Supplemental Materials

Molecular Biology of the Cell

Airoldi et al.

AIROLDI ET AL., SUPPLEMENTARY INFORMATION

1. Supplementary figures

Figure S1. Cell growth in different nitrogen-limited batch cultures. The final density of batch cultures grown in a range of nitrogen concentrations was determined using (A) proline, (B) arginine, (C) glutamine (D) ammonium, (E) allantoin and (F) urea as the sole nitrogen source. The concentration of each nitrogen-containing compound was varied such that the molarity of nitrogen was identical in each medium. A linear model (line) was fit to the data to estimate the yield.

Figure S2. Cell growth in different nitrogen-limited chemostat cultures. The steady-state density of chemostat cultures growing at different dilution rates in different nitrogen sources was determined for media containing (A) proline, (B) arginine, (C) glutamine (D) ammonium, (E) allantoin and (F) urea as the sole nitrogen source. In all chemostats the feed medium contained 800μM nitrogen. The final density for batch cultures grown in 800μM nitrogen using the same nitrogen sources (i.e. data from Figure 1) is shown on each plot for reference at D = 0 hr^{-1}.

Figure S3. Reproducibility of the growth rate response of gene expression. Comparison of gene expression response to growth rate in the current study with data reported in (8). The magnitude of response to growth rate is in good agreement between the two studies (n = 5,542 genes, Pearson correlation = 0.78). The gray dotted line demarks x = y values.

Figure S4. Perturbation of nitrogen limited chemostat by simultaneous addition of glutamine and proline. We modeled the change in cell density upon addition of a mixture of 20μM glutamine and 40μM proline as a continuous function of time (blue line) and predicted the instantaneous growth rate on the basis of global gene expression at discrete time points (red dots).

Figure S5. Dynamics of cell size in response to transient relief from nitrogen limitation. The distribution of cell size was measured in response to a large and small pulse of nitrogen to nitrogen-limited chemostats. We detected a transient increase in median cell size, measured by a Coulter counter, in response to a pulse of (A) 400μM glutamine but not (B) 40μM glutamine. