Timescale of degradation-driven protein level fluctuation in the yeast *Saccharomyces cerevisiae*

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Abstract

Generating robust, predictable perturbations in cellular protein levels will advance our understanding of protein function and enable control of physiological outcomes in biotechnology applications. Previous studies have shown that controlling RNA transcription achieves perturbations in protein levels over a timescale of several hours. Here, we demonstrate the potential for harnessing the protein degradation machinery to achieve robust, rapid control of a specific protein level in the yeast *Saccharomyces cerevisiae*. Using a light-driven protein degradation machinery and red fluorescent proteins as reporters, we show that under constant transcriptional induction, repeated triangular fluctuations in protein levels can be generated by controlling the protein degradation rate. Consistent with previous results using transcriptional control, we observed a continuous decrease in the magnitude of fluctuations as the modulation frequency increased, indicating low-pass filtering of input perturbation. However, compared to hour-scale fluctuations observed using transcriptional control, modulating the protein degradation rate enabled five to ten minute-scale fluctuations. Our study demonstrates the potential for repeated control of protein levels by controlling protein degradation rate, at timescales much shorter than that achieved by transcriptional control.

Introduction

Extensive research led to insights on how the dynamics and complexity of intracellular regulatory networks are organized to maintain a cellular response (Huang and Ingber 2000, Goldberg 2007, Huang and Ingber 2007, Vogel and Marcotte 2012, Ciechanover 2017). One form of regulation is protein turnover, which refers to the continual renewal or replacement of intracellular proteins. The output of this process is the steady-state intracellular protein concentration, maintained dynamically by continuous production and degradation. The steady-state shifts in response to extracellular stimuli or pathophysiologic conditions, which cause changes in production or degradation rates (Baracos 2000, Pratt, Petty et al. 2002, Mizushima and Klionsky 2007, Hinkson and Elias 2011, Ciechanover 2017, Kneppers, Langen et al. 2017). A powerful approach for gaining fundamental insights for such complex systems would be a bottom-up approach, in which the protein turnover is controlled using external stimuli.

Although production and degradation both contribute to protein turnover, control strategies have largely focused on gene transcription. Multitude of factors involved in transcriptional regulation and its impact on biology and disease have been well characterized (Hill and Treisman 1995, Tautz 2000, Bintu, Buchler et al. 2005, Lee and Young 2013). Numerous previous studies have demonstrated control of transcription using optical and chemical approaches to modulate protein levels (Bennett, Pang et al. 2008, Milias-Argeitis, Summers et al. 2011, Toettcher, Gong et al. 2011, Uhlendorf, Miermont et al. 2012, Melendez, Patel et al. 2014, Milias-Argeitis, Rullan et al. 2016). On the other hand, the effect of degradation process on protein turnover control has been far less characterized. In nature, regulation of protein levels by modulating the degradation rate have been discovered in several proteins with high importance in physiology and drug

development (Hellmann and Estelle 2002, Burger and Seth 2004, Varshavsky 2008). For instance, the tumor suppressor protein p53 is kept on at a low steady-state level by Mdm2 that mediates its turnover through protein degradation (Adams 2003, Zilfou and Lowe 2009, Brady and Attardi 2010). Upon DNA damage by γ radiation, the steady-state is perturbed, and p53 turnover exhibits an oscillatory behavior indicating that state transitions are occurring repeatedly (Geva-Zatorsky, Rosenfeld et al. 2006). These examples demonstrate the robustness of regulating degradation rates to control protein levels.

A major finding from controlling transcription was the experimental observation of low-pass filtering of transcriptional induction in the yeast *S. cerevisiae* (Bennett, Pang et al. 2008). In this previous study, Bennett and colleagues modulated the transcription rate of a fluorescent protein by controlling the extracellular concentration of glucose (transcriptional repressor) over a constant level of galactose (transcriptional inducer) using a microfluidic system (Bennett, Pang et al. 2008). Protein turnover faithfully responded to slow glucose fluctuations, while being insensitive to high-frequency fluctuations. Such low-pass filtering behavior may be important in preventing distortion of cellular response by high-frequency noise signals from the stochastic intracellular environment.

