Single-cell zeroth-order protein degradation enhances the robustness of synthetic oscillator

Wilson W Wong[1], Tony Y Tsai[2], James C Liao[1*]
1. Department of Chemical and Biomolecular Engineering, 2. Department of Biomathematics,
University of California, Los Angeles, California, USA
*email: liaoj@ucla.edu

Introduction

One of the most characterized protein degradation systems in *Escherichia coli* is the AAA+ protease family (e.g., ClpXP and ClpAP). These proteases recognize and degrade *ssrA*-tagged proteins. The *ssrA* tag and its variants were fused to many proteins to reduce their half-lives for various synthetic circuits. *In vivo* data[1] showed that *ssrA*-tagged proteins display a first-order degradation kinetics, suggesting a relatively high K_m, while *in vitro* data[2] showed a much lower K_m (75nM) , leading to a zeroth-order kinetics. Since protein degradation is an important step in synthetic oscillators, this discrepancy prompted us to examine the kinetic order of proteolysis in detail. Interestingly, we discovered a discrepancy between single-cell and population measurements that is due to a wide distribution of the protein expression level. Using the measured zeroth order kinetics for protein degradation in a model analysis, we found that the synthetic gene-metabolic oscillator we previously developed was much more robust.

The ssr-A tagged protein degradation exhibits a zeroth-order kinetics in single cell measurement

To determine the kinetic order of proteolysis, we fused two different *ssrA* tags, LAA and ASV, to the Green Fluorescent Protein (GFP), and expressed them under an IPTG-inducible promoter in glucose medium. The LAA tag is naturally found in *E. coli* and the ASV tag is a modified version of LAA, with a longer half-life[1]. We acquired single-cell and population measurement using quantitative time-lapse fluorescent microscopy. Interestingly, the degradation dynamics of the LAA-tagged GFP for individual cells displayed a zeroth-order kinetics (Fig. 1A), indicating that the protein level is significantly higher than the K_m of the protease in Michaelis-Menten kinetics. However, when measured in a bulk solution, the degradation dynamics exhibited a first-order kinetics (Fig. 1B), indicating the protein level is much lower than the K_m. In contrast, GFP tagged with ASV displays first-order degradation kinetics at both the single-cell and the population levels (Figs. 1C and D).

Discrepancy between single-cell and population measurements is explained by wide distribution of protein or protease levels

In the LAA experiment, we found that the initial protein level displayed a long-tailed distribution with a range of 160 fold. Averaging the single cell data produced a first order kinetics, consistent with bulk results. Therefore, we tested the hypothesis that wide

distribution of initial protein level resulted from protein expression noise causes this discrepancy. We developed a stochastic model based on the Gillespie algorithm to simulate proteolysis with various combination of protein and protease distribution. Together with analytical derivation[4], we concluded that the wide distribution of protein or protease level could both distort the proteolysis kinetic in population average, while long-tailed distribution of initial protein level along is sufficient to produce the apparent first-order kinetics at the population level from the zeroth-order single-cell dynamics.

