are non-enzymatically degraded or diluted. Now, enzyme rhythms can increase the average flux even at a constant substrate level. (Figure 6). As a third model variant, we consider a loop-shaped pathway with compounds serving as input and output (Figure 7). Also in this case, spontaneous enzyme waves can be beneficial. The principal synergy (Figure 7(c)) shows a positive local maximum, reflecting a dynamic resonance of the metabolic system. Slower or faster spontaneous oscillations would lead to a lower benefit. The optimal frequency – the one at which enzyme rhythms, at a constant enzyme cost, provide the largest increase in flux – could be expected to provide the largest selection advantage in evolution. We can see this as a new type of pattern formation in time, arising not from dynamics, but from the economics of cellular resources. For model details, see www.metabolic-economics.de/enzyme-rhythms/.

2.5 Periodic economic demands and economic potentials

We now come back to enzyme adaptation to external metabolite rhythms and study their optimality conditions in a more abstract way. In a quadratic expansion around a steady reference state, and assuming

\footnote{In this model, enzyme activities are allowed to change fast (with a characteristic time of 1 s). This assumption will be critically revised in Figure 8.}
In contrast to the model in Figure 5, pathway intermediates are diluted, non-enzymatically degraded, or lost by passive diffusion through cell membranes (which happens to gaseous compounds such as H₂S or acetaldehyde). Enzyme rhythms can reduce this loss, even in the absence of external rhythms, and increase the metabolic benefit. Like in Figure 5, we assume that enzyme levels can oscillate fast without extra cost.

Figure 6: Beneficial spontaneous enzyme rhythm. In contrast to the model in Figure 5, pathway intermediates are diluted, non-enzymatically degraded, or lost by passive diffusion through cell membranes (which happens to gaseous compounds such as H₂S or acetaldehyde). Enzyme rhythms can reduce this loss, even in the absence of external rhythms, and increase the metabolic benefit. Like in Figure 5, we assume that enzyme levels can oscillate fast without extra cost.

that our solution respects all constraints, the optimality conditions read

\[ 0 = \frac{\partial f}{\partial \bar{u}} = g_u + G_u x \Delta \bar{x} + G_{uu} \Delta \bar{u} - (h_u + H_{uu} \Delta \bar{u}) \]

\[ 0 = \frac{\partial f}{\partial \tilde{u}} = g_{\tilde{u}} + G_{\tilde{u}x} \Delta \tilde{x} + G_{\tilde{u}\tilde{u}} \Delta \tilde{u} - (h_{\tilde{u}} + H_{\tilde{u}\tilde{u}} \Delta \tilde{u}). \]  

(8)

The derivatives \( g_u, G_{ux}, G_{uu}, \ldots \) refer to the benefit and cost functions \( g \) and \( h \) and to the unperturbed reference state. The terms \( g_u \) and \( g_{\tilde{u}} \), called static and periodic enzyme demands, and the terms \( h_u \) and \( h_{\tilde{u}} \), called static and periodic enzyme burdens, are also benefit and cost gradients. However, the circle \( \circ \) reminds us that these are not gradients \( g_u \) and \( h_u \) (or \( g_{\tilde{u}} \) and \( h_{\tilde{u}} \)) in the reference state, but gradients in the periodic state. Written in terms of enzyme demand and burden, the optimality conditions (8) simply read \( \dot{g}_u = h_u \) and \( \dot{g}_{\tilde{u}} = h_{\tilde{u}} \). Enzyme demand and burden can also be defined outside the range of our quadratic approximation. For general periodic states, with benefit and cost functionals given by \( g(\bar{u}, \tilde{u}) \) and \( h(\bar{u}, \tilde{u}) \), we define them as

\[ \dot{g}_u = \frac{\partial g}{\partial \bar{u}}, \quad \dot{g}_{\tilde{u}} = \frac{\partial g}{\partial \tilde{u}}, \quad \dot{h}_u = \frac{\partial h}{\partial \bar{u}}, \quad \dot{h}_{\tilde{u}} = \frac{\partial h}{\partial \tilde{u}}. \]  

(9)

Again, the optimality conditions \( \frac{\partial f}{\partial \bar{u}} = \frac{\partial g}{\partial \bar{u}} - \frac{\partial h}{\partial \bar{u}} = 0 \) and \( \frac{\partial f}{\partial \tilde{u}} = \frac{\partial g}{\partial \tilde{u}} - \frac{\partial h}{\partial \tilde{u}} = 0 \) can be written as \( \dot{g}_u = \dot{h}_u \) and \( \dot{g}_{\tilde{u}} = \dot{h}_{\tilde{u}} \). What can we learn from this? If our expansion point is an enzyme-optimal reference state, we know that \( g_u = h_u \); and if the fitness functional is invariant against time shifts, we obtain \( g_{\tilde{u}} = h_{\tilde{u}} = 0 \). In these cases, the first-order terms in Eq. (8) cancel out and can be ignored. Solving for \( \Delta \bar{u} \) and \( \Delta \tilde{u} \), we reobtain Eqs (6) and (7). Finally, if the cost functional is also linear (i.e., \( H_{uu} = 0 \)), then \( \dot{h}_{\tilde{u}} \) will vanish as well, and we obtain the simple optimality condition \( \dot{g}_u = 0 \), i.e., the periodic demand of each enzyme must vanish.

What else can we know about enzyme demands? Since they refer to enzymes, they can be visualised as variables in the network, and the network structure is in fact reflected in their values. To see this, we define
a similar type of variables for metabolites, called economic potentials. The definition is as follows. The
function \( g \) describes the overall benefit, evaluated in the dynamic state of the model. For an independent
metabolite \( i \), we consider a small virtual exchange flux \( \varphi_i \) producing this metabolite, and define the static
and periodic economic potentials as \( w_{ci} = \frac{\partial g}{\partial x_i} \) and \( \tilde{w}_{ci} = \frac{\partial g}{\partial \tilde{x}_i} \), for static and periodic variations of
the exchange flux. The (static and periodic) enzyme burdens and the (static and periodic) economic potentials
of their reactants are linked by the economic balance equation (derivation in SI [54,6])

\[
\begin{pmatrix}
\dot{h}_u \\
\dot{h}_\tilde{u}
\end{pmatrix} = \begin{pmatrix}
\dot{g}_u \\
\dot{g}_\tilde{u}
\end{pmatrix} = \begin{pmatrix}
\tilde{E}_u^\pi & \tilde{E}_{\tilde{u}}^\pi \\
E_u^\pi & E_{\tilde{u}}^\pi
\end{pmatrix} \dot{\Delta} w_c + b_c
\]

(10)

with effective elasticities \( \tilde{E}_g^\pi \) defined for the periodic state. Here, \( \Delta w_{ci} \) denotes the economic potential
difference along reaction \( l \), and the (static and periodic) flux gains \( b_c \) and \( \tilde{b}_c \) are defined as the direct
derivatives of the benefit functional with respect to metabolic fluxes. Close to a steady reference state, an
expansion of the elasticity coefficients yields the formula

\[
\begin{pmatrix}
Dg(\bar{u}) h_u \\
Dg(\bar{u}) h_{\tilde{u}}
\end{pmatrix} = \begin{pmatrix}
Dg(\bar{v}) g_u \\
Dg(\bar{v}) g_{\tilde{u}}
\end{pmatrix} = \begin{pmatrix}
Dg(\bar{v}) \tilde{g}(\bar{v}) \\
Dg(\bar{v}) g(\bar{v})
\end{pmatrix} \dot{\Delta} w_c + b_c
\]

(11)

where \( \bar{u} = \bar{u}^{\text{ref}} + \Delta \bar{u} \) contains the average enzyme activities, \( \dot{\bar{v}} = \bar{v}^{\text{ref}} + \bar{E}_c^{\pi} \Delta \bar{c} + \bar{E}_{\tilde{c}}^{\pi} \Delta \bar{\tilde{c}} \) is the vector
of fluxes in the central state, and \( \dot{\tilde{v}} = \bar{E}_{\tilde{c}}^{\pi} \tilde{c} + \bar{E}_{\tilde{\tilde{c}}}^{\pi} \tilde{\tilde{c}} \). Eq. (10), for periodic states, shows that static and
periodic economic variables may affect each other. Applying this formula to the reference state itself, we
can set \( \dot{\bar{v}} = \bar{v} \) and \( \dot{\tilde{v}} = 0 \) and obtain the uncoupled equations

\[
\begin{align*}
\dot{h}_u &= g_u = Dg(v/\bar{u}) \dot{\Delta} w_c + b_c \\
\dot{h}_{\tilde{u}} &= \dot{g}_u = Dg(v/\bar{u}) \dot{\Delta} w_c + \tilde{b}_c.
\end{align*}
\]

(12)

If the cost function does not explicitly depend on time, the periodic enzyme burden must vanish (\( \dot{h}_{\tilde{u}} = 0 \)).
For more details, see SI [54,6]

2.6 Bounds on amplitudes and posttranslational enzyme rhythms

Until here, we saw how optimal enzyme profiles can be predicted from synergies, which are given in the
matrices \( F_{\tilde{x}_i} \) and \( F_{\tilde{u}_i} \). However, if the solutions \( \bar{u}_t \) and \( \tilde{u}_t \) from Eqs (5) and (7) violate a constraint, these
equations will not even apply. This can happen, e.g., if an enzyme amplitude exceeds the enzyme’s central
value (which immediately happens for enzymes that are inactive in the reference state). Also some other
questions remained open: can any desired enzyme rhythms be realised by gene expression? And, otherwise,
could cells realise their rhythms by other mechanisms such as posttranslational modification? How should
both mechanisms be combined? To answer these questions, we need to think about amplitude constraints.
What are the relevant constraints for our profile \( u(t) = \bar{u} + \Delta \bar{v} + \text{Re}(\bar{u} e^{i \omega t}) \)? Maximal amplitude vectors
\( \bar{p}_{\text{max}} \) and constraints between \( \bar{u} \) and \( \tilde{u} \) are obtained as follows. First, to prevent negative enzyme activities,
the shifts \( \Delta \bar{u}_t \) must not be smaller than \( -\bar{u}_t \) and the amplitude \( |\bar{u}_t| \) must not exceed the average level
\( \bar{u}_t + \Delta \bar{u}_t \). Therefore, oscillating enzymes must show positive average levels. More constraints arise when
Figure 7: Spontaneous rhythm in a loop-shaped pathway. In the reference state, a flux along the loop is driven by an inflow from metabolite X1 and an outflow to metabolite X2. (a) Network structure and reference flow. (b) Enzyme and metabolite rhythm. The optimal spontaneous enzyme rhythm is shown by blue arrows. Enzyme amplitudes are phase-shifted along the loop. Brown arrows show the corresponding metabolite rhythm. (c) Principal synergy (i.e., maximal fitness curvature) depending on frequency. The local maximum with a positive synergy value suggests an incentive for spontaneous oscillations at this frequency.

enzyme rhythms are caused by rhythmic mRNA profiles: then, enzyme rhythms at high frequencies are restricted to very small amplitudes (see appendix C and SI S1.4). With these constraints, we obtain an optimality problem

\[
\text{Maximise } \Delta F(\Delta \bar{u}, \bar{u}) \text{ subject to } |\bar{u}| \leq \bar{p}^{\text{max}}(\bar{u}, \omega)
\]  

where \(\bar{u} = \bar{u}^{\text{ref}} + \Delta \bar{u}\), the vector \(|\bar{u}|\) contains the absolute values from \(\bar{u}\), \(\bar{p}^{\text{max}}(\bar{u}, \omega)\) describes frequency-dependent amplitude bounds. If a solution is such that all constraints are inactive, the constraints can be ignored and Eqs (6) or (7) can be used instead. This is the case, e.g., for adaptive rhythms with small amplitudes \(\bar{x}\), all enzymes being active in the reference state. In all other cases, the constraints will affect the solution; if an enzyme hits a constraint, this will have secondary effects on other enzymes, and thus on the entire optimal state (see SI S4.5).

Mathematically, active constraints can be treated by Lagrange multipliers, which lead to extra terms in the solution for \(\Delta \bar{u}\) and \(\bar{u}\). Instead of Eqs (6) and (7), we obtain (proof in SI S4.5)

\[
\Delta \bar{u} = -F^{-1}_{\bar{u}u} \left[ F_{\bar{u}x} \Delta \bar{x} - \mu - Dg(\bar{p}^{\text{max}}(\bar{u}, \omega)) \gamma \right]
\]

\[
\bar{u} = -F^{-1}_{\bar{u}u} \left[ F_{\bar{u}x} \bar{x} - Dg \left( \frac{\bar{u}}{|\bar{u}|} \right) \gamma \right].
\]  

The signs of the Lagrange multipliers (in vectors \(\mu\) and \(\gamma\)) reflect the types of constraints: \(\mu_i < 0\) for inactive enzymes (with \(\bar{u}_i = 0\)), \(\mu_i > 0\) for enzymes whose central values hit upper bounds, and \(\mu_i = 0\) otherwise. Likewise, \(\gamma_i > 0\) holds for all enzymes with active amplitude constraints, and \(\gamma_i = 0\) otherwise. Thus, these terms only appear if the corresponding constraints are active: if no enzymes hits a constraint, all Lagrange multipliers vanish and we reobtain our simple formulae (6) and (7). However, any extra term, even for a single enzyme, will affect the profiles of other enzymes through the matrix multiplication. If enzymes hit their amplitude constraints, the corresponding elements in \(\gamma\) lead to a coupling between \(\Delta \bar{u}\)
(a) Enzyme amplitudes (assuming that activity changes can be fast)

(b) Enzyme amplitudes (combination of slow gene expression changes and fast covalent modification)

Figure 8: Optimal enzyme rhythms realised by gene expression and posttranslational regulation. (a) Optimal enzyme amplitudes in a linear pathway (see Figure 5) depending on frequency \( f = \omega / (2 \pi) \). The central curve shows the average enzyme activity; the grey band indicates the amplitude. We assume that any enzyme rhythm (even at high frequencies) can be realised by gene expression. (b) The same model, assuming that expression changes can only produce limited amplitudes, and that the desired amplitudes are achieved by enzyme modification. Light grey areas refer to expression amplitudes, blue areas refer to amplitudes by posttranslational modifications (for details, see Figure 14). The total amplitude (outer envelope) is given by their sum. Even in this simple model, complex optimal control strategies can arise. At low-frequency perturbations, the enzymes are almost in phase with the external perturbation (phases not shown); at high frequencies, enzymes 3 and 4 oscillate in opposite phase. At medium frequencies (\( \approx 0.5 \, \text{s}^{-1} \) for enzyme 3 and \( \approx 0.1 \, \text{s}^{-1} \) for enzyme 4), the two enzymes flip their phases and their amplitudes become very small.

and \( \tilde{u} \); only for high frequencies, the term in the first equation becomes very small and the coupling can be neglected. In the second equation, the phase angles of \( \tilde{u} \) appear again on the right-hand side in the term \( D_S \left( \frac{\tilde{u}}{|\tilde{u}|} \right) \). Therefore, the equation does not yield \( \tilde{u} \) directly, but a self-consistent solution needs to be found.

Until here, enzyme rhythms, at a fixed average enzyme activity, were assumed to be cost-neutral. The only exception was models in which a non-linear fitness function and fitness-averaged fitness functional are used. However, if we allow for posttranslational modifications, rhythms can become costly. This is the reason: whenever a desired enzyme amplitude \( |\tilde{u}| \) exceeds the maximal protein amplitude \( \tilde{p}^{\text{max}} \), the difference \( |\tilde{u}| - \tilde{p}^{\text{max}} \) must be achieved posttranslationally, at an effective cost proportional to the amplitude difference. To capture this cost in numerical optimisation, we introduce, for each enzyme, an auxiliary variable \( \eta \) describing the difference between desired enzyme amplitude and maximal possible
protein amplitude. This is the amount by which the average enzyme concentration must be increased (see appendix D). The resulting model allows for fast, large oscillations, but penalises them by effective costs; thus, enzyme modification rhythm will only be used if they pay off. For example, optimal enzyme rhythms for the linear pathway from Figure 5 can be realised by expression and posttranslational regulation (Figure 8). As expected, slow enzyme rhythms (on the time scale of hours) are mainly realised by expression changes, while fast oscillations are mainly realised posttranslationally. Due to cost optimality, expression rhythms and posttranslational modification rhythms should always act in parallel (i.e., posttranslational inhibition should take place when enzymes expression is low).

By considering constraints, we obtain more realistic predictions and new types of qualitative behaviour. Amplitude constraints are important if enzymes are not (or very weakly) expressed in the reference state or if fast oscillations are required (faster than the typical time scale of protein dynamics, which in the case of growing cells is roughly given by the cell-cycle time). First, an increase in enzyme amplitudes may require an increase in the central values. In particular, enzymes that are inactive in the reference state can only start oscillating if the central value rises to a positive value, which implies an additional cost (see appendix D). Nevertheless, a rhythmic activation of (statically) inactive enzymes can be beneficial, for instance, where storage reactions are used to buffer fluctuations in external supply. Second, protein amplitudes are limited because of the slow timescale of protein production and degradation. At higher frequencies, only very small protein amplitudes will be possible and post-translational modification (e.g., phosphorylation) may be required for larger oscillations. In our models, allosteric regulation is usually captured by enzymatic rate laws and will not be optimised. However, if desired, allosteric regulation can also be treated as a different kind of post-translational modification.

2.7 Optimal enzyme rhythms are shaped by pairwise synergies

Given a metabolic model, we know how to predict optimal enzyme rhythms. The formulae for optimal enzyme profiles are summarised in the appendix, and results for a number of example models are discussed on www.metabolic-economics.de/enzyme-rhythms/. Can we also tell what typical patterns will emerge in the enzyme profiles, and how they are shaped by fitness objective, external conditions, metabolic dynamics, and enzyme constraints? To understand phenomena such as sequential activation or coregulation in metabolic pathways, we need general rules that apply to arbitrarily complex systems. Such rules cannot be derived by solving individual example models numerically. However, our formulae for small-amplitude oscillations enable us to understand general patterns in enzyme profiles. Enzyme rhythms will be shaped by fitness synergies and by constraints on individual enzyme amplitudes. To obtain an optimal profile, we first consider the pairwise synergies and then search for the global behaviour that combines them in the most beneficial way. There are three types of synergies: enzymes/environment synergies (F_{xx}), enzyme/enzyme synergies (F_{uu} off-diagonal elements), and enzyme self-synergies (F_{uu} diagonal elements). Synergies can arise in different ways, for example: (i) Synergy through supply. An oscillating parameter may create time windows in which enzymes experience higher (or lower) substrate levels and, thus, become more efficient. (ii) Synergy through exclusion. In other cases, parameter rhythms can create time windows in which certain processes should be avoided (for instance, phases in which reactive oxygen species (ROS) levels are high, which makes DNA replication problematic. (iii) Synergy through cost. In models with nonlinear
cost functions, the upregulation of an enzyme can increase the cost pressure and create an incentive to downregulate other enzymes. An emerging rhythm in one enzyme will promote rhythms of opposite phase in other enzymes.

