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Response to temporal parameter fluctuations in biochemical networks

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Abstract

Metabolic response coefficients describe how variables in metabolic systems, like steady state concentrations, respond to small changes of kinetic parameters. To extend this concept to temporal parameter fluctuations, we define spectral response coefficients that relate Fourier components of concentrations and fluxes to Fourier components of the underlying parameters. It is also straightforward to generalize other concepts from metabolic control theory, such as control coefficients with their summation and connectivity theorems. The first-order response coefficients describe forced oscillations caused by small harmonic oscillations of single parameters: they depend on the driving frequency and comprise the phases and amplitudes of the concentrations and fluxes. Close to a Hopf bifurcation, resonance can occur: as an example, we study the spectral densities of concentration fluctuations arising from the stochastic nature of chemical reactions. Second-order response coefficients describe how perturbations of different frequencies interact by mode coupling, yielding higher harmonics in the metabolic response. The temporal response to small parameter fluctuations can be computed by Fourier synthesis. For a model of glycolysis, this approximation remains fairly accurate even for large relative fluctuations of the parameters.

Keywords: Response coefficient; Fourier analysis; Metabolic control analysis; Correlated fluctuations; Resonance; Mode coupling

1. Introduction

Biochemical reaction networks, which implement both metabolism and signalling in cells, are subject to permanent perturbations. The velocities of single chemical reactions depend on kinetic parameters like rate constants or enzyme activities. These parameters may fluctuate due to external changes like temperature shifts, but also due to internal processes, for instance, changes of cell size and energy demand that go along with the cell cycle. Moreover, reaction rates show stochastic fluctuations (Gillespie, 1977, 2000) which play a role if only few molecules are present (McAdams and Arkin, 1997; Thattai and van Oudenaarden, 2001) as in cell signalling or in the control of gene expression. How will the dynamics of the entire biochemical network respond to such permanent, fluctuating perturbations of the individual reaction velocities?

It is well known that shifts of the kinetic parameters can have dramatic effects on the behaviour of metabolic systems: at bifurcation points, the system may undergo qualitative changes, for instance switch between stationarity, oscillations, and chaos. Usually, however, a small change of the parameters will only shift a steady state or deform a limit cycle (Demin et al., 1999; Reijenga et al., 2002). Metabolic control analysis (MCA) (Fell, 1992; Heinrich and Schuster, 1996; Hofmeyer, 2001) describes how a static parameter change will alter the system's metabolic variables, such as stationary metabolic concentrations or fluxes, or the system trajectories (Ingalls and Sauro, 2003). If the parameters are changed by a small amount, the resulting shift of the metabolic variables is approximately

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proportional to the parameter shift, and the linear coefficients are called the metabolic response coefficients (Heinrich and Schuster, 1996). For larger perturbations, a quadratic approximation involving second-order response coefficients has been proposed (Höfer and Heinrich, 1993).

How can we describe the effects of parameter fluctuations in time? Demin et al. (1999) assumed that each reaction velocity is the product of a static enzyme concentration and an oscillatory turnover rate: the Fourier components of the system's oscillations were then expanded with respect to static enzyme concentrations, for fixed oscillations of the external parameters. Along a slightly different line, Ingalls (2004) and Liebermeister (2004) analysed how a stable system responds to small harmonic oscillations of single parameters. A harmonic perturbation will lead to forced harmonic oscillations of all metabolic variables, each with a certain amplitude and phase shift. The oscillations of parameters and system variables are related to each other by frequency-dependent, complex functions termed the spectral response coefficients (Liebermeister, 2004). It turns out that this generalization of MCA to oscillatory perturbations requires only a slight modification of the existing formulae. A thorough treatment for linearized systems has been given in Ingalls (2004).

We extend this idea to general nonlinear systems and define spectral response coefficients by differentiating Fourier components of metabolic variables with respect to the Fourier components of the parameters: the first and second derivatives are then termed the spectral response coefficients of first and second order. For small parameter perturbations, the spectral response coefficients can be used to approximate the frequency spectrum of the metabolic variables. The respective time courses can then be obtained by Fourier synthesis. In this article, we first review responses to static parameter changes and linear systems with temporal parameter perturbations. Then, the spectral response are defined in Section 4. Section 5 is devoted to spectral control coefficients. In the remainder, we discuss how perturbations of certain frequencies can be amplified by resonance. Resonance can also occur with stochastic parameter fluctuations, giving rise to a peak in the spectral density of concentration fluctuations. We conclude the article with two illustrating examples: the propagation of perturbations along a linear reaction chain and a model of glycolysis with oscillating energy storage.

Mathematical notation: (1) Vectors and matrices are denoted by bold face letters. (2) If a subscript or superscript appears twice in a formula, as in $A_{ik}B_{kl}$, it is summed over by convention. (3) Functionals are written with square and round brackets: if a functional h maps the functions $f_1(\cdot),\ldots,f_n(\cdot)$ to a function $g: x \to g(x)$, then $h[f_1(\cdot),\ldots,f_n(\cdot)](x)$ denotes $g(x)$. (4) $\mathbf{I} = (\delta_{ik})$ denotes the identity matrix, while $\delta_\alpha(\omega) := \delta(\omega - \alpha)$ is Dirac's delta distribution. (5) Oscillations are described by circular frequencies (Greek letters), e.g. $\omega = 2\pi/T$, where T is the period. (6) If $x(t)$ is a time course, then $\hat{x}_\omega := \hat{x}(\omega)$ denotes its Fourier transform at frequency ω, $x(\cdot)$ denotes the entire function, and $\hat{x}(\cdot)$ denotes the Fourier transform as a function.

2. Static response coefficients

A thorough treatment of the metabolic response coefficients can be found in Fell (1992), Heinrich and Schuster (1996) and Hofmeyer (2001). As a reminder, let us briefly recall some basic definitions: the metabolite concentrations $x_l(t)$ in a biochemical reaction network follow the differential equations

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{x}(t) = \mathbf{N}\mathbf{v}(\mathbf{x}(t), \mathbf{p}), \tag{1}$$

given here in vectorial form. The velocities of the chemical reactions are given by the kinetics functions $v_k(\mathbf{x}, \mathbf{p})$ where the kinetic parameters are denoted by p_m. Each column of the stoichiometric matrix $\mathbf{N}$ contains the stoichiometric coefficients of a chemical reaction, describing the amounts of metabolites that are consumed and produced in this reaction. If the metabolite concentrations are constrained by conservation relations, then $\mathbf{N}$ does not have full row rank. In this case, we follow (Reder, 1988) and represent the system by a set of independent metabolites: first, we reorder $\mathbf{N}$ such that its top part $\mathbf{N}_R$ consists of a maximal set of linearly independent rows. Then $\mathbf{N}$ is split into the product $\mathbf{N} = \mathbf{L}\mathbf{N}_R$ where $\mathbf{N}_R$ is called the reduced stoichiometric matrix and $\mathbf{L}$ is called the link matrix.

The derivatives of the reaction kinetics v_k with respect to metabolite concentrations and kinetic parameters are called the unscaled reaction elasticities

$$\varepsilon^S_{kl} := \frac{\partial v_k}{\partial x_l}, \quad \varepsilon^P_{km} := \frac{\partial v_k}{\partial p_m},$$

$$\varepsilon^{SS}_{klj} := \frac{\partial^2 v_k}{\partial x_l \partial x_j}, \quad \varepsilon^{SP}_{klm} := \frac{\partial^2 v_k}{\partial x_l \partial p_m}, \quad \varepsilon^{PP}_{kmn} := \frac{\partial^2 v_k}{\partial p_m \partial p_n}. \tag{2}$$

The Jacobian matrix for the independent metabolites reads $\mathbf{M}^0 = \mathbf{N}_R\varepsilon^S\mathbf{L}$. We assume that with a parameter vector $\mathbf{p}^0$, the system exhibits a stable steady state $\mathbf{s}(\mathbf{p}^0)$ fulfilling

$$0 = \mathbf{N}\mathbf{v}(\mathbf{s}(\mathbf{p}^0), \mathbf{p}^0). \tag{3}$$

In the following, we shall assume that the steady state remains stable in a neighbourhood Ω_p around the unperturbed parameters.[1] The steady state concentrations and metabolic fluxes at parameters $\mathbf{p} \in \Omega_p$ are

[1]This is the case if the kinetics functions can be continuously differentiated twice with respect to both concentrations and parameters.

described by the functions $\mathbf{s}(\mathbf{p})$ and $\mathbf{j}(\mathbf{p}) := \mathbf{v}(\mathbf{s}(\mathbf{p}), \mathbf{p})$, respectively. A small static change $\Delta\mathbf{p} = \mathbf{p} - \mathbf{p}^0$ of the parameters will shift the steady state. The resulting change $\Delta\mathbf{s} = \mathbf{s}(\mathbf{p}) - \mathbf{s}(\mathbf{p}^0)$ of metabolite concentrations can be written as a Taylor expansion

$$\Delta s_l = R^S_{lm}\Delta p_m + \tfrac{1}{2}R^{S,2}_{lmn}\Delta p_m \Delta p_n + \mathcal{O}((\Delta\mathbf{p})^3) \tag{4}$$

of the parameter changes. The metabolic response coefficients (Heinrich and Schuster, 1996; Höfer and Heinrich, 1993)

$$R^S_{lm} := \frac{\partial s_l(\mathbf{p})}{\partial p_m},$$

$$R^{S,2}_{lmn} := \frac{\partial^2 s_l(\mathbf{p})}{\partial p_m \partial p_n} \tag{5}$$

are the derivatives of the steady state concentrations with respect to the parameters. Flux response coefficients R^J_{km} and $R^{J,2}_{kmn}$ for the steady state flux $\mathbf{j}$ are defined accordingly. It is important not to confuse the elasticities ε^P_{km}, which are used for expanding the single reaction velocities at fixed concentrations, with the flux response coefficients R^J_{km} used to expand the stationary fluxes: the former refer to a local property of an isolated reaction velocity, while the latter describe a global property of the entire system. Also note that in this article, elasticities as well as control and response coefficients are used in their unscaled form.

3. Parameter fluctuations in a linear system

The concept of response coefficients described above is now extended to temporally varying parameters $\mathbf{p}(t) = \mathbf{p}^0 + \Delta\mathbf{p}(t)$ that fluctuate around the unperturbed values $\mathbf{p}^0$. Before tackling general metabolic systems in Section 4, let us study a simple linear system in detail. We consider a single metabolite $x(t)$ that is produced with a rate $k_1 p(t)$ and linearly degraded with a rate constant k_2. The parameter $p(\cdot)$ describes the fluctuating concentration of a precursor metabolite. The concentration $x(t)$ follows the differential equation

$$\frac{\mathrm{d}}{\mathrm{d}t}x(t) = ax(t) + bp(t), \tag{6}$$

where we have set $b = k_1$ and $a = -k_2$. If we impose the initial condition $x(t_0) = x_0$, the solution reads

$$x(t) = \mathrm{e}^{a(t-t_0)}x_0 + \int_{t_0}^{t} \mathrm{e}^{a(t-t')}bp(t')\,\mathrm{d}t'. \tag{7}$$

The first term depends on the initial value x_0 but not on the time-dependent parameter $p(\cdot)$, and it vanishes if we impose an initial condition at $t_0 = -\infty$. The integration kernel $K^S(t,t') = b\mathrm{e}^{a(t-t')}\Theta(t-t')$ in the second term is called the pulse-response function.[2]

We shall now try to reobtain result (7) from the Fourier-transforms of $x(t)$, $p(t)$, and the pulse-response function. The second term of Eq. (7), for $t_0 \to -\infty$, will be called $s[p(\cdot)](t)$. Hence, the differential equation (6) defines a mapping

$$p(\cdot) \xrightarrow{ODE} s[p(\cdot)](\cdot). \tag{8}$$

Together with the Fourier transformation

$$\hat{s}_\omega := \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{-\mathrm{i}\omega t}s(t)\,\mathrm{d}t, \tag{9}$$

$$\hat{p}_\omega := \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{-\mathrm{i}\omega t}p(t)\,\mathrm{d}t \tag{10}$$

fulfilling

$$p(t) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{\mathrm{i}\omega t}\hat{p}_\omega\,\mathrm{d}\omega, \tag{11}$$

we can map a parameter spectrum to the corresponding concentration spectrum via the combined mappings

$$\hat{p}(\cdot) \xrightarrow{FT} p(\cdot) \xrightarrow{ODE} s(\cdot) \xrightarrow{FT} \hat{s}(\cdot).$$