Here we applied a light-activated protein degradation machinery (Renicke, Schuster et al. 2013) in *S. cerevisiae* to study the dynamics of protein level fluctuations driven by changes in the degradation rate. In this approach, a reporter protein is fused to a blue light-activated degradation signal (photo-sensitive degron, psd), which represents a protein with controllable degradation rates (**Fig. 1**). We take advantage of the light-controlled degradation machinery to analyze the frequency-dependent fluctuations in protein turnover, induced by varying frequency of light inputs that modulate the protein degradation. Square-wave blue light with varying frequencies was

delivered to *S. cerevisiae* cells constitutively expressing psd and Δ psd constructs (**Fig. 1**). Using this system, we observed that upon application of low-frequency square waves of light, the protein concentration oscillates with the same corresponding frequency. When the blue light input frequency increases, the amplitude of oscillation decreases, characteristic of a low-pass filter response. However, compared to previously reported transcriptionally controlled fluctuations, the response to degradation-driven fluctuations was faster, enabling protein fluctuations on the order of 10 minutes. This suggests that compared to controlling gene expression, regulating protein degradation allows a much faster response in protein levels.

We applied the system identification approach to model the impact of light-activated degradation on protein turnover. System identification is widely used to study the response of a system under time-varying stimulations to construct models of dynamical systems from the measured input and output data (Ljung 1998). We defined a model for the protein turnover based on system identification to better understand its low-pass filtering characteristics. We modeled the dynamic of protein turnover with protein production and degradation as inputs and protein level as the output. Based on our experimental observation on how the protein level changes under different input fluctuations, we adaptively estimated the model parameters. The estimated model parameters were used for the frequency-domain analysis, which allowed quantitative comparison of degradation driven low-pass filtering to previous transcriptional control results.

Figure 1.

Figure 1. Schematic of photo-sensitive degron (psd) constructs used in this study. In the psd construct (a), a protein degradation signal sequence derived from the ornithine decarboxylase is fused to the blue-light sensing light-oxygen-voltage (LOV) domain from *Arabidopsis thaliana*. Upon blue light activation, the degradation signal accessibility increases, resulting in faster protein degradation. The Δ psd construct (b), contains all components of psd except the degradation signal sequence.

Results and Discussion

Low-pass filtering of degradation-driven protein level fluctuations in the yeast *S. cerevisiae*

The psd module (**Fig. 1**) comprises of light oxygen voltage 2 domain from *Arabidopsis thaliana* (*AtLOV2*), fused with a derivative of the carboxy-terminal degnon of murine ornithine decarboxylase (ODC), enabling the control of ubiquitin-independent proteasomal degradation rate upon blue light illumination (Renicke, Schuster et al. 2013). While *AtLOV2* is sensitive and responsive to blue light, the ODC degradation signal is responsible for increased protein degradation (**Fig. 1**). To observe the effect of fluctuations in protein degradation rate on protein turnover, we applied pulses of blue light and monitored the fluorescence emission from the red fluorescent protein tagRFP fused to psd (**Fig. 1a**). To validate protein degradation due to the ODC degradation signal, we generated a control construct that lacks the signal sequence, termed Δ psd (**Fig. 1b**). The expression of these constructs in yeast *S. cerevisiae* was continuously driven using the constitutive GAL_{1-10} promoter under galactose. To validate continuous expression, we measured the *tagRFP* transcript level by extracting the mRNA from yeast cells and performing a quantitative polymerase chain reaction (qPCR) of the transcript (**Fig. 2a**, see **Supp. Fig. 1** and **Supp. Table 1** for RNA quality control and primers used). We illuminated the cells with maximum blue light intensity used in this study (light power density of 0.027 mW/mm^2 ($106.02 \text{ } \mu\text{mol/m}^2\text{s}$)) and the longest duration ($1/f = 100 \text{ min}$ where f indicates illumination frequency) to assess the effect of illumination on gene expression. The mRNA was extracted from cells at the start of the experiment and at the end of each illumination cycle (see inset in **Fig. 2a**). Two primer sets that bind to different regions of *tagRFP* were used for amplification, along with primers for two previously validated housekeeping genes, *TFC1* and *UBC6* (Teste, Duquenne et al. 2009), as a reference. Expression of *tagRFP* remained unchanged for 200 minutes and a significant increase was detected between 200 to 300 minutes (**Fig. 2a**, $p = 0.014$ and 0.023 , student's t-test comparing Δ Ct at 200 min. to that at 300 min. for the two *tagRFP* primer sets, respectively ($n = 3$ independent

