molecular systems biology

Queueing up for enzymatic processing: Correlated signaling through coupled degradation

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 02 May 2011

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the four referees who agreed to evaluate your manuscript. As you will see from the reports below, the referees find the topic of your study of potential interest. They raise, however, some important concerns, which, I am afraid to say, preclude its publication in its present form.

In general, the reviewers felt that this work made a potentially important contribution to our understanding of the coupling that can arise from saturated degradation in synthetic systems. They were, however, not convinced of the endogenous relevance of this phenomenon -- a point independently raised by three of the reviewers. The third reviewer specifically indicated that this issue should be directly tested, and suggests a possible experimental design. In addition, the reviewers had a series of more specific concerns, including requests for additional detail in several cases, particularly on the different purposes of the stochastic vs deterministic models.

The editor notes that the current manuscript is too long for our Report format (limited to 3 Figures and 22,000 characters without the Methods). As such, we ask that the revised work be formatted as a full-length Article; please see our Instructions to Authors for the format guidelines (www.nature.com/msb/authors). At your discretion, this format would allow you to include additional Figures, or to move portions of the Supp. Methods, to the main manuscript.

Also, with your revised manuscript, we ask that you provide machine-readable versions of your mathematical models in a common format (SBML is preferred when appropriate). We also encourage you to submit models to a public repository like BioModels or JWS Online, and to provide numerical versions of the experimental results as "figure source data" (e.g.

<http://tinyurl.com/365zpej>). This sort of figure-associated data may be particularly appropriate for Fig 2. Guidelines have been pasted at the end of this email.

If you feel you can satisfactorily deal with these points and those listed by the referees, you may wish to submit a revised version of your manuscript. Please attach a covering letter giving details of the way in which you have handled each of the points raised by the referees. A revised manuscript will be once again subject to review and you probably understand that we can give you no guarantee at this stage that the eventual outcome will be favorable.

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Yours sincerely,

Editor
Molecular Systems Biology

Referee reports

Reviewer #1 (Remarks to the Author):

The manuscript by Cookson et al. is a part of a series of studies (Mather et al 2010, Mather et al 2011) on a stochastic queueing model that describes the simultaneous degradation of different proteins that compete for the same enzyme(s). In the overloaded regime, the protein levels increase synchronously which results in temporal cross-correlations between concentrations of different protein species. The model makes both qualitative and quantitative descriptions. The latter one includes predictions about mean values and coefficient of variations. The recently published study by Mather et al 2010 presents the theory. The current manuscript presents experiments designed to support the theory. It is well written and analyses an interesting phenomenon.
One concern is that the authors do not make clear what their stochastic and deterministic models can or cannot predict. Why is there a need for both models? What is the connection between the stochastic queuing model and the deterministic ODE model?
1) The stochastic queuing model as presented in Cookson et al describes steady-state distributions and consequently cannot account for time-dependent, dynamic processes. In particular, it cannot be used to study a time-dependent driving force as illustrated in Fig 1d and it cannot address Experiments 2 and 3 and the corresponding findings illustrated in Fig 3 and 4. Due to this limitation, Cookson et al introduce the ODE model. If so, this should be stated. In any case, the motivation for using the ODE model should be clarified.
2) With the simulation in Fig 1b the authors try to demonstrate that the ODE and the queuing model yield consistent results in the limit of high protein levels. Page 19, Figure caption 1, we find "... dashed lines show the deterministic mass action solution [ODE model] which becomes accurate as the number of proteins of each type becomes large". Does this statement only hold for the special parameters used in Fig 1b? Where is the proof that the ODE model and the queuing model yield the same results in the limit of high protein levels?
3) The stochastic queuing model cannot (and does not intend to) explain the transcription rates. However, it also does not explain the sigmoid curves obtained in the first experiment and shown in Fig 2c. The authors fit the sigmoid Hill function (18) to the experimental curve and three parameters that shift that curve in y-direction. That is, the data are completely explained by a sigmoid function S(dox)+c(k) where c(k) is a constant that differs for k=1 AXA=0.8, k=2 AXA=1 and k=3 AXA=1.5. The experimentally observed sigmoid CFP curves are used for calibration of the model. Predictions