Let us consider a parameter time course with the Fourier spectrum $\hat{p}(\cdot)$. The Fourier components of the concentration and parameter time courses, at frequency ω, will be denoted by $\hat{s}_\omega[\hat{p}(\cdot)]$ and $\hat{p}_\omega[\hat{p}(\cdot)]$, respectively. We define the spectral response coefficient as the functional derivative (indicated by a curved δ) of $\hat{s}_\omega$ with respect to the Fourier component $\hat{p}_\alpha$

$$R^S(\omega,\alpha) := \frac{\delta\hat{s}_\omega[\hat{p}(\cdot)]}{\delta\hat{p}_\alpha} = \lim_{h\to 0}\frac{\hat{s}_\omega[\hat{p}(\cdot) + h\delta_\alpha(\cdot)] - \hat{s}_\omega[\hat{p}(\cdot)]}{h}. \tag{12}$$

To compute it, we insert $x(t) = s[p(\cdot)](t)$ into Eq. (6), Fourier-transform the equation, and obtain

$$\mathrm{i}\omega\hat{s}_\omega[\hat{p}(\cdot)] = a\hat{s}_\omega[\hat{p}(\cdot)] + b\hat{p}_\omega[\hat{p}(\cdot)]$$

$$\Rightarrow 0 = -(a - \mathrm{i}\omega)\hat{s}_\omega[\hat{p}(\cdot)] - b\hat{p}_\omega[\hat{p}(\cdot)] \quad \text{for all } \omega. \tag{13}$$

Differentiating this equation with respect to a Fourier component $\hat{p}_\alpha$ yields

$$0 = -(a - \mathrm{i}\omega)\frac{\delta\hat{s}_\omega[\hat{p}(\cdot)]}{\delta\hat{p}_\alpha} - b\frac{\delta\hat{p}_\omega[\hat{p}(\cdot)]}{\delta\hat{p}_\alpha} = -(a - \mathrm{i}\omega)R^S(\omega,\alpha) - b\delta_\alpha(\omega), \tag{14}$$

$$\Rightarrow R^S(\omega,\alpha) = -(a - \mathrm{i}\omega)^{-1}b\delta_\alpha(\omega), \tag{15}$$

which is the Fourier transform of the pulse-response function.

[2] $\Theta(t-t')$ denotes the Heaviside function, defined by $\Theta(t-t') = 1$ for $t \geqslant t'$, $\Theta(t-t') = 0$ for $t < t'$.

Just to check this result, let us try to reobtain a solution $s(t)$ for a given time course $p(\cdot) = p^0(\cdot) + \Delta p(\cdot)$ with static $p^0(t) = p^0$. The general solution reads

$$x(t) = \mathrm{e}^{a(t-t_0)}x_0 + s[p^0(\cdot)](t) + s[\Delta p(\cdot)](t), \tag{16}$$

$$= \mathrm{e}^{a(t-t_0)}x_0 + s^0(t) + \Delta s(t), \tag{17}$$

where the second term yields $s^0(t) = -p^0 b/a(1 - \mathrm{e}^{a(t-t_0)})$. The first two terms vanish for $t_0 \to -\infty$. To compute the third term $\Delta s(t)$, which is due to the perturbation, we approximate

$$\Delta\hat{s}_\omega \approx \int_{-\infty}^{\infty} R^S(\omega,\alpha)\Delta\hat{p}_\alpha \,\mathrm{d}\alpha = -(a - \mathrm{i}\omega)^{-1} b \Delta\hat{p}_\omega. \tag{18}$$

Fourier synthesis of Eq. (18) yields

$$\Delta s[p(\cdot)](t) \approx \int_{-\infty}^{t} \mathrm{e}^{a(t-t')} b\, \Delta p(t')\,\mathrm{d}t', \tag{19}$$

which is actually the exact solution (compare Eq. (8)) and (8), because the Fourier transform is also linear.

The above results hold in general for metabolic systems with linear differential equations. In particular, we may linearize a metabolic system around a stable steady state, that is, replace the kinetics functions by linear approximations and obtain the linear equation system

$$\frac{\mathrm{d}}{\mathrm{d}t}\Delta\mathbf{x}_{ind} = \mathbf{N}_R(\varepsilon^S\mathbf{L}\Delta\mathbf{x}_{ind} + \varepsilon^P\Delta\mathbf{p}) \tag{20}$$

for the vector $\mathbf{x}_{ind}$ of independent metabolites. Setting $A = \mathbf{N}_R\varepsilon^S\mathbf{L}$ and $B = \mathbf{N}_R\varepsilon^P$, we can treat this equation system just like the above example. The pulse-response function and the spectral response coefficients matrix for all metabolites read

$$\mathbf{K}^S(t-t') = \mathbf{L}\mathrm{e}^{\mathbf{N}_R\varepsilon^S\mathbf{L}(t-t')}\mathbf{N}_R\varepsilon^P\Theta(t-t'), \tag{21}$$

$$\mathbf{R}^S(\omega,\alpha) = -\mathbf{L}(\mathbf{N}_R\varepsilon^S\mathbf{L} - \mathrm{i}\omega\mathbf{I})^{-1}\mathbf{N}_R\varepsilon^P\delta_{\alpha(\omega)}. \tag{22}$$

Formula (22) will also remain valid for stable nonlinear systems.

4. Spectral response coefficients

We shall now generalize the concept of spectral response coefficients to general metabolic networks described by a nonlinear equation system

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{x}(t) = \mathbf{N}\mathbf{v}(\mathbf{x}(t),\mathbf{p}(t)) \tag{23}$$

for all $t \in \mathrm{I}\mathbb{R}$. We still assume that (i) for the parameter set $\mathbf{p}^0$, there is a stable steady state $\mathbf{s}^0 = \mathbf{s}(\mathbf{p}^0)$, that (ii) the steady state remains stable in a neighbourhood of $\mathbf{s}^0$ and $\mathbf{p}^0$, and that (iii) the Jacobian matrix $\mathbf{M}^0 = \mathbf{N}_R\varepsilon^s\mathbf{L}$ for the independent metabolites has full rank.

4.1. Standard solution

First, we need to establish a unique mapping between the time courses of parameters and variables that does not depend on the choice of initial conditions. For static parameters $\mathbf{p}(t) = \mathbf{p}^0$, there exist trajectories from different initial points that converge to the steady state concentrations $\mathbf{s}^0 = \mathbf{s}(\mathbf{p}^0)$, and there may also be other solutions outside the basin of attraction. Among all these solutions, we choose the constant time course $\mathbf{s}(t) = \mathbf{s}^0$ as the "standard" solution. Fluctuating parameters $\mathbf{p}(t) = \mathbf{p}^0 + \Delta\mathbf{p}$ will lead to perturbed time courses. In this case, a standard solution $\mathbf{s}(t) = \mathbf{s}^0 + \Delta\mathbf{s}(t)$ will be defined as follows: for each initial time t_0, we first set

$$\mathbf{p}^*(t,t_0) := \begin{cases} \mathbf{p}^0 & : t < t_0, \\ \mathbf{p}^0 + \Delta\mathbf{p}(t) & : t \geqslant t_0. \end{cases} \tag{24}$$

Let $\mathbf{s}(t,t_0)$ be the (unique) solution of Eq. (23) for the parameter time course $\mathbf{p}^*(t,t_0)$ and $s(t',t_0) = \mathbf{s}^0$ for $t' < t_0$. The standard solution for $\mathbf{p}(t)$ is defined by

$$\mathbf{s}(t) := \lim_{t_0\to-\infty} \mathbf{s}(t,t_0) \tag{25}$$

if this limit (with respect to the L^∞ norm) exists and fulfils the system equations (23). We restrict our analysis to bounded, sufficiently small $\Delta\mathbf{p}(\cdot)$ for which such a standard solution exists.

4.2. Fourier transforms

We next assume that the perturbation $\Delta\mathbf{p}(t)$ is so small that also $\mathbf{s}[\mathbf{p}(\cdot)](t)$ remains bounded. With Fourier components defined by

$$\hat{p}_{m\omega} := \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{-\mathrm{i}\omega t} p_m(t)\,\mathrm{d}t, \tag{26}$$

$$\hat{s}_{l\omega} := \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{-\mathrm{i}\omega t} s_l(t)\,\mathrm{d}t, \tag{27}$$

$$\hat{j}_{k\omega} := \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{-\mathrm{i}\omega t} j_k(t)\,\mathrm{d}t, \tag{28}$$

the time courses of parameters, metabolite concentrations, and fluxes can be described by Fourier synthesis

$$p_m(t) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{\mathrm{i}\omega t}\hat{p}_{m\omega}\,\mathrm{d}\omega, \tag{29}$$

$$s_l(t) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{\mathrm{i}\omega t}\hat{s}_{l\omega}\,\mathrm{d}\omega, \tag{30}$$

$$j_k(t) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{\mathrm{i}\omega t}\hat{j}_{k\omega}\,\mathrm{d}\omega. \tag{31}$$

For keeping the formula clear, we write the frequencies of Fourier transforms as Greek subscripts. Note the all time courses and their Fourier transforms are

represented by tempered distributions. For instance, we may consider a harmonic oscillation $f(t) = e^{i\alpha t}$, the Fourier transform of which is the delta distribution $f_\omega = \sqrt{2\pi}\delta_\alpha(\omega)$.

4.3. Definitions

The standard solution of equation system (23), written as a functional of $\mathbf{p}(\cdot)$, is called $\mathbf{s}[\mathbf{p}(\cdot)](t)$. The corresponding reaction velocities are denoted by $\mathbf{j}[\mathbf{p}(\cdot)](t) := \mathbf{v}(\mathbf{s}[\mathbf{p}(\cdot)](t), \mathbf{p}(t))$. In analogy to Eq. (8), the Fourier components of concentrations and reaction velocities can be written as functionals of the Fourier-transformed parameters

$$\hat{s}_{l\omega} : \hat{\mathbf{p}}(\cdot) \to \hat{s}_{l\omega}[\hat{\mathbf{p}}(\cdot)],$$

$$\hat{j}_{k\omega} : \hat{\mathbf{p}}(\cdot) \to \hat{j}_{k\omega}[\hat{\mathbf{p}}(\cdot)]. \tag{32}$$

In analogy to Eqs. (5) and (12), we define the spectral concentration response coefficients of first and second order by the functional derivatives

$$R^S_{lm}(\omega, \alpha) := \frac{\delta \hat{s}_{l\omega}[\hat{\mathbf{p}}(\cdot)]}{\delta \hat{p}_{m\alpha}}, \tag{33}$$

$$R^{S,2}_{lmn}(\omega, \alpha, \beta) := \frac{\delta^2 \hat{s}_{l\omega}[\hat{\mathbf{p}}(\cdot)]}{\delta \hat{p}_{m\alpha} \delta \hat{p}_{n\beta}} \tag{34}$$

if these derivatives exist. Spectral flux response coefficients are defined analogously:

$$R^J_{lm}(\omega, \alpha) := \frac{\delta \hat{j}_{l\omega}[\hat{\mathbf{p}}(\cdot)]}{\delta \hat{p}_{m\alpha}}, \tag{35}$$

$$R^{J,2}_{lmn}(\omega, \alpha, \beta) := \frac{\delta^2 \hat{j}_{l\omega}[\hat{\mathbf{p}}(\cdot)]}{\delta \hat{p}_{m\alpha} \delta \hat{p}_{n\beta}}. \tag{36}$$

In analogy to the static case, matrices of spectral flux and concentration control coefficients are defined as

$$C^S_{lm}(\omega, \alpha) := \frac{\delta \hat{s}_{l\omega}[\hat{\mathbf{p}}(\cdot)]}{\delta \hat{p}_{m\alpha}} \left(\frac{\partial v_m}{\partial p_m}\right)^{-1}, \tag{37}$$

$$C^J_{km}(\omega, \alpha) := \frac{\delta \hat{j}_{k\omega}[\hat{\mathbf{p}}(\cdot)]}{\delta \hat{p}_{m\alpha}} \left(\frac{\partial v_m}{\partial p_m}\right)^{-1}, \tag{38}$$

where each parameter p_m acts specifically on a single reaction velocity v_m.