138 culture preparations)). This result indicates that the GAL_{L1-10} promoter allows continuous
139 expression of the tagRFP constructs, unhindered by light illumination.

140

Figure 2.

Figure 2. Experimental validation and observation of degradation-driven protein level fluctuations in yeast expressing tagRFP fused to psd or Δ psd. (a) qPCR analysis of *tagRFP* transcripts during blue light illumination (inset indicates illumination timepoints). Two sets of primers (primer sets 1 and 2) that bind to different regions of *tagRFP* were used, and previously validated housekeeping genes *TFC1* and *UBC6* were used as references. Δ Ct values were calculated by subtracting the Ct of *tagRFP* from the average of the Ct of the two reference genes. *: $p < 0.05$, student's t-test comparing Δ Ct at 200 min. to that at 300 min. Error bars indicate standard deviation from $n = 3$ independent culture preparations. **(b)** Normalized fluorescence level of tagRFP-psd and tagRFP- Δ psd under dark (top panels); tagRFP- Δ psd illuminated with blue light cycles (bottom panels). Solid lines indicate the mean fluorescence intensities and lighter lines indicate standard deviation from $n = 10$ to 15 colonies of cells. **(c)** Fluorescence of tagRFP-psd under varying frequencies of blue light illumination. f denotes the temporal frequency and $1/f$ indicates cycle period. Solid lines indicate the mean fluorescence intensities and lighter lines indicate standard deviation from $n = 10$ to 15 colonies. **(d)** Experiment for $1/f = 20$ min. was repeated with 25 colonies of cells. Same intensity of blue light (power density of 0.027 mW/mm^2 ($106.02 \text{ } \mu\text{mol/m}^2\text{s}$)) was used in all panels. In panels (b) and (c), data for each illumination frequency was obtained in separate experiments using independent cultures of cells.

Since the cell cycle of *S. cerevisiae* is on the order of 100 min. (Herskowitz 1988), we observed cell division during the repeated illumination cycles (**Supp. Fig. 2**). Proteins in each cell are divided into daughter cells, resulting in dilution of proteins. One approach to quantify the entire amount of proteins throughout cell division is to sum the fluorescence intensity from a colony of cells originating from mother cells (Talia, Skotheim et al. 2007). In agreement with previous studies, we found that summing the fluorescence intensity of a colony of cells results in continuous increase (red line in **Supp. Fig. 2**), while the fluorescence intensity divided by the area of cells fluctuates as the cells divide (blue line in **Supp. Fig. 2**). To capture the change in protein level decoupled from cell division, we used the sum of fluorescence intensities of a colony of cells as a measure of protein levels throughout this study.

We then tested the effect of pulses of blue light on yeast cells expressing tagRFP-psd or tagRFP-Δpsd. In the absence of blue light, the normalized fluorescence level of tagRFP-psd and tagRFP-Δpsd (see **Materials and Methods** section for calculation) showed a continuous increase over 300 min. (**Fig. 2b**). The fluorescence level of tagRFP-Δpsd was generally higher than that of tagRFP-psd (**Fig. 2b**), likely due to the fact that the LOV domain conformation is in equilibrium between active and inactive states even under dark (Strickland, Yao et al. 2010), resulting in slight background degradation even without blue illumination. As anticipated, the level of tagRFP-Δpsd was unresponsive to two distinct frequencies of illumination (**Fig. 2b**, $f = 1/100$ mins and $f = 1/80$ mins, respectively in experiments 1 and 2). In contrast, fluorescence level of tagRFP-psd showed distinct fluctuations with varying frequency of blue light (**Fig. 2c**). We repeated the experiment 5 times, each at a distinct illumination frequency (**Fig. 2c**). Fluctuation in protein level was clearly visible up to illumination frequency of $1/f = 40$ mins, but not at the highest illumination frequency tested ($1/f = 20$ mins) (**Fig. 2c**). When the experiment for $1/f = 20$ mins was repeated with a larger