of the queuing model can then be found in Fig 2d and S2a namely the YFP levels and in S2b: the covariance of CFP.
4) As mentioned above the steady-state queuing model presented in Cookson et al is by definition about steady-states and therefore cannot speak to experiments 2 and 3 and the results shown in Figs 3 and 4. However, the authors introduce an ODE model that can deal with that situation. Why is it not used to explain the data?
5) Related to issue 4): on page 11 the authors state "By taking a quantitative approach to determining ... we were able to generate a more precise numerical queuing model". In fact, the authors applied their model only to one out of three experiments. Experiments 2 and 3 were not quantitatively addresses.
6) In the manuscript some information is missing.
6a) How did the authors draw Fig 1b? It seems that they used Eqs. 8 to 13. This should be stated explicitly
6b) How did the authors compute Fig 1c and S2b? Probably, they used the expression for covariance derived in Mather et al 2010. In any case, the formula should be presented somewhere in this manuscript.
6c) In order to predict the covariance functions in Fig S2b, was there a need for fitting some new parameters? In other words, was the calibration using the sigmoid experimental response curves of Fig 2c sufficient to determine all relevant parameters?

7) The author's findings might be of particular importance in the context of synthetic biology. What is the real biological significance (other than for synthetic biology) of correlating otherwise unconnected pathways via limited supply of shared enzymes? Is this only relevant in extreme situations such as stress response in bacteria? Does the cell purposefully correlate the pathways in response to stress, or is this just a mere side effect? Under what conditions the mammalian proteosome system can be overloaded?

Reviewer #2 (Remarks to the Author):

This is an elegant piece of work with significant implications for quantitative analysis of cellular networks. The basic idea is that, when an enzyme is overloaded, it will create indirect coupling between its substrates. Guided by modeling, the authors experimentally demonstrated the presence of this mechanism for the protease ClpXP. They further demonstrated its implication for propagating oscillating signals and for coupling dynamics of different gene circuits. Both computational and experimental aspects of the work were well done.
While this idea is highly intuitive and likely generally applicable for other proteases, such coupling effect has largely been neglected in quantitative analysis of cellular networks (natural or synthetic). As such, the present study represents a major contribution. I recommend its publication at MSB with some minor revisions/clarifications, which should strengthen the paper.

1) I thought the results in Figure 4 are very interesting. However, I wish to see more time courses from different cells. Also, it will be very helpful to present the corresponding modeling results, showing how induction of CFP-LAA would slow down or destroy the oscillations. A salient feature of the presented data is that the coupling also reduced the amplitude of the GFP oscillations - I wonder if this could be predicted from modeling as well.
Related to this, on page 10, the authors wrote "some cells produced irregular oscillations ...; while others ...". But only one trace was shown for each scenario.

2) In the context of synthetic biology, this study represents another example where introduction of synthetic gene circuits could cause unexpected perturbations to the host cell, which could in turn impact the dynamics of synthetic gene circuits. The authors should note a related study from the You lab (Tan et al, 2009, Nature Chemical Biology), which demonstrates generation of bistability in part due to circuit-mediated growth retardation.

3) In the last paragraph of page 8, the authors made a set of arguments on how this mechanism would be physiologically relevant and cited DNA damage response as an example. I am not particularly sure of the significance of these arguments, even though they sound plausible. As noted above, it's quite intuitive such queueing-mediated coupling would happen whenever a critical enzyme becomes limiting. When it occurs (and to what extent) has to be determined case by case,

which requires quantitative measurements of the process of interest. In the absence of such data, I would suggest removing or tuning down these arguments. They don't appear to be critical for the main points of the paper at any rate.