4.4. Computing the response and control coefficients

The spectral response and control coefficients can be computed from the stoichiometric matrix and the elasticity matrices. With the definitions

$$\mathbf{C}^S(\omega) := -\mathbf{L}(\mathbf{N}_R \varepsilon^s \mathbf{L} - i\omega \mathbf{I})^{-1} \mathbf{N}_R, \tag{39}$$

$$\mathbf{C}^J(\omega) := \varepsilon^s \mathbf{C}^S(\omega) + \mathbf{I}, \tag{40}$$

the matrices of control coefficients read

$$\mathbf{C}^S(\omega, \alpha) = \mathbf{C}^S(\omega)\delta_\alpha(\omega), \tag{41}$$

$$\mathbf{C}^J(\omega, \alpha) = \mathbf{C}^J(\omega)\delta_\alpha(\omega). \tag{42}$$

With the further definitions

$$\mathbf{R}^S(\omega) := \mathbf{C}^S(\omega)\varepsilon^P, \tag{43}$$

$$\mathbf{R}^J(\omega) := \mathbf{C}^J(\omega)\varepsilon^P, \tag{44}$$

$$\Gamma_{kmn}(\alpha, \beta) := \varepsilon^{SS}_{kqr} R^S_{qm}(\alpha) R^S_{rn}(\beta) + \varepsilon^{SP}_{krm} R^S_{rn}(\beta) + \varepsilon^{SP}_{kqn} R^S_{qm}(\alpha) + \varepsilon^{PP}_{kmn}, \tag{45}$$

the spectral response coefficients can be expressed as

$$\mathbf{R}^S(\omega, \alpha) = \mathbf{R}^S(\omega)\delta_\alpha(\omega), \tag{46}$$

$$\mathbf{R}^J(\omega, \alpha) = \mathbf{R}^J(\omega)\delta_\alpha(\omega), \tag{47}$$

$$R^{S,2}_{lmn}(\omega, \alpha, \beta) = \frac{1}{\sqrt{2\pi}} C^S_{lk}(\omega) \Gamma_{kmn}(\alpha, \beta) \delta_{\alpha+\beta}(\omega), \tag{48}$$

$$R^{J,2}_{lmn}(\omega, \alpha, \beta) = \frac{1}{\sqrt{2\pi}} C^J_{lk}(\omega) \Gamma_{kmn}(\alpha, \beta) \delta_{\alpha+\beta}(\omega). \tag{49}$$

The detailed derivation of these formulae is given in the appendix. The term $\delta_\alpha(\omega)$ in Eqs. (46) and (47) implies that, to first order, a harmonic perturbation of frequency α yields a pure harmonic response of the same frequency. To second order (Eqs. (48) and (49)), modes of different frequencies are coupled: two perturbations of frequencies α and β lead to a second-order response of frequency $\omega = \alpha + \beta$. For static perturbations with $\alpha = \beta = 0$, the above formulae turn into the well-known results for static response coefficients. The factor $1/\sqrt{2\pi}$ reflects the arbitrary prefactor chosen for the Fourier transformations in Eqs. (26)–(28), and it disappears if the prefactor $1/2\pi$ is chosen for the Fourier transform (26)–(28). Moreover, we will see below that it cancels out in the Fourier synthesis used to reobtain time courses.

4.5. Response to temporal parameter fluctuations

With the spectral response coefficients, we can approximately solve the system (23), given a parameter perturbation $\Delta\mathbf{p}(t)$. In analogy to Eq. (4), we approximate the Fourier spectrum of concentration fluctuations around a static standard time course $\mathbf{s}^0(t) = \mathbf{s}^0$ by

$$\Delta \hat{s}_{l\omega} \approx \int_{-\infty}^{\infty} R^S_{lm}(\omega, \alpha) \Delta \hat{p}_{m\alpha} \, d\alpha + \frac{1}{2} \iint_{-\infty}^{\infty} R^{S,2}_{lmn}(\omega, \alpha, \beta) \Delta \hat{p}_{m\alpha} \Delta \hat{p}_{n\beta} \, d\alpha \, d\beta. \tag{50}$$

With Eqs. (46) and (47), this formula simplifies to

$$\Delta\hat{s}_{l\omega} \approx R^S_{lm}(\omega)\Delta\hat{p}_{m\omega} + \frac{1}{2}\int_{-\infty}^{\infty} R^{S,2}_{lmn}(\omega,\alpha)\Delta\hat{p}_{m\alpha}\Delta\hat{p}_{n(\omega-\alpha)}\,\mathrm{d}\alpha \quad (51)$$

with the shortcut $R^{S,2}_{lmn}(\omega,\alpha) := 1/\sqrt{2\pi}C^S_{lk}(\omega)\Gamma_{kmn}(\alpha,\omega-\alpha)$. Flux spectra can be computed accordingly by using the flux response coefficients. Approximate time courses $\Delta s_l[\mathbf{p}(\cdot)](t)$ and $\Delta j_k[\mathbf{p}(\cdot)](t)$ can be reconstructed from the Fourier spectrum by Fourier synthesis, Eq. (30).

How will a metabolic system respond to harmonic oscillations of a single parameter? Let us consider the following parameter perturbation

$$\Delta p_m(t) = \Delta p_m \mathrm{e}^{\mathrm{i}\alpha t}$$

and its Fourier transform

$$\Delta\hat{p}_{m\omega} = \sqrt{2\pi}\Delta p_m \delta_\alpha(\omega).$$

By using Eqs. (30) and (51), the response is approximated by

$$\Delta s_l(t) \approx R^S_{lm}(\alpha)\mathrm{e}^{\mathrm{i}\alpha t}\Delta p_m + \tfrac{1}{2}C^S_{lj}(2\alpha)\Gamma_{jmm}(\alpha,\alpha)\mathrm{e}^{\mathrm{i}2\alpha t}(\Delta p_m)^2. \quad (52)$$

The second-order terms oscillate with the frequency 2α. Instead of the complex exponential, a sine function $\Delta p_m \sin(\mathrm{i}\alpha t)$ could be used to describe the perturbation. Note that this function contains Fourier components at α and $-\alpha$, giving rise to second-order responses at frequencies $\omega = 2\alpha$ and $\omega = 0$. The resulting time course, though, is just the real part of (52). Eq. (52) also shows that the prefactor $1/\sqrt{2\pi}$ in the second-order term is cancelled during Fourier synthesis. Thus for static perturbations with frequencies $\alpha = \beta = 0$, the spectral response and control coefficients yield the same results as traditional MCA.

5. Spectral control coefficients

The metabolic control coefficients (Heinrich and Schuster, 1996) quantify the influence of certain chemical reactions, irrespective of the particular parameter perturbed. Based on the spectral response coefficients, it is straightforward to define spectral control coefficients, as has been pointed out by Ingalls (2004) and Liebermeister (2004). Here we introduce second-order spectral control coefficients along with their summation theorems.

5.1. Second-order control coefficients

Second-order control coefficients for static perturbations have been introduced in Höfer and Heinrich (1993). Along the same lines, we may want to split the second-order response coefficients into a product

$$R^{Y,2}_{lmn}(\omega,\alpha,\beta) = C^{Y,2}_{lij}(\omega,\alpha,\beta)\varepsilon^P_{im}\varepsilon^P_{jn}, \quad (53)$$

where Y stands for either concentrations or fluxes, and $C^{Y,2}_{lij}$ is the tensor of second-order control coefficients. This separation is only feasible with reaction-specific parameters p_i that appear as prefactors in the reaction kinetics, hence $v_i(\mathbf{x},\mathbf{p}) = p_i w_i(\mathbf{x})$. In this case, the elasticity matrix ε^P is diagonal and the second-order control coefficients are defined by

$$C^{Y,2}_{lij}(\omega,\alpha) := \frac{\delta^2 \hat{y}_{l\omega}[\hat{\mathbf{p}}(\cdot)]}{\delta\hat{p}_{i\alpha}\delta\hat{p}_{i\beta}}\left(\frac{\partial v_i}{\partial p_i}\right)^{-1}\left(\frac{\partial v_j}{\partial p_j}\right)^{-1} = R^{Y,2}_{lij}(\omega,\alpha,\beta)(\varepsilon^P_{ii})^{-1}(\varepsilon^P_{jj})^{-1}. \quad (54)$$

They read

$$C^{Y,2}_{lij}(\omega,\alpha,\beta) = \frac{1}{\sqrt{2\pi}}\delta_{\alpha+\beta}(\omega)C^Y_{lk}(\omega) \quad (55)$$

$$[\varepsilon^{SS}_{kqr}C^S_{qi}(\alpha)C^S_{rj}(\beta) + \delta_{ki}v_i^{-1}\varepsilon^S_{ir}C^S_{rj}(\beta) + \delta_{kj}v_j^{-1}\varepsilon^S_{jq}C^S_{qi}(\alpha)]. \quad (56)$$

This can be shown as follows: the decomposition $v_i(\mathbf{x},\mathbf{p}) = p_i w_i(\mathbf{x})$ implies that ε^{PP}_{lmn} vanishes and ε^{SP}_{lmn} can be factorized into $\varepsilon^{SP}_{lmn} = \varepsilon^S_{lm}\,\varepsilon^P_{ln}v_l^{-1}$, so $\Gamma_{kmn}(\alpha,\beta)$ reads

$$\Gamma_{kmn}(\alpha,\beta) = [\varepsilon^{SS}_{kqr}C^S_{qi}(\alpha)C^S_{rj}(\beta) + \varepsilon^S_{kr}\delta_{ki}v_k^{-1}C^S_{rj}(\beta) + \varepsilon^S_{kq}\delta_{kj}v_k^{-1}C^S_{qi}(\alpha)]\varepsilon^P_{im}\varepsilon^P_{jn}. \quad (57)$$

Together with Eqs. (48) and (49) follows Eq. (53).

5.2. Summation and connectivity theorems

Just like the static control coefficients, the spectral control coefficients fulfil summation and connectivity theorems.[3] We recall here the theorems for first-order response coefficients as given by Ingalls (2004), which generalize the well-known theorems for static control coefficients. The Eqs. (39) and (40) imply the summation theorems

$$\mathbf{C}^J(\omega)\mathbf{K} = \mathbf{K}, \quad (58)$$

$$\mathbf{C}^S(\omega)\mathbf{K} = 0, \quad (59)$$

where $\mathbf{K}$ denotes a matrix of stationary fluxes fulfilling $\mathbf{NK} = 0$ and thus $\mathbf{N}_R\mathbf{K} = 0$. Moreover, Eqs. (39) and (40) yield the connectivity theorems

$$\mathbf{C}^J(\omega)\varepsilon^S\mathbf{L} = -\mathrm{i}\omega\varepsilon^S\mathbf{L}(\mathbf{N}_R\varepsilon^S\mathbf{L} - \mathrm{i}\omega\mathbf{I})^{-1}, \quad (60)$$

$$\mathbf{C}^S(\omega)\varepsilon^S\mathbf{L} = -\mathbf{L}(\mathbf{I} - \mathrm{i}\omega(\mathbf{N}_R\varepsilon^S\mathbf{L} - \mathrm{i}\omega\mathbf{I})^{-1}). \quad (61)$$

[3] The theorems of MCA state linear relations among control coefficients. Each theorem concerns the control exerted by certain sets of reactions. A summation theorem concerns reactions in a stationary flux mode. As the stationary modes do not depend on the reaction kinetics, the summation theorems refer only to the stoichiometric structure of the network. A connectivity theorem concerns a set of all reactions with kinetics depending on a certain metabolite concentration.

