Modelling oscillating living systems: Cell energy metabolism as weighted networks of nonautonomous oscillators

Joe Rowland Adams and Aneta Stefanovska

Abstract Oscillations are a common feature throughout life, forming a key mechanism by which living systems can regulate their internal processes and exchange information. To understand the functions and behaviours of these processes, we must understand the nature of their oscillations. Studying oscillations can be difficult within existing physical models that simulate the changes in a system's masses through autonomous differential equations. We discuss an alternative approach that focuses on the phases of oscillating processes and incorporates time as a key consideration. We will also consider the application of these theories to the cell energy metabolic system, and present a novel model using weighted nonautonomous Kuramoto oscillator networks in this context.

1 Introduction

It is increasingly clear that a wide variety of biological processes are rhythmic in nature, from glycolysis within a cell to the heart pumping blood throughout the body [6, 31]. Replicating this fluctuating behaviour poses a challenge to many traditional modelling methods, which can rely upon approximations of the system as thermodynamically closed and linear, and which examine the system asymptotically in time. Such models may only generate oscillations at particular parameter selections and modulations, oscillate with a high degree of stability, and exist in a steady state within most of their parameter space. This is in contrast to much of what has been observed of oscillating living systems, where the oscillations continually fluc-

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tuate in their frequency and amplitude, and continue until the death of the system itself [2,5,6,9,13,14,18,19,24–26,28–30,35–37]. We will present and discuss a different approach that rethinks how fluctuating biological systems are best modelled, applied to the cell energy metabolic system.

2 Principles of an alternative approach

In table 2 we outline the key principles that form our method for modelling oscillating living systems, which we discuss further in this section.

Table 1 Summary of the principles informing our modelling approach contrasted to those of mainstream approaches

Mainstream principles	Our principles
Open systems can be modelled as perturbed closed systems	Open systems can only be fully represented by open models
Oscillations result from instability of a dynamical system	Oscillations are inherent to the dynamics of open systems. Living systems continuously exchange energy and matter with the environment and each process is characterized by self-sustained oscillations on a certain time-scale
Nonlinear systems can be recombined from linear systems	Nonlinear systems are best understood by nonlinear models
Time variation in living systems is often due to noise, and can be averaged out over asymptotic time	Time variation in living systems is often deterministic, and must be modelled as nonautonomous to reflect the full system dynamics

It is easy to see that biological systems are open: without being able to exchange mass and energy across its boundary a cell would die, the blood would not be oxygenated by the lungs, and neurons would not receive the energy they need to fire [8, 23, 31, 40]. While it can be mathematically simpler to treat these systems as closed off to their environment, doing so is not modelling them in their healthy, existing state, but instead a dead or dying one. The first principle of our approach is therefore to allow the modelled system to be open. Attempting to model transfers of mass in an open system can be distinctly difficult. Tracking each unit of mass throughout the entire system necessitates the inclusion of processes that may otherwise not need to be considered, and are often challenging, if not impossible, to measure experimentally in their living states.

Oscillations can often be considered as a perturbation of a system away from its 'natural' steady state. However, an attempt to remove oscillations from an otherwise oscillatory system would be equivalent to destroying the system itself: oscillations not only allow a compartmentalisation of otherwise conflicting processes, but play a significant role in the exchange of information and regulation throughout living systems [37]. Therefore we instead treat them as an intrinsic result of the openness of living systems.

While modelling systems' interactions linearly also simplifies the mathematics, it does not reflect the biological reality. Biological systems endemically exhibit transitions in behaviour disproportionate to environmental changes [7], and so we propose to model them as nonlinearly interacting phase oscillators [32].

The fourth key principle of our approach is that living systems should be studied according to the time scales in which they actually exist and function. Analysing the properties of a system in an asymptotic time frame can erase dynamics that exist for only short times. Lucas et al., for example, demonstrated that nonautonomous phase oscillators may synchronise intermittently, and that this is missed when using asymptotic methods [21].

This variation of frequency of oscillation is seen throughout biology [2, 19, 25, 37], and hence our model considers nonlinearly interacting phase oscillations with nonautonomous frequencies, analysed on finite time scales.