4) Reference Mather 2009 is published in PRL not Biophys J.
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Reviewer #3 (Remarks to the Author):

Using a series of synthetic systems, Cookson et al. demonstrate that direct competition for a limited enzymatic resource (in this study, ClpXP) can result in indirect coupling of protein concentration. The results presented, both in single-cells and in bulk culture, provide convincing evidence of this phenomenon. The data are discussed clearly and the figures provide strong evidence that direct competition is occurring in these experiments. The authors fit their data using a framework based on queuing theory, however, the advantages of this framework over classic models of substrate competition in enzymatic processes are not discussed. While the authors provide striking evidence of indirect coupling in their synthetic system, it is not clear that such coupling occurs in natural systems (see specific points below). Despite this, the mechanism of coupling presented is novel and should be of great interest to the synthetic biology community. Although this work is suitable, in principle, for publication in Molecular Systems Biology, several concerns (detailed below) need to be addressed.

Specific concerns:
Page 6: "In healthy cells, the burden on ClpXP appears to be relatively low, with the capacity to handle an increase in tagged proteins of about 3-fold without overloading the available protease machinery." This statement is misleading as written; the work referenced appears to demonstrate that the tmRNA tagging system is under-loaded in these conditions, not the protease system.

Pages 6, 9, 11: "For example, under DNA damage conditions some critical proteins that are targets of ClpXP can increase in abundance by over 10-fold...there are likely tens of thousands of proteins competing..." These statements are too strong given the data, as none of these references show that the proteolysis system is saturated under these conditions. Indeed, Neher et al. found that under conditions of stress, some proteins exhibited lower levels of degradation. However, they went on to show that this decreased degradation was not the result of competition. Further, the example of ribosomal subunit proteins rplJ and rplO is misleading. Although these are abundant proteins, and are substrates for ClpXP, only a small fraction of the total pool of ribosomal proteins are likely degraded, even under conditions of stress.

The authors should directly test if stress conditions over-load the protease system using their CFP-LAA producing strain. By producing low levels of CFP-LAA (e.g. [ARA] ~0.2-0.4), they could start with an under-loaded system (from Figure 1). They could then subject the cell to stress. An increase in CFP-LAA detected would be consistent with overburdening the protease system. Non-specific effects of the stress condition of CFP-LAA could be controlled for using either the untagged CFP variant or a clpP- strain.

Figure 2: The text describes this as Plac/ara driving production of CFP, however the figure indicates that GFP is produced.

Page 8: The calculated in vivo degradation rate (76-152 CFP/min/ClpX) is ~100x greater than degradation rates of similar substrates measured in vitro. Farrell et al. (2005) proposed that this discrepancy could be accounted for by rapid substrate degradation before CFP folding. Given the relatively slow rates of CFP/YFP folding, it seems likely that in the under-burdened regime, most proteins are degraded before folding. However, in the over-burdened regime, many of the proteins would be degraded after having folded (resulting in degradation up to 100x slower).

The model does not currently appear to account for this discrepancy and it is not obvious to this reviewer how incorporation of different degradation rates in these different regimes would affect the fits presented. It would be useful for the authors to address the discrepancy in the observed degradation rate and comment (in the main text) how this parameter affects the model.

Page 12: Throughout the paper, the authors convincingly demonstrate that competition for a limited resource, such as ClpXP, can result in correlated behavior. A brief discussion of other limited enzymatic resources (e.g. transcription factors, enzymes used in post-translational modification, etc.) that could be used to generated coupled behavior would greatly extend the generality of the paper.

Supplementary Information page 3: The controls demonstrating equal antibody affinity for GFP, CFP, and YFP should be described.

Reviewer #4 (Remarks to the Author):

To study the cross-talk between two networks that are independent except for having common degradation mechanisms, the authors investigate a synthetic gene network that is composed of two different fluorescence proteins that are expressed independently but have identical degradation tags. The tags are recognized by ClpXP. Under the condition that the number of ClpXP is limited, they found significant correlations in the two fluorescence protein concentrations. Their experimental results were explained by using a stochastic model of gene expression. In their previous publication, they showed that the model was directly related to mathematical queuing theory. In this manuscript, they verified the theoretical prediction based on the stochastic model and furthermore provide a novel insight on designing synthetic circuits by using shared post-translational processes. The paper is well written and the length of the paper is appropriate. There are no major issues with the manuscript and it is recommended for publication after the following minor revisions.

