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The steady-state assumption in oscillating and growing systems

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HIGHLIGHTS

- New mathematical foundation of steady-state assumption based on averages.
- Applies to oscillating and growing systems.
- Does not require quasi-steady-state assumption.
- Pinpoints unintuitive effects in the integration of metabolite concentrations.
- Can be used to approximate growth maximization in dynamic metabolic network models.

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ABSTRACT

The steady-state assumption, which states that the production and consumption of metabolites inside the cell are balanced, is one of the key aspects that makes an efficient analysis of genome-scale metabolic networks possible. It can be motivated from two different perspectives. In the time-scales perspective, we use the fact that metabolism is much faster than other cellular processes such as gene expression. Hence, the steady-state assumption is derived as a quasi-steady-state approximation of the metabolism that adapts to the changing cellular conditions.

In this article we focus on the second perspective, stating that on the long run no metabolite can accumulate or deplete. In contrast to the first perspective it is not immediately clear how this perspective can be captured mathematically and what assumptions are required to obtain the steady-state condition.

By presenting a mathematical framework based on the second perspective we demonstrate that the assumption of steady-state also applies to oscillating and growing systems without requiring quasi-steady-state at any time point. However, we also show that the average concentrations may not be compatible with the average fluxes.

In summary, we establish a mathematical foundation for the steady-state assumption for long time periods that justifies its successful use in many applications. Furthermore, this mathematical foundation also pinpoints unintuitive effects in the integration of metabolite concentrations using nonlinear constraints into steady-state models for long time periods.

1. Introduction

A rather frequently used assumption for metabolic network modelling is that the production and consumption of internal metabolites must balance (steady-state assumption). This assumption lies at the core of many metabolic network analysis techniques such as flux balance analysis (FBA) (Varma and Palsson, 1994; Orth et al., 2010), elementary flux mode analysis (Schuster and Hilgetag, 1994), metabolic control analysis (Heinrich and Schuster, 1998) or gene intervention studies (Hädicke and Klamt, 2011; Burgard et al., 2003).

Given the stoichiometric matrix S of a metabolic network, we call a vector of reaction rates (fluxes) $\mathbf{w}$ a steady-state flux if it satisfies

$$S\mathbf{w} = \mathbf{0}. \tag{SS}$$

In this paper we provide a new, mathematically sound derivation of the steady-state condition using flux averages over time. This derivation does not require any underlying theory on

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dynamics, like oscillations, in metabolic networks. While the biological motivation of our approach, as detailed in Section 1.2, is well known (Fell, 1997; Steuer and Junker, 2009; Knoke et al., 2008; Schuster and Fell, 2007; Palsson, 2015), the mathematical foundation presented here strengthens the existing approaches that study metabolism using steady-state fluxes.

The steady-state assumption, as used in metabolic network analysis, is usually mathematically derived from a quasi-steady-state perspective. This perspective is however not always applicable, as pointed out in Song and Ramkrishna (2009). Therefore, our mathematical derivation presented here does not use the quasi-steady-state argument. We nevertheless outline the quasi-steady-state perspective below for the sake of comparison.

1.1. Classical derivation based on the quasi-steady-state assumption

To illustrate the differences between the existing theory and our new derivation, we first recall how the steady-state assumption is mathematically derived in the quasi-steady-state perspective.

Given a kinetic model

$$\dot{\mathbf{c}}(t) = S\mathbf{v}(t), \quad \mathbf{v}(t) = f(\mathbf{e}(t), \mathbf{c}(t)) \tag{KM1}$$

that describes the dynamics of the internal metabolite concentrations $\mathbf{c}$, reaction rates $\mathbf{v}$ and enzyme concentrations $\mathbf{e}$, we assume that the dynamics of the metabolism can be approximated by a quasi-steady-state solution with respect to the enzyme dynamics. A quasi-steady-state solution of (KM1) is a tuple of time-dependent functions $(\mathbf{c}, \mathbf{v}, \mathbf{e})$ such that

$$\mathbf{0} = S\mathbf{v}(t), \quad \mathbf{v}(t) = f(\mathbf{e}(t), \mathbf{c}(t)) \quad \text{for all } t \geq 0. \tag{QSS}$$

Note that in the QSS solution the enzyme and metabolite concentrations can still change over time (the constraint on the metabolite concentrations $\dot{\mathbf{c}}(t) = S\mathbf{v}(t)$ is dropped) while fluxes transition from one metabolic steady-state to another, and are therefore not constant.