At $\omega = 0$, the well-known theorems for static control coefficients (Heinrich and Schuster, 1996) are reobtained.

Besides the connectivity theorems, there hold also the relations

$$\mathbf{C}^J(\omega)(\varepsilon^s\mathbf{L} - \mathrm{i}\omega\mathbf{N}_R^+) = -\mathrm{i}\omega\mathbf{N}_R^+, \tag{62}$$

$$\mathbf{C}^S(\omega)(\varepsilon^s\mathbf{L} - \mathrm{i}\omega\mathbf{N}_R^+) = -\mathbf{L}, \tag{63}$$

where $\mathbf{N}_R^+ := \mathbf{N}_R^T(\mathbf{N}_R\mathbf{N}_R^T)^{-1}$ is the pseudoinverse of $\mathbf{N}_R$. In general, $\mathbf{N}_R^+$ is not sparse, so these formula do not describe local relations. At $\omega = 0$, again the static connectivity theorems are reobtained.

The summation theorems for second-order control coefficients follow immediately from Eqs. (55) and (59). They read

$$C_{lij}^{Y,2}(\omega, \alpha, \beta)k_i^{(1)}k_j^{(2)} = 0, \tag{64}$$

where $\mathbf{k}^{(1)}$ and $\mathbf{k}^{(2)}$ are arbitrary kernel vectors fulfilling $\mathbf{N}\mathbf{k}^{(1)} = \mathbf{N}\mathbf{k}^{(2)} = 0$. The theorem (64) is a generalized form of the static summation theorems from Höfer and Heinrich (1993).

6. Resonance

In certain systems, oscillatory perturbations can lead to resonance, that is, to strong responses around a certain frequency. The reason for this can be seen from the first-order response coefficients, namely from the eigenvalues of the matrix $\mathbf{Q}(\omega) := (\mathbf{N}_R\varepsilon^s\mathbf{L} - \mathrm{i}\omega\mathbf{I})^{-1}$ in Eq. (39). The Jacobian matrix $\mathbf{M}^0 = \mathbf{N}_R\varepsilon^S\mathbf{L}$ determines how the system behaves after small deviations from the steady state: complex eigenvalues with negative real part correspond to exponentially damped oscillations. As long as all eigenvalues have negative real parts, the steady state remains stable. If upon a parameter change, a complex eigenvalue crosses the imaginary axis, then the respective oscillatory mode becomes unstable and a limit cycle may appear. This phenomenon is called a supercritical Hopf bifurcation.

What happens if such a mode is driven by harmonic perturbations? The Jacobian and $\mathbf{Q}(\omega)$ have the same eigenvectors: for each eigenvalue $\lambda = \kappa + \mathrm{i}\omega_0$ of the Jacobian, $\mathbf{Q}(\omega)$ has a corresponding eigenvalue $\lambda^*(\omega) = 1/(\kappa + \mathrm{i}(\omega_0 - \omega))$. Just below a Hopf bifurcation, where $\kappa < 0$ and $|\kappa|$ is small, $|\lambda^*(\omega)|$ can become quite large around $\omega = \omega_0$ and thus show a resonance around $\omega = \omega_0$. This resonance will also be visible in the response coefficients, which are linear combinations of all eigenvalues of $\mathbf{Q}(\omega)$. We can conclude that near a Hopf bifurcation, even if the system is still stable, a damped oscillatory mode can become sensitive to oscillatory perturbations of frequency ω_0.

Example 6.1. For illustration, let us study the smallest biochemical system with Hopf bifurcation (Wilhelm and Heinrich, 1995) shown in Fig. 1, top left. The system equations and the stoichiometric matrix read

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} x_1 \\ x_2 \\ x_3 \end{pmatrix} = \begin{pmatrix} (k_1 q - k_4)x_1 - k_2 x_1 x_2 \\ -k_3 x_2 + k_5 x_3 \\ k_4 x_1 - k_5 x_3 \end{pmatrix}, \tag{65}$$

$$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & -1 & 0 \\ 0 & 0 & -1 & 0 & 1 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix}. \tag{66}$$

Setting all rate constants $k_l = 1$ (with dimensionless time), the external metabolite concentration q is a bifurcation parameter with the critical value $q = 3$. For $q = 2$, the system exhibits a stable steady state at $\mathbf{x} = (111)^T$. The eigenvalues of the Jacobian

$$\mathbf{M}^0 = \begin{pmatrix} 0 & -1 & 0 \\ 0 & -1 & 1 \\ 1 & 0 & -1 \end{pmatrix}$$

are shown in the complex plane in Fig. 1, top right. The upper circle corresponds to a damped oscillatory mode with frequency (imaginary part) ω_0. Upon a parameter shift, it may become unstable at a similar frequency (triangle). The lower left diagram in Fig. 1 shows $|\lambda^*(\omega)| = |(\kappa + \mathrm{i}(\omega_0 - \omega))^{-1}|$ as a function of the excitation frequency ω with its resonance peak around $\omega = \omega_0$.

7. Stochastic parameter fluctuations

Until here, we considered fixed time courses of the parameter fluctuations, but the analysis can also be extended to stochastic fluctuations or, that is, realizations of a stochastic process. In this section, we shall consider a linearized biochemical system with parameter perturbations given by independent Gaussian noise. Without loss of generality, we assume that the noise has unit variance when expressed in the chosen physical units. The concentration fluctuations $\Delta\mathbf{x}(t)$ follow a stochastic process obeying the Langevin equation

$$\mathrm{d}\Delta\mathbf{x}(t) = \mathbf{N}\varepsilon^S\Delta\mathbf{x}(t)\,\mathrm{d}t + \mathbf{N}\varepsilon^P\,\mathrm{d}\mathbf{w}(t), \tag{67}$$

where the $w_k(t)$ are independent Wiener processes, each related to one of the parameters. This stochastic differential equation can also be written in the symbolic form

$$\frac{\mathrm{d}}{\mathrm{d}t}\Delta\mathbf{x}(t) = \mathbf{N}\varepsilon^S\Delta\mathbf{x}(t) + \mathbf{N}\varepsilon^P\Delta\mathbf{p}(t), \tag{68}$$

where the parameter perturbations $\Delta p_k(t)$ are uncorrelated Gaussian white noises of unit variance. This equation is a stochastic analog of Eq. (20). The solution

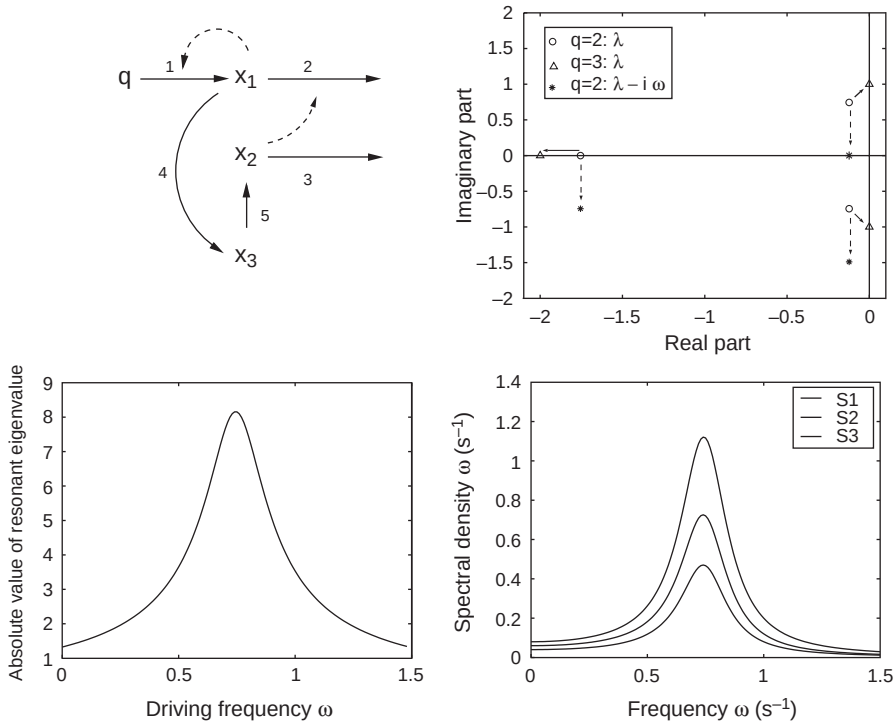

Fig. 1. Resonant response in a small biochemical system (Wilhelm and Heinrich, 1995). Top left: Network topology. Solid arrows indicate chemical reactions while dashed arrows denote positive regulatory interactions. Top right: Spectrum of the Jacobian matrix $\mathbf{M}^0$. The eigenvalues of $\mathbf{M}^0$ (circles) are shown in the complex plane, for the value $q = 2$ of the bifurcation parameter. At $q = 3$, the pair of complex eigenvalues crosses the imaginary axis (triangles, solid arrows) and the steady state becomes unstable, giving rise to stable oscillations. The upper complex eigenvalue (for $q = 2$) is $\lambda = \kappa + \mathrm{i}\omega_0$ with $\kappa \approx -0.12, \omega_0 \approx 0.74$. Stars indicate the eigenvalues for $q = 2$, shifted by $-\mathrm{i}\omega$ (dashed arrows). At $\omega \approx \omega_0$, $|\lambda - \mathrm{i}\omega|$ becomes small. Bottom left: the matrix $\mathbf{Q}(\omega) = (\mathbf{M}^0 - \mathrm{i}\omega)^{-1}$ has the same eigenvectors as $\mathbf{M}^0$. The graph shows the absolute value of the eigenvalue $\lambda^*(\omega) = (\lambda - \mathrm{i}\omega)^{-1}$ as a function of the excitation frequency ω. For $\omega \approx \omega_0$, $|\lambda^*(\omega)|$ becomes resonant as $|\lambda - \mathrm{i}\omega|$ becomes small. Bottom right: The resonance is also visible in the spectral densities of concentration fluctuations due to small molecule numbers (see Section 7).

$\Delta\mathbf{s}(t)$ and the corresponding reaction velocities $\Delta\mathbf{j}(t) = \varepsilon^S \Delta\mathbf{s}(t) + \varepsilon^P \Delta\mathbf{p}(t)$ are merged into a vector

$$\Delta\mathbf{y} = \begin{pmatrix} \Delta\mathbf{s} \\ \Delta\mathbf{j} \end{pmatrix}$$

and accordingly, we set

$$R^Y(\omega) = \begin{pmatrix} R^S(\omega) \\ R^J(\omega) \end{pmatrix}.$$

Correlated fluctuations can be described by the covariance functions $\mathrm{cov}(\Delta Y_i(t), \Delta Y_j(0))$ or by their Fourier transforms, the spectral densities $\mathscr{S}^Y_{ij}(\omega)$. The diagonal element $\mathscr{S}^Y_{ii}(\omega)$ describes the square amplitude of fluctuations in y_i at frequency ω. The matrix $\mathscr{S}^Y(\omega)$ can be computed from the spectral response coefficients (see Knobloch and Kwakernaak, 1985):

$$\mathscr{S}^Y(\omega) = R^Y(\omega)\mathscr{S}^P(R^Y(\omega))^\dagger = R^Y(\omega)(R^Y(\omega))^\dagger. \qquad (69)$$

The symbol † denotes the matrix adjoint, that is, the complex conjugate of the transposed matrix. The second equality follows from the fact that the spectral density $\mathscr{S}^P$ of the white noises $p_k(t)$ is just the identity matrix.

As a fundamental example, we shall now study the intrinsic stochastic fluctuations of chemical reactions. On a microscopic level, the state of a well-mixed chemical system can be described by discrete molecule numbers, which are increased or decreased by discrete reaction events. Under certain conditions (Gillespie, 2000), the molecule numbers can be approximated by continuous random variables $\bar{x}_i$ following the chemical Langevin equation

$$\frac{\mathrm{d}}{\mathrm{d}t}\bar{x}_i(t) = N_{ik} a_k(\bar{\mathbf{x}}(t)) + N_{ik}\sqrt{a_k(\bar{\mathbf{x}}(t))}\eta_k(t). \qquad (70)$$

The propensity function a_k describes the probability per time that the kth reaction will occur in the next infinitesimal time interval. Apart from describing molecule numbers instead of concentrations, Eq. (70) resembles the deterministic Eq. (23) for biochemical networks, with an additional stochastic term accounting for the fluctuations. If the molecule numbers and thus the propensities a_k are small, then fluctuations may become important. For large molecule numbers, one may neglect the noise term, express the molecule numbers $\bar{x}_i$ by concentrations, and reobtain the deterministic model (23) for the average concentrations.