In global enzyme rhythms, all these synergy effects come together. Knowing the synergies, we may try to predict which global behaviour would maximize the total fitness (see Figure 9). This step from pairwise synergies to an optimal global rhythm resembles the calculation of forced oscillations in dynamical systems. If a dynamical system is perturbed by external oscillations, the resulting forced oscillations depend both on the coupling to the external perturbation and on the system’s internal dynamics (i.e., which would also be visible, e.g., in damped oscillations after a short perturbation). In dynamical systems, local interactions are reflected in the global dynamics. Mathematically, the local interactions between neighbouring metabolites are represented by the sparse Jacobian matrix, from which global behaviour (propagating waves or steady
(a) Network and fluxes  (b) Synergies on network  (c) Enzyme synergies  (d) Synergy phase plot

Figure 10: Synergies in the loop pathway (Figure 7), shown by synergy phase plots. (a) Network and optimal enzyme rhythms. (b) Synergies between enzyme rhythms. Enzymes are shown as nodes, their synergies are shown as arcs. The absolute values are indicated by arc colors (dark: large); phase shifts are shown by position and shape of the arrowheads. The position of the arrowhead along the edge represents the phase shift in units of $\frac{2\pi}{2\pi}$. An arrowhead close to a node indicates a small phase shift and points to the node that should peak later (e.g., the arrowhead between $u_1$ and $u_2$ shows that $u_1$ should peak slightly before $u_2$). A dot near the centre of an edge indicates that the enzymes should have opposite phases (see, e.g., the edge between $u_1$ and $u_3$). Self-synergies are not shown. (d) Synergy phase plot. Enzymes are arranged on a circle according to their optimal phases. Arcs are drawn as in (a).

State changes (state changes) can then be derived. In kinetic models, for example, enzymes, metabolites, and reactions are directly linked by stoichiometric coefficients and reaction elasticities, whereas the global behaviour (including the propagation of perturbations) is described by periodic response coefficients, derived from the inverse Jacobian. In enzyme optimisation, there is a similar transition from “local” to “global”. We start from pairwise fitness synergies, which can be seen as local (namely, pairwise) economic effects between enzymes (even though they are already based on global metabolic dynamics). Then we compute the optimal global enzyme profile. The interplay between synergies in a global rhythm can be illustrated by synergy phase plots (see Figure 10). The oscillating parameters (external metabolite and enzyme activities) are shown on the network, and pairwise synergies are represented by arcs with arrows. In a second plot, the enzymes are shown on a circle, arranged by their optimised peak times; in a good arrangement, strong arrows will point in clockwise direction.

How can we find such a good arrangement of enzyme activities? From intuition alone, it would be hard to predict which resulting rhythm would be optimal, and by which synergy effects it will be dominated. Optimal enzyme rhythms must be self-consistent, i.e., each enzyme must be adapted to the rhythms created by external metabolite and all enzymes, which in turn are optimally adapted. The resulting self-consistent state can be seen as the result of many rounds of hypothetical adaptation: if a parameter starts oscillating, it enforces metabolic oscillations to which all enzymes will have to adapt. The resulting rhythms cause further oscillations, promote further enzyme rhythms, and so on. If this process converges, the sum of all adaptations yields the self-consistent global enzyme rhythm. Mathematically, the multiplication with a matrix inverse in Eqs (6) and (7) is exactly a way to compute this infinite series of adaptations (see SI S2.4). A similar matrix inversion appears in metabolic control analysis, when control matrices (describing global, long-term influences) are computed from the Jacobian matrix (which describes local, short-term interactions).
Any enzyme rhythm in a metabolic system will evoke dynamic behaviour, and will thus provide opportunities for further adaptations. For example, a peak in one enzyme activity will create time-shifted peaks in metabolite levels, and during these peaks, other enzymes will be able to work more efficiently. The same will be true for a sequence of such peaks or for an enzyme rhythm. Whenever some coordinated enzyme behaviour provides a way to increase fitness, there will be an incentive for cells to show this behaviour.

### 2.8 Strategies for optimal enzyme regulation

Should organisms behave homeostatically, or should they use changes in the environment as an opportunity to run different processes at different times? Should biochemical processes within cells be synchronised or separated in time, and in which order should they be arranged in metabolic cycles? From a functional perspective, organisms can be expected to do whatever fits their needs (i.e., what improves their evolutionary fitness). In winter, many trees lose their leaves and many animals hibernate, living on storage compounds they produced in the warmer seasons. The production of wood in trees varies depending on the season, and flowering is season-dependent. We can think of the yearly cycle as one process, which has evolved (and is under selective pressure) as a whole. Then, the behaviour in each phase should be adapted to the environment and to the behaviour in past and future phases. We can think of daily rhythms (e.g., the management of glucose by the liver) in a similar way.

To explain biological behaviour in terms of function, we could assume different types of strategies: by “instantaneous” strategies (“In each moment, do what is best under the current conditions”) or by “scheduling” strategies (“Shift each process to the moment in which the external conditions are best”). Here, we assumed “holistic” strategies (“Phase all processes in time such that no further rearrangement would provide an advantage”). In a holistic strategy, the aim would be an arrangement in which all resources are used most efficiently, material for each phase is prepared by previous processes, side products would be reused in following processes, and as little material as possible remains unused. Such strategies correspond to an ecological thinking.

If temporal behaviour is optimised as a whole, the resulting behaviour may involve anticipation. In metabolism, anticipation means that enzyme activities are not passively adapted to their current substrate levels (as in mechanistic substrate activation, or as the notion of just-in-time production may suggest). On the contrary, they actively shape the metabolite profiles and create future conditions under which enzymes will be able to act more efficiently. We can even see this in the “just-in-time” activation of a linear chain: if the goal were to produce some end product very fast, then all enzymes would be immediately activated. Then, however each enzyme would be used less efficiently, and converting most of the substrate into product would take longer. The sequential activation, in contrast, delays the production of internal metabolites, generates large concentration gradients within the pathway, and makes enzymes more efficient once they are activated.

To understand optimal enzyme rhythms, two levels of description – a dynamic metabolic model and an enzyme optimality problem – must be combined (see Figure 9, top and bottom). The two levels require different kinds of reasoning. Dynamic phenomena are described causally: an enzyme perturbation affects a reaction rate, the reaction rate affects reactant levels, the reactant levels affect other reaction rates,
and so on. Thus, in a pathway of irreversible reactions, perturbations would propagate down the pathway, from cause to effect. The enzyme allocation problem forces us to think in reverse. Starting from a desired fitness effect, we infer the necessary metabolic behaviour (e.g., changes in fluxes) and the required enzyme profiles behind them. Thus, we trace the causal effects of enzymes in opposite direction, both along pathways and in time. This inverted causation is what I call “promoting”. “Promoting a rhythm” does not mean “causing a rhythm dynamically”. It means “creating fitness incentives for a certain enzyme rhythm to occur”, considering the dynamical and functional consequences that this rhythm will have. Any process that is causally affected by an enzyme (even indirectly) can promote adaptations of that enzyme.

To promote a rhythm in an enzyme B, an enzyme A need not have a dynamic effect on B, and maybe not even on the reaction catalyzed by B. Maybe A and B just influence some common, fitness-relevant process C. This same form of inverted metabolic control can be used to study optimal adaptations of steady states [11], instead of predicting the effects of enzyme changes (as in MCA), we consider a desired effect and search for enzyme changes that would realise this effect in an optimal way. When studying rhythms, the inversion becomes even more obvious because the effects of enzyme rhythms are “travelling” in the network, and can be traced back in time.

The results suggest a deep analogy relation between enzyme expression and enzyme function. The expression patterns of proteins are not random, but reflect their involvement in metabolic pathways or other cellular systems. Complex-forming proteins, or enzymes involved in the same metabolic pathway, are often coexpressed and their temporal order of expression may reflect the sequential complex assembly [15] or the sequence of enzymatic steps in a pathway [15]. Thus, the expression profiles of enzymes, in their synchronous or sequential activation, seem to “portray” the metabolic network topology. This makes sense: if the components of a protein complex, or a pathway, were not expressed together, resources would be wasted. This suggests that a “portrayal relation” between protein expression and cellular networks provides evolutionary advantages, and that it can be understood by optimality models.

Mathematical models as considered here may clarify the reasons for coregulation in pathways, and thus relations between expression patterns and network structure. For example, we may see whether enzymes in a pathway should be controlled proportionally, or only those that exert strong flux control. In metabolic modelling, “portrayal” relations between enzyme profiles (treated as a control system) and metabolism (the system to be controlled) have been predicted for a number of scenarios. For steady states, Klipp and Heinrich [15] showed that optimal enzyme activities are proportional to the scaled flux control coefficients, i.e., to how strongly each enzyme affects the metabolic flux. A similar result holds for long-term enzyme adaptations to static external changes: here, the enzyme adaptation reflects fitness synergies, which follow from metabolic response coefficients [15]: enzymes that have strong effects on beneficial state variables and whose adaptation would be cheap should be strongly adapted. The sequential activation strategies predicted in [12, 13] relate enzyme activation to the sequence in a metabolic pathway. We now saw that very similar phenomena obtain also for periodic enzyme profiles: optimal enzyme rhythms show coordinated patterns, and the order of activation reflects the order in which metabolic perturbations will propagate in the network.

What is the link between enzyme profiles, metabolic control, and network structure in these theories? In the formulae described here, optimal enzyme profiles depend on periodic perturbations in the environment,
on how the network transmits such perturbations, and on the dependence of cell fitness on state variables. These three factors determine the external perturbations and the synergy matrices in our formulae. The second factor, the transmission of perturbations, is described by periodic response and control coefficients. These coefficients are closely related to network structure: first, they follow from the Jacobian matrix, which directly connects neighbouring metabolites, by matrix inversion; second, stoichiometric matrix and elasticity matrix, control coefficients are directly related through the summation and connectivity theorems of MCA [19]. Thus, there is a direct mathematical link between network structure, forward control, and the shape of optimal enzyme rhythms.

## 3 Discussion

Biological rhythms are often described as sequences of discrete phases, like sleep and wake. Also metabolic rhythms could be modelled as a periodic sequence of steady states with different static enzyme profiles [20]. However, metabolic changes are often continuous rather than abrupt. Metabolic rhythms that are caused by expression changes will be smooth on the metabolic timescale (see SI S1.4), and even sudden extracellular changes would look smooth after having propagated through the metabolic network. Here I generally assumed that metabolic rhythms are smooth and their amplitudes are small, and I focused on sine-wave functions for a number of reasons. In control theory, optimal control profiles can be found by solving the Riccati equation, a differential equation that is integrated backwards in time, starting from the final system state. To obtain periodic solutions, initial and final states would have to be matched. One way to do this is to Fourier-expand all curves (of control and state variables) and to solve an optimality problem for the Fourier coefficients. This leads to the approach proposed here: rhythms are described by smooth, small-amplitude sine-wave oscillations around a (possibly shifted) static reference state, with shifts, phases, and amplitudes to be optimised. Eqs (6) and (7) show how optimal global behaviour emerges from the fitness synergies of single enzymes with sine-wave profiles. Based on the result for sine waves, optimal time profiles for non-harmonic periodic perturbations can be computed by Fourier synthesis [15].

The mechanisms of protein production and degradation act as a low-pass filter and leads to smooth enzyme profiles. Profiles with short peaks could yield stronger allosynchrony effects, but need to be realised post-transcriptionally (i.e., implying effective extra costs).

For non-periodic control problems, other basis functions could be used, e.g., orthogonal polynomials.

By considering complex-valued frequencies, the approach could even be used to describe exponentially damped oscillations, as they may occur, for example, in models with dilution.
tions (for instance, biomass production rate minus the enzyme cost). Then, the static fitness function is translated into a fitness functional for time courses. I proposed two types of fitness functionals, representing different assumptions about cellular time scales. Both functionals are based on time averages. In a **state-averaged fitness**, the static fitness function is applied to the time-averaged metabolic state: enzymes can control fitness only by shifting the average concentrations and fluxes. With the **fitness-averaged fitness** (and nonlinear static fitness functions), fluctuations in state variables will affect the overall fitness. There are also more general fitness functionals in which state variables are averaged on some time scale \( \tau \) before the fitness is evaluated.

Thinking about optimality may improve our understanding of biological regulation. For example, according to our formulae, there are two main scenarios. First, there are systems in which spontaneous metabolic cycles can be beneficial. Whenever there is a positive principal synergy at a finite frequency, this shows that the reference states is fitness-unstable against certain enzyme rhythms and that certain enzyme rhythms can improve the system’s performance even in a constant environment. In this case, there is an incentive for spontaneous oscillations. Second, a system may be fitness-stable against spontaneous enzyme rhythms, but external rhythms may still promote rhythmic enzyme adaptation. Depending on model parameters, the same model may show both types of behavior. For example, a linear pathway with fast dilution may allow for spontaneous rhythms, while the same pathway with weak dilution shows only adaptive rhythms. The dependence of optimal strategies on a model parameter resembles a bifurcation. It is not a bifurcation in the usual dynamical sense (a change in dynamic behaviour caused by parameter changes), but a bifurcation regarding the choice of optimal strategies.

Optimality approaches to biological rhythms could be relevant for other fields of biology, especially for medical modelling. Periodic processes like glucose management in the liver could be better understood by assuming that enzyme activities are not only mechanically regulated, but also functionally adapted. Another usage of such models would be in finding optimal schemes for drug administration. In such applications, the distribution and effects of drugs in the body would be described by a mechanistic pharmacokinetics/pharmacodynamics model. Drug dosages, instead of enzyme activities, would be treated as control variables and the objective would be to maximise the intended effects of drugs while minimising their side effects. Such models could be used to find optimal times for drug administration during a day, or to plan combination therapies in which several drugs are administered at different times to exploit allosynchrony. For example: a first drug could make cells more susceptible to a second drug that is administered with a phase delay; or cancer cells could first be synchronised in their cell cycle or in a metabolic cycle before a second drug hits them in the right phase. Of course, such predictions could also be based on direct numerical optimisation. However, a theory that relates network structure, biochemical dynamics, and external interventions by general formulae may be helpful – because general laws will equally apply to large, complicated systems; because they can show how optimal global behaviour emerges from local fitness synergies, and what “design principles” may be behind beneficial enzyme rhythms; and because it yields general conditions for the existence of spontaneous beneficial oscillations.

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13 In some cases, enzyme cost does not matter: if linear enzyme cost functions or state-averaged fitness functionals are used, enzyme rhythms will be cost-neutral, so fitness is directly determined by metabolic benefit.

14 For averages on specific time scales, we can use a convolution functional \( f(t) = \int K(t - t') f(t') dt' \) with a kernel function \( K \) of width \( \tau \). The averaging may capture the fact that the system dynamics will be “blurred” in time by downstream biochemical pathways before the fitness advantage is realised. By choosing the extreme time scales \( \tau \to \infty \) or \( \tau = 0 \), we obtain our two functionals as limiting cases.
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Appendix

A Forced oscillations in biochemical networks

To study the benefit of enzyme rhythms, we need to know how oscillations propagate in metabolic networks. In this section, I recall some formulae from [11] and [15]. For the mathematical notation, e.g., regarding complex-valued vectors, see SI S5. A kinetic model is a system of rate equations $\frac{dc}{dt} = N r(c, p) - \lambda c$ with concentration vector $c$, stoichiometric matrix $N$ (for internal metabolites), and a flux vector $\mathbf{v} = r(c, p)$. The term $\lambda c$ describes dilution in growing cells, where $\lambda$ is the cell growth rate (or $\lambda = 0$ if there is no dilution). The vector $p$ contains two types of model parameters: external parameters, in a subvector $x$ (e.g., external concentrations) and control parameters in a subvector $u$ (e.g., enzyme activities). A state with a concentration vector $s$ satisfying the stationarity condition $0 = N v(s, p)$ is called a stationary or steady state. We consider a reference parameter vector $p_0$ and require that stable steady states $s(p)$ must exist for all parameter sets $p$ close to $p_0$. Rhythmic changes in external concentrations and in enzyme activities are then described by a time-dependent parameter vector

$$p(t) = p_0 + \text{Re}(\tilde{p} e^{i\omega t})$$

with a complex amplitude vector $\tilde{p}$ and circular frequency $\omega$ (see Figure 11). The parameter perturbation causes periodic concentration and flux changes, which propagate through the network as dampened waves. In a linear model, the resulting concentration and flux rhythms would be harmonic oscillations with frequency $\omega$. In nonlinear models, oscillations can evoke higher harmonics and shift the time-averaged state away from the reference state. Such nonlinear effects can be studied in a second-order approximation [15] (see Figure 3). Below, we neglect higher harmonics and focus on shifts of time averages (of metabolite concentrations, fluxes, and metabolic fitness) because only these effects matter in our fitness functionals.

The direct effect of reactant and enzyme rhythms on reaction rates can be described by spectral elasticities [15]. Since enzyme activities are prefactors in the rate laws $v_l = u_l r_l(c)$, enzyme rhythms alone (at constant reactant levels and a constant average enzyme activity) cannot lead to second-order effects such as higher harmonics or shifts of the average fluxes. However, reactant rhythms or combinations of reactant and enzyme rhythms can have such effects (see Figure S1). To compute a shift in an average flux, we consider two periodic parameters $a$ and $b$ (representing enzyme levels, reactant levels, or other quantities affecting the rate). Their time profiles read

$$a(t) = \bar{a} + \text{Re}(\bar{a} e^{i\omega t}), \quad b(t) = \bar{b} + \text{Re}(\bar{b} e^{i\omega t})$$

with complex amplitudes $\bar{a}$ and $\bar{b}$, frequency $\omega$, and a phase shift $\Delta \varphi = \varphi(\bar{a}) - \varphi(\bar{b})$. If $a$ peaks before $b$, $\Delta \varphi$ will be small and positive. For small-amplitude oscillations, the reaction rate can be approximated by

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

(17)
Box 3: Oscillations and complex amplitudes

(a) Harmonic timecourse

![Harmonic timecourse](image)

Enzyme level in time

- Momentary value: projection to x−axis

(b) Complex amplitude

![Complex amplitude](image)

- Movement of pointer in time
- Momentary value: projection to x−axis

(c) Phase−shifted rhythms

![Phase−shifted rhythms](image)

- Enzyme level
- Time

(d) Pointer snapshot (at t=0)

![Pointer snapshot](image)

- Imag
- Real

(e) Rhythm peak times

![Rhythm peak times](image)

- Peak times shown on "clock"

Figure 11: Enzyme rhythms described by complex amplitudes. (a) Harmonic enzyme profile. Starting from a constant reference state (straight solid line), the level is shifted by $\Delta \bar{u}$, and a harmonic oscillation with circular frequency $\omega = 2\pi/T$ (with period length $T$) and complex amplitude $\bar{u}$ is added. For illustration, the rhythm can be divided into phases (maximal, decreasing, minimal, increasing) according to phase angles (e.g., $[-\pi/4, \pi/4]$ for the “maximal” phase), but such discrete phases have little practical relevance in a smooth dynamics. (b) Instead, a harmonic oscillation is described by the real part of a complex exponential function $\bar{u} e^{i\omega t}$, visualised here as a rotating pointer. The complex amplitude $\bar{u}$ encodes the oscillation amplitude and the initial phase shift at time $t=0$. The real part (projection to x-axis) describes the time-dependent deviation $\Delta \bar{u}(t)$. (c) Oscillations with different phase shifts (peak times shown by arrows). (d) The same oscillations, shown as pointers at time $t=0$. (e) Peak times displayed on a “clock”. The vertical pointer corresponds to the curve peaking at $t=0$; the pointers for curves with later peak times follow in clockwise direction. Metabolite and flux rhythms can be described in the same way.

with reference flux $\bar{v}$ and reaction elasticities $E_a, E_b, E_{aa}, E_{ab},$ and $E_{bb}$, and the resulting shift reads

$$\Delta \langle \bar{v} \rangle_t \approx \frac{1}{2} E_{\bar{a}\bar{a}} |\bar{a}|^2 + E_{\bar{a}\bar{b}} |\bar{a}| |\bar{b}| \cos(\Delta \phi) + \frac{1}{2} E_{\bar{b}\bar{b}} |\bar{b}|^2$$

(18)

with the periodic second-order elasticities $E_{\bar{a}\bar{a}} = \frac{1}{2} E_{aa}, E_{\bar{a}\bar{b}} = \frac{1}{2} E_{ab},$ and $E_{\bar{b}\bar{b}} = \frac{1}{2} E_{bb}$. If parameter $a$ describes an enzyme activity $u$ and parameter $b$ describes a reactant level $x$, we can set $E_{\bar{a}\bar{a}} = E_{\bar{a}\bar{u}} = 0,$ $E_{\bar{a}\bar{b}} = E_{\bar{a}\bar{x}} = \frac{1}{2} E_{ux},$ and $E_{\bar{b}\bar{b}} = \frac{1}{2} E_{xx},$ and the shift reads

$$\Delta \langle \bar{v} \rangle_t \approx E_{\bar{a}\bar{x}} |\bar{a}| |\bar{x}| \cos(\Delta \phi) = \frac{E_x}{2u} |\bar{a}| |\bar{x}| \cos(\Delta \phi).$$

(19)
Figure 12: Optimality problem for enzyme rhythms. The metabolic state (fluxes and metabolite levels are the state variables) is influenced by external parameters and enzyme levels. A fitness function (given by the difference of metabolic benefit and enzyme cost) needs to be maximised. Whenever an enzyme profile would be able to increase the fitness, there is an incentive for realising this profile. The scheme can refer to steady states (with static external and enzyme profiles) or dynamic behaviour (with external and enzyme rhythms).