number of colonies cells, the fluctuation was discernable albeit markedly reduced (**Fig. 2d**). Based on these findings, we conclude that the output fluctuations observed in the experiment are the result of light-induced increase in protein degradation rate mediated by the psd.

The reduction in fluctuations in protein levels at higher input illumination frequency is characteristic of low-pass filtering (Antoniou 2016), and indicate that the input perturbation driving protein degradation has been filtered out at these higher frequencies. To analyze the low-pass behavior of the degradation driven protein turnover, we determined the time delay between the input blue light illumination frequency and output protein fluorescence intensity using cross-correlation. We calculated the cross-correlation (see **Supplementary Information** for calculation) for experiments using tagRFP-psd shown in **Fig. 2c**. Since the protein level decreases upon illumination, a minimum in the cross-correlation occurs (**Fig. 3**, indicated by arrows). Based on the minimum value for each experiment, the time delay between the input and output signals ranged from 1 to 15 minutes (see **Supplementary Information** for calculation). We find that the time at which minima occur shortens as the input frequency fluctuations increases, characteristic of a low-pass filtering system.

Figure 3.

Figure 3. Cross-correlation between input blue light illumination and protein fluorescence intensity. The curves are cross-correlations resulted from four experiments with varying input frequencies indicated in the inset. The arrows show the minimum (after time 0) of each curve. Note that the minimums do not directly indicate the time delay, but allow the calculation of the time delay as shown in the Supplementary Information.

Degradation-driven fluctuations in a faster maturing fluorescent protein.

Fluorescent proteins contain a chromophore formed through autocatalytic maturation (Wachter 2007, Craggs 2009, Subach and Verkhusha 2012). The fluorescence is only detected after maturation, effectively causing a slowdown of apparent protein production rate. Although not tested, a shift in protein maturation time is expected to impact the low-pass filtering behavior

when transcription rate is modulated as in Bennett *et al.*'s work (Bennett, Pang et al. 2008). In contrast, when protein degradation rate is modulated, as in our experiments, a shift in protein maturation time should not affect the low-pass filtering behavior.

To test this, we replaced tagRFP in tagRFP-psd with the fluorescent protein mCherry (mCherry-psd), which has a significantly shorter maturation time compared to that of tagRFP (maturation half-time of ~40 minutes for mCherry vs. ~100 minutes for TagRFP (Merzlyak, Goedhart et al. 2007)). We then repeated the experiment shown in **Fig. 2c** using mCherry-psd and monitored the mCherry fluorescence in colony numbers similar to that in **Fig. 2d** (25 to 30 colonies having 4 to 8 cells at the beginning of the experiment). Clear fluctuations in mCherry fluorescence were visible, down to illumination frequency of $1/f = 20$ min (**Fig. 4a**). We also conducted more experiments with higher frequencies of input ($1/f = 10$ min and $1/f = 5$ min) and detected small but distinguishable fluctuations (**Fig. 4b** and see **Supp. Fig. 4** for extended experiments). We observed consistent decrease in the amplitude of output fluctuations upon increasing the frequency of degradation fluctuation, characteristic of a low-pass filtering behavior.

Figure 4.

Figure 4. Experimental observation of degradation-driven protein level fluctuations in yeast expressing mCherry fused to psd. (a) Fluorescence of mCherry-psd under varying frequencies of blue light illumination. (b) Fluorescence of mCherry-psd under higher frequency illumination. For frequency $1/f = 20$ min., experiment in panel (a) was repeated. For all panels, solid lines indicate the mean fluorescence intensities and lighter lines indicate standard deviation from $n = 25$ to 30 colonies. Blue light intensity was 0.027 mW/mm^2 ($106.02 \text{ } \mu\text{mol/m}^2\text{s}$) for all panels.