1. CFP is mis-spelled as GFP in Fig.2b (y-axis and legend).

2. Figure 2d and Figure 3c look quite different: In Figure 3c, there is no flat plateau in the low expression region. This seems to imply that Figure 3c is operated in the burdened state only (at least away from the uncorrelated state). As shown in the correlation graph (the inset graph), the correlations are still significant for all time. But in the figure caption, it was stated that "....representing transitions between the under and overburdened states." Maybe the issue here is the choice of the word "under-burden", of which the meaning is not clear.

3. The figure 4 shows how the slow-down in the degradation can affect the dynamics. The similar mechanisms related to degradation and its effect on the dynamics was studied theoretically in gene regulatory networks, regarding to retroactivity [Del Vecchio et al MSB 2008] and fan-out [Kim and Sauro, BME 2010]. It would be appropriate to reference these articles.

1st Revision - authors' response 26 July 2011

1st Editorial Decision 24 October 2011

Thank you again for submitting your revised work to Molecular Systems Biology. We have now heard back from two of the three referees who agreed to evaluate your revised study, and we have decided to render a decision now to avoid further delay. As you will see, the referees felt that the revisions made had satisfied their main concerns, and they are now largely supportive of publication. The first reviewer, however, has some remaining minor concerns and makes suggestions for modifications, which we ask you to address in a final revision of this work.

When preparing your revision we ask that you also address the following format and content issues:

1. Please provide machine-readable versions of your mathematical models in a common format (SBML is preferred when appropriate). We also strongly encourage you to submit models to a public repository like BioModels or JWS Online, and to incorporate the relevant accession numbers the methods section of your manuscript.

2. Molecular Systems Biology generally requires that authors provide the underlying data for all key experiments. To help authors present such data in a useful manner, we provide a new functionality that allows readers to directly download the 'source data' associated with selected figure panels (e.g. <http://tinyurl.com/365zpej>). We strongly encourage you to provide the numerical data underlying the experimental results presented in Figures of the main manuscript (particularly, Figures 3 and 4). Please see our Instructions of Authors for more details on preparation and formatting of figure source data (<http://www.nature.com/msb/authors/index.html#a3.4.3>).

Thank you for submitting this paper to Molecular Systems Biology.

Sincerely,

Editor - Molecular Systems Biology
msb@embo.org

Referee reports:

Reviewer #1 (Remarks to the Author):

Review: The revised manuscript by Dr Hasty and colleagues (2nd round):

Most of the comments have been satisfactory addressed. I recommend the manuscript for publication, provided the following minor issues are fixed:

1. Clarification: The supplement states that the ODE approximation of the stochastic queuing model was used to fit the parameters. But do fitted curves in Fig 3 actually depict simulations of the stochastic model as mentioned in the figure legend or the ODE approximation?
2. In the same context, the supplement states that the ODEs approximate the stochastic model well. However, this statement requires a justification, at least the inclusion of a supplementary figure that compares the ODE approximation and the full stochastic model for the fitted parameters. This can be done readily.
p5: It should be stated more clearly that the balance point depends on lambda1 and lambda2.
p6: sigma^s should be explained better.
p6: Figure 2b does not resemble an ultrasensitive Hill function (Hill exponent > 1). The response looks more like a shifted Michaelis Menten (Hill exponent = 1).
p8: Fig. 3a,b should be compared with Fig 2b. A short comment suffices.

p8: A figure showing the actual correlations (not just mean) should be included.

Reviewer #3 (Remarks to the Author):

The edited manuscript by Cookson et al. satisfactorily addresses my prior concerns. The work provides an interesting, quantitative model to explain previously observed phenomenon and, in my opinion, warrants publication in Molecular Systems Biology

2nd Revision - authors' response 11 November 2011

