Period-Amplitude Co-variation in Biomolecular Oscillators

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Abstract
The period and amplitude of biomolecular oscillators are functionally important properties in multiple contexts. For a biomolecular oscillator, the overall constraints in how tuning of amplitude affects period, and vice versa, are generally unclear. Here we investigate this co-variation of the period and amplitude in mathematical models of biomolecular oscillators using both simulations and analytical approximations. We computed the amplitude-period co-variation of ten benchmark biomolecular oscillators as their parameters were individually varied around a nominal value, cataloguing instances where an increase in period is accompanied by an increase or decrease in amplitude as well as more complex co-variations. To account for the amplitudes of the many biomolecular species that may be part of the oscillation circuit, we use a power norm-based amplitude metric, finding an increase of instances where an increase in period correlates with an increase in this amplitude metric. Finally, we calculate “scaling laws” of period-amplitude co-variation for a subset of these benchmark oscillators, finding that as period increases the amplitude increases or remains constant. These results should help to understand the amplitude-period co-variation of oscillators in biomolecular as well as other contexts.

1 Introduction
Oscillatory behaviour in biomolecular systems span multiple scales of space and time. An important example is that of circadian rhythms, which are present in both eukaryotes and prokaryotes. The classical studies of the genes period and timeless in Drosophila melanogaster provided a genetic context to these oscillations [1]. More recently, biochemical investigations have proved invaluable in investigating the interactions between the proteins KaiABC that are at the core of the cyanobacterial circadian clock [2, 3]. In addition to these examples of naturally occurring oscillatory behaviour, significant effort has been devoted to designing oscillators for synthetic biology applications inside cells and in biochemical contexts. Examples of synthetic oscillators include the repressor oscillator [4] and Smolen oscillator in cells, the repressilator and its variations in cell extracts [6], as well as oscillators in vitro [7, 8] (please see [9] for more examples). In these investigations of analysis and design of oscillators, mathematical models have played an important role in understanding the underlying principles.

An important guiding principle for oscillator investigations have been the presence of a negative feedback with an additional delay-causing mechanism, either explicitly through a time delay such as due to one or more intermediate steps or due to a positive feedback [10–13]. Systems-level studies on these oscillators have characterized the extent of robustness of different mechanistic realizations of oscillations [14, 15]. These have also addressed various aspects of oscillator function. For example, a recent study showed that the amplitude-period co-variation in an oscillator can depend on underlying mechanisms [16]. In particular, it was argued that a combination of positive and negative feedback enables a broad range of periods for a fixed amplitude, in contrast to mechanisms based on negative feedback alone. These studies represent important work towards connecting the structure and function of natural as well as synthetic biomolecular oscillators.

There are at least three interesting aspects of the amplitude-period co-variation in oscillators. First, as mentioned above, the amplitude-period co-variation has different qualitative characteristics depending on whether the oscillator has both positive and negative feedback or only negative feedback [16]. Second, an experimental study noted the correlation between amplitude and period [7]. Third, from energetic considerations related to protein production and degradation, it seems less favourable to have both large amplitude and small period. Given these, whether there are constraints and/or patterns in the amplitude-period co-variation in biomolecular oscillator models is generally unclear.

Here, we investigate the amplitude-period co-variation in biomolecular oscillator models. For this, we use computational models of an array of benchmark biomolecular oscillators — Repressilator, Pentilator, Goodwin oscillator, Van der Pol oscillator, Fitzhugh-Nagumo oscillator, Frzilator, Cyanobacterial circadian oscillator, Metabolator, a mixed
feedback oscillator and Meyer-Stryer model of calcium oscillations — and compute the trajectories for different values of parameters starting from a nominal parameter set, varying one parameter at a time. We find that the co-variation

![Figure 1: Possible period-amplitude co-variations in biomolecular oscillators.](image)

of the peak amplitude and the period can be increasing, decreasing, constant or a combination of these trends (Fig. 1). To account for the multiple states that typically constitute these oscillator models, we define an amplitude metric based on a power norm and find that these display, relatively, an enhanced increasing trend. Finally, we approximate a subset of the models analytically to calculate "scaling laws" for the amplitude-period co-variation, finding an increasing trend. These results provide insight into the amplitude-period co-variation of biomolecular oscillators as well as a reference for parameters that can be tuned to achieve desired amplitude and frequency.