This formula resembles Eq. (3) (with $\tilde{E}_{\tilde{u}} \tilde{x}$ replacing the prefactor $k/2$).

In a single reaction, reactant and enzyme rhythms can act synergistically, and their effects on the average flux can be described by second-order spectral elasticities. Rhythms in metabolic networks, driven by rhythms of enzymes and external metabolites, can be described in a similar way. The relation between an applied enzyme or external rhythm and the resulting flux and concentration rhythms is described by periodic response coefficients (see SI 1.3). If a state variable $s$ (metabolic flux or internal concentration) is influenced by oscillating parameters $a(t)$ and $b(t)$, a second-order expansion for $s(t)$ yields three terms: forced oscillations with frequencies $\omega$, forced oscillations with frequency $2\omega$, and a shift of the central value. This shift

$$
\Delta \langle s \rangle \approx \frac{1}{2} R_{aa}^s(\omega) \tilde{a}^2 + \frac{1}{2} R_{bb}^s(\omega) \tilde{b}^2 + \frac{1}{2} \text{Re} \left( e^{-i\Delta \varphi} R_{ab}^s(\omega) \right) |\tilde{a}| |\tilde{b}|,
$$

(20)

again, consists of three terms: two terms represent self-synergies of $a$ and of $b$ (describing, e.g., the dynamic self-inhibition of an enzyme); the third term describes a synergy between the parameters. Its magnitude and sign depend on the phase shift $\Delta \varphi$. Eq. (20) contains no first-order term, because 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 a model with many periodic parameters (amplitude vector $\tilde{p}$), we can sum over all pairwise synergies (each described by Eq. (20)) and obtain the total shift

$$
\Delta \langle y \rangle \approx \frac{1}{2} \tilde{p}^\dagger R_{pp}^s(\omega) \tilde{p}.
$$

(21)

The symbol $\tilde{p}^\dagger$ denotes the adjoint (i.e., complex conjugate transpose). The synergy matrix $R_{pp}^s(\omega)$ is Hermitian and can be computed from the stoichiometric matrix $N$ and the first-and second-order elasticities (for details, see SI 1.3). Its elements, called second-order periodic response coefficients (see SI 1.3), resemble the second-order spectral control coefficients from 15 except for a scaling factor. In a second-order approximation, there are no fitness synergies between rhythms of different frequencies.
Fitness functions for metabolic dynamics

To study optimal enzyme allocation, we need to define a fitness function to be optimised. Our fitness functions score enzyme profiles in two ways: by the benefit achieved in metabolism, and by the cost for maintaining the enzyme levels. For a single reaction, we would consider a benefit function \( g(u, x) = b(v(u, x)) \), a cost function \( h(u) \), and define the fitness function \( f(u, x) = g(u, x) - h(u) \). For an entire network, we consider a fitness function (see Figure 12)

\[
f(u) = b(s(u)) - h(u),
\]

with a metabolic benefit function \( b(s) \) scoring the state variable vector \( s \) (containing flux vector \( v \) and concentration vector \( c \)), and a cost function \( h(u) \) scoring the control vector \( u \). The control variables can be enzyme activities or other variables, including the dilution rate in growing cells [11]. In a pathway model, the benefit and cost functions \( b(s) \) and \( h(u) \) describe how the pathway contributes to cell fitness, as assumed by the modeller. Cost functions for protein expression can be determined experimentally [21, 22], e.g., by measuring growth deficits after an artificial expression of non-functional proteins. In our models, the cost must be an increasing (linear or nonlinear) function of the protein levels. Next, benefit and cost are expanded to second order

\[
\begin{align*}
\Delta s & \approx \Delta s + \frac{1}{2} \Delta s^\top \mathbf{B}_{ss} \Delta s, \\
\Delta \bar{u} & \approx \Delta \bar{u} + \frac{1}{2} \Delta \bar{u}^\top \mathbf{H}_{uu} \Delta \bar{u},
\end{align*}
\]

where \( \mathbf{b}_{s}, \mathbf{B}_{ss}, \mathbf{h}_{u}, \) and \( \mathbf{H}_{uu} \) are the gradients and curvature matrices of the functions \( b \) and \( h \). In models with external parameters \( x_j \) (e.g., external concentrations), the steady-state fitness

\[
f(u, x) = b(s(u, x)) - h(u)
\]

contains \( x \) as an extra argument. In static optimality problems for \( u \), the parameters \( x_j \) are given and control parameters \( u_l \) must be chosen to maximise \( f(u, x) \), locally and under all given constraints. The synergy matrices contain the curvatures of the fitness function Eq. (24) and are given by

\[
\begin{align*}
\mathbf{F}_{ux} &= \mathbf{b}_{s}^\top R_{s}^{u} + R_{x}^{u} \mathbf{B}_{ss} R_{s}^{u}, \\
\mathbf{F}_{uu} &= \mathbf{b}_{s}^\top R_{s}^{x} + R_{x}^{u} \mathbf{B}_{ss} R_{s}^{x} - \mathbf{H}_{uu}.
\end{align*}
\]

If we consider an enzyme-optimal state and omit all inactive reactions from the model, the fitness gradient \( f_u = (\partial f / \partial u_l) \) must vanish and the synergy matrix \( \mathbf{F}_{uu} \) must have negative eigenvalues.

Now we consider optimal adaptations to periodic perturbations. We assume that all parameters oscillate around their reference values:

\[
\begin{align*}
x(t) &= x^{ref} + \text{Re}[\tilde{x} \, e^{i\omega t}], \\
u(t) &= u^{ref} + \text{Re}[\tilde{u} \, e^{i\omega t}].
\end{align*}
\]
In a second-order approximation, these perturbations may shift the average metabolic state and the average fitness. The time average of a variable $s$ can be expanded, using Eq. (20), as

$$\langle s \rangle \approx s(\mathbf{x}_\text{ref}, \mathbf{u}_\text{ref}) + \frac{1}{2} \left( \mathbf{\tilde{x}} \mathbf{\tilde{u}}^\top \mathbf{R}^s_{\tilde{x}\tilde{x}}(\omega) \mathbf{R}^s_{\tilde{u}\tilde{x}}(\omega) \right) \langle \mathbf{\tilde{x}} \rangle \langle \mathbf{\tilde{u}} \rangle.$$  

(27)

When assigning fitness values to enzyme profiles $\mathbf{u}(t)$, we can choose between two types of fitness functionals. In the state-averaged fitness functional, we apply the fitness function (22) to the time-averaged state:

$$F^{(S)} = b(\langle s(t) \rangle) - h(\langle \mathbf{u}(t) \rangle).$$  

(28)

In the fitness-averaged fitness functional, we first apply the fitness function (22) and then take the time average:

$$F^{(F)} = \langle b(\mathbf{u}(t)) \rangle - h(\langle s(t) \rangle).$$  

(29)

In more general fitness functionals, the fitness would be evaluated on specific time scales. Based on our fitness function and our fitness functional ($F^{(S)}$ or $F^{(F)}$), we can now compute the fitness effects of parameter rhythms. Given a periodic perturbation of external concentrations $x_j$ and enzyme activities $u_l$, the resulting fitness change can be approximated by a quadratic function of the amplitude vectors $\mathbf{\tilde{x}}$ and $\mathbf{\tilde{u}}$. The synergy matrices (which determine this quadratic function) can be computed from the periodic response coefficients and the fitness derivatives. For the state-averaged fitness Eq. (28), they read

$$F^{(S)}_{\tilde{x}\tilde{x}} = b_s^\top \mathbf{R}^s_{\tilde{x}\tilde{x}}(\omega),$$

$$F^{(S)}_{\tilde{u}\tilde{x}} = b_s^\top \mathbf{R}^s_{\tilde{u}\tilde{x}}(\omega).$$  

(30)

For the fitness-averaged fitness Eq. (29), they contain additional terms:

$$F^{(F)}_{\tilde{x}\tilde{x}} = b_s^\top \mathbf{R}^s_{\tilde{x}\tilde{x}}(\omega) + \frac{1}{2} (\mathbf{R}^\tilde{s}_{\tilde{x}\tilde{x}}(\omega))^\top \mathbf{B}_{ss} \mathbf{R}^\tilde{s}_{\tilde{x}\tilde{x}}(\omega),$$

$$F^{(F)}_{\tilde{x}\tilde{u}} = b_s^\top \mathbf{R}^s_{\tilde{u}\tilde{x}}(\omega) + \frac{1}{2} (\mathbf{R}^\tilde{s}_{\tilde{u}\tilde{x}}(\omega))^\top \mathbf{B}_{ss} \mathbf{R}^\tilde{s}_{\tilde{u}\tilde{x}}(\omega) - \frac{1}{2} \mathbf{H}_{uv}. $$  

(31)

For slow oscillations ($\omega \approx 0$), we can approximate $\mathbf{R}^\tilde{s}_{\tilde{x}\tilde{x}} \approx \mathbf{R}^s_{\tilde{x}\tilde{x}}$, $\mathbf{R}^\tilde{s}_{\tilde{u}\tilde{x}} \approx \mathbf{R}^s_{\tilde{u}\tilde{x}}$, and $\mathbf{R}^\tilde{s}_{\tilde{x}\tilde{u}} \approx \mathbf{R}^s_{\tilde{x}\tilde{u}}$. Eq. (31) yields the same synergy matrices as Eq. (25), but with a prefactor of 1/2. This prefactor makes sense: periodic synergy matrices, even in the limit $\omega \to 0$, do not refer to constant parameter shifts in one direction, but to (infinitely slowly) alternating perturbations, where only $\sqrt{1/2}$ of the maximal amplitude is realised on average.

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15In a functional $F = \langle f \int K(t-t')s(t')dt' \rangle_t$, state variables $s(t)$ are convoluted with a kernel function (temporal width $\tau$), a fitness function is applied, and the resulting fitness values are averaged over time.
C Optimal enzyme profiles

To predict optimal enzyme profiles, we first need to know how small (static or periodic) variations of enzyme activities and external parameters would affect fitness. According to our fitness expansion Eq. (5), all necessary information is contained in the synergy matrices (30) and (31). Based on these matrices, and ignoring any further constraints on enzyme profiles, we can predict optimal enzyme profiles for a number of cases (see Figures 4 and 13).

1. Criterion for optimal steady states. Given the constant external parameters $x_j$, an enzyme profile $u$ is an interior optimum of $f$ if the fitness gradient $f_u(x, u)$ vanishes, the synergy matrix $F_{uu}(x, u)$ is negative definite (i.e., all eigenvalues are negative), and all enzyme activities $u_l$ are positive. In this case, the parameter set $(x, u)$ is called enzyme-balanced. An enzyme profile is a boundary optimum if some of the enzymes form an internal optimum while other enzyme activities vanish and the slopes $\partial f / \partial u_l$ for these enzymes are negative.

2. Optimal adaptation to static external perturbations. If $(x^{\text{ref}}, u^{\text{ref}})$ is an enzyme-balanced parameter set and $\Delta \bar{x}$ a static perturbation of $x^{\text{ref}}$, a static enzyme change $\Delta \bar{u}^{\text{opt}}$ leading to an enzyme-balanced state $(x^{\text{ref}} + \Delta \bar{x}, u^{\text{ref}} + \Delta \bar{u}^{\text{opt}})$ is called the optimal adaptation to $\Delta \bar{x}$. For small perturbations $\Delta \bar{x}$, the optimal adaptation can be approximated by [11]

$$
\Delta \bar{u}^{\text{opt}}(\Delta \bar{x}) \approx A_x^{u} \Delta \bar{x}
$$

with the adaptation matrix $A_x^{u} = -F_{uu}^{-1} F_{ux}$. The synergy matrices $F_{uu}$ and $F_{ux}$ are given by Eq. (25), and $F_{uu}$ is invertible because our initial state is assumed to be optimal. Eq. (32) can be proven easily: in an optimal adaptation, the fitness gradient must vanish before the perturbation ($f_u(x^{\text{ref}}, u^{\text{ref}}) = 0$) and after the adaptation ($f_u(x^{\text{ref}} + \Delta \bar{x}, u^{\text{ref}} + \Delta \bar{u}) = 0$). By expanding these equations to first order and equating them, we obtain the condition $\Delta f_u = F_{uu} \Delta \bar{u} + F_{ux} \Delta \bar{x} = 0$; solving for $\Delta \bar{u}$ yields Eq. (32).

As an example, consider a single reaction. The fitness function reads $f(u, x) = g(u, x) - h(u)$. If the cost function is not too steep, the optimal enzyme activity $\bar{u}^{\text{opt}}(x) = \arg \max_u f(u, \bar{x})$ is positive (see SI S3.1 for an example model). Since the fitness function is negatively curved, any spontaneous enzyme variation would lead to a fitness loss. As the substrate level increases from $\bar{x}$ to $\bar{x} + \Delta \bar{x}$, the optimal enzyme activity also increases. The optimal adaptation to a small change $\Delta \bar{x}$

$$
\Delta \bar{u}^{\text{opt}} \approx -f_{uu}^{-1} f_{ux} \Delta \bar{x}
$$

depends on the fitness curvatures (also called synergies) $f_{uu} = \frac{\partial^2 f}{\partial u^2} < 0$ and $f_{ux} = \frac{\partial^2 f}{\partial u \partial x} > 0$ in the initial optimal state. A positive increase $\Delta \bar{x}$ leads to a positive adaptation $\Delta \bar{u}^{\text{opt}}$, and the fitness increases by $\Delta f = f_{ux} \Delta \bar{u}^{\text{opt}} \Delta \bar{x} + \frac{1}{2} f_{uu} \Delta \bar{u}^{2 \text{opt}} = -\frac{1}{2} f_{uu}^{-1} f_{ux}^2 \Delta \bar{x}^2 > 0$.

3. Optimal adaptation to external oscillations. Optimal adaptations to external rhythms (with amplitude vector $\bar{x}$) can be computed in a similar manner. The optimal enzyme rhythm reads

$$
\bar{u}^{\text{opt}}(\bar{x}) \approx \bar{A}_x^{u} \bar{x}.
$$
Figure 13: This overview shows possible fitness landscapes for a single enzyme within a larger metabolic system. Axes refer to the central level $\bar{u}$ and to the amplitude $|\tilde{u}|$ of the enzyme (the phase angle is assumed to be optimally chosen in all cases). (a) Unperturbed system and systems under static or periodic external perturbation. The fitness optimum (red dot) is shifted by perturbations. (b) System in which the enzyme become active under rhythmic perturbations. In the reference state, constraints (diagonal lines) force the enzyme to be inactive. Upon periodic perturbation (right), an enzyme rhythm becomes profitable even if it entails a costly increase of the central value. (c) Enzyme rhythms realised by protein expression and posttranslational modification. Diagonal lines indicate constraints for protein rhythms. The optimal rhythm on the left can be realised by expression changes alone. In the centre (where the constraint is tighter, e.g., due to a higher frequency), only a suboptimal rhythm can be realised. On the right, we assume that posttranslational modification rhythms can be added. Now any enzyme amplitudes can be realised, but at a cost; this is why the optimum deviates from the nominal maximum of the fitness landscape (centre of the circle). (d) Systems in which the reference state is fitness-unstable against enzyme rhythms. In the first case (left), the amplitude will increase spontaneously until it hits a bound. In the second case (right), the solution diverges. In reality, very large enzyme values would become costly. In the model, this could be captured by constraining the maximal enzyme activities.

The periodic adaptation matrix $\tilde{A}^u = -F_{\tilde{u}\tilde{u}}^{-1}F_{\tilde{u}x}$ follows from the synergy matrices $F_{\tilde{u}\tilde{u}}$ and $F_{\tilde{u}x}$ for periodic perturbations (Eqs 30 and 31).

4. **Fitness change** By inserting the optimal adaptations $\Delta \tilde{u}_{\text{opt}}$ or $\tilde{u}_{\text{opt}}$ into Eq. (5), we obtain the overall fitness change resulting from perturbation and adaptation. For periodic perturbations $\tilde{x}$, this fitness change reads $\Delta F = \tilde{u}_{\text{opt}}^\dagger F_{\tilde{x}\tilde{x}}^{-1} F_{\tilde{u}\tilde{x}} = -\tilde{x}^\dagger F_{\tilde{x}\tilde{x}}^{-1} F_{\tilde{u}\tilde{x}} \tilde{x}$. 

31
5. **Beneficial spontaneous oscillations.** An enzyme rhythm $\tilde{u}$ alone (without external perturbation) would change the fitness by

$$\Delta F \approx \frac{1}{2} \tilde{u}^\dagger F_{\tilde{u}\tilde{u}} \tilde{u}. \quad (35)$$

If a vector $\tilde{u}$ yields a positive fitness change $\Delta F$, it represents a beneficial “spontaneous” enzyme rhythm. This holds for any eigenvector of $F_{\tilde{u}\tilde{u}}$ with a positive eigenvalue. Since $F_{\tilde{u}\tilde{u}}$ is Hermitian, it has real-valued eigenvalues and its eigenvectors span the whole space of possible amplitude vectors. For beneficial spontaneous enzyme rhythms to exist, the reference state must be fitness-stable against any static enzyme changes but fitness-unstable against certain enzyme rhythms. This means: all eigenvalues of $F_{\tilde{u}\tilde{u}}$ must be negative and some eigenvalue of $F_{\tilde{u}\tilde{u}}(\omega)$, for some frequency $\omega \neq 0$, must be positive.

To find the small-amplitude rhythm with the highest fitness advantage, we compute the eigenvector $\tilde{u}^{\text{opt}}$ with the largest eigenvalue (called principal fitness synergy $\sigma(\omega)$). This eigenvector represents the best enzyme rhythm at a given norm $|\tilde{u}|$, and its elements define the relative amplitudes and phases. With larger amplitudes, optimal enzyme rhythms will also be shaped by amplitude constraints and cannot be simply obtained from the eigenvectors. If there are beneficial enzyme rhythms at different frequencies, the one with the highest principal synergy $\sigma$ will be most beneficial, and thus most likely to appear in evolution. If $\sigma$ has a maximum at $\omega = 0$, there is no clear selection advantage to oscillations of finite frequency.