System identification and frequency-domain analysis.

To quantitatively assess the low-pass filtering, we constructed a mathematical model for degradation-driven protein turnover using the difference equation approach we previously described (Shin 2016). The model equation can be expressed as:

$$y(n) = p_y y(n-1) + p_{pro} u_{pro}(n-1) + p_{deg} u_{deg}(n-1) \quad (1)$$

where n is the discrete-time index, $y(n)$ is the normalized fluorescence intensity at time n , $u_{pro}(n)$ is the normalized amount of galactose (transcription inducer) at time n , $u_{deg}(n)$ is the normalized intensity of blue light (degradation rate controller) at time n . Parameters p_y , p_{pro} , and p_{deg} indicate endogenous degradation, production, and light-induced degradation, respectively. These parameters can be adaptively estimated from the experimental data using the normalized least mean square (NLMS) algorithm, one of commonly used adaptive system identification algorithms (Sayed 2011). Assuming constant protein production, as indicated by relatively consistent transcription (**Fig. 2a**), eq. 1 can be simplified by approximating $u_{pro}(n)$ to 1:

$$y(n) = p_y y(n-1) + p_{pro} + p_{deg} u_{deg}(n-1) \quad (2)$$

The above equation represents a multiple-input single-output (MISO) system that can be transformed into the frequency domain using the z -transform.

$$Y(z) = \frac{p_{deg}}{z-p_y} U_{deg}(z) + \frac{p_{pro} z^2}{(z-1)(z-p_y)} \quad (3)$$

Eq. 3 illustrates the relationship between the single output $Y(z)$ and two independent inputs (blue light intensity, $U_{deg}(z)$, and the normalized galactose concentration which is assumed to be constant at 1). The equation can be re-expressed in matrix form:

$$Y(z) = \begin{bmatrix} \frac{p_{deg}}{z-p_y} & \frac{p_{pro} z^2}{(z-1)(z-p_y)} \end{bmatrix} \begin{bmatrix} U_{deg}(z) \\ 1 \end{bmatrix} = [H_1(z) \quad H_2(z)] \begin{bmatrix} U(z) \\ 1 \end{bmatrix} \quad (4)$$

$$H_1(z) = \frac{p_{deg}}{z-p_y} \quad (5)$$

$$H_2(z) = \frac{p_{pro} z^2}{(z-1)(z-p_y)} \quad (6)$$

where $H_1(z)$ shows the relationship between the output $Y(z)$ (protein fluorescence intensity) and input $U_{deg}(z)$ (blue light intensity). The unknown parameter values p_y and p_{deg} in $H_1(z)$ were estimated using the NLMS adaptive system identification algorithm (See **Supplementary Information** and **Supp. Fig. 5**). The Bode plot in **Fig. 5** (indicated as ‘Model’) shows the amplitude and phase shift response of $H_1(z)$, calculated based on the mean and standard deviation of estimated parameter values acquired from each experiment (See **Supplementary Information**). The amplitude responses clearly show low-pass filtering, indicated by a steady decrease in the amplitude of the fluctuations as input blue light illumination frequency increases (**Fig. 5a**). We performed a statistical comparison of experimental results (tagRFP-psd vs. mCherry-psd) as well as experiments to the model (tagRFP-psd vs. model, mCherry-psd vs. model). The summary of statistical comparisons in **Table 1** show that the model captured the amplitude and phase shift responses well. As for amplitude response, no significant difference was found when comparing model vs. tagRFP-psd as well as model vs. mCherry-psd (**Fig. 5a** and **Table 1**). When comparing phase shift response of model vs. tagRFP-psd, significant difference was found only for at $1/f = 100$ min and $1/f = 80$ min, but not at other frequencies (**Fig. 5b** and **Table 1**). Phase shift response of model vs. mCherry-psd was not significantly different at all illumination frequencies (**Fig. 5b** and **Table 1**). When comparing tagRFP-psd vs. mCherry-psd, mCherry-psd showed significantly smaller amplitude response at frequencies $1/f = 80$ min, $1/f = 60$ min, and $1/f = 40$ min (**Fig. 5a** and **Table 1**), but no significant difference was found for phase shift (**Fig. 5b** and **Table 1**). These results show that both tagRFP-psd and mCherry-psd showed low-pass filtering with similar time delay, and confirm that the difference in maturation time between tagRFP and mCherry do not cause a change in the low-pass filtering behavior. Comparing the Bode plot of our experiments with the previous study using transcriptional control (Bennett, Pang et al. 2008), we found that the