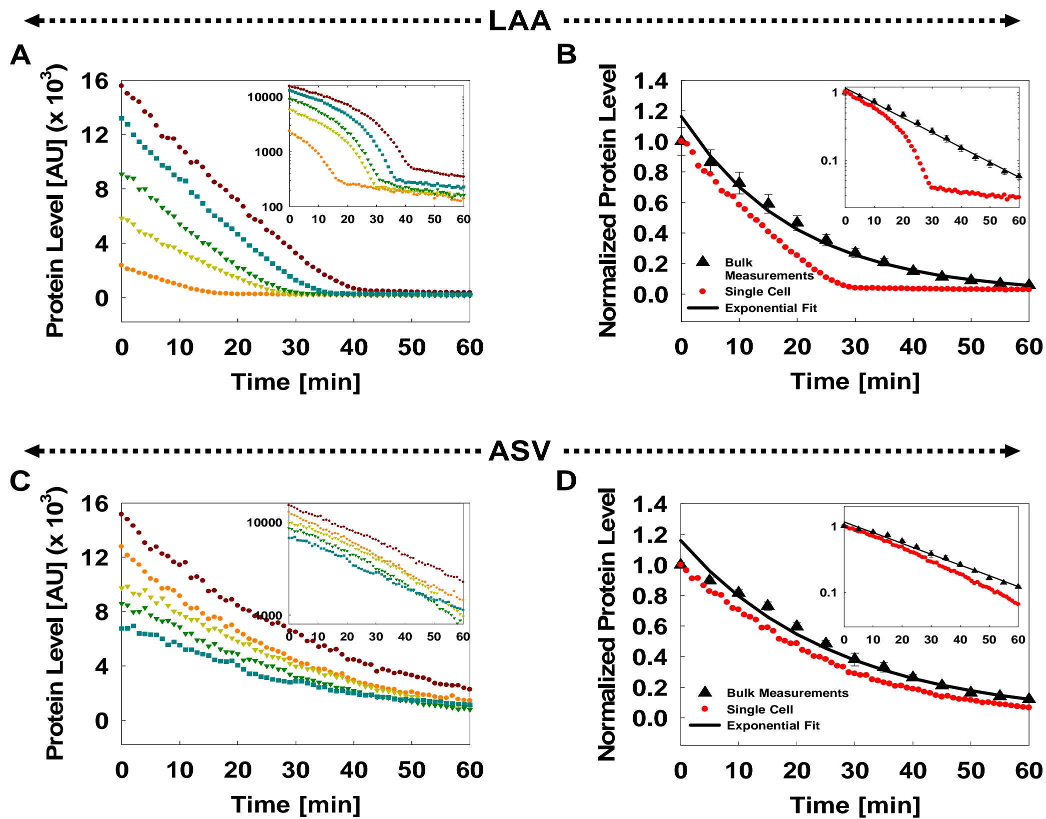

Figure 1: Experimental results reveal discrepancy of protein degradation dynamics between single-cells and population level. (A, C) Time-course measurements of protein degradation for individual cells. The kinetics is zeroth-order for LAA-tagged GFP (A) and is first-order for ASV-tagged GFP (C). These data are representative of more than four independent experiments. Insets: when plotted on the semi-log scale, the single-cell degradation dynamics is curved for LAA, because it is zeroth order, whereas the dynamics is linear for ASV. (B, D) The bulk degradation kinetics measured by fluorescence plate reader. The kinetics measured in bulk (▲) is first order for both LAA and ASV. The error bar represents average of four samples. The solid line is an exponential fit of the data. The kinetics of one of the single-cell measurements is also plotted for comparison (●). The data are normalized against the initial time point to facilitate comparison. Insets: when plotted on the semi-log scale, the bulk degradation kinetics is close to linear (first-order), whereas the single-cell dynamics is curved (zero-order) for LAA only.

Zeroth-order proteolysis enhances the robustness of gene-metabolic oscillators

To investigate the effects of proteolysis kinetic on synthetic oscillators, we examined the model of metabolator[3], a synthetic gene-metabolic oscillator (Fig 2A). We modified the first order proteolysis term in the original model with the Michaelis-Menten kinetics for protein

degradation, $R_d = \frac{k_d[GFP]}{K_m + [GFP]}$, where K_m equals 75nM[2]. Thus, the current model[4] includes a protein degradation kinetics that falls in the zeroth-order regime and a first-order protein dilution due to cell growth. Fig 2B and C showed that when zeroth order proteolysis is incorporated in the model, the oscillatory region enlarges significantly, making the design of the synthetic oscillator more robust.