2 Results

2.1 Simulation of Oscillations

We considered previously developed mathematical models of ten benchmark oscillators — Repressilator [4], Penti-lator [16], Goodwin oscillator [17], Van der Pol oscillator [18], Fitzhugh-Nagumo oscillator [19, 20], Frzilator [21], Cyanobacterial circadian oscillator [2, 3], Metabolator [22], Mixed feedback oscillator [23] and Meyer-Stryer model of calcium oscillations [24]. Although Van der Pol oscillator is not a biomolecular oscillator, we note that it is a benchmark nonlinear oscillator and corresponding period-amplitude co-variation is worth investigating. These models are based on ordinary differential equations (Please see supplementary material). To obtain the oscillatory trajectories, these equations were simulated using the MATLAB solvers ode15s and ode23t. For each oscillator, we plotted the trajectories by varying one parameter at a time over a twofold range around the nominal value. These are shown in a red-blue colour map with the X-axis representing time, Y-axis representing parameter value, and the colour representing the amplitude of oscillation. We also show oscillatory trajectories picked for three values in the parameter regime (Please see supplementary material : Figs. 1-10 A). To find the period and amplitude, we first numerically confirm the existence of oscillations. The amplitude is calculated from the peak-to-peak amplitude. The period is calculated from the average time-interval between five crests.
| Oscillator/Parameters | Peak-peak Amplitude | Period | $M_p$ Metric |
|-----------------------|---------------------|--------|--------------|
| **Repressilator**     |                     |        |              |
| $\gamma$              | Increases           | Increases| Increases    |
| $k_b$                 | Decreases           | Increases| Decreases    |
| $k_m$                 | Complex$^a$         | Decreases| Decreases    |
| $k_p$                 | Increases           | Decreases| Complex$^a$  |
| $\tau$                | Decreases           | Increases| Increases    |
| **Pentilator**        |                     |        |              |
| $\gamma$              | Increases           | Increases| Increases    |
| $k_b$                 | Complex$^a$         | Increases| Decreases    |
| $k_m$                 | Decreases           | Decreases| Decreases    |
| $k_p$                 | Constant            | Decreases| Decreases    |
| $\tau$                | Decreases           | Increases| Increases    |
| **Goodwin Oscillator**|                     |        |              |
| $k_1$                 | Increases           | Increases| Increases    |
| $k_2$                 | Decreases           | Constant| Increases    |
| $k_3$                 | Decreases           | Constant| Constant     |
| $k_4$                 | Increases           | Decreases| Complex$^a$  |
| $k_5$                 | Increases           | Decreases| Complex$^a$  |
| $k_6$                 | Increases           | Decreases| Complex$^a$  |
| **Van der Pol Oscillator** |               |        |              |
| $\mu$                 | Constant            | Increases| Constant     |
| $\omega$              | Constant            | Decreases| Increases    |
| **Fitzhugh-Nagumo oscillator** |           |        |              |
| $\theta$              | Decreases           | Complex$^b$| Decreases    |
| $\gamma$              | Constant            | Complex$^b$| Increases    |
| $\omega$              | Constant            | Complex$^b$| Increases    |
| $\phi$                | Constant            | Decreases| Constant     |
| **Frzilator**         |                     |        |              |
| $\phi$                | Increases           | Decreases| Increases    |
| $d_f$                 | Constant            | Constant| Decreases    |