We consider a volume Ω_n such that a concentration of 1 mol/l is equivalent to n molecules:[4] if $N_A \approx 6.022 \times 10^{23}\,\mathrm{mol}^{-1}$ denotes Avogadro's constant, then $N_A\Omega_n$ is n l/mol. To treat the intrinsic fluctuations as parameter perturbations, we introduce metabolite concentrations $x_i := (N_A\Omega)^{-1}\bar{x}_i$ and reaction velocities $v_k(\mathbf{x}) := (N_A\Omega)^{-1}a_k(N_A\Omega\mathbf{x})$. We assume that without the stochastic term in Eq. (70), the mean concentrations would be in steady state. Then we include the fluctuations η_k as virtual parameters p_k into the kinetics and rewrite Eq. (70) as

$$\frac{\mathrm{d}}{\mathrm{d}t}x_i(t) = N_{ik}v_k^*(\mathbf{x}(t), \mathbf{p}(t))$$

$$\text{with } v_k^*(\mathbf{x}, \mathbf{p}) := v_k(\mathbf{x}) + \sqrt{(N_A\Omega)^{-1}v_k(\mathbf{x})}p_k. \tag{71}$$

By linearizing the kinetics v_k around $\mathbf{x}^0$ and setting $\Delta\mathbf{x}(t) := \mathbf{x}(t) - \mathbf{x}^0$, we obtain

$$\frac{\mathrm{d}}{\mathrm{d}t}\Delta\mathbf{x}(t) = \mathbf{N}\varepsilon^S\Delta\mathbf{x}(t) + \mathbf{N}\varepsilon^P\mathbf{p}(t)$$

$$\text{with } \varepsilon^P := (N_A\Omega)^{-1/2}\mathrm{diag}(\mathbf{v}(\mathbf{x}^0))^{1/2}, \tag{72}$$

which is just the Langevin equation (68). The system size appears as a prefactor $n^{-1/2}$ in the elasticity matrix ε^P, and, as a consequence, in the spectral response coefficients.

Example 7.1. We consider again the reaction system described by Eq. (66) and compute the spectral densities of concentration fluctuations. We keep the parameter values $k_i = 1$, now assuming that time is measured in seconds and concentrations are measured in mol/l. With $n = 100$, for instance, the external concentration $q = 2\,\mathrm{mol/l}$ corresponds to a (fixed) number of 200 molecules, while the steady-state concentrations $x_1 = x_2 = x_3 = 1\,\mathrm{mol/l}$ correspond to 100 molecules of each species. The corresponding volume is a cube of 5.5 nm edge length. The diagonal elements of ε^P read $(N_A\Omega_n)^{-1/2}(\sqrt{2}, 1, 1, 1, 1)^T$, and the spectral density for concentration fluctuations is

$$\begin{aligned}\mathscr{S}^S &= (N_A\Omega_n)^{-1}C^S(\omega)\mathrm{diag}(\mathbf{v})(C^S(\omega))^\dagger \\ &= n^{-1}(\mathbf{M}^0 - \mathrm{i}\omega)^{-1}\mathbf{N}\mathrm{diag}(\mathbf{v})\mathbf{N}^T \\ &\quad \times(\mathbf{M}^0 + \mathrm{i}\omega)^{-1^T}\,\mathrm{l/mol}.\end{aligned} \tag{73}$$

The physical unit of the spectral density itself is s^{-1} because $\mathbf{v}$ is measured in mol/(l s). Fig. 1, bottom right, shows that the resonance in the response coefficients leads to a strong resonance peak in the spectral densities.

[4] To do so, we first consider a volume $\Omega_1 = 1/(N_A\,\mathrm{mol})$ liters $\approx 1.66 \times 10^{-24}$ l. If the concentration of a metabolite is 1 mol/l, then the volume Ω_1 will contain one molecule of it on average. We then set $\Omega_n = n\Omega_1$.

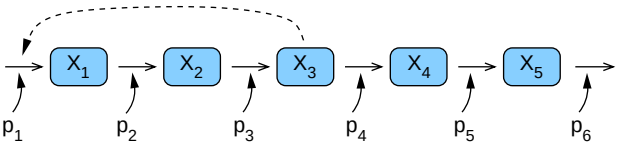

Fig. 2. Linear reaction chain with metabolite concentrations $x_1, \ldots, x_5$. The reaction velocities are controlled by parameters p_i (solid arrows). In a second version of this example, the first reaction is inhibited by metabolite 3 (dashed arrow).

8. Examples

8.1. Linear chain

We shall now study how forced oscillations propagate through a chain of chemical reactions (see Fig. 2). We consider small perturbations $p_l(t)$ of the kinetic parameters around constant standard values, leading to small deviations $x_l(t)$ and $v_l(t)$ of concentrations and fluxes, respectively. The differential equations for these deviations read

$$\frac{\mathrm{d}}{\mathrm{d}t}x_l = v_l - v_{l+1}. \tag{74}$$

The linearized reaction kinetics with forward elasticities ε^S_{+l} and backward elasticities $-\varepsilon^S_{-l}$ read

$$v_1 = -\varepsilon^S_{-1}x_1 + \varepsilon^P_1 p_1,$$

$$v_l = \varepsilon^S_l x_{l-1} - \varepsilon^S_{-l}x_l + \varepsilon^P_l p_l \quad \text{for } 2 \leqslant l \leqslant n-1,$$

$$v_n = \varepsilon^S_n x_{n-1} + \varepsilon^P_n p_n. \tag{75}$$

Fig. 3 shows the first-order spectral response coefficients for a chain length $n = 5$ and irreversible kinetics with $\varepsilon^S_i = 1, \varepsilon^S_{-i} = 0$. For simplicity, the parameter elasticities ε^P_l were also chosen to be 1, so the response coefficients equal the control coefficients. The response coefficients were computed according to Eqs. (46) and (47). The oscillatory perturbation of reaction 1 leads to a traveling wave of metabolite and flux oscillations. Due to the irreversible kinetics, the wave propagates only in forward direction, with an exponentially decreasing amplitude and a constant phase shift between subsequent reactions. The parameters inside the chain also influence the substrate of their reaction, with a large phase shift, similar to a negative influence. The response coefficients decrease with ω, so the chain acts as a low-pass filter. If the reactions are reversible, the eigenvalues split and the wave propagates in both forward and backward direction (not shown).

Now we introduce a feedback term between the metabolite concentration x_3 and the reaction velocity v_1 (Fig. 2, dashed arrow):

$$v_1 = \varepsilon^S_1 x_1 - \varepsilon^S_{-1}x_1 - \varepsilon^S_{\mathrm{fb}}x_3. \tag{76}$$

The value of $\varepsilon^S_{\mathrm{fb}}$ (here, for simplicity, 1) originates from a linearization of the reaction kinetics with respect to x_3.

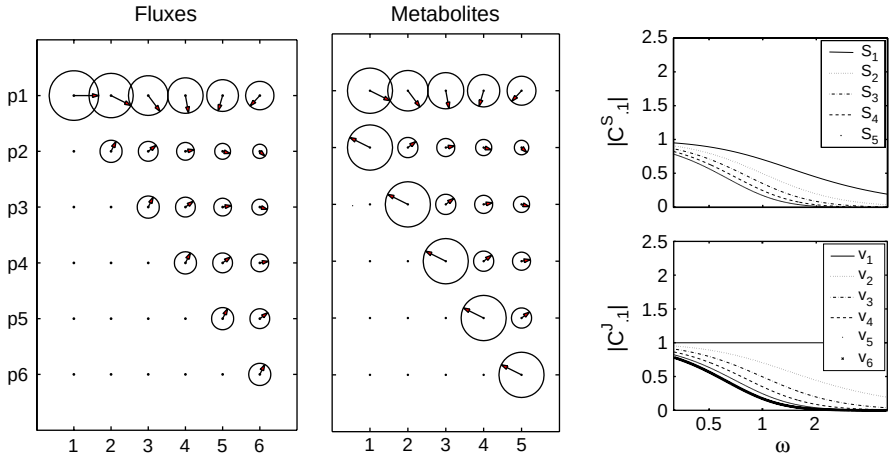

Fig. 3. Spectral response coefficients for the linear reaction chain (Fig. 2, without feedback). All reactions are irreversible (Eqs. (75), with $\varepsilon^S_l = 1, \varepsilon^S_{-l} = 0$). With $\varepsilon^P_l = 1$, response and control coefficients are identical. Left: Matrix $\mathbf{R}^J(\omega)$ of flux response coefficients at frequency $\omega = 0.5$. The response coefficients are complex numbers, with absolute values shown by circle radii (arbitrarily scaled) and phase angles indicated by arrows. Each row corresponds to the perturbation of a parameter p_i. The columns show the response of the different fluxes. The perturbations give rise to damped travelling waves. Centre: The same, for concentration response coefficients $\mathbf{R}^S(\omega)$. The columns correspond to the metabolites. Right: Dependence on the driving frequency for a perturbation of p_1. Right top: Concentration response coefficients to the first parameter x_1, as a function of ω. Right bottom: The same, for flux response coefficients.

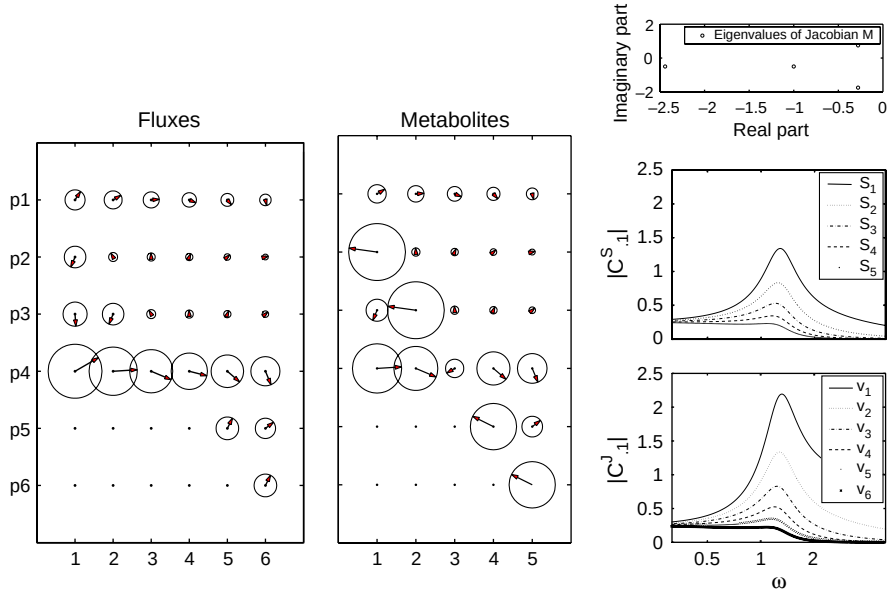

Fig. 4. Spectral response coefficients in an irreversible linear reaction chain (see Fig. 2) with feedback inhibition (Eq. (76), compare Fig. 3). Left: Due to the negative feedback loop, an acceleration of reaction 4 by p_4 yields a strong response in the entire chain. Right: A complex eigenvalue of the Jacobian close to the imaginary axis (top) leads to a resonance of flux response coefficients (centre) and concentration response coefficients (bottom).

The results for this system are shown in Fig. 4: via to the feedback loop, the parameters $p_2, \ldots, p_5$ control the whole chain. The pair of complex eigenvalues of the Jacobian shows that the system is just below a Hopf bifurcation, giving rise to resonance behaviour, so the chain acts as a band pass filter.