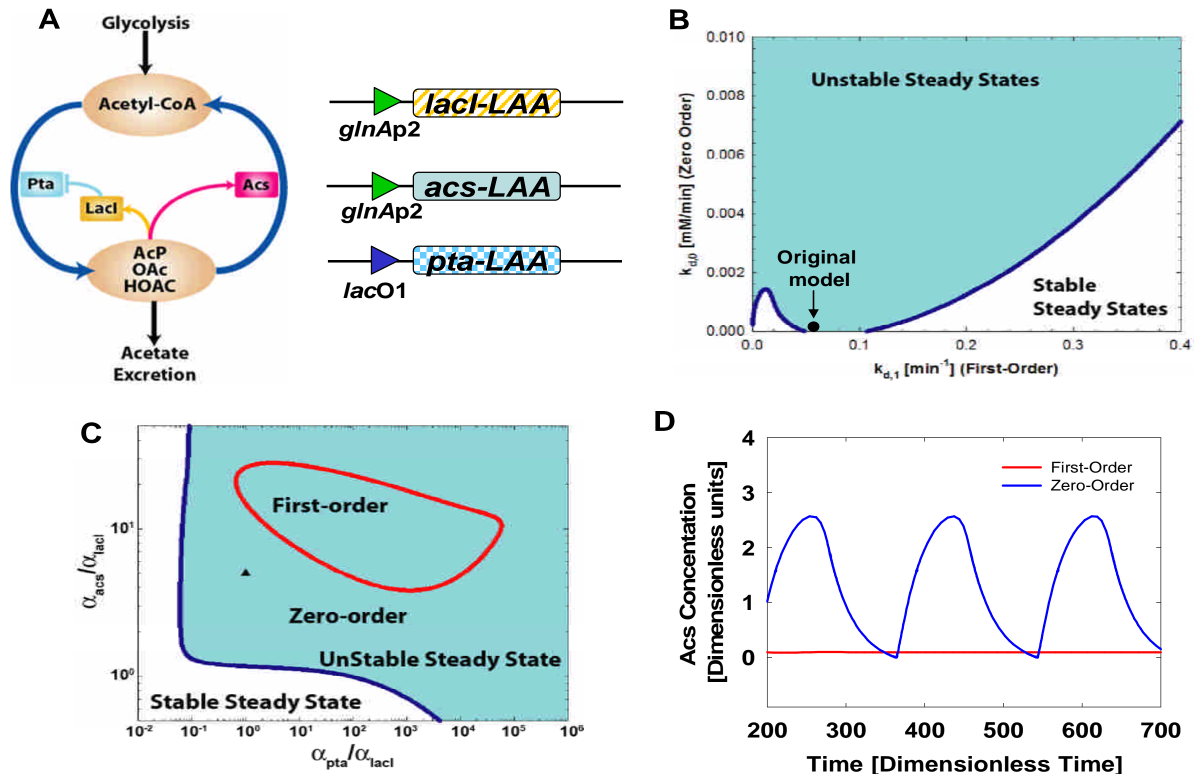

Figure 2: Effect of zero-order degradation kinetics on the metabolator[3]. (A) Network diagrams of the metabolator. The promoter glnAp2 is activated by AcP. The promoter lacO1 is repressed by LacI. All proteins are tagged with the LAA degradation tag. (B) Phase diagram of the zeroth-order degradation rate, kd,0, and first-order degradation rate, kd,1, for the metabolator model. The presence of zeroth-order protein degradation enlarges the parameter space for oscillation. When the zero-order degradation rate is zero (x-axis), the current model reduces to the original model. The point on the x-axis indicates the degradation rate used in the original metabolator model. (C) Phase diagram of the relative copy numbers acs and pta to lacI when the degradation kinetics is zeroth- and first-order. (D) Oscillation dynamics using parameters that lay outside of the first-order region, but inside the zero-order region

Reference

1. Andersen, J.B. (1998) New unstable variants of green fluorescent protein for studies of transient gene expression in bacteria. *Appl. Environ. Microbiol.*, **64**, 2240-2246.
2. Hersch, G.L., Baker, T.A. and Sauer, R.T. (2004) SspB delivery of substrates for ClpXP proteolysis probed by the design of improved degradation tags. *Proc Natl Acad Sci U S A*, **101**, 12136-12141.
3. Fung, E., Wong, W.W., Suen, J.K., Bulter, T., Lee, S.G. and Liao, J.C. (2005) A synthetic gene-metabolic oscillator. *Nature*, **435**, 118-122.
4. Wong, W.W., Tsai, T.Y., Liao, J.C. (2007) Single-cell zeroth order protein degradation enhances the robustness of synthetic oscillator. *Mol. Sys. Bio.*, 3:130