same amplitude of fluctuations (e.g., an amplitude of around 0.3 which is equivalent to -10.5 dB) was achieved at approximately 3-fold higher frequencies (0.06 rad/min) in degradation-driven control (achieved at frequency of 0.02 rad/min), indicating a much faster timescale. Taken together, these results demonstrate that degradation driven control is a robust approach for controlling protein levels at minutes timescale.

Although we demonstrated this capability in the yeast *S. cerevisiae*, we anticipate the timescale determined here will apply to many other cell types, considering the UPS is widely conserved among eukaryotes. In fact, light-sensitive degradation using the psd has been successfully demonstrated in cell types including human, and other mammalian cells (Sun, Zhang et al. 2017, Baaske, Gonschorek et al. 2018). Moreover, light-activation is highly suitable for feedback control since the intensity, frequency, and location of illumination can be instantly modulated. Since we demonstrated that controlling the degradation rate is sufficient to drive protein levels under constant transcription, we anticipate this approach to allow generating protein oscillations previously not achievable using transcriptional control.

Figure 5.

Figure 5. Model and experimental comparison of low-pass filtering using Bode plots. (a) Amplitude response and **(b)** phase shift response from tagRFP-psd, mCherry-psd, and model calculations as a function of input frequency. Data points indicate median and error bars indicate interquartile range (IQR) from four colonies of cells. Statistical analysis of the results is found in Table 1.

300 **Table 1.**

Set Group	f = 2π/100 (1/f = 100 min)		f = 2π/80 (1/f = 80 min)		f = 2π/60 (1/f = 60 min)		f = 2π/40 (1/f = 40 min)		f = 2π/20 (1/f = 20 min)	
	Amplitude	Phase shift	Amplitude	Phase shift	Amplitude	Phase shift	Amplitude	Phase shift	Amplitude	Phase shift
	Median (IQR)									
Model	-12.691 (3.352)	2.586 (0.027)	-14.579 (4.359)	2.554 (0.030)	-20.007 (4.805)	2.510 (0.008)	-23.572 (4.840)	2.444 (0.010)	-25.096 (4.847)	2.300 (0.016)
tagRFP-psd	-11.537 (0.642)	3.016 (0.204)	-11.293 (0.288)	2.906 (0.236)	-16.055 (0.440)	2.985 (0.157)	-17.9937 (0.446)	2.435 (0.550)	-35.654 (0.446)	2.199 (0.471)
mCherry-psd	-15.686 (2.608)	2.953 (0.173)	-15.935 (1.590)	2.710 (0.196)	-22.007 (3.538)	2.251 (0.209)	-25.2071 (4.433)	2.435 (0.314)	-34.437 (4.433)	2.042 (0.314)
P-value	0.095	0.024*	0.023*	0.030*	0.024*	0.115	0.023*	0.981	0.351	0.701

301

302 *Difference in median value of model, tagRFP-psd, and mCherry-psd assessed using Kruskal-Wallis test, P-value < 0.05

303 Gray highlighted cells in each column indicate pairs with significant difference as assessed using NEMENYI test for multiple pairwise comparisons between the

304 groups, P-value < 0.05

305

306

307 **Conclusions**

308 Here we demonstrated robust generation of controlled protein level fluctuations in the yeast *S.*
 309 *cerevisiae* using a blue-light induced protein degradation machinery. We found that by monitoring
 310 the total fluorescence of colonies of cells, protein fluctuations with minutes timescale can be
 311 reproducibly generated. By using two red fluorescent proteins with distinct maturation time, we
 312 show that the delay in protein production rate does not change the protein level fluctuation
 313 generated by modulating the protein degradation rate. The results show that controlling protein
 314 degradation rates under constant transcription as a means for modulating protein levels,
 315 demonstrating a clear pathway for feedback control of protein levels with minutes timescale.