8.2. Glycolysis model

As a biological example, we studied forced oscillations in the glycolysis model of Hynne et al. (2001). The model describes the production and consumption of energy in a suspension of yeast cells. The variables represent metabolite concentrations inside the cells (17 metabolites) and in the growth medium (5 metabolites), while the 63 parameters comprise the kinetic constants of the reactions, as well as the Glucose concentration in the inflowing medium. Their values were determined in Hynne et al. (2001) at the onset of glycolytic oscillations. Model equations and parameter values were taken from the JWS online model database (Olivier and Snoep, 2004).

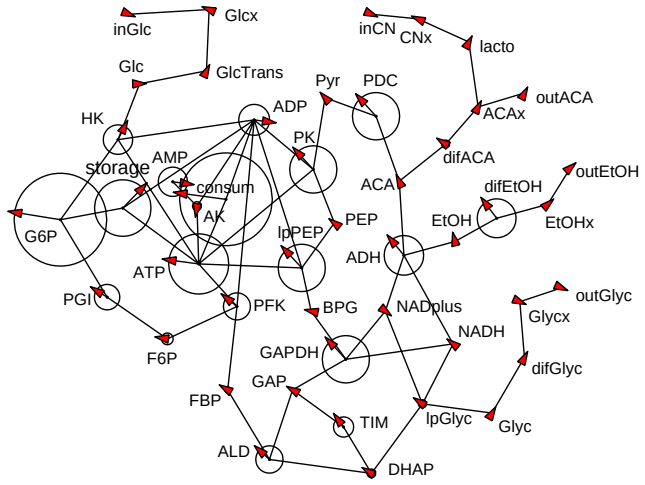

Fig. 5. Linear response coefficients in the glycolysis model of Hynne et al. (2001). The rate constant k_{22} of the energy storage reaction G6P + ATP → ADP (denoted by *storage*) is perturbed by a harmonic oscillation with a period of 10 min. The model topology is shown by straight lines between the reactions and metabolites. As in Figs. 3 and 4, circles with arrows represent complex response concentration response coefficients $\mathbf{R}^S$ and $\mathbf{R}^J$ (arbitrary scaling).

For a low Glucose concentration of 5.0 mM in the inflowing medium, we obtain a stable steady state. Now the rate constant k_{22} for the energy storage reaction $\mathrm{G6P} + \mathrm{ATP} \to \mathrm{ADP}$ is perturbed by oscillations of frequency $\alpha = 2\pi/(10\,\mathrm{min})$ and a maximal relative amplitude of 30% around the standard value $2.2593\,\mathrm{mM}^{-1}\,\mathrm{min}^{-1}$. Fig. 5 shows the resulting linear response coefficients of concentrations (left diagram, $\mathbf{R}^S(\omega)$ computed from Eq. (46)) and fluxes (right diagram, $\mathbf{R}^J(\omega)$ computed from Eq. (47)).

The detailed shape of the forced oscillations is shown in Fig. 6. For the exact numerical simulation, the system was initialized at its steady state (indicated by the straight line) and then run for about 30 periods. The solid curves show the time courses during one oscillation period after this tuning. The numerical results are compared to approximations from Eq. (52). For the parameters chosen, the first-order response coefficients (dotted lines) yield a close approximation: deviations from sine waves are partly captured by the second-order approximation (dashed lines). Despite the relatively large parameter variation, the second-order effects remain rather small.

9. Discussion

Some subsystems of cells show stable oscillations or other intrinsic dynamics. Others behave rather lazily, relaxing towards a stable stationary state. When MCA is applied to the latter, they are usually described in isolation while in reality, they are coupled to the rest of the cell and thus subject to permanent parameter perturbations. Another inevitable source of perturbations are the stochastic fluctuations of reaction velocities. How will such parameter perturbations, which can be oscillatory, transient, or stochastic, propagate along a biochemical network that is close to a stable steady state? To address this question, we generalized MCA from steady states to entire time courses, using functional analysis instead of usual vector analysis.

Instead of directly studying the time courses of parameters, concentrations, and fluxes, we represented them in a Fourier basis of harmonic oscillations. This leads to particularly simple results for linearized systems (see Ingalls, 2004 and Liebermeister, 2004), in which the propagation of parameter perturbations in time[5] and the response to oscillatory perturbations are two sides of a coin. The response to perturbations can be described by a pulse-response function, that is, the system's response to a δ-like (infinitely short) perturbation of a parameter. The response to general perturbations can be expressed by convolving this pulse-response function with the time course of the parameter. Working with Fourier components has the advantage that the convolution translates to a simple multiplication in frequency-space, where the spectral response coefficients are just a Fourier-transforms of the pulse-response function. A second, quite popular approach in the control theory of linear systems is the Laplace transformation: a time-invariant linear system can be described by its transfer function $H(\cdot)$, the Laplace-transformed of the system's pulse-response function. Due to the close relation between Laplace and Fourier transformation, transfer function and first-order spectral response coefficients are related by $H(\mathrm{i}\omega) = R^S(\omega, \omega)$.

For general nonlinear systems, we defined the spectral response coefficients by functional derivatives of the Fourier transforms: the resulting first-order response coefficients are identical to those defined in Ingalls (2004) and Liebermeister (2004) for linearized systems. Our method is closely related to traditional MCA: the spectral response coefficients are computed from the Fourier-transformed differential equation $\mathrm{i}\omega\,\mathbf{s}(\omega) = \mathbf{N}\mathbf{v}(\omega)$, in a similar manner as the static response coefficients can be derived from the stationarity condition $0 = \mathbf{N}\mathbf{v}$ (see Heinrich and Schuster, 1996). Due to the left-hand side of the differential equation, a term $\mathrm{i}\omega\mathbf{I}$ is added to the Jacobian matrix. As a consequence, the control and response coefficients become complex and frequency-dependent. With the modified control coefficients matrix $C^S(\omega)$, it is straightforward to generalize various results from MCA to non-zero frequencies. For

[5]The temporal response to perturbations of metabolite concentrations, instead of parameters, has been studied before: Heinrich and Reder (1991) studied the relaxation from a perturbed state towards the steady state. The respective pulse-response function, in the form of a correlation matrix, was expanded with respect to paths through the network in Rojdestvenski and Cottam (2000). Steuer et al. (2003) studied the overall covariance between metabolite concentrations due to local stochastic perturbations of the concentrations.

instance, modular systems can be studied, for each frequency separately, in a similar way as in standard modular response theory (Bruggeman et al., 2002). The spectral control coefficients fulfil summation and connectivity theorems, just like their static counterparts: the summation theorems remain unchanged, while the connectivity theorems contain an additional, frequency-dependent term.

Our method is well suited for large systems: in contrast to numerical simulation, no differential equations need to be solved, and the steady state has to be computed only once.[6] Given the steady state, the response coefficients for each frequency can be calculated by matrix operations, the most time-consuming part being the numerical inversion of the Jacobian. For computing the Fourier synthesis, however, this matrix inversion has to be repeated for each frequency considered. Like in static MCA, the expansion with response coefficients is only valid for small parameter changes. For larger perturbations, it is not guaranteed that (1) a unique standard solution can be defined, (2) this solution can be Fourier-transformed, (3) the response coefficients exist, and (4) the second-order approximation yields satisfying results. We may assume, however, that these assumptions hold for small perturbations $\Delta\mathbf{p}(\cdot)$, and our simulations for the glycolysis model confirmed this even for considerable perturbations. For linearized systems, theory ensures that the spectral response coefficients exist and yield the exact solution.

We can conclude that a stable biochemical system, subject to small parameter perturbations, acts as a frequency filter: a harmonic parameter oscillation leads to a forced oscillation of system variables with the same frequency, but with different phases and amplitudes. Biochemical systems may show specific frequency-dependent behaviour: near a supercritical Hopf bifurcation, resonance can occur and drive the system out of the region where the linear approximation was valid. Some systems, like the irreversible linear chain, will act as a low-pass filter extracting the slow harmonic part from incoming oscillations. On the other hand, nonlinearities may lead to mode-coupling, adding harmonics to an incoming sine wave, as it has been found experimentally in the photosynthesis system of plants and bacteria (Nedbal et al., 2003).

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[6]All numerical calculations in this work have been done with Matlab. The scripts can be obtained from the author.

Appendix A. Derivation of spectral response coefficients

A.1. Spectral elasticities

For given vector-valued $\mathbf{x}(t)$ and $\mathbf{p}(t)$, the Fourier components of a reaction kinetics v_k are defined by

$$\hat{v}_{k\omega}[\mathbf{x}(\cdot),\mathbf{p}(\cdot)] := \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{-\mathrm{i}\omega t} v_k(\mathbf{x}(t),\mathbf{p}(t))\,\mathrm{d}t. \tag{77}$$

We define the spectral elasticities by the functional derivatives

$$\varepsilon^S_{km}(\omega,\alpha) := \frac{\delta \hat{v}_{k\omega}[\mathbf{x}(\cdot),\mathbf{p}(\cdot)]}{\delta \hat{x}_{m\alpha}},$$
$$\varepsilon^P_{km}(\omega,\alpha) := \frac{\delta \hat{v}_{k\omega}[\mathbf{x}(\cdot),\mathbf{p}(\cdot)]}{\delta \hat{p}_{m\alpha}}, \tag{78}$$

$$\varepsilon^{SS}_{kmn}(\omega,\alpha,\beta) := \frac{\delta^2 \hat{v}_{k\omega}[\mathbf{x}(\cdot),\mathbf{p}(\cdot)]}{\delta \hat{x}_{m\alpha}\delta \hat{x}_{n\beta}},$$
$$\varepsilon^{SP}_{kmn}(\omega,\alpha,\beta) := \frac{\delta^2 \hat{v}_{k\omega}[\mathbf{x}(\cdot),\mathbf{p}(\cdot)]}{\delta \hat{x}_{m\alpha}\delta \hat{p}_{n\beta}},$$

$$\varepsilon^{PP}_{kmn}(\omega,\alpha,\beta) := \frac{\delta^2 \hat{v}_{k\omega}[\mathbf{x}(\cdot),\mathbf{p}(\cdot)]}{\delta \hat{p}_{m\alpha}\delta \hat{p}_{n\beta}}. \tag{79}$$

The spectral elasticity $\varepsilon^S_{km}(\omega,\alpha)$, for instance, describes how the Fourier component of the kth reaction velocity at frequency ω responds to a small oscillatory perturbation of concentration x_m with frequency α. The reaction is viewed in isolation, that is, the rest of the network is kept fixed at its unperturbed state. If the unperturbed time courses $\mathbf{x}^0(\cdot)$ and $\mathbf{p}^0(\cdot)$ are static, the spectral elasticities read

$$\varepsilon^S_{km}(\omega,\alpha) = \varepsilon^S_{km}\delta_\alpha(\omega), \tag{80}$$

$$\varepsilon^P_{km}(\omega,\alpha) = \varepsilon^P_{km}\delta_\alpha(\omega), \tag{81}$$

$$\varepsilon^{SS}_{kmn}(\omega,\alpha,\beta) = \frac{1}{\sqrt{2\pi}}\varepsilon^{SS}_{kmn}\delta_{\alpha+\beta}(\omega), \tag{82}$$

$$\varepsilon^{SP}_{kmn}(\omega,\alpha,\beta) = \frac{1}{\sqrt{2\pi}}\varepsilon^{SP}_{kmn}\delta_{\alpha+\beta}(\omega), \tag{83}$$

$$\varepsilon^{PP}_{kmn}(\omega,\alpha,\beta) = \frac{1}{\sqrt{2\pi}}\varepsilon^{PP}_{kmn}\delta_{\alpha+\beta}(\omega). \tag{84}$$