6. **Bounds for enzyme amplitudes**

The amplitudes $|\tilde{p}|$ of protein profiles $\bar{p} + \text{Re} [\tilde{p} e^{i\omega t}]$ are constrained for several reasons (see Figure 14): (i) $|\tilde{p}| \leq \bar{p}$: The amplitude cannot be larger than the average value because otherwise, protein levels would become negative. (ii) A simple model of protein production and degradation (with degradation constant $\kappa$) yields an even tighter constraint $|\tilde{p}| \leq \sqrt{\frac{\kappa}{\kappa + \omega}} \bar{p}$ (see SI S1.4). At $\omega = 0$, the two constraints are identical (see Figure 14 (a)). (iii) A third constraint arises as follows: the time derivatives of the protein concentration, $d\tilde{p}/dt = -\bar{p} \omega \sin(\omega t)$, reaches a maximal negative value $-\bar{p} \omega$ in a moment when the protein level is given by $\bar{p}$, implying a degradation rate $\kappa \bar{p}$. Since the rate of decrease cannot exceed the degradation rate, we obtain the constraint $\tilde{p} \leq \bar{p} / \kappa$. (iv) Due to limited space in cells, we can put upper bounds on maximal protein levels $\bar{p} + |\tilde{p}|$ or, alternatively, the average level $\bar{p}$. With all these constraints, each protein amplitude $|\tilde{p}|$ has an upper bound $\tilde{p}^{\text{max}}(\bar{p}, \omega) = \min \left( \sqrt{\frac{\kappa}{\kappa + \omega}}, \frac{\kappa}{\omega} \right) \bar{p} = \gamma(\omega) \bar{p}$. The maximal relative amplitude $\gamma = \tilde{p}^{\text{max}} / \bar{p}$ depends on the frequency and on the effective degradation constant $\kappa$; at frequencies $\omega \gg \kappa$, it comes close to zero.

7. **The role of constraints in enzyme optimisation**

In the fitness expansion Eq. (5), there are no synergy terms between enzyme rhythms and static changes, or between enzyme rhythms at different frequencies. Therefore, the optimal adaptations to external rhythms of different frequencies should be additive. However, if there are constraints on enzyme activities, the overall solution (i.e., optimal enzyme rhythms, summed over all frequencies) must respect the constraints (e.g., on maximal enzyme activities). We may compute a preliminary solution by summing the solutions for individual frequencies, and check whether all constraints are satisfied. If they are not, a solution containing all frequencies needs to be determined by a joint optimisation with constraints.

8. **Enzymes that become active in periodic states**

In some models, enzymes may remain inactive in
the optimal reference state, but start oscillating in periodic states. Due to the amplitude constraints, the average levels of these enzymes must increase with the amplitude. In this case, static and periodic adaptations cannot be treated separately, and the constrained optimality problem Eq. (13), must be solved even for small oscillations. As usually, there are two cases: (i) The reference state is fitness-stable against enzyme oscillations, and enzyme rhythms must be promoted by external oscillations. (ii) The reference state is fitness-unstable against enzyme oscillations (involving an increase in average enzyme activities); in our second-order expansion, the resulting oscillations will become large and will hit the bounds for maximal enzyme activities or amplitudes.

9. **Non-optimal enzyme profiles and the resulting fitness loss.** Non-optimal enzyme profiles lead to a fitness loss. For example, a profitable enzyme rhythm running in opposite phase will become costly. The fitness loss caused by non-optimal enzyme profiles can be quantified using Eq. (5) (see Figure 4 (c)): with a mismatch \( \tilde{u}_{\text{mis}} = \tilde{u} - \tilde{u}_{\text{opt}} \) between optimal and actual amplitude vector, we obtain the fitness change

\[
\Delta F_{\text{mis}} \approx \text{Re}[\tilde{x}^\dagger F_{\tilde{x}\tilde{x}} \tilde{u}_{\text{mis}} + \tilde{u}_{\text{opt}}^\dagger F_{\tilde{u}\tilde{u}} \tilde{u}_{\text{mis}} + \tilde{u}^\dagger \ast F_{\tilde{u}\tilde{u}} \tilde{u}_{\text{mis}}] = \text{Re}[\tilde{u}^\dagger \ast F_{\tilde{u}\tilde{u}} \tilde{u}_{\text{mis}}] 
\]

(36)

(note that \( \tilde{x}^\dagger F_{\tilde{x}\tilde{x}} + \tilde{u}_{\text{opt}}^\dagger F_{\tilde{u}\tilde{u}} = 0 \) in the reference state). If a model is fitness-stable against enzyme rhythms, the synergy matrix \( F_{\tilde{u}\tilde{u}} \) is negative definite, so Eq. (36) describes a fitness loss.

The calculations of optimal enzyme rhythms has been implemented in a computational workflow. Matlab code and models are available at [www.metabolic-economics.de/enzyme-rhythms/](http://www.metabolic-economics.de/enzyme-rhythms/).

### D Extensions of the theory

The calculation of optimal enzyme rhythms can be extended to other cases:

1. **Other kinds of constraints.** In the optimisation of \( \Delta \tilde{u} \) and \( \tilde{u} \) by Eq. (13), we would also consider constraints on metabolite concentrations, fluxes, the sum of enzyme activities, or time averages of these quantities\(^{16}\), and we may predefine periodic profiles of metabolite concentrations or fluxes\(^{17}\). More generally, constraints of the form \( M \tilde{x} = \xi \) could be used to predefine linear combinations of periodic fluxes or concentrations. Alternatively, one could employ soft constraints, implemented by penalty terms in the fitness function.

2. **Other control variables.** So far, we assumed that our control variables \( u_l \) represent enzyme activities, while the state variables represent metabolite levels. The formalism can also be used differently. For example, the kinetic model could include enzyme levels as dynamic variables, while other variables, such

\(^{16}\)Aside from negative enzyme activities, which are always excluded, we can also exclude negative metabolite levels by using a first-order expansion: the approximative constraint \( c + R^*_l \tilde{u} + R^*_x \tilde{x} \geq 0 \) excludes enzyme profiles for which the model would become inconsistent (because our Taylor expansion would yield meaningless results).

\(^{17}\)Here is an example. One could predefine the amplitude and phase of the cellular ATP/ADP ratio (possibly, based on experiments), and a model would have to realise this ratio in a linear approximation (i.e., \( R_{\text{ATP/ADP}} \tilde{u} \) would be constrained to a predefined complex number).
as mRNA levels, transcription factor activities, drug dosages, or the cell growth rate (which determines dilution in the metabolic model) could be treated, instead, as control variables.

3. Frequency-dependent cost. Enzyme cost in bacteria can be defined by measured growth deficits after an artificial expression of protein. In experiments, the high growth deficit was found to be high directly after the perturbation, and it decreased after some hours. Possibly, ribosome levels or other cellular resources had gotten out of balance and the cell needed time to return to a balanced state. If similar adjustments occur during metabolic oscillations, there will be higher costs during any increase in enzyme levels, and lower costs for the same enzyme levels later on. Thus, our cost function would not only depend on enzyme levels, but also on their changes in time. Enzyme oscillations would come at an extra cost, and fast oscillations would be more costly because ribosome adaptation would be less able to follow fast periodic changes. To describe this effect in models, we would need to assume frequency-dependent enzyme cost functions. However, when biological rhythms are slow and predictable, as in day-night cycles, cells may be able to anticipate future ribosome demands, and there may be no such frequency-dependent costs.

4. Periodic fitness functions. So far, the rate equations and fitness functions in our models were not explicitly time-dependent; time dependencies were only caused by oscillating parameters. However, there are good reasons to assume time-dependent fitness functions in models. Typically, a model will describe a cellular pathway, which in reality is embedded in a larger cell. Let us assume that this cell is subject to a time-independent fitness function and to periodic external perturbations. In an optimally adapted state, all cellular subsystems will oscillate and thus provide dynamic perturbations to our pathway. At the same time, other cellular pathways will also have periodically changing requirements for whatever our pathway produces: thus, effectively, we need to assume a time-dependent fitness function for our pathway of interest. Generally, to flexibly zoom into a system and describe its parts by effective models, we need to allow for periodic fitness functions. As soon as the time-shift invariance of our fitness functionals is broken, new terms may appear in the fitness expansion Eq. (5): there can be first-order terms resulting from periodic enzyme activities, as well as synergy terms linking static shifts and amplitudes. The existing terms in the fitness expansion would change as well, and perturbations of different frequencies could show synergy effects.

5. Oscillations involving covalent modification and allosteric regulation. If enzyme rhythms are realised by periodic gene expression, their amplitudes will be limited, and for high frequencies, the maximal amplitudes become very small (Figure 14 (a)). To obtain large, fast oscillations, enzyme activities need to be modulated, for example, by covalent modification such as phosphorylation (Figure 14 (b)-(d)). However, inhibited (or incompletely activated) enzyme molecules become less efficient on average. To compensate for this (i.e., to keep the average enzyme activity constant when applying periodic posttranslational inhibition), the time-average protein level must be increased. To implement this effect in models, we distinguish between an enzyme’s concentration \( p(t) \) (concentration of enzyme molecules, appearing in the cost function) and activity \( u(t) \) (concentration of enzyme molecules in the active state, appearing in the rate laws). To express the new cost in terms of \( u \), we apply the following rationale: if a desired enzyme amplitude can be realised by protein expression alone, there is no need for posttranslational regulation, enzyme and protein curves are identical, and the enzyme cost function is
Figure 14: Amplitude constraints for enzyme rhythms can be overcome by periodic posttranslational inhibition. (a) Constraints for protein amplitudes (with an average enzyme concentration $\bar{p}$ and protein degradation constant $\kappa = 1$). The blue region shows feasible protein amplitudes $\tilde{p}_{\text{max}}(\omega)/\bar{p}$ at different frequencies. (b) If a desired activity profile $u(t)$ respects the amplitude constraints for protein levels, the oscillation can be directly realised by periodic expression. (c) If the desired amplitude is too large, it cannot be realised by expression alone, and additional posttranslational regulation is needed. (d) To realise the desired amplitude, the cell may increase the average enzyme concentration (left) and then inhibit the enzyme periodically by posttranslational modification (right). Colours correspond to the amplitudes shown in Figure 8. The inhibition amplitude and the required increase in average enzyme concentration are both given by $q = |\tilde{u}| - \tilde{p}_{\text{max}}$, the difference between desired activity amplitude and allowed protein amplitude. The higher protein level leads to an extra cost, which can be attributed, effectively, to the periodic inhibition. It is proportional to the inhibition amplitude.

given by the protein cost function with $p$ replaced by $u$. If the desired enzyme amplitudes is too large, the deviation between the desired enzyme amplitude $\tilde{u}$, and the maximal protein amplitude $\tilde{p}_{\text{max}}$ is described by an auxiliary variable $q_l = \max(0, |\tilde{u}| - \tilde{p}_{\text{max}}(\bar{p}, \omega))$ (see Figure 14), and the optimisation problem is formulated in terms of $\bar{u}$, $\tilde{u}$, $p$, and $q_l$ (see SI S2.6). Instead, the resulting extra cost can be attributed, effectively, to the posttranslational inhibition itself and will be proportional to the inhibition amplitude.
Supplementary information

This supplementary text contains additional material about metabolic dynamics, example models, and mathematical derivations. Section S1 describes how periodic perturbations in single reactions can be described by spectral elasticities; how biochemical systems respond to small static or periodic perturbations and how these responses can be computed by response coefficients; how static, spectral, and periodic response coefficients are computed; and how periodic changes in transcript and protein levels are related. Section S2 describes the prediction of optimal enzyme profiles in time. In section S3, simple example models are discussed. Section S4 contains proofs and derivations, followed by a description of the mathematical notation (with lists of mathematical symbols in Tables S1 and S2).

S1 Dynamics of metabolic oscillations

The steady state of kinetic models can be shifted by static parameter changes. Periodic parameter changes, instead, drive forced oscillations. These dynamic responses can be computed in a second-order approximation [23, 24, 15].

S1.1 Expansion of periodic reaction rates

Enzymatic rate laws describe the rates of reactions as functions of substrate, product, and enzyme levels. They can be linearly expanded with the expansion coefficients called reaction elasticities. A reaction elasticity is the derivative between the reaction rate and a variable that directly determines this rate, in a given (maybe non-stationary) metabolic state. The unscaled reaction elasticities of a rate law \( \nu_k(c, p) \), defined as

\[
E_{v_k}^{c_m} = \frac{\partial \nu_k}{\partial c_m} \quad E_{v_k}^{p_m} = \frac{\partial \nu_k}{\partial p_m} \quad E_{v_k}^{c_m,c_n} = \frac{\partial^2 \nu_k}{\partial c_m \partial c_n} \quad E_{v_k}^{c_m,p_n} = \frac{\partial^2 \nu_k}{\partial c_m \partial p_n} \quad E_{v_k}^{p_m,p_n} = \frac{\partial^2 \nu_k}{\partial p_m \partial p_n}
\]

(S1)

allow us to Taylor-expand the rate law around a given reference state. This notion can be extended to periodic states. Sine-wave oscillations in the reactant and enzyme levels lead to a periodic state, in which reaction rates oscillate. Shifts and amplitudes of the rate can be computed, to second order, with the help of periodic elasticities (see [15] and Figure S1). In the model in Figure 2, we considered mass-action kinetics, i.e., a rate law that is linear in both \( u \) and \( x \). Using sine functions as an ansatz for enzyme profiles, we can compute the optimal amplitude and phase (in this case, we would obtain a rhythm in phase with the substrate rhythm). If a rate law is nonlinear in \( x \), computing the optimal amplitude would be difficult. However, for small perturbations, we can expand the rate law around the reference state and obtain an
with the mixed elasticity $E$ will matter for periodic enzyme adaptation. This term resembles the rate law in our mass-action model, oscillation period $T$, reactant profiles vanishing time averages, and the third term does not depend on $E$ so $X; in reversible reactions, $Y$ will peak after $X$ (phase shift $0 < \varphi < \pi$), and the enzyme will peak before X.

The prefactor $E$ contains the reactant amplitudes and $x$. To model oscillations, we now consider harmonic enzyme profiles $\tilde{x}$. To determine the flux change caused by $u(t)$, averaged over an oscillation period $T$. This flux change depends only on the last term because the first two terms have vanishing time averages, and the third term does not depend on $u$. Thus, only the last term in Eq. (S2) will matter for periodic enzyme adaptation. This term resembles the rate law in our mass-action model, with the mixed elasticity $E_{ux} = \frac{1}{2} E_x$ replacing the rate constant $k$; the average rate shift due to enzyme adaptation reads

$$\Delta(v)_t \approx \Re\left\{ \frac{1}{2} E_{ux} \tilde{u} \tilde{x} \right\} = \frac{1}{2} E_{ux} |\tilde{u}| |\tilde{x}| \cos(\varphi). \tag{S3}$$

The prefactor $E_{ux} = \frac{1}{2} E_x$, called periodic second-order elasticity, relates flux shifts to enzyme and substrate amplitudes. For reactions with several substrates and products, we obtain

$$\Delta(v)_t \approx \frac{1}{2} \Re\left\{ \tilde{u} E_{ux} \tilde{x} \right\}, \tag{S4}$$

where $\tilde{x}$ contains the reactant amplitudes and $E_{ux} = \frac{1}{2} E_x = \frac{1}{2} E_x$ is the row vector of periodic enzyme-reactant elasticities. As an example, consider a reaction $X \rightleftharpoons Y$ with elasticities $E_X > 0$ and $E_Y \leq 0$. With reactant amplitudes $\tilde{x}$ and $\tilde{y}$, the flux shift caused by an enzyme profile with the complex amplitude $\tilde{u}$ reads $\Delta(v)_t = \Re\left\{ \frac{1}{2} \Re\left[ \tilde{u} (E_X \tilde{x} + E_X \tilde{y}) \right] \right\}$. To maximise the flux at a fixed amplitude $|\tilde{u}|$, the enzyme must be in phase with $E_X \tilde{x} + E_Y \tilde{y}$. In an irreversible reaction ($E_Y = 0$), the enzyme must be in phase with $X$; in reversible reactions, $Y$ will peak after $X$ (phase shift $0 < \varphi < \pi$), and the enzyme will peak before $X$.  

Figure S1: Periodic reaction rate caused by substrate oscillations. The rate law (black curve) relates substrate levels ($x$-axis) to reaction rates ($y$-axis). A harmonic substrate oscillation (red curve, where the $y$-axis symbolises time) leads to a non-harmonic rate oscillation (blue curve, where $x$-axis symbolises time). The minimal and maximal concentrations correspond to minimal and maximal rates (dashed lines), and the average concentration corresponds to the median rate (dotted line), which differs from the average rate (blue straight line). In a second-order approximation, the flux shift can be determined from the substrate amplitude with the help of periodic elasticities (see Eq. (S3)). The substrate oscillation, at a constant substrate level, decreases the average flux. A flux increase could be achieved, instead, with a sigmoidal Hill kinetics (not shown).
S1.2 Effective elasticities for periodic metabolic states

In a periodic metabolic state, substrate, product, and enzyme levels will show periodic profiles; in such a state, we can define periodic-state elasticities, describing how (time-average rates or amplitudes of reaction rates are affected by additional (static or periodic) variations of these profiles. Using periodic elasticity matrices (marked by a circle), the reaction rate profile can be expanded to first order as (proof in section S4.2)

\[
\begin{pmatrix}
\delta \nu \\
\delta \nu\\
\end{pmatrix} =
\begin{pmatrix}
\frac{\delta E}{\delta \nu} & \frac{\delta E}{\delta \nu} \\
\frac{\delta E}{\delta \nu} & \frac{\delta E}{\delta \nu} \\
\end{pmatrix}
\begin{pmatrix}
\delta c \\
\delta \bar{c}\\
\end{pmatrix} +
\begin{pmatrix}
\frac{\delta E}{\delta \nu} & \frac{\delta E}{\delta \nu} \\
\frac{\delta E}{\delta \nu} & \frac{\delta E}{\delta \nu} \\
\end{pmatrix}
\begin{pmatrix}
\delta c \\
\delta \bar{c}\\
\end{pmatrix}.
\] (S5)

Close to a static reference state, the effective periodic enzyme elasticities are given by

\[
\begin{pmatrix}
\frac{\delta E}{\delta \nu} & \frac{\delta E}{\delta \nu} \\
\frac{\delta E}{\delta \nu} & \frac{\delta E}{\delta \nu} \\
\end{pmatrix}
\begin{pmatrix}
\delta c \\
\delta \bar{c}\\
\end{pmatrix} =
\begin{pmatrix}
Dg(\frac{\delta \bar{c}}{\delta \nu}) Dg(\frac{\delta \bar{c}}{\delta \nu})^{-1} - 1 \\
Dg(\frac{\delta \bar{c}}{\delta \nu}) Dg(\frac{\delta \bar{c}}{\delta \nu})^{-1} - 1 \\
\end{pmatrix} +
\begin{pmatrix}
Dg(\frac{\delta \bar{c}}{\delta \nu}) Dg(\frac{\delta \bar{c}}{\delta \nu})^{-1} - 1 \\
Dg(\frac{\delta \bar{c}}{\delta \nu}) Dg(\frac{\delta \bar{c}}{\delta \nu})^{-1} - 1 \\
\end{pmatrix}.
\] (S6)

where \( \bar{c} \) is the average enzyme level, \( \bar{c} = v_{ref} + E_{c} \Delta \bar{c} + E_{x} \Delta \bar{x} \) is the flux vector in a hypothetical “central state”, and \( \Delta = E_{c} \Delta \bar{c} + E_{x} \Delta \bar{x} \).