3 Modelling a cell's energy metabolism

Our model brings together these four principles to examine the oscillations of the energy metabolism of a single cell. The focus of this model is the production of ATP, a key molecule in maintaining cellular functions, by glycolysis, consuming glucose, and mitochondrial oxidative phosphorylation (OXPHOS), consuming oxygen [8, 40]. We build on the work of Lancaster et al. [20], who modelled each metabolic process as a singular nonautonomous phase oscillator. This model is based on the theory of chronotaxic systems, which characterises nonautonomous oscillations as a method for stabilising against external perturbations [34]. We extend this to include multiple oscillators of each process, transforming the glycolytic and OXPHOS processes into weighted networks of Kuramoto oscillators [17]. We also incorporate the findings of Lucas et al. [21], deterministically varying the frequencies of the oscillations.

This model is represented diagrammatically in figure 1. It consists of four main elements – two weighted Kuramoto networks of phase oscillators representing glycolysis and OXPHOS, and two sets of phase oscillators driving these networks, representing glucose and oxygen.

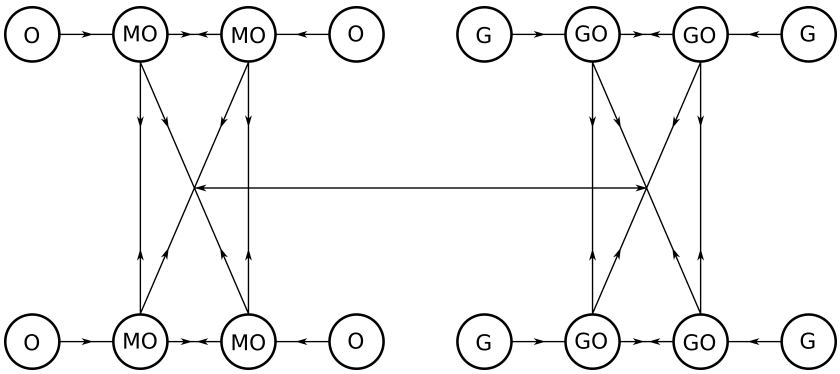

Fig. 1 Oscillator model diagram, where each circle represents a glycolysis (GO), glucose (G), mitochondrial OXPHOS (MO) or oxygen (O) oscillator, and each line a coupling.

That these processes are oscillatory has been extensively established by experimentation, and further, that they may do so nonautonomously [2, 5, 6, 9, 13, 14, 18–20, 24–26, 28–30, 35–37]. The networks of the model reflect the fact that glycolysis occurs in a cell distributed throughout the cytosol, undergoing multiple different reactions simultaneously, and that these reactions appear to communicate through the exchange of acetaldehyde molecules [10, 16, 22, 29, 39]. Similarly, cells contain multiple mitochondria, each undergoing OXPHOS, communicating through molecular exchange, common regulation and inter-mitochondrial nano tunnels [3, 4, 12, 18, 30, 38]. The weighting of these networks, such that neighbouring oscillators influence one another more strongly than those more separated, reflects the spatial distances between these individual processes, and the diffusive nature of their molecular-exchange-driven communications.

We now introduce the mathematical formulation of these elements, beginning with the concept of phase oscillators. These are derived from ordinary differential equations that exhibit self-sustaining oscillations in their state dynamics. Phase, in this circumstance, is defined as the position of the equation along its oscillatory cycle at a given time. The frequency here refers to the velocity of this phase, which we allow to vary in time. We choose to focus on phase as the building blocks of our model, initially discarding the amplitude of the oscillations. This is because at a microscopic level the oscillator is a unit defined with a phase only, while the amplitude is built at a mesoscopic level, resulting from the mean field of the network.

The oscillators' phase can be further defined in the immediate region around its oscillations in state space through the use of isochrons. Isochrons connect all points in the region adjacent to a stable cycle with the one point on the cycle that, after a time, will first meet the perturbed points back on the cycle as the perturbation decays. Thus all these points are defined by the same phase [27, 32].

For nonautonomous oscillators we may also make this extension of definition, by considering each state in time as an autonomous system of slightly different frequency to the ones proceeding and following it. So long as the cycle of each autonomous system exists in the region of attraction of the system proceeding it, we

may define the former's phase via the isochrons of the latter system. This assumption hence requires that the change in the oscillator's frequency over time remains small in comparison to the frequency itself [15].