316

317 **Materials and Methods**

318 **Cloning and expression of a photo-sensitive degron in yeast**

319 The photo-sensitive degron (psd) module used in this study has been previously described by
 320 Renicke and colleagues (Renicke, Schuster et al. 2013). A red fluorescent reporter protein
 321 (tagRFP) was fused to the N-terminus of *AtLOV2*. The tagRFP-psd construct was cloned into the
 322 pRS316 plasmid (Huang and Shusta 2005) (a gift from Dr. Eric Shusta) using restriction enzymes
 323 NotI and BsrGI (New England Biolabs) under the galactose inducible GAL_{1-10} promoter. Psd
 324 without the ODC degron (Δ psd) was generated using polymerase chain reaction and cloned using
 325 the same restriction sites. The resulting plasmids were named pRS316-tagRFP-psd and pRS316-
 326 tagRFP- Δ psd respectively. The plasmids were transformed into the yeast *Saccharomyces*

cerevisiae strain BJ5464 (BJ α , α *ura3-52 trp1 leu2 Δ 1 his3 Δ 200 pep4:: HIS3 prb1 Δ 1.6R can1 GAL*) cells, using a commercially available kit (Zymo Research). Transformed cells were grown in SD-CAA medium (0.1 M sodium phosphate, pH 6.0, 6.7 g/L yeast nitrogen base, 5.0 g/L casamino acids, 20.0 g/L glucose), supplemented with tryptophan (0.04 g/L) at 30 °C on an orbital shaker (250 rpm).

qPCR gene expression analysis

Briefly, cells containing pRS316-tagRFP- Δ psd plasmid were grown in SD-CAA medium supplemented with tryptophan (0.04 g/L) at 30 °C on an orbital shaker (250 rpm) overnight. Yeast cells grown overnight were diluted to OD₆₀₀ of 1, and the medium was replaced with the induction medium, SG-CAA (0.1 M sodium phosphate, pH 6.0, 6.7 g/L yeast nitrogen base, 5.0 g/L casamino acids, 20.0 g/L galactose) supplemented with tryptophan (0.04 g/L) and incubated at 16 °C on an orbital shaker (250 rpm) for 20 hrs. Then cells were diluted 5-fold in SG-CAA (supplemented with tryptophan) and grown at 30 °C on an orbital shaker (250 rpm) for period of 400 mins with blue light illumination with period of $1/f = 100$ mins. Cells (10 mL of culture) were collected at the end of each 100 min illumination cycle (blue light power density of 0.027 mW/mm² (106.02 μ mol/m²s)), and stored at 4 °C until analysis. RNA extraction was performed right after samples collecting samples using RNeasy Mini Kit (Qiagen cat# 74104) according to manufacturer's protocols. Quality of purified RNAs were checked by measuring the ratio of absorbance at 260 nm to 280 nm (A260/A280) by using the NanoDrop ND-1000 (Nanodrop Technologies). Integrity and concentration of RNA were assessed by using the automated electrophoresis station Agilent TapeStation 4200 (Bio-Rad).