S1.3 Metabolic response coefficients (static and periodic)

Given a kinetic model in a steady reference state, the static and periodic response coefficients can be computed as follows. With a state vector \( s_{i}(p) = (s_{j}(p)) \) as a function of the system parameters, the static response coefficients [23, 24] are defined as

\[
P_{s_{i}}^{p_{m}} = \frac{\partial s_{i}}{\partial p_{m}}, \quad P_{s_{i}}^{p_{m}p_{n}} = \frac{\partial^{2} s_{i}}{\partial p_{m} \partial p_{n}}.
\] (S7)

Using the control matrices [25]

\[
C^{s} = -L(N_{R}E_{c}L)^{-1}N_{R}, \quad C^{l} = E_{c} C^{s} + I,
\] (S8)

the response matrices can be written as [24]

\[
\begin{align*}
R^{s}_{p} &= C^{s} E_{p} \\
R^{s}_{pp} &= C^{s} \Gamma
\end{align*}
\]

where \( \Gamma = E_{cc}(R^{s} \otimes R^{s}) + E_{cp}[R^{s} \otimes I] + E_{pc}[I \otimes R^{s}] + E_{pp} \).

(S9)

The spectral response coefficients relate the Fourier components of periodic parameters to Fourier components of the responding periodic state variables [15]. With the spectral control matrices

\[
\begin{align*}
\tilde{C}^{s}(\omega) &= -L(N_{R}E_{c}L - i\omega I)^{-1}N_{R} \\
\tilde{C}^{l}(\omega) &= E_{c} \tilde{C}^{s}(\omega) + I
\end{align*}
\] (S10)
they can be written as
\[
\begin{align*}
\tilde{R}^s_p(\omega) &= \tilde{C}^s(\omega) E_p \\
\tilde{R}^s_{pp}(\omega) &= \frac{1}{\sqrt{2\pi}} \tilde{C}^s(0) \cdot \Gamma(\omega, -\omega) \\
\tilde{R}^s_{pp}(\omega) &= \frac{1}{\sqrt{2\pi}} \tilde{C}^s(2\omega) \cdot \Gamma(\omega, \omega)
\end{align*}
\]

where \( \Gamma(\alpha, \beta) = E_{cc}\left[\tilde{R}^s_p(\alpha) \otimes \tilde{R}^s_p(\beta)\right] + E_{cp}\left[\tilde{R}^s_p(\alpha) \otimes I\right] + E_{pc}\left[I \otimes \tilde{R}^s_p(\beta)\right] + E_{pp}. \) (S11)

The first-order spectral response coefficients (in the matrix \( \tilde{R}^s_p(\omega) \)) link perturbations and effects of the same frequency \( \omega \). The second-order spectral response coefficients (in the tensors \( R^s_{pp}(\omega) \) and \( \tilde{R}^s_{pp}(\omega) \)) describes changes of \( y \) at frequencies 0 (static shift) and 2\( \omega \) (second harmonic). Details are given in [15]. The spectral response coefficients refer to complex Fourier components themselves. However, the concentration curves in our models are given by the real parts of complex exponentials: \( p(t) = \bar{p} + \text{Re}(\tilde{p} e^{i\omega t}) \). Therefore, our expansion coefficients are not given by spectral response coefficients, but by the (almost identical) periodic response coefficients. The first-order spectral and periodic coefficients are in fact identical; the second-order periodic coefficients contain a prefactor \( \sqrt{\frac{2}{\pi}} \) (see section S4.3 for a derivation):

\[
\begin{align*}
R^s_{pp}(\omega) &= \sqrt{\frac{2}{\pi}} R^s_{pp}(\omega), \\
\tilde{R}^s_{pp}(\omega) &= \frac{\sqrt{2\pi}}{2} \tilde{R}^s_{pp}(\omega).
\end{align*}
\] (S12)

If oscillations are very slow (\( \omega \approx 0 \)), periodic response coefficients can be approximated by static response coefficients. The second-order coefficients, however, must be divided by 2:

\[
\begin{align*}
R^s_p(\omega \approx 0) &\approx R^s_p \\
R^s_{pp}(\omega \approx 0) &\approx \frac{1}{2} R^s_{pp} \\
\tilde{R}^s_{pp}(\omega \approx 0) &\approx \frac{1}{2} \tilde{R}^s_{pp}.
\end{align*}
\] (S13)

### S1.4 Periodic mRNA and protein levels

Protein rhythms can be realised by periodic gene expression. However, a protein rhythm will not look exactly like its mRNA counterpart, but will be phase-shifted and have a smaller relative amplitude (unless there is “active”, time-dependent protein degradation). At high frequencies, the protein amplitudes become very small. To relate mRNA and protein rhythms mathematically, we assume that protein production is proportional to current mRNA level and that protein is linearly degraded or diluted. Regulated protein degradation is not considered in this simple model. The protein level \( p(t) \) will follow the differential equation

\[
\frac{dp(t)}{dt} = \alpha m(t) - \kappa p(t)
\] (S14)
with mRNA concentration $m(t)$, rate constant $\alpha$ for protein production, and rate constant $\kappa$ for protein degradation (possibly including enzyme dilution); the rate constants may differ between proteins. With Eq. (S14), a harmonic mRNA profile $m(t) = \bar{m} + \text{Re}(\tilde{m} e^{i\omega t})$ leads to a harmonic protein profile $p(t) = \bar{p} + \text{Re}(\tilde{p} e^{i\omega t})$ with curve parameters

$$\tilde{p} = \frac{\alpha}{\kappa} \bar{m}, \quad \tilde{p} = \frac{\alpha}{\kappa + i\omega} \bar{m}. \quad \text{(S15)}$$

In turn, to realise a protein profile $p(t) = \bar{p} + \text{Re}(\tilde{p} e^{i\omega t})$, one would need an mRNA profile with

$$\bar{m} = \frac{\kappa}{\alpha} \bar{p}, \quad \bar{m} = \frac{\kappa + i\omega}{\alpha} \bar{p}. \quad \text{(S16)}$$

To avoid negative mRNA levels, the curve parameters must satisfy $\bar{m} \leq \bar{m}$, which implies the inequality $|\tilde{p}| \leq \bar{p}^{\text{max}}(\bar{p}, \omega) = (1 + \omega/\kappa)^{-1/2} |\bar{p}|$ for protein amplitudes. The phase shift between mRNA and protein peaks is given by $\arctan(\omega/\kappa)$; for $\omega \approx 0$, mRNA and protein will oscillate in phase; for high frequencies, the protein peaks after the mRNA with a phase shift of $\pi/2$. Proteins with a slow turnover ($\kappa \gg \omega$) show weak oscillations and large phase shifts. In contrast, if oscillations are much slower than protein turnover ($\omega \ll \kappa$), the phase shifts can be neglected.

### S2 Economics of metabolic oscillations

To predict optimal enzyme profiles under external perturbation, we need to define a fitness function. Here we consider fitness as a function of external parameters $x_j$ and enzyme activities $u_l$, and compute its first and second derivatives.

#### S2.1 How static and periodic perturbations affect fitness

A steady state, resulting from constant external parameters $x_j$ and enzyme activities $u_l$, is described by a vector $s(x, u) = \left( s(x, u) \right)$ (the existence of a stable steady state is required for the theory to apply). Starting from reference parameters $(x^{\text{ref}}, u^{\text{ref}})$ and considering static parameter perturbations $(x) = (x^{\text{ref}}) + (\Delta x)$, the state variables can be expanded as:

$$\Delta s \approx \left( R_x \Delta x \right) + \frac{1}{2} \left( R_{xx} \Delta x \Delta u \right). \quad \text{(S17)}$$

We now consider the fitness function $f = b(s(x, u)) - h(u) = g(x, u) - h(u)$. The benefit and cost changes can be expanded to second order:

$$g \approx g(u^{\text{ref}}) + b_x \cdot \Delta s + \frac{1}{2} \Delta s^\top B_{xx} \Delta s$$

$$h \approx h(u^{\text{ref}}) + h_u \cdot \Delta u + \frac{1}{2} \Delta u^\top H_{uu} \Delta u. \quad \text{(S18)}$$
By inserting the expansions (S17) and (S18) into the fitness function, we obtain the deviation-dependent fitness \( f(x^{\text{ref}} + \Delta \bar{x}, u^{\text{ref}} + \Delta \bar{u}) \). Differentiating by \( \Delta \bar{x} \) and \( \Delta \bar{u} \), we obtain the gradients and the Hessian matrices ("fitness synergy matrices")

\[
\begin{align*}
  f_{x}^\top &= b_s^\top R_x^s \\
  f_{u}^\top &= b_s^\top R_u^s - h_u^\top \\
  F_{u x} &= b_s^\top \cdot R_{u x}^s + R_u^s R_{u x}^s B_{s s} R_x^s \\
  F_{u u} &= b_s^\top \cdot R_{u u}^s + R_u^s R_{u u}^s - H_{u u}.
\end{align*}
\] (S19)

We now consider external and enzyme parameters that oscillate around their reference state (without any shift of average values):

\[
\begin{align*}
  x(t) &= x^{\text{ref}} + \text{Re}[\tilde{x} e^{i\omega t}] \\
  u(t) &= u^{\text{ref}} + \text{Re}[\tilde{u} e^{i\omega t}].
\end{align*}
\] (S20)

While the time average of \( u \) is simply \( u^{\text{ref}} \), the average metabolic state \( \bar{s} \) is expanded by Eq. (21) with the help of second-order periodic response coefficients:

\[
\Delta \bar{s} \approx \frac{1}{2} \left( R_{\bar{s} \tilde{u}}^s \begin{pmatrix} \begin{pmatrix} \begin{pmatrix} \tilde{x} \\ \tilde{u} \end{pmatrix} \otimes \begin{pmatrix} \tilde{x} \\ \tilde{u} \end{pmatrix} \end{pmatrix} \right) \right).
\] (S21)

There is no first-order term because \( R_{\bar{s} \tilde{x}}^s \) and \( R_{\bar{s} \tilde{u}}^s \) vanish. Importantly, the second-order expansion contains no interaction between static and periodic perturbation parameters. Therefore, we can use Eqs (S17) and (S21) separately.

### S2.2 Fitness functionals for periodic metabolic time courses

To assess the fitness changes caused by metabolic rhythms, we need to score them by fitness functionals. One possibility is to apply a static fitness function \( f = b(s(x, u)) + h(u) \) to time-averaged state variables ("state-averaged fitness"). Since the time-averaged enzyme activities remain unchanged (\( \Delta \bar{u} = 0 \)), benefit and cost can be expanded as

\[
\begin{align*}
  g(\langle s \rangle_t) &\approx b(\bar{s}) + b_s \cdot \Delta \bar{s} + \frac{1}{2} \Delta \bar{s}^\top B_{s s} \Delta \bar{s} \\
  h(\langle u \rangle_t) &= h(\bar{u}).
\end{align*}
\] (S22)

With Eq. (S21), the derivatives of \( f = g(\langle s \rangle_t) - h(\langle u \rangle_t) \) read

\[
\begin{align*}
  f_{x}^\top &= 0 \\
  f_{u}^\top &= 0 \\
  F_{u x} &= b_s^\top \cdot R_{u x}^s \\
  F_{u u} &= b_s^\top \cdot R_{u u}^s.
\end{align*}
\] (S23)
In an alternative fitness functional ("fitness-averaged fitness"), fitness is evaluated in each time point and then averaged over time. This leads to additional terms in the fitness Hessians. To derive these terms, we expand the time courses $s(t)$ and the fitness in each time point to second order, integrate over one oscillation period (duration $T$) and collect all first- and second-order terms. For the cost term, we obtain:

$$\langle h(u) \rangle_t = \frac{1}{T} \int_0^T h(u^\text{ref} + \text{Re}[\tilde{u}e^{i\omega t}]) \, dt$$

$$\approx \frac{1}{T} \int_0^T h(u^\text{ref}) + h(u) \cdot \text{Re}[\tilde{u}e^{i\omega t}] + \frac{1}{2}(\tilde{u}e^{i\omega t})^\dagger H_{uu}(\tilde{u}e^{i\omega t}) \, dt$$

$$= h(u^\text{ref}) + \frac{1}{4} \tilde{u}^\dagger H_{uu} \tilde{u}.$$  \hfill (S24)

The first-order term, integrated over time, cancels out. The benefit term is computed in a similar way:

$$\langle g(u) \rangle_t = \langle g(u) \rangle + \frac{1}{4} \tilde{s}^\dagger B_{ss} \tilde{s}$$

where $\tilde{s} = R_s^s \tilde{x} + R_u^s \tilde{u}$.

Together, the time-averaged fitness terms

$$\langle g(u) \rangle_t \approx g(u^\text{ref}) + b_s \cdot \Delta \bar{x} + \frac{1}{2} \Delta \bar{x}^\dagger B_{ss} \Delta \bar{x} + \frac{1}{4} \tilde{s}^\dagger B_{ss} \tilde{s}$$

$$\langle h(u) \rangle_t = h(u^\text{ref}) + \frac{1}{4} \tilde{u}^\dagger H_{uu} \tilde{u}$$  \hfill (S25)

yield the first and second order derivatives of $F = \langle g(s) - h(u) \rangle_t$:

$$f_x = 0$$

$$f_u = 0$$

$$F_{\bar{x}\bar{x}} = b_x^\dagger R_{\bar{x}\bar{x}}^s + \frac{1}{2} R_{\bar{u}\bar{u}}^s B_{ss} R_{\bar{x}\bar{x}}^s$$

$$F_{\bar{u}\bar{u}} = b_u^\dagger R_{\bar{u}\bar{u}}^s + \frac{1}{2} R_{\bar{u}\bar{u}}^s B_{ss} R_{\bar{u}\bar{u}}^s - \frac{1}{2} H_{uu}.$$  \hfill (S26)

Since $R_{\bar{x}\bar{x}}^s (\omega = 0) = \frac{1}{2} R_{\bar{x}\bar{x}}^{pp}$, the frequency-dependent synergy matrices $F_{\bar{x}\bar{x}}$ and $F_{\bar{u}\bar{u}}$ are given by the static synergy matrices $F_{\bar{x}\bar{x}}$ and $F_{\bar{u}\bar{u}}$, divided by a factor of 2. What happens if stationary and periodic perturbations are applied together? Since there are no mixed second derivatives ("fitness synergies") between stationary and periodic perturbations (proof in S4.4), their effects can simply be added. The derivatives of our fitness $F(x^\text{ref}, u^\text{ref}, \Delta \bar{x}, \Delta \bar{u}; \bar{x}, \bar{u})$ with respect to $\Delta \bar{x}, \Delta \bar{u}, \bar{x}$, and $\bar{u}$ are directly given by Eqs (S23) and (S25).

### S2.3 Optimal enzyme rhythms that realise a desired metabolic rhythm

How can we realise a desired metabolic rhythm by rhythmic enzyme profiles? Or, more precisely, if an external rhythm is given (with amplitude vector $\tilde{x}$) and certain rhythms of state variables are desired (with amplitude vectors $\tilde{c}$ and $\tilde{v}$), which enzyme amplitudes $\tilde{u}$ will realise these rhythms as precisely as
possible and at a minimal enzyme cost? The optimal enzyme profiles must be self-consistent: each enzyme profile must be adapted to the dynamic metabolic state, which arises from the enzyme profiles themselves. Such inverse problems can be hard to solve. However, approximations based on MCA, as used in [11] for steady states, make the problems tractable. To compute the amplitude vector \( \tilde{u} \), we need to “invert” the propagation of oscillations in networks. In a first-order expansion, this is straightforward. For sine-wave perturbations of a single frequency, the propagation of perturbations in the network can be described by first-order periodic response coefficients. Now we defined a goal, for instance, to realise a predefined metabolite profile \( \tilde{c} = R_{\tilde{c}} \tilde{u} + R_{\tilde{x}} \tilde{x} \). If the response matrix \( R_{\tilde{c}} \) is invertible, we can achieve this by setting \( \tilde{u} = R_{\tilde{c}}^{-1} \left[ \tilde{c} - R_{\tilde{x}} \tilde{x} \right] \). In other cases, the metabolic behaviour may not be precisely realisable, or may be realisable by more than one enzyme profile. In the first case, we employ a least-squares optimisation; in the second case, we add a regularisation term to the optimality objective (e.g., a term \( \tilde{u}^{\dagger} \tilde{u} \) favouring small-amplitude profiles\(^{18} \)). The optimality problem for the enzyme amplitude vector \( \tilde{u} \) reads

\[
\text{Minimise } ||(\tilde{c} - R_{\tilde{c}} \tilde{x}) - R_{\tilde{c}} \tilde{u}|| + \tilde{u}^{\dagger} \tilde{u},
\]

(S27)

possibly with constraints on enzyme or metabolite profiles. Eq. (S27) holds for harmonic time profiles \( c(t) \) to be realised. To realise non-harmonic time courses \( c(t) \), we may split them into Fourier components, determine the optimal enzyme profile for each component, and overlay these profiles by Fourier synthesis to obtain the enzyme time course \( u(t) \). This procedure, however, may fail if Fourier modes are effectively coupled by active constraints (e.g., arising from upper or lower bounds on individual enzyme levels).

