September 26, 2014

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Thank you for your email on September 24th, 2014, inviting us to submit a revised version of our manuscript, "Amplitude metrics for cellular circadian bioluminescence reporters" (MS: 2014BIOPHYSJ304278R) by Peter C. St. John, Stephanie R. Taylor, John H. Abel, and Francis J. Doyle III.

We thank the reviewers for their second round of comments on the manuscript, in particular Reviewer #2 for their continued attention to detail. We believe addressing these remaining concerns and suggestions have improved the polish of the manuscript.

Please see our detailed responses to the reviewers below:

Reviewer #1

The authors have largely responded you my concerns.

Typos and suggestions

- p. 2: "fail to capture the collective dynamics of a population oscillators"

This typo has been addressed, the sentence now reads:

> **Page 2**
>
> Ordinary differential equation (ODE) models of gene regulation are capable of describing the amplitude and phase-resetting behavior of single cells, but fail to capture the collective dynamics of a population **of** oscillators.

- mu is defined a few lines after it is first used in Eq. (20). Perhaps define it first?

This change has also been made.

> **Page 7**
>
> To define an amplitude change metric for such a case, we compare a perturbed trajectory $x(t)$ to a phase-shifted limit cycle reference $y(t)$,

for which $x(t) \to y(t)$ for sufficiently long times. Since $x(t)$ approaches the reference as $t \to \infty$, **the means of both trajectories are equal and can be calculated by**

$$\mu := \int_0^{2\pi} \frac{x^\gamma(\theta)}{2\pi}\, d\theta. \tag{20}$$

The amplitude change metric is defined as

$$\begin{aligned} \Delta A(x(t), y(t)) &:= \int_0^\infty (x(t)-\mu)^2 - (y(t)-\mu)^2\, dt \\ &= \int_0^\infty h(t)\, dt. \end{aligned} \tag{21}$$

Reviewer #2

The revised manuscript becomes much more clear. The authors addressed most of my concerns in their revision. However, I have still couple concerns regarding the manuscript.

1) In the previous review, I was concerned about using the Gillesplie algorithm for the model with non-elementary reactions because recent studies have clearly showed the inaccuracy of doing that (Thomas, Straube and Grima BMC Syst Biol (2012), Agarwal et al. J Chem Phy (2012) and Kim, Josic and Bennett Biophy J (2014)). Regarding this concern, the author showed that their continuous approximation also works for the model based on mass-action kinetics (Model 2). But, in the manuscript, the approximation is more accurate for Model 2 than other models, which are based on non-elementary reactions. This indicates that the inaccuracy coming from the Gillespie algorithm with non-elementary reaction appears to contribute the error seen in Model 1 and 3. Furthermore, I am mostly concerned that this manuscript can mislead the audience that using Gillespie algorithm with non-elementary reactions is justified. Please, clearly discuss the limitation in this approach.

We acknowledge there has been a great deal of literature concerning the accuracy of using the Gillespie stochastic simulation algorithm for non-elementary propensity functions. In K. R. Sanft, *et al*, *IET Syst. Biol.* (2011), the authors conclude that the Michealis-Menten approximation overestimates the noise of the corresponding full system of equations. In L. A. Widmer, *et al*, *J. Chem. Phys.* (2013), the authors prove that the noise of the full system is bounded between $3/4 \to 1$ of the noise estimated by the Michealis-Menten approximation. For hill-type kinetics, the full system can take an arbitrary level of noise relative to the approximated system (L. A. Widmer, Master's Thesis, 2013).

However, the most important consideration here is that the full system of equations require additional parameter values to be chosen; by choosing these parameter values, the noise of the full system can be changed relative to the system with non-elementary propensity functions. Since the values of these expanded parameters are not specified in the models used, we simply fit the noise characteristics of the approximated model (using the volume parameter) to yield physiologically realistic desynchronization rates. Therefore, the validity of whether or not these approximated solutions closely represent the noise characteristics of the full solutions does not play a large role in determining the accuracy of the continuous approximation.

To address the larger point, we discuss reasons why the continuous approximations are less

accurate for the larger model than the smaller model. We acknowledge that the method is only completely accurate in the limit of no noise in the underlying oscillator. When noise is present, the 'unperturbed' population consists of oscillators not only with a distribution of phases, but also with the states of many oscillators far from the deterministic limit cycle (this can be seen in the left plot of supplemental movies S1 & S2). As a result, the responses of oscillators not directly on the limit cycle to a pulse can differ from their limit cycle counterparts, decreasing the accuracy of the method for systems with high noise. In practice, however, it takes a considerable amount of noise before the continuous predictions deviate substantially from the stochastic populations. It is also important to consider that the larger model consists of many more state variables than the mass-action model, only one of which is shown in Fig. S3. Therefore, the difference in accuracy likely comes from increased deviation about the deterministic limit cycle in 8 spatial dimensions, rather than from non-elementary propensity functions.