| $k_c$                 | Decreases           | Decreases| Decreases    |
| $d_e$                 | Increases           | Increases| Complex$^a$  |
| $k_e$                 | Decreases           | Decreases| Decreases    |
| $d_e$                 | Constant            | Constant| Constant     |
| **Cyanobacteria circadian oscillator** |               |        |              |
| $k_{1UT}$             | Constant            | Constant| Constant     |
| $k_{2TD}$             | Constant            | Constant| Constant     |
| $k_{3TD}$             | Constant            | Constant| Constant     |
| $k_{4US}$             | Constant            | Constant| Constant     |
| $k_{5TU}$             | Increases           | Constant| Decreases    |
| $k_{6DT}$             | Constant            | Constant| Constant     |
| $k_{7DS}$             | Decreases           | Decreases| Decreases    |
| $k_{8SU}$             | Increases           | Increases| Increases    |
| $k_{9UT}$             | Increases           | Decreases| Increases    |
| $k_{10TD}$            | Increases           | Decreases| Decreases    |
| $k_{11SD}$            | Increases           | Increases| Increases    |
| $k_{12US}$            | Decreases           | Decreases| Decreases    |
| $k_{13TU}$            | Decreases           | Constant| Decreases    |
| $k_{14DT}$            | Increases           | Increases| Increases    |
| $k_{15De}$            | Increases           | Increases| Increases    |
| $k_{16SL}$            | Decreases           | Decreases| Decreases    |
| $K_{1/2}$             | Decreases           | Decreases| Decreases    |
| Oscillator/Parameters | Metabolator | Peak-peak Amplitude | Period | $M_p$ Metric |
|-----------------------|-------------|---------------------|-------|--------------|
| $V_{gly}$             | Constant    | Decreases           | Increases |                |
| $k_{TCA}$             | Decreases   | Constant            | Decreases |                |
| $k_1$                 | Complex     | Decreases           | Increases |                |
| $k_{m,1}$             | Constant    | Constant            | Constant |                |
| $k_2$                 | Complex     | Decreases           | Complex$^a$ |                |
| $k_{m,2}$             | Decreases   | Constant            | Constant |                |
| $k_{Ack,f}$           | Increases   | Increases           | Increases |                |
| $k_{Ack,r}$           | Complex$^a$ | Decreases           | Decreases |                |
| $C$                   | Constant    | Constant            | Constant |                |
| $H^+$                 | Constant    | Constant            | Constant |                |
| $K_{eq}$              | Constant    | Constant            | Constant |                |
| $k_3$                 | Constant    | Constant            | Constant |                |
| $HOAC_E$              | Constant    | Constant            | Constant |                |
| $K_{g,1}$             | Complex$^a$ | Decreases           | Increases |                |
| $n$                   | Increases   | Increases           | Constant |                |
| $K_{g,2}$             | Complex$^a$ | Increases           | Constant |                |
| $K_{g,3}$             | Complex$^a$ | Decreases           | Increases |                |
| $\alpha_0$            | Complex$^a$ | Constant            | Constant |                |
| $\alpha_1$            | Complex$^a$ | Increases           | Decreases |                |
| $\alpha_2$            | Complex$^a$ | Decreases           | Constant |                |
| $\alpha_3$            | Complex$^a$ | Decreases           | Increases |                |
| $k_d$                 | Complex$^a$ | Decreases           | Increases |                |