S2.4 Global enzyme rhythms, described as a series of hypothetical adaptations

Given the periodic fitness synergies between model parameters, how can we determine a globally optimal enzyme rhythm? The solution must be self-consistent, i.e., optimal (temporal and network-wide) enzyme profile must be optimally adapted not only to external perturbations, but also to their own metabolic effects. Such self-consistent solutions can be understood by assuming a series of hypothetical adaptations. If an external parameter oscillates, it creates a direct incentive for enzyme oscillations; for each enzyme, the optimal relative amplitude and phase shift are defined by the complex fitness synergy between enzyme and oscillating parameter. As soon as the enzymes realise these amplitudes, their own rhythms give rise to new synergy effects, now determined by the elements of \( F_{\tilde{u}\tilde{u}} \). These synergies require an additional adaptation, which creates new incentives for enzyme adaptation, and so on. By adding all adaptations, we obtain exactly the optimal adaptation predicted by Eq. (6). Let us show this mathematically. We first consider an unperturbed reference state (which must be fitness-stable against enzyme oscillations), which is then perturbed by an external parameter with amplitude \( \tilde{x} \). The elements of \( F_{\tilde{u}\tilde{u}} \) describe fitness synergies between the external parameter and individual enzymes; if enzyme \( l \) starts oscillating with complex amplitude \( \tilde{u}_l \), there will be a synergy term \( \tilde{u}_l^* F_{\tilde{u}_l\tilde{u}_l} \tilde{x}_l \) and a self-synergy term \( \frac{1}{2} F_{\tilde{u}_l\tilde{u}_l} \tilde{u}_l^2 \). The self-synergy \( F_{\tilde{u}_l\tilde{u}_l} \) is a real number and must be negative (otherwise, there could be beneficial spontaneous oscillations of enzyme \( l \), which contradicts our assumption). The optimal enzyme rhythm – the rhythm that maximises

\(^{18}\text{Oscillations of different enzymes could also be weighted differently; in models with non-linear investment function and fitness-averaged fitness functional, the cost term itself could be used for regularisation.}\)
the sum of both terms – reads
\[ \tilde{u}_l^{(1)} = -\frac{F_{\tilde{u}_l \tilde{x}}}{F_{\tilde{u}_l \tilde{u}_l}} \tilde{x}. \] (S28)

This is the direct (or “first-level”) adaptation. Now we assume that all enzymes realise this direct adaptation. In the next adaptation step, we recalculate the adaptation of our enzyme \( l \); with the existing enzyme rhythms, the benefit now reads \[ \tilde{x} F_{\tilde{u}_l \tilde{x}} + \sum_{j \neq l} \tilde{u}_j F_{\tilde{u}_j \tilde{u}_l} \tilde{u}_l, \] and we obtain the (first and second-level) adaptation
\[ \tilde{u}_l^{(2)} = -\frac{1}{F_{\tilde{u}_l \tilde{u}_l}} \left[ F_{\tilde{u}_l \tilde{x}} \tilde{x} + \sum_{j \neq l} F_{\tilde{u}_l \tilde{u}_j} \tilde{u}_j^{(1)} \right] = -\frac{1}{F_{\tilde{u}_l \tilde{u}_l}} \left[ F_{\tilde{u}_l \tilde{x}} \tilde{x} - \sum_{j \neq l} F_{\tilde{u}_l \tilde{u}_j} \frac{F_{\tilde{u}_j \tilde{x}}}{F_{\tilde{u}_j \tilde{u}_j}} \tilde{x} \right]. \] (S29)

If we iterate this process, we obtain an infinite series of higher-level adaptations, and if this series converges, the self-consistent solution will satisfy
\[ \tilde{u}_l^{(\infty)} = -\frac{1}{F_{\tilde{u}_l \tilde{u}_l}} \left[ F_{\tilde{u}_l \tilde{x}} \tilde{x} + \sum_{j \neq l} F_{\tilde{u}_l \tilde{u}_j} \tilde{u}_j^{(\infty)} \right]. \] (S30)

Since \( F_{\tilde{u}_l \tilde{u}_l} \) is invertible by assumption, we can solve for the self-consistent enzyme rhythm:
\[ \tilde{u}^{(\infty)} = -F_{\tilde{u}_l \tilde{u}_l}^{-1} F_{\tilde{u}_l \tilde{x}} \tilde{x}. \] (S31)

The last step can also be differently shown. We assume that the self-consistent optimal enzyme rhythm is given by Eq. (S31). To break this down into a series of adaptation terms, we split our matrix \( F_{\tilde{u}_l \tilde{u}_l} \) into a matrix \( A \) containing the diagonal elements and a matrix \( B \) containing the off-diagonal elements, and rewrite \( F_{\tilde{u}_l \tilde{u}_l} = A + B = A \left[ I + A^{-1} B \right] \). Inserting this into Eq. (S31), we obtain
\[ \tilde{u}^{(\infty)} = -\left[ A \left[ I + A^{-1} B \right]^{-1} \right] F_{\tilde{u}_l \tilde{x}} \tilde{x} \]
\[ = -\left[ I + A^{-1} B \right]^{-1} A^{-1} F_{\tilde{u}_l \tilde{x}} \tilde{x} \]
\[ = -\left[ I - A^{-1} B + A^{-1} B A^{-1} B - \ldots \right] A^{-1} F_{\tilde{u}_l \tilde{x}} \tilde{x} \]
\[ = \left[ -A + A^{-1} B A^{-1} + A^{-1} B A^{-1} B A^{-1} - \ldots \right] F_{\tilde{u}_l \tilde{x}} \tilde{x}. \] (S32)

This is exactly the expansion series obtained above.

### S2.5 Enzymes that are only induced by rhythmic states

So far we assumed, for our optimal static reference state, that all enzymes are active. If all enzyme levels are optimal and none of them hits a bound (in this case, the bound at \( u_l = 0 \)), the optimality condition tells us that \( f_l = 0 \). Now let us abandon this assumption and consider enzymes that are inactive in the optimal reference state (\( u_l = 0 \); see Figure 13 (b)). Representing the inactive and active enzymes by
subvectors with subscripts I and A, we obtain the optimality condition

\[ f_{u_A} = 0, \quad f_{u_I} < 0. \]  \hspace{1cm} (S33)

The situation is illustrated in Figure 13: an active enzyme is shown in Figure 13 (a), left; an inactive enzyme is shown in Figure 13 (b), left. We can now expand the fitness function around the reference state like in Eq. (5). With the non-vanishing fitness gradient in the reference state, our expansion of the fitness function will contain a first-order term for \( \Delta u \). For generality, we now even allow for a periodically varying fitness function, yielding a gradient \( \tilde{f}_u \) for enzyme amplitudes \( \tilde{u} \). Moreover, instead of referring to an unperturbed reference state, we allow for an already perturbed reference state (with external perturbation \( \tilde{x} \)). The perturbation will lead to a second-order term \( \text{Re}(\tilde{x}^T F_{\tilde{x}\tilde{u}} \tilde{u}) \). Since this term is linear in \( \tilde{u} \), we can include it into the gradient \( \tilde{f}_u \) and obtain the effective, complex-valued gradient \( \tilde{f}_u = f_u + F_{\tilde{u}\tilde{x}} \tilde{x} \) for enzyme rhythms.

To predict optimal enzyme profiles, we now expand the fitness function, close to our (static or already perturbed) reference state, to first order in \( \Delta \bar{u} \) and \( \tilde{u} \) (where irrelevant terms are omitted):

\[ \Delta F(\Delta \bar{u}, \tilde{u}) = f_u \cdot \Delta \bar{u} + \text{Re}([f_u + F_{\tilde{u}\tilde{x}} \tilde{x}] \cdot \tilde{u}). \]  \hspace{1cm} (S34)

These terms will dominate the fitness value close to the reference state. We now ask, for our system under external perturbation \( \tilde{x} \) and with a gradient \( f_u \) representing rhythmic changes of fitness requirements, whether the enzyme profile should deviate from its static reference values. That is, we maximise Eq. (S34) under the constraints

\[ 0 \leq u^{\text{ref}} + \Delta \bar{u} \leq u^{\text{max}}, \quad |\tilde{u}| \leq \bar{u} = u^{\text{ref}} + \Delta \bar{u} \]  \hspace{1cm} (S35)

for static and periodic enzyme variations, or more general amplitude constraints \( |\tilde{u}| \leq \bar{u}^{\text{max}}(\bar{u}, \omega) \), where the vector \( |\tilde{u}| \) contains the magnitudes of the elements of \( \tilde{u} \). This optimality problem can be solved numerically. To understand why certain enzymes show stay inactive or start oscillating, it is helpful to reformulate the cost function: instead of stating that an enzyme oscillation would imply an increasing average enzyme level (and that this increase is costly), we introduce an effective cost for enzyme oscillations that captures this effect. This effective cost concerns only enzymes that were originally inactive, and increases linearly with absolute enzyme amplitudes (which makes it non-differentiable in the reference state). To derive a formula for this cost, we reconsider the previous optimality problem and assume, as the only constraints, a simple amplitude constraint for previously inactive enzymes: \( \forall i : u_i^{\text{ref}} = 0 \Rightarrow |\tilde{u}_i| \leq \bar{u}_i \). According to Eq. (S33), any purely static change \( \Delta \bar{u} \) would be costly. However, rhythms in the previously inactive enzymes require an increase in their average values: for matching average values and amplitudes, we express \( \Delta \bar{u} \) in terms of \( \tilde{u} \) as \( \Delta \bar{u}_I = |\tilde{u}|, \Delta \bar{u}_A = 0 \). By inserting this choice of \( \Delta \bar{u} \) into Eq. (S34), we obtain an effective amplitude-dependent fitness function

\[ F^{\text{eff}}(\tilde{u}) = f_u^T |\tilde{u}| + \text{Re}([f_u + F_{\tilde{u}\tilde{x}} \tilde{x}] \cdot \tilde{u}) \]  \hspace{1cm} (S36)

\( \text{The relation describes the minimal possible increase in average levels; below this, negative enzyme activities would arise. A larger increase, on the contrary, would be costly and unnecessary (unless our solution hits other constraints, which we excluded here).} \)
(since \( f_{\alpha} = f_{\alpha} \) and \( f_{\alpha A} = 0 \)). The first term is the effective cost of oscillations, induced by the amplitude constraint. What vectors \( \tilde{u} \) will maximise this fitness change? Given a vector \( |\tilde{u}| \), the second term will be maximal when the corresponding elements of \( \tilde{u} \) and \( f_{\alpha} + F_{\alpha A} \tilde{x} \) have the same phases, so the optimal phases of \( \tilde{u} \) are given by the phases of \( f_{\alpha} + F_{\alpha A} \tilde{x} \). Thus, we can write the effective fitness of an enzyme rhythm in terms of the real-valued amplitudes \( |\tilde{u}| \):

\[
F^{\text{eff}}(\tilde{u}) = f_{\alpha}^T |\tilde{u}| + |f_{\alpha} + F_{\alpha A} \tilde{x}|^T |\tilde{u}| = |f_{\alpha} + F_{\alpha A} \tilde{x}|^T |\tilde{u}|. \tag{S37}
\]

When optimising \( |\tilde{u}| \) with this linear fitness function, the amplitudes must be bounded. This leads to a constrained linear programming problem for the real-valued amplitude vector \( |\tilde{u}| \). For example, to study small oscillations (which do not hit any bounds), we can restrict our amplitude vectors to a small norm \( |\tilde{u}| = \varepsilon \). Maximising the fitness Eq. (S37) under this constraint is simple: the resulting \( \tilde{u} \) is given by the vector \( f_{\alpha} + |f_{\alpha} + F_{\alpha A} \tilde{x}| \), scaled to length \( \varepsilon \). Thus, in our linear approximation, optimal complex enzyme amplitudes will given by \( f_{\alpha} + |f_{\alpha} + F_{\alpha A} \tilde{x}| \), multiplied by some scaling factor. As shown before, the phases of \( \tilde{u} \) are given by the phases of \( f_{\alpha} + F_{\alpha A} \tilde{x} \).

**S2.6 Fast enzyme rhythms and the effective cost of posttranslational regulation**

So far, we assumed that enzyme activities \( u_l \) are directly given by enzyme concentrations, i.e., protein levels \( p_l \). In reality, enzyme activity can also be regulated by posttranslational modifications (e.g., phosphorylation). Posttranslational regulation allows cells to generate fast, strong enzyme rhythms that cannot achieved by gene expression alone. To describe how both mechanisms should be combined in optimal enzyme rhythm, we model the two variables separately: the enzyme activity \( u_l \), representing the concentrations of active enzyme, and the protein level \( p_l \) representing the total concentration of (active plus inactive) enzyme. The fraction of active enzyme molecules (i.e., molecules capable of catalysing the reaction) is called \( p_l = u_l / p_l \). To realise an enzyme activity \( u_l \) (appearing in the rate laws), a protein level \( p_l = u_l / p_l \) (scored by our enzyme cost function) will be needed. In a static enzyme optimisation, a value of \( p_l = 1 \) would be optimal, protein levels and enzyme activities should have identical values, and no posttranslational modifications are needed. In periodic optimality scenarios, posttranslational modifications become crucial because large, fast oscillations cannot be realised without them. Of course, then, enzyme activities will be temporarily lower than the protein levels, and to keep average enzyme activities constant, the average protein levels must be increased. This makes the oscillations, effectively, costly.

To account for this in models, we can either introduce protein variables \( (p, \bar{p}) \) as extra variables and relate them to enzyme activities \( (\bar{u}, \bar{u}) \) by explicit constraints; or, we analyse these constraints and translate \( h(p) \) into an effective cost function \( h^{\text{eff}}(\bar{u}, \bar{u}) \). In both cases, to score an enzyme activity profile by its cost, we ask how this profile could be realised at a minimal cost by combining expression changes and protein turnover, or by regulated protein degradation; however, this may come at an even higher cost.
modification. We assume that protein expression is costly, while protein modification, by itself, comes for free. Then, given a desired enzyme activity profile \(u(t)\), we search for the cheapest protein profile \(p(t)\) that can realise these enzyme activities, and consider the cost of this protein profile. We consider posttranslational inhibition only (an "incomplete activation" could be treated similarly). Now I briefly discuss the two possibilities.

1. **Explicit constraints** We formulate our optimality problem in terms of control variables \(\bar{u}, \bar{\dot{u}}, \bar{p}, \bar{\dot{p}}, q\), and of given perturbation parameters \(x\) and \(\dot{x}\). Benefit and cost are given by functions \(g(\bar{u}, \bar{\dot{u}}, x, \dot{x})\) and \(h(\bar{p}, \bar{\dot{p}})\) and expanded to second order around the reference state. The auxiliary vector \(q\) is given by \(q = \max(0, |\bar{u}| - \bar{p}^{\text{max}})\). At an oscillation frequency \(\omega\), we obtain the optimality problem

Maximize \[ g(\bar{u}, \bar{\dot{u}}, x, \dot{x}) - h(\bar{p}, \bar{\dot{p}}) \]
subject to
\[
\begin{align*}
0 & \leq \bar{p} \leq \bar{p}^{\text{max}} \\
0 & \leq \bar{\dot{u}} \leq \bar{\dot{p}} \\
q & = \bar{p} - \bar{\dot{u}} \\
|\bar{u}| & \leq \bar{u} \\
|\bar{\dot{p}}| & \leq \bar{p}^{\text{max}}(\bar{p}, \omega) \\
0 & \leq |\bar{\dot{p}}| \\
|\bar{\dot{p}}| & = |\bar{\dot{u}} - q| \\
\varphi(\bar{u}) & = \varphi(p) \\
\end{align*}
\]

where \(\varphi(\cdot)\) denotes phase angles. The inequalities are redundant and imply that \(q \geq 0\). Close to a static reference state (which may not be enzyme-optimal), benefit and cost functions can be replaced by the quadratic approximation.

2. **Effective cost function** Here, enzyme activities \(u_t\) are used as the main variables; from the given protein cost function \(h(p)\), we derive an effective cost function \(h(\bar{u}, \bar{\dot{u}})\), scoring the enzyme activity profiles (see Figure 14). If there were no posttranslational modifications, enzyme activities \(u_t\) and protein levels \(p_t\) would be identical, and for an activity profile \((\bar{u}, \bar{\dot{u}})\), we would obtain the following costs and constraints. With linear costs or state-averaged cost functions, the cost is given by a function \(H = h(\bar{u}) = \sum_t h_{u_t} \bar{u}_t\). There are two constraints: \(|\bar{u}_t| \leq \bar{u}_t\), to prevent negative values, and \(|\bar{\dot{u}}_t| \leq \bar{p}_t^{\text{max}}(\omega, \bar{u}_t)\), representing the maximal amplitude obtainable from transcription and translation. In contrast, if posttranscriptional modifications are possible, enzyme activities \((u, \dot{u})\) and protein levels \((p, \dot{p})\) can differ. We assume that, for optimality reasons, enzyme activity rhythms are realised by expression changes whenever possible. If the desired activity amplitude is too large, the missing part \(q_t = |\bar{u}_t| - \bar{p}_t^{\text{max}}\) is realised posttranslationally. This requires a periodic inhibition with amplitude \(q_t\) and in phase with the desired \(\bar{u}_t\). This inhibition, by itself, would reduce the average enzyme activity by \(q_t\); this must be compensated by increasing the protein mean value \(\bar{u}_t\), again by \(q_t\). Thus, for a desired enzyme amplitude \(\bar{u}_t\), we set \(q_t = |\bar{u}_t| - \bar{p}_t^{\text{max}}, |\dot{p}_t| = |\bar{u}_t| - q_t\), and \(\bar{p}_t = \bar{u}_t + q_t\). With a linear protein cost function \(h(p)\), we obtain the effective cost and constraints

\[
H^{\text{eff}}(\bar{u}, \bar{\dot{u}}) = h(\bar{u} + |\bar{u}| - \bar{p}^{\text{max}}(\bar{u}, \omega)), \quad |\bar{u}_t| \leq \bar{u}_t, \quad |\bar{u}_t| \leq \bar{p}_t^{\text{max}}(\bar{u}_t + q_t, \omega) + q_t. \quad (S39)
\]
Formulae for $\tilde{p}_i^{\text{max}}$, e.g., obtained from a simple model of translation, are described in appendix C of the article. They all have the form $\tilde{p}_i^{\text{max}}(\bar{p}_i, \omega) = \gamma(\omega) \bar{p}_i$, where the prefactor $\gamma(\omega)$ decreases with frequency $\omega$.

S3 Models

S3.1 Single reaction with predefined substrate and product rhythm

Optimal enzyme rhythms in a single reaction have been discussed in the article. For illustration, I now revisit this calculation with two changes in the model. First, I consider a reversible rate law (i.e., the rate is affected by substrate and product levels), and second, I use cosine functions instead of complex exponentials (which is mathematically equivalent). I first focus on dynamics and derive the synergies between reactant and enzyme rhythms; then I consider optimal enzyme rhythms, where fitness is given by the average flux minus a cost that increases quadratically with the enzyme activities.