Having defined phase in the region of nonautonomous cycles, we can consider methods of coupling oscillators. Because our approach focuses on the frequencies and phases of the systems involved, phase coupling is used to model the effects of the biological processes on one another. Through this form of coupling, oscillatory systems perturb one another's phase in a backwards or forwards direction, depending on the comparative directions of oscillation of the two systems. Too strong coupling, however, can perturb the phase beyond the region defined by isochrons. Therefore in order for the perturbed system to remain in the region of its original cycle, where phase is defined, we must further require that that the coupling generating the perturbation is only weak [11, 27, 32].

We may now consider the equations of the model. First, the glycolysis and OXPHOS intra-network connections are defined as

$$\begin{aligned}\dot{\theta}_{GONi} &= \frac{K_{GO}}{N}\sum_{j=1}^{N} W_{ij}\sin\left(\theta_{GOj}-\theta_{GOi}\right)\\ \dot{\theta}_{MONi} &= \frac{K_{MO}}{M}\sum_{j=1}^{M} W_{ij}\sin\left(\theta_{MOj}-\theta_{MOi}\right),\end{aligned} \tag{1}$$

where the subscript GO represents the glycolytic network and MO the OXPHOS, N the number of glycolytic oscillators, M the number of OXPHOS oscillators, K_X the relevant network coupling strength and θ_X the phase.

The weighting of edges within the glycolytic and mitochondrial networks consists of more heavily weighting shorter edges, where the nodes are positioned equidistantly around a ring. Mathematically, for $i \leq \frac{N}{2}$

$$W_{ij} = \begin{cases} \dfrac{W}{|i-j|}, & \text{for } j \in \left[1, i+\frac{N}{2}-1\right] \\ \dfrac{W}{|j-N-i|}, & \text{for } j \in \left[i+\frac{N}{2}, N\right], \end{cases} \tag{2}$$

and for $N \geq i > \frac{N}{2}$

$$W_{ij} = \begin{cases} \dfrac{W}{|i-j|}, & \text{for } j \in \left[i-\frac{N}{2}+1, N\right] \\ \dfrac{W}{|j+N-i|}, & \text{for } j \in \left[1, i-\frac{N}{2}\right], \end{cases} \tag{3}$$

where i denotes the index of the node under consideration, j the index of the node at the other end of the corresponding edge, N the number of nodes in the network, W a constant to be chosen, and W_{ij} the resulting weighting of the edge connecting nodes i and j.

Next, the glucose and oxygen driving are defined as,

$$\begin{aligned}\dot{\theta}_{GOGi} &= \varepsilon_G \sin(\theta_{GOi} - \theta_{Gi}) \\ \dot{\theta}_{MOOi} &= \varepsilon_O \sin(\theta_{MOi} - \theta_{Oi}),\end{aligned} \tag{4}$$

where the subscript G represents the glucose driving and O the oxygen, and ε_X represents the coupling strength of the relevant driving.

Finally, the inter-network interactions arise through coupling each network to the mean field of the other [33], such that

$$\begin{aligned}\dot{\theta}_{GOMOi} &= F_{GO} r_{MO} \sin(\Psi_{MO} - \theta_{GOi}) \\ \dot{\theta}_{MOGOi} &= F_{MO} r_{GO} \sin(\Psi_{GO} - \theta_{MOi}).\end{aligned} \tag{5}$$

Here F_X is the intra-network coupling strength, r_X the Kuramoto order parameter, where $r_X e^{i\phi} = \frac{1}{N}\sum_{k=1}^{N} e^{i\theta_{Xk}}$ and ϕ is the phase of the mean field arising from the network, such that $r_X = 1$ indicates a totally ordered network, while $r_X = 0$ a totally disordered one. Further, the average phase of network X is $\Psi_X = \frac{1}{N}\sum_{i=1}^{N} \theta_{Xi}$.