**Mixed feedback oscillator**

| $\alpha$ | Increases | Increases | Increases |
|----------|-----------|-----------|-----------|
| $\sigma$ | Complex$^a$ | Constant | Increases |
| $\gamma_x$ | Decreases | Decreases | Decreases |
| $\gamma_y$ | Increases | Decreases | Increases |
| $\tau_y$ | Increases | Increases | Constant |

**Meyer-Stryer model of calcium oscillations**

| $c_1$ | Increases | Decreases | Decreases |
|-------|-----------|-----------|-----------|
| $c_2$ | Increases | Increases | Increases |
| $c_3$ | Constant  | Decreases | Decreases |
| $c_4$ | Complex$^a$ | Complex$^a$ | Decreases |
| $c_5$ | Increases | Increases | Increases |
| $c_6$ | Decreases | Decreases | Decreases |
| $c_7$ | Increases | Increases | Decreases |
| $K_1$ | Increases | Increases | Increases |
| $K_2$ | Decreases | Decreases | Decreases |
| $K_3$ | Increases | Increases | Increases |
| $R$   | Complex$^a$ | Complex$^a$ | Decreases |

$^a$ First increases then decreases.

$^b$ First decreases then increases.

Constant is defined as variations within ±5% from nominal values.
Figure 2: Co-variation of period and peak-peak amplitude for biomolecular oscillators. In each plot, the point of intersection of the curves is the center of the black circle which corresponds to the nominal parameter set and the radius is 10% of oscillation period for nominal parameter set. Circle shaped markers represent the largest value of the corresponding parameter. a) Repressilator. b) Pentilator. c) Goodwin oscillator. d) Van der Pol oscillator. e) Fitzhugh-Nagumo oscillator. f) Frzilator. g) Cyanobacteria circadian oscillator. h) Metabolator. i) Mixed feedback oscillator. j) Meyer-Stryer model of calcium oscillations.
Figure 3: Co-variation of period and amplitude metric ($M_p$) for biomolecular oscillators. In each plot, the point of intersection of the curves is the center of the black circle which corresponds to the nominal parameter set and the radius is 10% of oscillation period for nominal parameter set. Circle shaped markers represent the largest value of the corresponding parameter. a) Repressilator. b) Pentilator. c) Goodwin oscillator. d) Van der Pol oscillator. e) Fitzhugh-Nagumo oscillator. f) Frzilator. g) Cyanobacteria circadian oscillator. h) Metabolator. i) Mixed feedback oscillator. j) Meyer-Stryer model of calcium oscillations.
2.2 Co-variation of Period and Peak Amplitude

We begin by computing the co-variation of the period and peak amplitude. As mentioned above, we start from the nominal parameter set and individually vary each parameter in a two-fold range around its nominal value. These results are plotted in Supplementary (Supplementary Figs. 1-10 B). A zoomed in version of these trends are shown in Fig. 2. The overall trends are summarized in Table 1 (Columns 2 and 3). The rows where the peak-peak amplitude and period exhibit like co-variation are shaded for clarity.

2.3 Co-variation of Period and a Power Norm-based Amplitude Metric

In the above computations, we computed the peak amplitude based on one variable, such as the protein concentration in the Goodwin oscillator or an mRNA concentration in the Repressilator. Most oscillator models, however, have more than one variable. To take this into account, we consider amplitude metrics based on power norm [25].

Consider a limit-cycle oscillatory system $\dot{x}(t) = f(x, p)$, where states $x \in \mathbb{R}^n$ and parameter set $p \in \mathbb{R}^m$, then we define the power norm in one-cycle duration of $T$.

**Definition 1. Metric $M_p$.** For the system defined above, the root mean square norm in one-cycle duration of $T$, is given by,

$$M_p = \sqrt{\frac{1}{T} \int_0^T \left( \sum_{i=1}^n x_i^2(t) \right) dt}, \quad (1)$$

where $x_i$ are the states in differential equation of the oscillator model. Based on this definition, we computed the co-variation of the period and this amplitude metric. The full simulations are shown in Supplementary (Supplementary Figs. 1-10 C). A zoomed in version is shown in Fig. 3.

The results of these calculations are also summarized in Table 1 (columns 3 and 4) . As before, the rows where the period and this amplitude metric exhibit like co-variation are shaded for clarity.

These results help to understand constraints in amplitude-period variations in different biomolecular oscillators.

2.4 Co-variation scaling laws

In the above sections we have computed the co-variation of the period and amplitude metrics for different benchmark oscillators as individual parameters are varied. Next, we investigate this aspect further by focusing on particular oscillators to get analytical approximation.

Consider the repressilator, one of the most important benchmark for oscillator designs. As parameters are varied individually around their default values, the co-variation of period and peak-peak amplitude take on different shapes. For example, for the maximal production rate $\gamma$, both amplitude and period increase simultaneously (Fig. 2a). In contrast, for the protein degradation rate constant $k_p$, the amplitude increases as the period decreases. For the RNA degradation constant $k_m$, the shape is more complicated. When we consider the amplitude metric based on the power norm, these shapes change, with the dominant trend that the amplitude and period increase/decrease simultaneously (Fig. 3a). These trends provide an understanding of the co-variation of period and amplitude.