We will demonstrate this for $\varepsilon^S_{km}(\omega,\alpha)$ and $\varepsilon^{SS}_{kmn}(\omega,\alpha,\beta)$. The spectral elasticities $\varepsilon^S_{km}(\omega,\alpha)$ read

$$\begin{aligned}\frac{\delta \hat{v}_{k\omega}[\mathbf{x}(\cdot),\mathbf{p}(\cdot)]}{\delta \hat{x}_{m\alpha}} &= \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} \mathrm{e}^{-\mathrm{i}\omega t}\frac{\delta}{\delta \hat{x}_{m\alpha}} \\ &\quad v_k\left(\frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty}\mathrm{e}^{\mathrm{i}\gamma t}\hat{x}_{1\gamma}\,\mathrm{d}\gamma,..,\mathbf{p}(t)\right)\mathrm{d}t \\ &= \frac{1}{2\pi}\int_{-\infty}^{\infty}\mathrm{e}^{-\mathrm{i}\omega t}\frac{\partial v_k(\mathbf{x}(t),\mathbf{p}(t))}{\partial x_m}\mathrm{e}^{\mathrm{i}\alpha t}\,\mathrm{d}t.\end{aligned} \tag{85}$$

For static $\mathbf{x}^0(t) = \mathbf{x}^0$ and $\mathbf{p}^0(t) = \mathbf{p}^0$, this yields

$$\varepsilon^S_{km}(\omega,\alpha) = \frac{\partial v_k(\mathbf{x},\mathbf{p})}{\partial x_m}\frac{1}{2\pi}\int_{-\infty}^{\infty} e^{-i\omega t}e^{i\alpha t}\,dt = \varepsilon^S_{km}\delta_\alpha(\omega). \quad (86)$$

Likewise, the second derivatives $\varepsilon^{SS}_{kmn}(\omega,\alpha,\beta)$ for static $\mathbf{x}^0(t)$ and $\mathbf{p}^0(t)$ read

$$\begin{aligned}&\frac{\delta^2\hat{v}_{k\omega}[\mathbf{x}(\cdot),\mathbf{p}(\cdot)]}{\delta\hat{x}_{m\alpha}\delta\hat{x}_{n\beta}}\\ &= \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} e^{-i\omega t}\frac{\delta^2}{\delta\hat{x}_{m\alpha}\delta\hat{x}_{n\beta}}\\ &v_k\left(\frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} e^{i\gamma t}\hat{x}_{1\gamma}\,d\gamma,..,\mathbf{p}(t)\right)dt\\ &= \frac{1}{(2\pi)^{3/2}}\int_{-\infty}^{\infty} e^{-i\omega t}\frac{\partial^2 v_k(\mathbf{x}(t),\mathbf{p}(t))}{\partial x_m\delta x_n}e^{i\alpha t}e^{i\beta t}\,dt\\ &= \frac{\partial^2 v_k}{\partial x_m\partial x_n}\frac{1}{\sqrt{2\pi}}\delta_{\alpha+\beta}(\omega) = \frac{1}{\sqrt{2\pi}}\varepsilon^{SS}_{kmn}\delta_{\alpha+\beta}(\omega). \quad (87)\end{aligned}$$

The delta distributions in Eqs. (80) and (81) show that, to linear order, an oscillatory perturbation of a parameter leads to a velocity oscillation at the same frequency. The reason for this is that the derivatives in Eq. (78) are invariant under a shift of time and that the Fourier basis consists of eigenvectors of the time-shift operator, so Fourier components of different frequencies cannot be mixed. Likewise, to second order, two perturbations of frequencies α and β yield a velocity oscillation of frequency $\alpha+\beta$. The factor $1/\sqrt{2\pi}$ in Eqs. (82)–(84) is due to the convention chosen for the Fourier transformations in Eqs. (26)–(28) with $1/\sqrt{2\pi}$ as a prefactor.

A.2. Computing the first-order spectral response coefficients

To derive Eqs. (46) and (47) for the spectral response coefficients, we first assume that no conservation relations hold, so $\mathbf{N}$ has full rank. The differential Eq. (23) in the form

$$\frac{d}{dt}\mathbf{s}[\mathbf{p}(\cdot)](t) = \mathbf{N}\mathbf{j}[\mathbf{p}(\cdot)](t) \quad (88)$$

is Fourier-transformed:

$$0 = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{\infty} e^{i\omega t}(i\omega\hat{s}_{l\omega}[\hat{\mathbf{p}}(\cdot)] - N_{lk}\hat{j}_{k\omega}[\hat{\mathbf{p}}(\cdot)])\,d\omega \quad (89)$$

$$\Rightarrow 0 = i\omega\hat{s}_{l\omega}[\hat{\mathbf{p}}(\cdot)] - N_{lk}\hat{j}_{k\omega}[\hat{\mathbf{p}}(\cdot)] \quad \text{for all } \omega. \quad (90)$$

Eq. (90) is differentiated with respect to the Fourier component of p_m at frequency α

$$\begin{aligned}0 &= \frac{\delta}{\delta\hat{p}_{m\alpha}}(i\omega\hat{s}_{l\omega}[\hat{\mathbf{p}}(\cdot)] - N_{lk}\hat{j}_{k\omega}[\hat{\mathbf{p}}(\cdot)])\\ &= i\omega R^S_{lm}(\omega,\alpha) - N_{lk}R^J_{km}(\omega,\alpha). \quad (91)\end{aligned}$$

With

$$\hat{j}_{k\omega}[\hat{\mathbf{p}}(\cdot)] = \hat{v}_{k\omega}[\mathbf{s}[\mathbf{p}(\cdot)],\mathbf{p}(\cdot)] \quad (92)$$

and using the chain rule, $R^J_{km}(\omega,\alpha)$ can be expressed by

$$\begin{aligned}R^J_{km}(\omega,\alpha) &= \frac{\delta\hat{j}_{k\omega}[\hat{\mathbf{p}}(\cdot)]}{\delta\hat{p}_{m\alpha}}\\ &= \int_{-\infty}^{\infty}\frac{\delta\hat{v}_{k\omega}[\mathbf{s}[\mathbf{p}(\cdot)],\mathbf{p}(\cdot)]}{\delta\hat{x}_{n\gamma}}\frac{\delta\hat{s}_{n\gamma}[\hat{\mathbf{p}}(\cdot)]}{\delta\hat{p}_{m\alpha}}\,d\gamma\\ &\quad + \frac{\delta\hat{v}_{k\omega}[\mathbf{s}[\mathbf{p}(\cdot)],\mathbf{p}(\cdot)]}{\delta\hat{p}_{m\alpha}}\\ &= \int_{-\infty}^{\infty}\varepsilon^S_{kn}(\omega,\gamma)R^S_{nm}(\gamma,\alpha)\,d\gamma + \varepsilon^P_{km}(\omega,\alpha). \quad (93)\end{aligned}$$

Inserting this into Eq. (91) yields

$$\begin{aligned}0 &= i\omega R^S_{lm}(\omega,\alpha) - N_{lk}\int_{-\infty}^{\infty}\varepsilon^S_{kn}(\omega,\gamma)R^S_{nm}(\gamma,\alpha)\,d\gamma\\ &\quad - N_{lk}\varepsilon^P_{km}(\omega,\alpha). \quad (94)\end{aligned}$$

We assume that the unperturbed parameters $\mathbf{p}^0(\cdot)$ are static, thus yielding a static $\mathbf{s}^0(\cdot)$. Inserting Eqs. (80) and (81) into (94) yields

$$\begin{aligned}0 &= i\omega R^S_{lm}(\omega,\alpha) - N_{lk}\int_{-\infty}^{\infty}\varepsilon^S_{kn}\delta_\gamma(\omega)R^S_{nm}(\gamma,\alpha)\,d\gamma\\ &\quad - N_{lk}\varepsilon^P_{km}\delta_\alpha(\omega)\\ &= (i\omega\delta_{ln} - N_{lk}\varepsilon^S_{kn})R^S_{nm}(\omega,\alpha) - N_{lk}\varepsilon^P_{km}\delta_\alpha(\omega). \quad (95)\end{aligned}$$

Solving this for $R^S_{lm}(\omega,\alpha)$ yields, in matrix notation,

$$\mathbf{R}^S(\omega,\alpha) = -(\mathbf{N}\varepsilon^S - i\omega\mathbf{I})^{-1}\mathbf{N}\varepsilon^P\delta_\alpha(\omega). \quad (96)$$

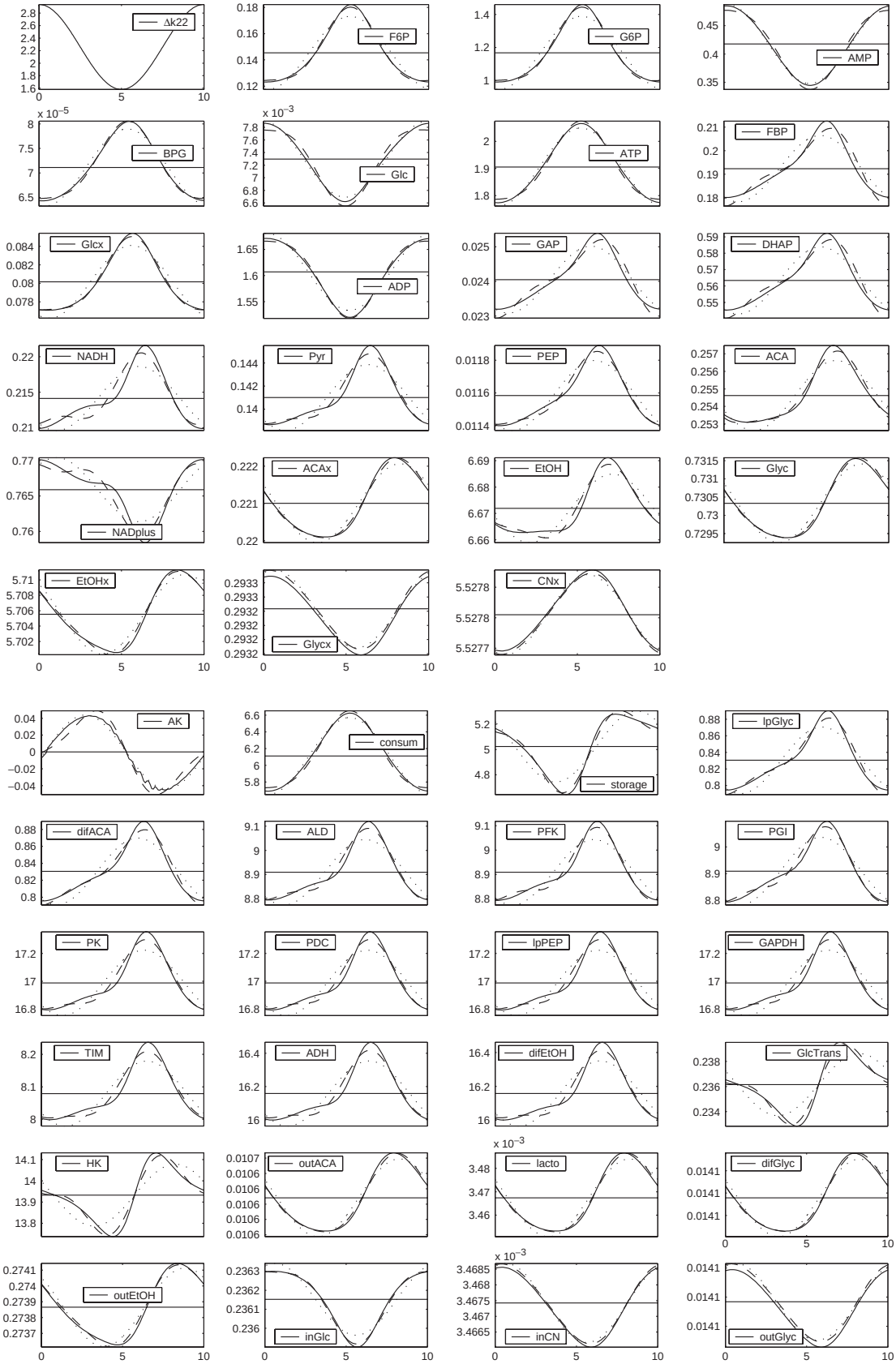
At this point, we have to account for possible conservation relations: if conservation relations hold among the metabolites, then $\mathbf{N}\varepsilon^S - i\omega\mathbf{I}$ will not be invertible. To fix this problem, we first restrict the analysis to the independent metabolites, replacing $\mathbf{N}$ by $\mathbf{N}_R$ and ε^s by $\varepsilon^s\mathbf{L}$. The matrix $\mathbf{N}_R\varepsilon^S\mathbf{L} - i\omega\mathbf{I}$ is guaranteed to be invertible because $\mathbf{N}_R\varepsilon^S\mathbf{L}$ is regular by assumption. Finally, the resulting response coefficients matrix for the independent metabolites must be premultiplied with $\mathbf{L}$ to yield a response coefficients matrix for all metabolites:

$$\mathbf{R}^S(\omega,\alpha) = -\mathbf{L}(\mathbf{N}_R\varepsilon^S\mathbf{L} - i\omega\mathbf{I})^{-1}\mathbf{N}_R\varepsilon^P\delta_\alpha(\omega). \quad (97)$$

From Eq. (93), together with Eqs. (80) and (81) follows

$$\mathbf{R}^J(\omega,\alpha) = \varepsilon^S\mathbf{R}^S(\omega,\alpha) + \varepsilon^P\delta_\alpha(\omega). \quad (98)$$

Why did we restrict our analysis to perturbation of steady states? For an expansion around a time-dependent $\mathbf{s}[\mathbf{p}^0](\cdot)$, the integral Eq. (94) may be solved by a Neumann integral series. This series, however, converges only for large $|\omega|$ and is numerically hard to handle, so we will not consider it further.