Consider a single reaction with substrate $X_1$ and product $X_2$ (concentrations $x_1$ and $x_2$). The reaction is catalysed by an enzyme with activity $u$ and reversible mass-action kinetics. With all rate constants set to 1, the rate law reads

$$v = u(x_1 - x_2). \quad (S40)$$

The reactant levels are predefined (i.e., our enzyme has no influence on reactant levels):

$$x_1(t) = \bar{x}_1 + \tilde{x}_1 \cos(\omega t)$$
$$x_2(t) = \bar{x}_2,$$ \hspace{1em} (S41)

and we consider enzyme profiles of the form

$$u(t) = \bar{u} + \tilde{u} \cos(\omega t + \varphi). \quad (S42)$$

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

$$v(t) = (\bar{u} + \tilde{u} \cos(\omega t + \varphi))(\bar{x}_1 + \tilde{x}_1 \cos(\omega t) - \bar{x}_2)$$
$$= \bar{u} (\bar{x}_1 - \bar{x}_2) + \tilde{u} \cos(\omega t + \varphi)(\bar{x}_1 - \bar{x}_2) + \bar{u} \tilde{x}_1 \cos(\omega t) + \tilde{u} \cos(\omega t + \varphi)\tilde{x}_1 \cos(\omega t). \quad (S43)$$

The last term describes the synergy between the periodic substrate and enzyme activities. With the formula $\cos(\alpha) \cos(\beta) = \frac{1}{2} [\cos(\alpha - \beta) + \cos(\alpha + \beta)]$, it can be rewritten as

$$\tilde{u} \tilde{x}_1 \cos(\omega t + \varphi) \cos(\omega t) = \tilde{u} \tilde{x}_1 \frac{1}{2} [\cos(\varphi) + \cos(2\omega t + \varphi)]. \quad (S44)$$
By averaging the flux (S43) over one oscillation period, we obtain

$$\bar{\nu} = \frac{1}{T} \int_0^T \nu(t) \, dt = \bar{u} (\bar{x}_1 - \bar{x}_2) + \frac{1}{2} \bar{u} \bar{x}_1 \cos(\varphi).$$  \tag{S45}$$

All other terms are periodic and thus average out. The first term in Eq. (S45) is simply the flux from our steady reference state; the second term describes a shift caused by the synergy of reactant and enzyme rhythms. In theory, the flux in this model could be infinitely increased by increasing the enzyme activity. However, high enzyme activities are costly, and we assume that their cost increases more than linearly (while the benefit, given by the flux, increases linearly with the enzyme level). The cost of an enzyme represents the burden of protein production and maintenance. For simplicity, we assume that it comprises a positive linear term (a fixed cost per enzyme molecule) and a quadratic term (representing an extra cost if many other enzyme molecules are present). Thus, the cost for an enzyme activity $u$ reads

$$h(u(t)) = \alpha u(t) + \frac{\beta}{2} u^2(t) \tag{S46}$$

with positive coefficients $\alpha$ and $\beta$. Averaged over time, it becomes

$$\langle h \rangle = \frac{1}{T} \int_0^T h(t) \, dt = \alpha \bar{u} + \frac{\beta}{2} \bar{u}^2 + \frac{\beta}{4} \bar{u}^2 = h(u_{\text{ref}}) + \frac{\beta}{4} \bar{u}^2.$$  \tag{S47}$$

Like in Eq. (S45), all single-cosine terms cancel out and the quadratic cosine term, averaged over an oscillation period, yields a factor of $\frac{1}{2}$. Compared to the reference state, the cost increases by $\frac{\beta}{4} \bar{u}^2$, which depends on cost curvature and oscillation amplitude. The fitness function is given by the flux (benefit) minus the enzyme cost, averaged over time:

$$F = \langle y \rangle - \langle h \rangle = \left( \bar{u}(\bar{x}_1 - \bar{x}_2) + \frac{1}{2} \bar{u} \bar{x}_1 \cos(\varphi) \right) - \left( \alpha \bar{u} + \frac{\beta}{2} \bar{u}^2 + \frac{\beta}{4} \bar{u}^2 \right).$$  \tag{S48}$$

We now assume a substrate rhythm $\bar{x}_1$, where the average reactant levels $\bar{x}_1 > \bar{x}_2$ are fixed (and the product level is assumed to be non-oscillating for simplicity). To maximise fitness, we choose the enzyme profile (defined by a pair $(\bar{u}, \tilde{u})$) that maximises $F$ under the constraints $\bar{u} \geq 0$, $|\tilde{u}| \leq \bar{u}$. To optimise $\bar{u}$ and $\tilde{u}$, we separate the terms that depend on time averages ($\bar{u}$) from those that depend on oscillations ($\tilde{u}$ and $\varphi$):

$$F = \left( (\bar{x}_1 - \bar{x}_2 - \alpha) \bar{u} - \frac{\beta}{2} \bar{u}^2 \right) + \left( \frac{1}{2} \bar{u} \bar{x}_1 \cos(\varphi) - \frac{\beta}{4} \bar{u}^2 \right).$$  \tag{S49}$$

To guarantee a fitness optimum, either benefit or cost function (or both) must be nonlinear. Here we assume a linear benefit function, so a (convex) nonlinear investment function is required. If our reaction were part of pathway model, the pathway flux (as a benefit) would typically be a concave, nonlinear function.
Neglecting the inequality constraints for $\bar{u}$ and $\tilde{u}$, we can maximise both terms separately and obtain

$$\bar{u}^{\text{opt}} = (\bar{x}_1 - \bar{x}_2 - \alpha)/\beta$$

$$\cos(\varphi^{\text{opt}}) = 1$$

$$\tilde{u}^{\text{opt}} = \bar{x}_1/\beta.$$  (S50)

For $\tilde{x} = 0$, we obtain the enzyme-optimal reference state ($\tilde{u}^{\text{opt}} = 0$). Now we have to check whether all constraints are satisfied. A solution for $\bar{u}$ will only be valid if $\alpha < \bar{x}_1 - \bar{x}_2$: i.e., already at very small enzyme activities, the enzyme benefit must exceed the cost; otherwise, a boundary optimum at $\bar{u} = 0$ is obtained (i.e., the enzyme is not expressed). Moreover, the optimum for $\tilde{u}$ holds whenever $\tilde{u}^{\text{opt}} \leq \bar{u}^{\text{opt}}$, otherwise, we obtain a boundary optimum at $\tilde{u} = \bar{u}^{\text{opt}}$.

S4 Proofs and derivations

S4.1 Enzyme rhythms can increase fluxes and evoke second harmonics

In a single reaction (see Figure 2 in the article), synchronous substrate and enzyme rhythms (represented by quadratic terms in $\tilde{u}$ and $\tilde{x}$ in Eq. (2)) lead to a static flux shift (frequency 0) and to a second harmonic (frequency $2\omega$):

$$\text{Re}(\tilde{u} e^{i \omega t}) \text{Re}(\tilde{x} e^{i \omega t}) = \frac{1}{2} (\tilde{u} e^{i \omega t} + \tilde{u}^* e^{-i \omega t}) \frac{1}{2} (\tilde{x} e^{i \omega t} + \tilde{x}^* e^{-i \omega t})$$

$$= \frac{1}{4} (\tilde{u} \tilde{x} e^{2i \omega t} + \tilde{u}^* \tilde{x} + \tilde{u} \tilde{x}^* + \tilde{u}^* \tilde{x}^* e^{-2i \omega t})$$

$$= \frac{1}{2} \text{Re}(\tilde{u} \tilde{x} e^{2i \omega t}) + \frac{1}{2} \text{Re}(\tilde{u}^* \tilde{x}).$$  (S51)

S4.2 Effective elasticities in periodic metabolic states

In periodic states, effective reaction elasticities can be used to describe changes of average fluxes and flux amplitudes caused by small (static or periodic) perturbations. To derive the formula (S5), we first consider a static reference state with internal metabolite levels $c_{\text{ref}}$, external metabolite levels $x_{\text{ref}}$, and enzyme levels $u_{\text{ref}}$. Close to this state, time-dependent reaction rates can be expanded as

$$v(t) \approx v_{\text{ref}} + \begin{pmatrix} E^c_x \\ E^c_u \\ E^x_x \\ E^x_u \\ E^u_x \\ E^u_u \end{pmatrix}^T \begin{pmatrix} \Delta c(t) \\ \Delta x(t) \\ \Delta u(t) \end{pmatrix} + \frac{1}{2} \begin{pmatrix} E^c_{cc} & E^c_{cx} & E^c_{cu} \\ E^c_{xc} & E^c_{xx} & E^c_{xu} \\ E^c_{uc} & E^c_{ux} & E^c_{uu} \end{pmatrix} \begin{pmatrix} \Delta c(t) \\ \Delta x(t) \\ \Delta u(t) \end{pmatrix}.  \quad (S52)$$
where \( v_{\text{ref}} = v(c_{\text{ref}}, x_{\text{ref}}, u_{\text{ref}}) \) and

\[
\begin{align*}
c(t) &= c_{\text{ref}} + \Delta c(t) \\
x(t) &= x_{\text{ref}} + \Delta x(t) \\
u(t) &= u_{\text{ref}} + \Delta u(t).
\end{align*}
\] (S53)

Specifically, we now consider a periodic state in which metabolite levels, external metabolite levels, and enzyme levels show periodic profiles

\[
\begin{align*}
c(t) &= c_{\text{ref}} + \Delta c + \text{Re}(\tilde{c} e^{i\omega t}) \\
x(t) &= x_{\text{ref}} + \Delta x + \text{Re}(\tilde{x} e^{i\omega t}) \\
u(t) &= u_{\text{ref}} + \Delta u + \text{Re}(\tilde{u} e^{i\omega t}).
\end{align*}
\] (S54)

Expanding the reaction rates to first order, we obtain the harmonic time dependence

\[
v(t) \approx v_{\text{ref}} + \tilde{\nu} + \text{Re}(\nu e^{i\omega t}).
\] (S55)

By equating this to the expansion Eq. \([S52]\), with the profiles \([S54]\) inserted, we obtain the expansion terms

\[
\Delta \tilde{\nu} = \left( \begin{array}{c} \tilde{E}^c \\ \tilde{E}^x \\ \tilde{E}^u \end{array} \right)^T \left( \begin{array}{c} \Delta \tilde{c} \\ \Delta \tilde{x} \\ \Delta \tilde{u} \end{array} \right) + \frac{1}{2} \left( \begin{array}{c} \tilde{c} \\ \tilde{x} \\ \tilde{u} \end{array} \right)^T \left( \begin{array}{ccc} E_{cc}^v & E_{cx}^v & E_{cu}^v \\ E_{xc}^v & E_{xx}^v & E_{xu}^v \\ E_{uc}^v & E_{ux}^v & E_{uu}^v \end{array} \right) \left( \begin{array}{c} \Delta \tilde{c} \\ \Delta \tilde{x} \\ \Delta \tilde{u} \end{array} \right) \] (S56)

We now consider our periodic state (defined by a steady reference state and additional changes \( \Delta c, \Delta x, \Delta u \) as well as \( \tilde{c}, \tilde{x}, \tilde{u} \)) and study the effects of small additional enzyme variations (defined by \( \delta \tilde{u} \) and \( \delta \tilde{u} \)). We expand the flux variation to first order

\[
\left( \begin{array}{c} \delta \tilde{v} \\ \delta \tilde{\nu} \end{array} \right) = \left( \begin{array}{cc} \tilde{E}^v_u & \tilde{E}^v_u \\ \tilde{E}^v_u & \tilde{E}^v_u \end{array} \right) \left( \begin{array}{c} \delta \tilde{u} \\ \delta \tilde{u} \end{array} \right),
\] (S57)

with expansion coefficients in the matrix still to be determined. From Eq. \([S56]\), and noting that \( E_{uu}^v = 0 \), we would obtain the expansion

\[
\begin{align*}
\delta v &= [\tilde{E}^v_u + \Delta \tilde{c}^T E_{cu}^v + \Delta \tilde{x}^T E_{xu}^v] \delta \tilde{u} + [\tilde{c}^T E_{cu}^v + \tilde{x}^T E_{xu}^v] \delta \tilde{u} \\
\delta \tilde{\nu} &= [\tilde{c}^T E_{cu}^v + \tilde{x}^T E_{xu}^v] \delta \tilde{u} + [\tilde{E}^v_u + \Delta \tilde{c}^T E_{cu}^v + \Delta \tilde{x}^T E_{xu}^v] \delta \tilde{u}.
\end{align*}
\] (S58)
By comparing this to Eq. \((S57)\) and noting that \(E^{u}_{c,i} = 0\), we obtain the effective elasticities

\[
\begin{align*}
E^{v}_{u} &= E^{v}_{u} + \Delta \bar{c} \Delta E^{v}_{x u} + \Delta \bar{x} \Delta E^{v}_{x u} \\
E^{c}_{u} &= E^{c}_{u} + \bar{c} \Delta E^{c}_{x u} + \bar{x} \Delta E^{v}_{x u}.
\end{align*}
\] (S59)

Since enzyme levels appear in the rate laws only as prefactors, we can replace effective elasticities with respect to reactant levels \(c\) with Fourier transformation, we adopt the prefactor convention from [15] and set periodic and spectral response coefficients differ only by a prefactor, which we will now derive. For the harmonic (real-valued) parameter perturbation \(E_{v}^{c} = \frac{\nu_{v}}{u_{i}}, \quad E_{c,i}^{v} = \frac{E_{c,i}^{v}}{u_{i}}\), and \(E_{x}^{v} = 0\) and thus rewrite the effective elasticities as

\[
\begin{align*}
\hat{E}^{v}_{u} &= \hat{E}^{v}_{u} = Dg(\bar{u})^{-1} (Dg(\nu_{v} + E_{c} \Delta \bar{c} + E_{x} \Delta \bar{x}) = Dg(\bar{u})^{-1} Dg(\bar{v}) \\
\hat{E}^{c}_{u} &= \hat{E}^{c}_{u} = Dg(\bar{u})^{-1} (Dg(E_{c} \Delta \bar{c} + E_{x} \Delta \bar{x}) = Dg(\bar{u})^{-1} Dg(\bar{v}),
\end{align*}
\] (S60)

where we defined \(\bar{v} = \nu_{v} + E_{c} \Delta \bar{c} + E_{x} \Delta \bar{x}\) and \(\hat{v} = E_{c} \Delta \bar{c} + E_{x} \Delta \bar{x}\). Altogether, we obtain the elasticity matrices

\[
\begin{pmatrix}
\hat{E}^{v}_{u} & \hat{E}^{c}_{u} \\
\hat{E}^{c}_{u} & \hat{E}^{c}_{u}
\end{pmatrix} = \begin{pmatrix}
(Dg(\bar{v}) Dg(\bar{u})^{-1} & Dg(\hat{v}) Dg(\bar{u})^{-1} \\
Dg(\hat{v}) Dg(\bar{u})^{-1} & Dg(\bar{v}) Dg(\bar{u})^{-1}
\end{pmatrix}.
\] (S61)

Effective elasticities with respect to reactant levels \(c\) or \(x\) can be derived in a similar way.

### S4.3 Periodic response coefficients Eq. \((S12)\)

Periodic and spectral response coefficients differ only by a prefactor, which we will now derive. For the Fourier transformation, we adopt the prefactor convention from [15] and set

\[
x(t) = \frac{1}{\sqrt{2\pi}} \int \tilde{x}(\omega) e^{i\omega t} dt, \quad \tilde{x}(\omega) = \frac{1}{\sqrt{2\pi}} \int x e^{-i\omega t} dt.
\] (S62)

A harmonic (real-valued) parameter perturbation

\[
\Delta p(t) = \frac{1}{2} \left[ p e^{i\omega t} + p^{*} e^{-i\omega t} \right]
\] (S63)

defined by a complex amplitude vector \(p\), has the Fourier transform

\[
\hat{p}(\alpha) = \sqrt{\frac{2\pi}{2}} \delta_{\alpha}(\omega) \hat{p} + \sqrt{\frac{2\pi}{2}} \delta_{\alpha}(-\omega) \hat{p}^{*}.
\] (S64)

The Fourier components of a state variable \(s\) can be approximated with the help of spectral response coefficients \(\hat{R}_{p}^{s}\), the functional derivatives between Fourier components [15]. In a first-order expansion, the Fourier components of \(s\), at frequency \(\alpha\), read

\[
\hat{s}(\alpha) \approx \hat{R}_{p}^{s}(\alpha) \hat{p}(\alpha) = \sqrt{\frac{2\pi}{2}} \delta_{\alpha}(\omega) \hat{R}_{p}^{s}(\omega) \hat{p} + \sqrt{\frac{2\pi}{2}} \delta_{\alpha}(-\omega) \hat{R}_{p}^{s}(-\omega) \hat{p}^{*}.
\] (S65)

By applying a reverse Fourier transformation, we obtain the temporal behaviour

\[
\Delta s(t) \approx \frac{1}{2} \left( \hat{R}_{p}^{s}(\omega) \hat{p} e^{i\omega t} + \hat{R}_{p}^{s}(-\omega) \hat{p}^{*} e^{-i\omega t} \right) = \text{Re}[\hat{R}_{p}^{s}(\omega) \hat{p} e^{i\omega t}].
\] (S66)
Here we used the fact that $\tilde{R}_p^s(\omega)$ and $\tilde{R}_p^s(-\omega)$ are complex conjugates. Thus, the periodic response coefficient, defined as the first-order expansion coefficient appearing in this formula, is given by the spectral response coefficient $\tilde{R}_p^s(\omega)$. Now we consider a second-order expansion. An expansion with spectral response coefficients yields additional terms in the Fourier transform, which need to be added to Eq. (565):

$$
\begin{align*}
\delta_\alpha(0) \tilde{R}_{pp}^s(\omega) \left( \frac{\sqrt{2\pi}}{2} \hat{p} \otimes \frac{\sqrt{2\pi}}{2} \hat{p}^* \right) \\
+ \frac{1}{2} \delta_\alpha(2\omega) \tilde{R}_{pp}^s(\omega) \left( \frac{\sqrt{2\pi}}{2} \hat{p} \otimes \frac{\sqrt{2\pi}}{2} \hat{p} \right) \\
+ \frac{1}{2} \delta_\alpha(-2\omega) \tilde{R}_{pp}^s(\omega) \left( \frac{\sqrt{2\pi}}{2} \hat{p}^* \otimes \frac{\sqrt{2\pi}}{2} \hat{p}^* \right)
\end{align*}
= \frac{2\pi}{4} \left( \delta_\alpha(0) \tilde{R}_{pp}^s(\omega) [\hat{p} \otimes \hat{p}^*] + \frac{1}{2} \delta_\alpha(2\omega) \tilde{R}_{pp}^s(\omega) [\hat{p} \otimes \hat{p}] + \frac{1}{2} \delta_\alpha(-2\omega) \tilde{R}_{pp}^s(\omega) [\hat{p}^* \otimes \hat{p}^*] \right) \tag{567}
$$

In the time domain (Eq. (522)), we obtain the additional terms

$$
\begin{align*}
... + \frac{\sqrt{2\pi}}{4} \left( \tilde{R}_{pp}^s(\omega)[\hat{p} \otimes \hat{p}^*] + \frac{1}{2} \tilde{R}_{pp}^s(\omega)[\hat{p} \otimes \hat{p}] e^{i2\omega t} + \frac{1}{2} \tilde{R}_{pp}^s(\omega)[\hat{p}^* \otimes \hat{p}^*] e^{-2\omega t} \right) \\
= ... + \frac{1}{2} \text{Re} \left( \frac{\sqrt{2\pi}}{2} \tilde{R}_{pp}^s(\omega)[\hat{p} \otimes \hat{p}^*] \right) + \frac{1}{2} \text{Re} \left( \frac{\sqrt{2\pi}}{2} \tilde{R}_{pp}^s(\omega)[\hat{p} \otimes \hat{p}] e^{2\omega t} \right). \tag{568}
\end{align*}
$$

The periodic response coefficients (i.e., the expansion coefficients in this formula) read

$$
\begin{align*}
R_{pp}^s(\omega) &= \frac{\sqrt{2\pi}}{2} \tilde{R}_{pp}^s(\omega), \\
\tilde{R}_{pp}^s(\omega) &= \frac{\sqrt{2\pi}}{2} \tilde{R}_{pp}^s(\omega). \tag{569}
\end{align*}
$$

Again, they are given by the spectral response coefficients, but with a prefactor $\sqrt{2\pi}$.

### S4.4 There are no fitness synergies between higher harmonics

Let us now see why rhythms of different frequencies can be treated separately. In our fitness expansion Eq. (5), fitness contributions at different frequencies appear independently; therefore, higher harmonics of enzyme rhythms can be optimised separately unless they are coupled by constraints. This will be shown now. For ease of notation, we merge all rhythmic parameters in a vector $p = (s)$. Our fitness functional $F$ depends on time-average values (e.g., average benefit $\langle b \rangle_t$, average cost $\langle h \rangle_t$, average concentrations and fluxes $\langle c \rangle_t$ and $\langle v \rangle_t$). We assume that the functional is invariant against time shifts, so a shifted profile $p(t + \Delta t)$ will yield the same fitness value as $p(t)$. After expanding $p(t)$ into a Fourier series $p(t) = p_0 + p_1 e^{i\omega t} + p_2 e^{i2\omega t} + ..$, we can write the functional as a function $f(p_0, p_1, p_2, ..)$. Now we expand $f$ into a power series around a reference state $p_{\text{ref}}$:

$$
f = f(p_{\text{ref}}) + f_p \cdot \Delta p + f_{p,\omega} \cdot \tilde{p}_{\omega} + f_{p,2\omega} \cdot \tilde{p}_{2\omega} + ..
$$

$$
= \begin{pmatrix}
\Delta \tilde{p}_p \\
\tilde{p}_{\omega} \\
\tilde{p}_{2\omega}
\end{pmatrix}^T \begin{pmatrix}
F_{pp} & F_{pp,\omega} & F_{pp,2\omega} & .. \\
F_{p\omega,pp} & F_{p\omega,\omega} & F_{p\omega,2\omega} & .. \\
F_{p2\omega,pp} & F_{p2\omega,\omega} & F_{p2\omega,2\omega} & .. \\
.. & .. & .. & ..
\end{pmatrix} \begin{pmatrix}
\Delta \tilde{p}_p \\
\tilde{p}_{\omega} \\
\tilde{p}_{2\omega}
\end{pmatrix} \tag{570}
$$

53
where $p(0) = p_{\text{ref}} + \Delta p$. Time-shift invariance, $F[p(t)] = F[p(t + \Delta t)]$, yields the condition

$$f(p(0), \tilde{p}(\omega); \cdots) = f(p(0), \tilde{p}(\omega) e^{i \omega \delta t}, \tilde{p}(2\omega) e^{i 2\omega \delta t}, \cdots). \tag{S71}$$

Thus, for time-shift invariance to be satisfied, Eq. (S70) must yield the same result if we replace $\tilde{p}(\omega) \rightarrow \tilde{p}(\omega) e^{i \omega \delta t}$, $\tilde{p}(2\omega) \rightarrow \tilde{p}(2\omega) e^{i 2\omega \delta t}$, and so on, for any choice of $\delta t$. This can only hold if all linear terms for periodic parameters (i.e., $f_{p,\omega}$, $f_{p,2\omega}$, ...) vanish and if all mixed quadratic terms for different frequencies (e.g., $F_{p,\omega}(p)$) vanish as well. For the non-mixed quadratic terms (e.g., $F_{p,\omega}(p)$), invariance holds because the factor $e^{i \omega \delta t}$ appears on both sides (once as a complex conjugate), and thus cancels out. We thus obtain an expansion of the form

$$f = f(p_{\text{ref}}) + \frac{1}{2} \Delta p \cdot F_{\text{pp}} \Delta p + \frac{1}{2} \Delta \tilde{p} \cdot F_{\tilde{p}\tilde{p}} \Delta \tilde{p} + \frac{1}{2} \Delta \tilde{p} \cdot F_{\tilde{p}\tilde{p}} \Delta \tilde{p} + \frac{1}{2} \Delta \tilde{p} \cdot F_{\tilde{p}\tilde{p}} \Delta \tilde{p} + \cdots \tag{S72}$$

Since the parameter vector $p$ comprises both $\mathbf{x}$ and $\mathbf{u}$, each quadratic term can be subdivided into separate terms for external-external, external-enzyme, and enzyme-enzyme synergies.