All RNA samples were diluted in RNase/DNase free water (Fisher Scientific cat# BP28191)) at the same concentration and a volume corresponding to 500 ng was added to iScript™ cDNA synthesis kit reagents (Bio-Rad cat# 1708890) for cDNA synthesis, per kit instructions. The reaction mix was incubated for 5 min at 25 °C, followed by 30 min at 46 °C and 5 min at 95 °C and hold at 4 °C. The cDNA samples were then stored at –80 °C.

qPCR was performed by using SsoAdvanced™ Universal SYBR Green Supermix (Bio-Rad cat# 1725270). 2 µl of cDNA was used per total of 20 µl reaction mixture as a template. *TFCI* and *UBC6* genes were used as housekeeping genes. See Supplementary Fig. 1 and Table 1 for RNA quality control and primers used. Cycling conditions included a polymerase activation step of 98°C for 2 min and 40 cycles of 98°C for 2 sec and annealing /extension at 60°C for 5 sec followed by melt curve analysis from 65 to 95°C in 0.5°C increments. Size of amplicons for each gene were verified on a 2% agarose gel. ΔCt values were calculated by subtracting the threshold cycle (Ct) of *tagRFP* from the average of the Ct of the two reference genes.

Light-induced degradation and imaging

Yeast cells grown overnight were diluted to OD₆₀₀ of 1, and the medium was replaced with the induction medium, SG-CAA (0.1 M sodium phosphate, pH 6.0, 6.7 g/L yeast nitrogen base, 5.0 g/L casamino acids, 20.0 g/L galactose) supplemented with tryptophan (0.04 g/L) at 16 °C on an orbital shaker (250 rpm) for 20 hrs. Cells were kept in the dark during the entire induction period to prevent unwanted degradation. To activate degradation, blue light (470 nm) was delivered at a power density of 0.027 mW/mm² (106.02 µmol/m²s) using a light emitting diode (LED, Thorlabs). For imaging tagRFP, green light (530 nm) was delivered at a power density of 0.69 mW/mm².

Extra precautions were taken to prevent exposure to light after induction until the experiment was completed. For imaging, cells were put into 96 wells plates coated with Concanavalin A (2 mg/mL, Sigma Aldrich) to attach the cells to the bottom of plates. Unbound cells were washed with SG-CAA medium.

To quantify the protein level, the sum of fluorescence intensity of each colony was measured over the experiment. To identify regions with colonies, ‘Otsu dark’ auto threshold method in ImageJ library was used. The mean fluorescence intensity over all colonies of all 5 experiments is defined as the normalization factor. The sum for each colony was then normalized to the normalization factor. Additionally, an offset specific to each experiment is used in all experiments’ dataset so that the first value starts at a normalized value of 1. In each experiment, measurements from at least 10 colonies were recorded and averaged.

LED intensities were controlled by an Arduino (See **Supplementary Information**) controlled using the μ Manager (Edelstein, Tsuchida et al. 2014). The system snaps images from all samples every minute while exposing psd and Δ psd samples to blue LED controlled via the Java programming language (See Supplementary Information). The image processing/analysis was done using the imageJ/ μ Manager libraries (See **Supplementary Information** for more details and the codes are available in Github (Mahrou 2021)).

Statistical analysis

To generate the Bode plot, the median phase and amplitude values from each of the 5 frequencies from 4 colonies of cells and median of 4 parameter values estimated for the model were calculated. The median values between the three datasets (Model, tagRFP-psd, and mCherry-psd) were compared using the Kruskal-Wallis test. When the median differences between the

groups were statistically significant (P -value <0.05), the NEMENYI test was also used to compare the median differences between groups of two. Statistical analyses were performed using real statistics in excel (Charles Zaiontz, www.real-statistics.com).

Acknowledgements

This work was funded by the University of Connecticut, the Alzheimer's Association (2019-AARG-NTF-640971), and NIH (1R21NS111358-01A1). We thank Bo Reese and Lu Li of Center for Genome Innovation within the Institute for Systems Genomics at the University of Connecticut for their help in the RNA quality assessment and qPCR analysis. The authors declare that they have no conflicts of interest with the contents of this article.

Author Contributions

B.M. designed and performed the experiments, derived the model and analyzed the data. A.P. cloned the psd constructs and conducted the qPCR gene expression analysis. M.H. conducted the statistical analysis. Y.C. and Y.S. supervised the project and assisted with the analysis and the interpretation of data and model. All authors discussed the results and contributed to the manuscript.

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