The four governing differential phase equations therefore are,

$$\begin{aligned}\dot{\theta}_{Gi} &= \omega_{Gi}(t) \\ \dot{\theta}_{Oi} &= \omega_{Oi}(t) \\ \dot{\theta}_{GOi} &= \omega_{GOi}(t) + \dot{\theta}_{GONi} - \dot{\theta}_{GOGi} + \dot{\theta}_{GOMOi} \\ \dot{\theta}_{MOi} &= \omega_{MOi}(t) + \dot{\theta}_{MONi} - \dot{\theta}_{MOOi} - \dot{\theta}_{MOGOi},\end{aligned} \tag{6}$$

where $\omega_X(t)$ is the time-varying natural frequency of oscillator X. The signs of the inter-network coupling terms are opposite to represent the inhibitory effects of OXPHOS on glycolysis, and the excitatory effects of glycolysis on OXPHOS [20].

A comparison between an output of this model and an experimental observation of cellular glycolysis in shown figure 2. The experimental data were obtained by Amemiya et al. [2], who optically measured the NADH fluorescence, a by-product of glycolysis, of batches of HeLa cells cultured under a variety of glucose starvation conditions. The model output is the combined Kuramoto order parameter of the glycolytic and OXPHOS networks, defined as

$$\Psi_{GOMO} = \frac{1}{(N+M)}\left(\sum_{i=1}^{N}\theta_{GOi} + \sum_{j=1}^{M}\theta_{MOj}\right).$$

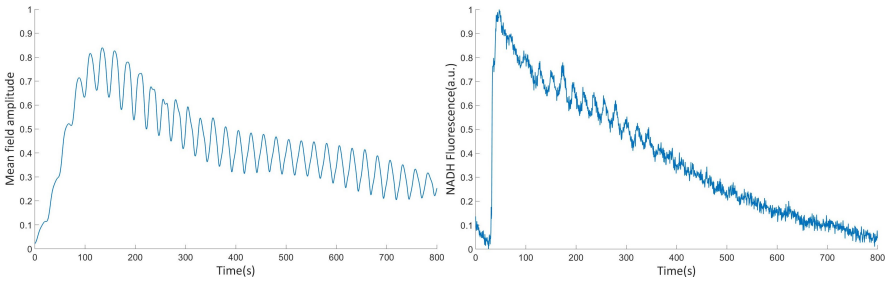

Fig. 2 Sample output of the model (left) and the NADH fluorescence of a single HeLa cell from the Amemiya et al experiment [2], normalised to within the range [0, 1] (right). The model output is represented by the combined Kuramoto order parameter of both the glycolytic and OXPHOS networks.

The parameter values are given in table 2.

Table 2 Parameters used in the simulation to generate the output displayed in figure 2

Parameter	Value(s)
ε_G	[0.1, 0.26]
ε_O	0.01
K_{GO}	1
K_{MO}	1
F_{GO}	0.05
F_{MO}	0.05
ω_G	[0.015, 0.065] Hz
ω_{GO}	[0.02, 0.04] Hz
ω_{MO}	[0.025, 0.075] Hz
ω_O	[0.02, 0.04] Hz
N	100
M	100
W	1

These results can be compared to the model of the same experiment by Amemiya et al. [1] who constructed a classical autonomous model of just the glycolytic process of a HeLa cell, in which mass was assumed to be conserved. Figure 2 in [1] presents an analogous output to what we have shown here. The model by Amemiya et al. involved 22 parameters in 7 governing equations, while our model relies on the 13 parameters of table 2 in the 4 governing equations shown in equation 6.

While the overall trend and oscillating nature of the model output in figure 2 are represented in the experimental data, we are undoing more analysis of the model to better replicate the oscillation death and frequency seen in the experiment. Further details of this simulation and analysis will be presented elsewhere.

4 Outlook

Modelling oscillating biological systems in their living state is a complex task. In order to reproduce every oscillation, variation of frequency, and different regime of stability a system offers, oscillations and nonautonomicity must be built in to the foundations of a model.

Using this approach, we can replicate oscillatory biological data in all its variety with only small changes to model parameters, that can themselves be matched to experimental measurements. Investigating the parameters at which various combinations of the oscillators of the model synchronise, and the transitions between these relationships, can also reveal a significant amount about a biological system. Each of these regimes can be understood as a healthy or pathological state of the system, revealing the breakdown of which mechanisms can be identified with which diseases [20].

Further, analysing the synchronisation of nonautonomous oscillator networks in finite time has already uncovered the new phenomenon of intermittent synchronisation [21]. Investigation of the metabolic model we have presented here, which introduces multiple networks and more complex forms of coupling, promises yet more unseen stabilisation behaviours.

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