Indeed, as Varma and Palsson put it, "this assumption is based on the fact that metabolic transients are typically rapid compared to cellular growth rates and environmental changes. The consequence of this assumption is that all metabolic fluxes leading to the formation and degradation of any metabolite must balance" (Varma and Palsson, 1994, p. 994). Similar reasons for assuming a quasi-steady-state for metabolism are obtained by comparing the time scale of metabolic processes (fast) to those of e.g. transcriptional regulation or cell cycle (slow) (Almquist et al., 2014; Heinrich and Schuster, 1996; Moreira dos Santos et al., 2004). Hence, it is assumed that at every time point the metabolite concentrations have converged to a steady-state and thus the quasi-steady-state assumption (QSS) follows (Schilling et al., 1999; Voss et al., 2003; Waldherr et al., 2015).

The quasi-steady-state assumption found successful applications in dynamic simulation models like dynamic flux balance analysis (dynamic FBA) (Mahadevan et al., 2002) and dynamic enzyme-cost flux balance analysis (Waldherr et al., 2015).

There are, however, situations when the quasi-steady-state assumption cannot be applied (Song and Ramkrishna, 2009; Behre and Schuster, 2009), which means the derivation above cannot be used. Therefore, the main result of this paper is a derivation that does not need this assumption.

Before we continue with our new mathematical approach, it is worth noting the difference between the steady-states in (QSS) and the global steady-state used in classical metabolic network analysis tools such as FBA.

Given (QSS), for every time point t, $\mathbf{v}(t)$ is a steady-state flux. Therefore, we consider the quasi-steady-state assumption a *time-local* property. From this the steady-state condition $S\mathbf{w} = 0$ as used in classical metabolic network analysis is derived. This simplification allows for an efficient analysis of metabolic networks, since metabolite concentrations and time do not need to be modelled anymore. For example, the constraint $S\mathbf{w} = \mathbf{0}$ is used in methods such as FBA to predict biomass yields and growth rates.

In FBA we use only one steady-state flux to describe the whole growth cycle. This is what we call a *time-global* steady-state flux. However, metabolic fluxes are not constant in time. For instance, during the cell cycle the cell goes through different phases (G_1, S, G_2 and M) during which the metabolic activity is different. Therefore, the metabolism can be considered to use different time-local steady-state fluxes that follow the division cycle. Since the sum of steady-state fluxes yields another steady-state flux (i.e., if $S\mathbf{w} = \mathbf{0}$ and $S\mathbf{v} = \mathbf{0}$, then $S(\mathbf{w} + \mathbf{v}) = \mathbf{0}$), by combining the time-local steady-state fluxes we can obtain a time-global steady-state flux for the whole growth cycle.

1.2. The perspective based on long time periods

However, we do not need time-local steady-states to obtain a time-global steady-state. For example the steady-state assumption is also often motivated by stating that no metabolite can accumulate or deplete on the long run (Fell, 1997). The aim of this paper is to provide a general mathematical framework based on this idea. In particular, we will generalize the approach used in Steuer and Junker (2009), and Knoke et al. (2008, 2010). They observe that, if after a time T no net change $\Delta\mathbf{c}(T) = \mathbf{0}$ has occurred in the metabolite concentrations, we obtain $S\int_0^T \mathbf{v}(t)dt = \mathbf{0}$. Hence, in this case, the average flux

$$\bar{\mathbf{v}}(T) := \frac{1}{T}\int_0^T \mathbf{v}(t)dt \tag{AVGV}$$

is also a steady-state flux. In contrast to the fluxes derived via the quasi-steady-state assumption, it applies globally over the time interval $[0, T]$. In particular, in cases where the quasi-steady-state assumption is not entirely justified (see e.g. Song and Ramkrishna, 2009), one can still obtain a time-global steady-state.

Building upon the ideas in Section 1.5.2 of Steuer and Junker (2009), we observe that, if we consider a long enough time period T, we do not necessarily need to come back to the same concentration. In order to obtain an average steady-state flux we only require that the concentrations stay bounded (see Fig. 1). While this is implied by physical laws, it should also happen because accumulation of metabolites in very high amounts is toxic for a cell. Therefore, on the long run, to avoid such toxicity, every metabolite should be produced, on average, at the same rate at which it is consumed (Fell, 1997). Moreover, even if deterministic chaos is rare in metabolic systems (Goldbeter et al., 2001), it is worth noting that the theory developed here is also applicable to chaotic and quasi-periodic systems if the attractor is bounded. Some ideas in this direction can be found in Knoke et al. (2008).

As already pointed out in Eker and Krummenacker (2013), if we consider long time periods, we also have to model the fact that molecule counts per cell change because of cell growth. Therefore, in the differential equation that models the change of concentrations in time we also need to consider an additional term that represents dilution via cell growth. Schuster et al. (2004) propose to neglect this term since it is anyway "small" compared to the intracellular fluxes.

Based on these observations, we present in Section 3 a mathematical perspective on the steady-state assumption that does not need the quasi-steady-state argument, but instead considers flux averages over time. Using this model we compute for how long we have to observe the system to obtain a sufficiently good