Δk22
F6P
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lpGlyc
difACA
ALD
PFK
PGI
PK
PDC
lpPEP
GAPDH
TIM
ADH
difEtOH
GlcTrans
HK
outACA
lacto
difGlyc
outEtOH
inGlc
inCN
outGlyc

A.3. Computing the second-order response coefficients

The second-order response coefficients $\mathbf{R}^{S,2}$ and $\mathbf{R}^{J,2}$ are computed in the same way: we first take the second derivative of Eq. (90):

$$0 = \frac{\delta^2}{\delta\hat{p}_{m\alpha}\delta\hat{p}_{n\beta}}(\mathrm{i}\omega\hat{s}_{l\omega}[\hat{\mathbf{p}}(\cdot)] - N_{lk}\hat{v}_{k\omega}[\hat{\mathbf{p}}(\cdot)])$$
$$= \mathrm{i}\omega R^{S,2}_{lmn}(\omega,\alpha,\beta) - N_{lk}R^{J,2}_{kmn}(\omega,\alpha,\beta). \quad (99)$$

To keep the formulae clear, we shall omit the functional arguments $[\hat{\mathbf{p}}(\cdot)]$ and $[\mathbf{s}[\mathbf{p}(\cdot)],\mathbf{p}(\cdot)]$ in the following. $R^{J,2}_{kmn}(\omega,\alpha,\beta)$ reads

$$R^{J,2}_{kmn}(\omega,\alpha,\beta) = \frac{\delta^2\hat{j}_{k\omega}}{\delta\hat{p}_{m\alpha}\delta\hat{p}_{n\beta}}$$
$$= \frac{\delta}{\delta\hat{p}_{m\alpha}}\left(\int_{-\infty}^{\infty}\frac{\delta\hat{v}_{k\omega}}{\delta\hat{x}_{r\gamma}}\frac{\delta\hat{s}_{r\gamma}}{\delta\hat{p}_{n\beta}}\,\mathrm{d}\gamma + \frac{\delta\hat{v}_{k\omega}}{\delta\hat{p}_{n\beta}}\right) \quad (100)$$
$$= \int_{-\infty}^{\infty}\left(\frac{\delta}{\delta\hat{p}_{m\alpha}}\frac{\delta\hat{v}_{k\omega}}{\delta\hat{x}_{r\gamma}}\right)\frac{\delta\hat{s}_{r\gamma}}{\delta\hat{p}_{n\beta}} + \frac{\delta\hat{v}_{k\omega}}{\delta\hat{x}_{r\gamma}}\left(\frac{\delta}{\delta\hat{p}_{m\alpha}}\frac{\delta\hat{s}_{r\gamma}}{\delta\hat{p}_{n\beta}}\right)\mathrm{d}\gamma + \frac{\delta}{\delta\hat{p}_{m\alpha}}\frac{\delta\hat{v}_{k\omega}}{\delta\hat{p}_{n\beta}} \quad (101)$$
$$= \int_{-\infty}^{\infty}\left(\int_{-\infty}^{\infty}\frac{\delta^2\hat{v}_{k\omega}}{\delta\hat{x}_{q\eta}\delta\hat{x}_{r\gamma}}\frac{\delta\hat{s}_{q\eta}}{\delta\hat{p}_{m\alpha}}\,\mathrm{d}\eta + \frac{\delta^2\hat{v}_{k\omega}}{\delta\hat{p}_{m\alpha}\delta\hat{x}_{r\gamma}}\right)\times\frac{\delta\hat{s}_{r\gamma}}{\delta\hat{p}_{n\beta}} + \frac{\delta\hat{v}_{k\omega}}{\delta\hat{x}_{r\gamma}}\frac{\delta^2\hat{s}_{r\gamma}}{\delta\hat{p}_{m\alpha}\delta\hat{p}_{n\beta}}\,\mathrm{d}\gamma + \int_{-\infty}^{\infty}\frac{\delta^2\hat{v}_{k\omega}}{\delta\hat{x}_{q\gamma}\delta\hat{p}_{n\beta}}\frac{\delta\hat{s}_{q\gamma}}{\delta\hat{p}_{m\alpha}}\,\mathrm{d}\gamma + \frac{\delta^2\hat{v}_{k\omega}}{\delta\hat{p}_{m\alpha}\delta\hat{p}_{n\beta}} \quad (102)$$
$$= \iint_{-\infty}^{\infty}\varepsilon^{SS}_{kqr}(\omega,\eta,\gamma)R^S_{qm}(\eta,\alpha)R^S_{rn}(\gamma,\beta)\,\mathrm{d}\eta\,\mathrm{d}\gamma + \int_{-\infty}^{\infty}(\varepsilon^{SP}_{krm}(\omega,\gamma,\alpha)R^S_{rn}(\gamma,\beta) + \varepsilon^{S}_{kr}(\omega,\gamma)R^{S,2}_{rmn}(\gamma,\alpha,\beta) + \varepsilon^{SP}_{kqn}(\omega,\gamma,\beta)R_{qm}(\gamma,\alpha))\,\mathrm{d}\gamma + \varepsilon^{PP}_{kmn}(\omega,\alpha,\beta). \quad (103)$$

If the unperturbed time courses $\mathbf{p}^0$ and $\mathbf{s}^0$ are static, inserting Eqs. (80)–(84) yields

$$R^{J,2}_{kmn}(\omega,\alpha,\beta) = \iint_{-\infty}^{\infty}\frac{1}{\sqrt{2\pi}}\varepsilon^{SS}_{kqr}\delta_{\omega-\gamma}(\eta)R^S_{qm}(\eta,\alpha)\times R^S_{rn}(\gamma,\beta)\,\mathrm{d}\eta\,\mathrm{d}\gamma + \int_{-\infty}^{\infty}\left(\varepsilon^{S}_{kr}\delta_{\gamma}(\omega)R^{S,2}_{rmn}(\gamma,\alpha,\beta) + \frac{1}{\sqrt{2\pi}}\varepsilon^{SP}_{krm}\delta_{\omega-\alpha}(\gamma)R^S_{rn}(\gamma,\beta) + \frac{1}{\sqrt{2\pi}}\varepsilon^{SP}_{kqn}\delta_{\omega-\beta}(\gamma)R_{qm}(\gamma,\alpha)\right)\mathrm{d}\gamma + \frac{1}{\sqrt{2\pi}}\varepsilon^{PP}_{kmn}\delta_{\alpha+\beta}(\omega)$$
$$= \frac{1}{\sqrt{2\pi}}\left[\int_{-\infty}^{\infty}\varepsilon^{SS}_{kqr}R^S_{qm}(\omega-\gamma,\alpha)R^S_{rn}(\gamma,\beta)\mathrm{d}\gamma + \sqrt{2\pi}\varepsilon^{S}_{kr}R^{S,2}_{rmn}(\omega,\alpha,\beta) + \varepsilon^{SP}_{krm}R^S_{rn}(\omega-\alpha,\beta) + \varepsilon^{SP}_{kqn}R_{qm}(\omega-\beta,\alpha) + \varepsilon^{PP}_{kmn}\delta_{\alpha+\beta}(\omega)\right].$$

Inserting Eqs. (46) for $\mathbf{R}^S(\omega,\alpha)$ yields

$$R^{J,2}_{kmn}(\omega,\alpha,\beta) = \frac{1}{\sqrt{2\pi}}[\varepsilon^{SS}_{kqr}R^S_{qm}(\alpha)R^S_{rn}(\beta)\delta_{\alpha+\beta}(\omega) + \sqrt{2\pi}\varepsilon^{S}_{kr}R^{S,2}_{rmn}(\omega,\alpha,\beta) + \varepsilon^{SP}_{krm}R^S_{rn}(\beta)\delta_{\alpha+\beta}(\omega) + \varepsilon^{SP}_{kqn}R_{qm}(\alpha)\delta_{\alpha+\beta}(\omega) + \varepsilon^{PP}_{kmn}\delta_{\alpha+\beta}(\omega)] \quad (104)$$
$$= \varepsilon^{S}_{kr}R^{S,2}_{rmn}(\omega,\alpha,\beta) + \frac{1}{\sqrt{2\pi}}\Gamma_{kmn}(\omega,\alpha,\beta)\delta_{\alpha+\beta}(\omega) \quad (105)$$

with the shortcut

$$\Gamma_{kmn}(\alpha,\beta) := \varepsilon^{SS}_{kqr}R^S_{qm}(\alpha)R^S_{rn}(\beta) + \varepsilon^{SP}_{krm}R^S_{rn}(\beta) + \varepsilon^{SP}_{kqn}R_{qm}(\alpha) + \varepsilon^{PP}_{kmn}. \quad (106)$$

Inserting this into Eq. (99) yields

$$0 = (\mathrm{i}\omega\delta_{lr} - N_{lk}\varepsilon^{S}_{kr})R^{S,2}_{rmn}(\omega,\alpha,\beta) - \frac{1}{\sqrt{2\pi}}N_{lk}\Gamma_{kmn}(\alpha,\beta)\delta_{\alpha+\beta}(\omega). \quad (107)$$

By solving this equation for $R^{S,2}$, using definition (39), and addressing possible conservation relations as above, we obtain

$$R^{S,2}_{jmn}(\omega,\alpha,\beta) = \frac{1}{\sqrt{2\pi}}C^S_{jk}(\omega)\Gamma_{kmn}(\alpha,\beta)\delta_{\alpha+\beta}(\omega). \quad (108)$$

Fig. 6. Forced oscillations in the glycolysis model Hynne et al. (2001). The rate constant k_{22} of the energy storage reaction G6P + ATP → ADP (denoted by *storage*) was perturbed by harmonic oscillations (compare Fig. 5). Top half: The top left diagram shows the perturbed parameter k_{22} as a function of time (in minutes). The remaining diagrams show the resulting forced oscillations of the metabolite concentrations around their steady state values (straight solid line). The metabolite concentrations are sorted by their relative response (i.e. oscillation amplitude divided by steady-state value). Solid curve: numerical solution (after a simulation of 30 oscillation periods). The other curves show first-order (dotted line) and second-order approximations (dashed line) calculated from the spectral response coefficients. The second-order term allows the curves to deviate from harmonic oscillations. Bottom half: Forced oscillations of the metabolic fluxes.

With Eq. (105) follows

$$R^{J,2}_{lmn}(\omega,\alpha,\beta) = \frac{1}{\sqrt{2\pi}}(\varepsilon^S_{lr}C^S_{jk}(\omega)+\delta_{lk})\Gamma_{kmn}(\alpha,\beta)\delta_{\alpha+\beta}(\omega)$$
$$= \frac{1}{\sqrt{2\pi}}C^J_{lk}(\omega)\Gamma_{kmn}(\alpha,\beta)\delta_{\alpha+\beta}(\omega). \qquad (109)$$

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