In general, there may be bounds such as on the maximum achievable amplitude for a given period. To understand this further, we used a framework for analytical approximations developed where a nonlinear oscillatory system is replaced by a sequence of linear operations for different parts of the oscillation cycle [26].

For the repressilator, the approximations to period ($T_{\text{approx}}$) and amplitude ($A_{\text{approx}}$) are given by,

$$T_{\text{approx}} = 3 \left[ \frac{2 \ln(2)}{k_m} + \frac{1}{k_p} \left( \ln(X) + \ln \left( \frac{X}{X - 1} \right) \right) \right],$$

$$A_{\text{approx}} = \frac{\gamma}{k_m}, \quad (2)$$

where $X = \frac{\tau \gamma \sqrt{k_b}}{k_p k_m}$.

The $A_{\text{approx}}$ is the maximal possible value of the mRNA concentration. As the minimum value is zero, $A_{\text{approx}}$ is the upper bound on the peak-peak amplitude.

These approximate scaling laws are plotted in Fig. 4a as parameters are varied individually. These show that the period and amplitude (peak-peak) should increase or decrease simultaneously, except in cases where the amplitude is constant. Therefore, the main trend from these scaling laws is that as amplitude increases the period also increases.

We repeated the above exercise for the Pentilator and the Goodwin oscillator. Period ($T_{\text{approx}}$) and amplitude ($A_{\text{approx}}$) approximations for Pentilator are given as,
Figure 4: Analytical approximation of peak-peak amplitude to period co-variations. Dashed lines are for the numerical solutions of the ODE whereas solid line curves for the analytical approximation for the period and peak-peak amplitude. Circle shaped markers represent the largest value of the corresponding parameter. a) Repressilator. b) Pentilator. c) Goodwin oscillator. Inset shows the zoomed version of the amplitude metric to period co-variation.

\[T_{\text{approx}} = 5 \left[ \frac{\ln(2)}{k_m} + \frac{1}{k_p} \left( \ln(X) + \ln \left( \frac{X}{X - 1} \right) \right) \right],\]

\[A_{\text{approx}} = \frac{\gamma}{k_m},\]

where \( X = \frac{\tau \gamma \sqrt{k_b}}{k_p k_m}. \)
For Goodwin oscillator, the approximated period ($T_{approx}$) and amplitude ($A_{approx}$) are

$$T_{approx} = 2 \ln(2) \left( \frac{1}{k_4} + \frac{1}{k_5} \right) + \frac{1}{k_6} \left[ \ln(X) + \ln \left( \frac{X}{X-1} \right) \right],$$

$$A_{approx} = k_1 \frac{k_2 k_3}{k_4 k_5 k_6},$$

where $X = \frac{k_1 k_2 k_3}{k_4 k_5 k_6}$. Using these approximations, we obtained similar conclusions for the Pentilator (Fig. 4b) and the Goodwin oscillator (Fig. 4c).

3 Conclusion

Understanding design principles underlying operation of biomolecular oscillators is an important challenge. Using computational models of benchmark oscillators — Repressilator, Pentilator, Goodwin oscillator, Van der Pol oscillator, Fitzhugh-Nagumo oscillator, Frzilator, Cyanobacterial circadian oscillator, Metabolator, a mixed feedback oscillator and Meyer-Stryer model of calcium oscillations — we present an investigation of the co-variation of period and amplitude. First, we plot the peak-to-peak amplitude versus the period as each parameter is individually varied in a range around the nominal value, finding a range of curves showing both mutually increasing as well as other trends. Second, noting that the peak-to-peak amplitude metric considers a single state, we adapted a metric based on the power of a multivariable output signal and plotted these against the period, finding a dominant trend that amplitude and period mutually increase. Third, we analytically approximate three oscillator models to obtain "scaling laws" for the period-amplitude co-variation, finding that the maximum amplitude and period mutually increase. These results provide an insight into the functioning of biomolecular oscillators as well as a reference to choose specified amplitude-period values for design applications.

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