We have endeavored to make these points more clear in the manuscript:

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Good agreement is seen between the continuous and stochastic simulations, indicating the proposed method is suitable for predicting the population-level responses for a variety of model types. **The slightly reduced accuracy seen in the larger Model 2 demonstrates a limitation of the method, which should be considered before applying the method to extreme cases. As Eq. 44 tabulates the response of the system from the limit cycle, systems which are perturbed from states far from the deterministic limit cycle will not be captured appropriately. Therefore, the difference in accuracy between Model 2 and Model 3 likely comes from increased deviation about the deterministic limit cycle in a greater number of spatial dimensions.**

Additionally, we caution readers against the unchecked use of the Gillespie algorithm for systems with non-elementary propensity functions:

Supplemental Info, Page 6

Model 1: Adapted from Novák & Tyson, 2008 (1). Model used for ARC demonstrations in Figs. 1-3, S1 ($P = 4$) and Fig. S2, Movies S1 & S2 ($P = 2$). **It should be noted that the use of the stochastic simulation algorithm for reactions with non-elementary propensities may inaccurately represent the noise of the full system. In this case, the volume of the stochastic simulations was chosen as $\Omega = 250$ to give a physiologically realistic desynchronization rate.**

2) Another concern is regarding the major result of the manuscript (Fig. 5). The author perturbed the degradation of Per mRNA parameter in response to light. However, this conflicts with the previous experimental studies, which have shown that light promotes the transcription of Per mRNA via CREB, not degradation.

This is a valid point, since our analysis captures the increase in *Per* mRNA following light pulses as a ***decrease*** in the degradation rate of *Per*. In the model, *Per* transcription is controlled by sev-

eral parameters: the maximum transcription rate, transcription rate kinetic constant, maximum degradation rate, degradation rate kinetic constant, and translation rate. Since we acknowledge that the model does not perfectly describe the true system, we tried to fit the model to data by searching the parameter ARCs for parameters and reporter states which gave the appropriate response. From these parameters, the maximum *Per* transcription rate and *Cry* mRNA emerged as the best choice given the response of the model and known experimental results:

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The next step in capturing the experimental data was finding a perturbation capable of desynchronizing the system. To find such a perturbation, we calculated population-level ARCs at several different knockdown strengths for each parameter in the model. We selected the degradation rate of *Per* mRNA from the feasible parameters, due to PER's known induction by CREB following photo-perturbation (46). The parameter was reduced by 28.5% during the light pulses, a value fit to maximize light-induced desynchrony. The output variable, PER2-luciferase in the experimental system, was chosen to be represented by *Cry1* mRNA - an E box activated gene that is buffered from the direct effects of the parameter perturbation.

We should emphasize that we are not predicting *Per* induction following light actually occurs via degradation, but rather that by acknowledging the model does not perfectly capture the experimental system, we simply seek a qualitatively accurate description of the underlying phenomena.

3) The key goal of this manuscript is distinguishing the single-cell level and population-level amplitude change. For this, the authors applied their tool to the experimental data (Fig. 5). However, Fig. 5 does not clearly show how their tool achieves such goal. Please provide additional figures. For instance, x_{ss} (steady-state perturbed trajectory) for perturbed and unperturbed cases, $p(\theta, t)$ for unperturbed case as well as perturbed case etc, so that readers can clearly see the difference of effect via single cell level and population level.

This is a good suggestion, and we have therefore added a figure to explicitly highlight the contributions from the population and single-cell level. Due to lack of space in the main text and desire not to further complicate the original figure, we have added this figure as Figure S4. A reference to the figure was added to the main text of the manuscript:

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The phase probability density function for the light-sensitive model trajectory is shown in Fig. 5C for several representative time points. ... **Amplitude change in this case is mediated both at the population and single-cell level, as indicated by their representative ARCs. The contributions from each source are summarized in Fig. S4.**

with the following figure and caption added to the supplementary material:

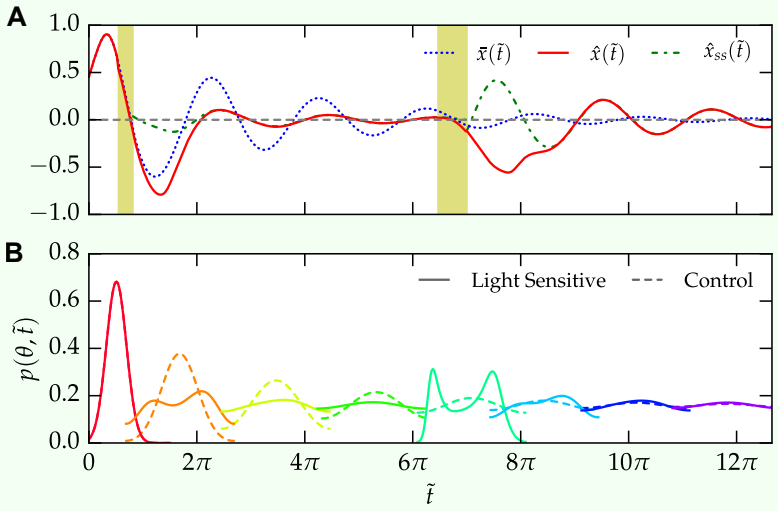

Figure S4: Evidence of single-cell and population-level amplitude change. (**A**) The simulated control trajectory as shown in Fig. 5 is plotted again as $\bar{x}(\tilde{t})$. The simulated light-sensitive trajector is also replotted as $\hat{x}(\tilde{t})$, which incorperates both single-cell and population-level effects. The difference between this trajectory and the steady-state perturbed trajectory following both pulses, $\hat{x}_{ss}(\tilde{t})$, demonstrates the effect of single-cell level amplitude change in this particular case. Single-cell amplitudes are greatly increased following the first pulse, and altered significantly following the second. (**B**) Comparison of the population densities for the control (dashed) and perturbed (solid) simulations. The perturbed population is greatly desynchronized following the first light pulse, as indicated by its flatness relative to the control trajectory. Following the second pulse, the perturbed population is more concentrated than the control, indicating higher amplitudes. These comparisons demonstrate the changes in population synchrony which occur due to the light pulse.