### S4.5 Optimal enzyme rhythms under constraints, Eq. (14)

To derive the optimal enzyme profiles under constraints, Eq. (14), we consider the quadratic optimality problem (13) with constraints. As constraints, we consider bounds for the central values $0 \leq \bar{u} \leq \bar{u}_{\text{max}}$ as well as amplitude constraints (either simple positivity constraints $|\bar{u}| \leq \bar{u}$ or frequency-dependent constraints based on protein production, $|\bar{u}| \leq \bar{p}^{\text{max}}(\bar{u}, \omega)$). The fitness function for control variables ($\Delta \bar{u}$, and $\bar{u}$) and external variables ($\Delta \bar{x}$, $\bar{x}$), with constant terms omitted, reads

$$F = \Delta \bar{u}^T F_{uu} \Delta \bar{u} + \frac{1}{2} \Delta \bar{u}^T F_{uu} \Delta \bar{u} + \text{Re} (\bar{u}^T F_{\bar{u} \bar{x}} \bar{x}) + \frac{1}{2} \bar{u}^T F_{\bar{u} \bar{u}} \bar{u}. \tag{S73}$$

The constraints $0 \leq \bar{u} \leq \bar{u}_{\text{max}}$, and $|\bar{u}| \leq \bar{p}^{\text{max}}(\bar{u}, \omega)$ can be described by setting

$$a = -\bar{u}$$

$$b = \bar{u} - \bar{u}_{\text{max}}$$

$$c = |\bar{u}| - \bar{p}^{\text{max}}(\bar{u}, \omega). \tag{S74}$$

and requiring $0 \leq a, b \leq 0, c \leq 0$. Using real-valued Lagrange multiplier vectors $\alpha$, $\beta$, and $\gamma$, we can reformulate the optimality problem and obtain the Kuhn-Tucker optimality conditions

$$0 = \frac{\partial F}{\partial \Delta \bar{u}} + \alpha \cdot \frac{\partial a}{\partial \Delta \bar{u}} + \beta \cdot \frac{\partial b}{\partial \Delta \bar{u}} + \gamma \cdot \frac{\partial c}{\partial \Delta \bar{u}}$$

$$0 = \frac{\partial F}{\partial \bar{u}} + \alpha \cdot \frac{\partial a}{\partial \bar{u}} + \beta \cdot \frac{\partial b}{\partial \bar{u}} + \gamma \cdot \frac{\partial c}{\partial \bar{u}} \tag{S75}$$
where all Lagrange multipliers are positive (or zero if the corresponding constraint is not active). After inserting Eq. (S73), we can rewrite Eq. (S75) as

\[
\begin{align*}
F_{ux} \Delta \bar{x} + F_{uu} \Delta \bar{u} &= -\alpha + \beta - \gamma \cdot \frac{\partial \bar{p}^{\text{max}}(\bar{u}, \omega)}{\partial \bar{u}}, \\
F_{\bar{u}x} \bar{x} + F_{\bar{u}u} \bar{u} &= \gamma \cdot \frac{\partial \bar{u}}{\partial \bar{u}}.
\end{align*}
\] (S76)

Noting that \(\frac{\partial \bar{p}^{\text{max}}(\bar{u}, \omega)}{\partial \Delta \bar{u}} = D_g(\bar{p}^{\text{max}}(\bar{u}, \omega))\) and \(\frac{\partial \bar{u}}{\partial \bar{u}} = D_g(\bar{u}/|\bar{u}|)\), and merging \(-\alpha\) and \(\beta\) into a vector \(\mu\), we obtain

\[
\begin{align*}
F_{ux} \Delta \bar{x} + F_{uu} \Delta \bar{u} &= \mu - D_g(\bar{p}^{\text{rel}}(\omega)) \gamma, \\
F_{\bar{u}x} \bar{x} + F_{\bar{u}u} \bar{u} &= D_g\left(\frac{\bar{u}}{|\bar{u}|}\right) \gamma.
\end{align*}
\] (S77)

where \(\mu_l < 0\) for inactive enzymes (i.e., \(\bar{u}_l = 0\)), \(\mu_l > 0\) for enzymes whose central value \(\bar{u}_l\) hits the upper bound, and \(\mu_l = 0\) otherwise. Similarly, \(\gamma_l > 0\) holds for all enzymes with active amplitude constraints, and \(\gamma_l = 0\) otherwise. Eq. (S77) can be solved for the optimal enzyme profiles

\[
\begin{align*}
\Delta \bar{u} &= -F_{uu}^{-1} \left[ F_{ux} \Delta \bar{x} - \mu - D_g(\bar{p}^{\text{rel}}(\omega)) \gamma \right], \\
\bar{u} &= -F_{\bar{u}u}^{-1} \left[ F_{\bar{u}x} \bar{x} - D_g\left(\frac{\bar{u}}{|\bar{u}|}\right) \gamma \right].
\end{align*}
\] (S78)

**S4.6 Economic balance equation for enzyme rhythms, Eqs (10) and (11)**

We now derive economic balance equations for periodic states. We consider a state in which rhythms \(\bar{x}\) and \(\bar{u}\) drive oscillations \(\bar{v}\) and \(\bar{c}\) and study the effects of small additional enzyme variations (\(\delta \bar{u}\) and \(\delta \bar{u}\)), whose effects on mass balances are compensated by periodic exchange fluxes (\(\delta \bar{v}, \delta \bar{c}\)). The compensation is chosen such that all metabolite profiles remain unchanged: \(\delta \bar{c} = 0, \delta \bar{c} = 0\). The fluxes, however, will change: to compute the variations, \(\bar{v}\) and \(\bar{v}\), we expand them in terms of \(\delta \bar{u}\) and \(\delta \bar{u}\):

\[
\begin{pmatrix}
\delta \bar{v} \\
\delta \bar{\bar{v}}
\end{pmatrix} = 
\begin{pmatrix}
\hat{E}_{\bar{u}}^v & \hat{E}_{\bar{u}}^{\bar{v}} \\
\hat{E}_{\bar{u}}^v & \hat{E}_{\bar{u}}^{\bar{v}}
\end{pmatrix}
\begin{pmatrix}
\delta \bar{u} \\
\delta \bar{u}
\end{pmatrix}.
\] (S79)

The rhythmic elasticities \(\hat{E}_{\bar{u}}^v\) refer to the periodic, unperturbed state (see S1.2). The virtual fluxes, in order to compensate the effects of \(\delta \bar{u}\) and \(\delta \bar{u}\) on all mass balances (i.e., to compensate the perturbed mass balances \(N \delta \bar{v}\) and \(N \delta \bar{c}\)), must read

\[
\begin{pmatrix}
\delta \bar{c} \\
\delta \bar{c}
\end{pmatrix} = -
\begin{pmatrix}
N & 0 \\
0 & N
\end{pmatrix}
\begin{pmatrix}
\delta \bar{v} \\
\delta \bar{\bar{v}}
\end{pmatrix} = -
\begin{pmatrix}
N & 0 \\
0 & N
\end{pmatrix}
\begin{pmatrix}
\hat{E}_{\bar{u}}^v & \hat{E}_{\bar{u}}^{\bar{v}} \\
\hat{E}_{\bar{u}}^v & \hat{E}_{\bar{u}}^{\bar{v}}
\end{pmatrix}
\begin{pmatrix}
\delta \bar{u} \\
\delta \bar{u}
\end{pmatrix}.
\] (S80)

We now defined the static and periodic enzyme demands \(\hat{g}_{\bar{u}}\) and \(\hat{g}_{\bar{u}}\) as the derivatives between the benefit functional and enzyme variations. Likewise, the static or periodic economic potentials \(\hat{w}_{\bar{c}}\) and \(\hat{w}_{\bar{c}}\) are
defined as derivatives between benefit functional and virtual fluxes:

\[
\dot{g}_\alpha = \frac{\partial g}{\partial u}, \quad \dot{g}_\beta = \frac{\partial g}{\partial \phi}, \\
\dot{w}_c = \frac{\partial g}{\partial \phi}, \quad \dot{w}_\tilde{c} = \frac{\partial g}{\partial \phi}.
\] (S81)

The total benefit change, caused by the compensated variation, can be expressed by the benefit changes due to the variations of enzyme levels and virtual fluxes:

\[
\delta g = \text{Re}\left[ \dot{g}_\alpha^\top \delta u + \dot{w}_c \delta \phi + \dot{w}_\tilde{c} \delta \phi \right]
\] (S82)

The benefit change can also be written in terms of the local flux variations (metabolite changes can be neglected because they are cancelled in the compensated variation):

\[
\delta g = \text{Re}\left[ b_v \delta \nu + b_\tilde{v} \delta \tilde{\nu} \right] = \left( b_v \right)^\top \left( \dot{E}_u^v \dot{E}_u^\nu \right) \left( \delta u \right) + \left( b_\tilde{v} \right)^\top \left( \dot{E}_u^\tilde{v} \dot{E}_u^\tilde{\nu} \right) \left( \delta \tilde{u} \right). \tag{S83}
\]

We now equate Eqs (S82) and (S83). Since the equality must hold for any choice of the vector \((\delta u, \delta \tilde{u})\), we obtain the economic rule

\[
\left( \begin{array}{c} \dot{g}_\alpha \\ \dot{g}_\beta \end{array} \right) = \left( \begin{array}{c} \dot{E}_u^v \\ \dot{E}_u^\tilde{v} \end{array} \right) \left( \begin{array}{c} \dot{E}_u^\nu \\ \dot{E}_u^\tilde{\nu} \end{array} \right)^\top \left( \begin{array}{c} b_v \\ b_\tilde{v} \end{array} \right) = \left( \begin{array}{c} \dot{E}_u^v \\ \dot{E}_u^\tilde{v} \end{array} \right) \left( \begin{array}{c} \dot{E}_u^\nu \\ \dot{E}_u^\tilde{\nu} \end{array} \right)^\top \left( \begin{array}{c} N^\top w_c + b_v \\ N^\top w_\tilde{c} + b_\tilde{v} \end{array} \right). \tag{S84}
\]

This balance equation holds generally. If our periodic state is close to a steady reference state, we can expand the effective elasticities, use Eq. (S6) from section S4.2, and obtain

\[
\frac{Dg(u)}{Dg(u)} \dot{h}_u = \frac{Dg(u)}{Dg(u)} \dot{h}_\beta = \left( \begin{array}{c} Dg(\dot{v}) \\ Dg(\dot{\tilde{v}}) \end{array} \right) = \left( \begin{array}{c} Dg(\dot{v}) \\ Dg(\dot{\tilde{v}}) \end{array} \right) \left( \begin{array}{c} \Delta w_c + b_v \\ \Delta w_\tilde{c} + b_\tilde{v} \end{array} \right), \tag{S86}
\]

where \(\dot{v}\) and \(u\) are the vectors of fluxes and enzyme levels in the central state, and \(\dot{\tilde{v}} = E_x^\nu \tilde{c} + E_x^\tilde{v} \tilde{x}\).
S5 Mathematical notation

Complex vectors and matrices Most formulae are written in matrix notation, with vectors, matrices, and tensors written in bold font. By default, all vectors, are column vectors. The adjoint $z^\dagger$ of a complex vector $z$ is defined as the complex conjugate transpose $z^\dagger = (z_1^*, ..., z_n^*)$ (i.e., a row vector; the star * indicates the complex conjugate). For convenience, we define a scalar product $\mathbf{a} \cdot \mathbf{b} = \mathbf{a}^\dagger \mathbf{b}$ for complex vectors. If a real-valued result is required, this must be stated explicitly as $\text{Re}(\mathbf{a} \cdot \mathbf{b})$. We obtain $\text{Re}(\mathbf{a} \cdot \mathbf{b}) = \text{Re}(\mathbf{a}) \cdot \text{Re}(\mathbf{b}) + \text{Im}(\mathbf{a}) \cdot \text{Im}(\mathbf{b})$ (with simple scalar products between real-valued vectors on the right). The scalar product between a vector and itself, $\mathbf{a} \cdot \mathbf{a} = \mathbf{a}^\dagger \mathbf{a}$, yields a positive real-valued number. Quadratic forms for complex vectors are written as $\mathbf{a}^\dagger \mathbf{M} \mathbf{b}$. If a matrix $\mathbf{M}$ is self-adjointed (i.e., Hermitian), the quadratic form $\mathbf{a}^\dagger \mathbf{M} \mathbf{a}$ yields a real-valued number. Tensor products $Y_{i mn} = \sum_l A_{il} B_{lm}^* C_{ln}$ are written as $Y = A \cdot B$; products $X_{i mn} = \sum_{pq} A_{ip} B_{pq}^* C_{qn}$ are written as $X = A [B \otimes C]$. The symbol $\otimes$ denotes the Kronecker product.

Complex amplitudes and complex derivatives Oscillations are described by the real parts of complex exponentials, for instance $a(t) = \text{Re}[\tilde{a} e^{i\omega t}] = |\tilde{a}| \cos(\omega t + \varphi(\tilde{a}))$, where $\varphi(z)$ is the phase angle of a complex number $z$. Oscillating vectors are written as $a(t) = \text{Re}[\tilde{a} e^{i\omega t}] = |\tilde{a}| \cos(\omega t + \varphi(\tilde{a}))$. Note that amplitudes and amplitude vectors, marked by a tilde, are complex-valued. Complex derivatives are written as follows. Let $f(z)$ be a function of a complex vector $z$. The first derivative is represented by a column vector $f_z = \left( \frac{\partial f}{\partial z_1}, ..., \frac{\partial f}{\partial z_n} \right)^\top$, where the complex derivatives of vector components $z_l = x_l + i y_l$ are defined by $\frac{\partial f}{\partial z_l} = \frac{\partial f}{\partial x_l} + i \frac{\partial f}{\partial y_l}$. For complex vectors $\mathbf{a}$ and $\mathbf{b}$, we obtain $\frac{\partial}{\partial \mathbf{b}} \text{Re}(\mathbf{a} \cdot \mathbf{b}) = \mathbf{a}$. The second derivatives form a matrix $F_{zz}$, where $F_{z_l z_k} = \left( \frac{\partial^2 f}{\partial z_l \partial z_k} \right)$. As an alternative notation, commonly used in metabolic control analysis, I also write $D_{zz}$, where $D$ can stand for $E$ (elasticity matrices) or $R$ (response coefficient matrices). Functions $f(z)$ of complex variables are Taylor-expanded to second order as $f(z) \approx f(0) + f_z \cdot z + \frac{1}{2} z^\dagger F_{zz} z$. For real-valued functions $f$, the real part needs to be taken: $f(z) \approx f(0) + \text{Re}(f_z \cdot z) + \frac{1}{2} z^\dagger F_{zz} z$.

Kinetic models Kinetic metabolic models are described in the methods section of the article. To account for conservation relations in the model [25], we split the stoichiometric matrix $\mathbf{N}$ into a product $\mathbf{N} = \mathbf{L} \mathbf{N}_R$, where $\mathbf{N}_R$ consists of linearly independent rows of $\mathbf{N}$. 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 |
| $v_r(u_l, c) = r_l(u_l, c)$ | mM/s | Reaction rate |
| $u_l$  | mM   | enzyme activity |
| $x_j$  |      | External parameter |
| $p_m$  |      | State parameter (enzyme activity or external parameter) |
| $s_i$  | mM   | Steady state concentration |
| $j_l$  | mM/s | Steady state flux (reaction rate) |
| $f(u)$ | D    | Fitness function: $f(u) = g(u) - h(u)$ |
| $g(u) = b(v(u), c(u))$ | D | Return function |
| $h(u)$ | D    | Investment function |
| $b_{ij}$ | D/(mM/s) | Flux demand $b_i^j = \partial b / \partial v_l$ |
| $\omega$ | s$^{-1}$ | Circular frequency |

$F^{(S)}(u) = f(\langle s(t) \rangle_t)$ D$^{-1}$ State-averaged fitness functional
$F^{(F)}(u) = f(\langle s(t) \rangle_t)$ D$^{-1}$ Fitness-averaged fitness functional

| I | Identity matrix |

Table S1: Mathematical symbols. The units of parameters $u_l$ and $x_l$ depend on the biological meaning of these parameters, which can vary from case to case. The unit D (Darwin) is a hypothetical fitness unit.

Steady states

$R_{sp} = (R_{pm}^{sn})$ 1st order response coefficients for a state variable $s$
$R_{pp} = (R_{pm,pn}^{pn})$ 2nd order response tensor ("synergy tensor")
$C_s, C_j$ Control coefficients for concentrations and fluxes
$E_c = (E_{li})$ Unscaled elasticity matrix
$E_{cc}, E_{cp}, E_{pc}, E_{pp}$ 2nd order elasticity tensors
$F_{xu}, F_{uu}$ Static fitness synergy matrices (Hessian matrices with respect to $\Delta \tilde{u}$ and $\Delta \tilde{x}$)

Periodic states

$C(\omega), C^{\prime}(\omega)$ Spectral control coefficients for concentrations and fluxes
$R^s(\omega)$ 1st order spectral response matrix
$R^s_{pp}(\omega), R^s_{pp}(\omega)$ 2nd order spectral response tensor for output $y$ at frequencies $0$ and $2\omega$
$R^p_{pp}(\omega), R^p_{pp}(\omega)$ 2nd order periodic response tensor for output $y$ at frequencies $0$ and $2\omega$
$F_{xa}, F_{ua}$ Periodic fitness synergy matrices (Hessian matrices with respect to $\Delta \tilde{u}$ and $\Delta \tilde{x}$)

Table S2: Symbols for metabolic control analysis. All MCA coefficients (elasticities, response and control coefficients) are used in their unscaled form.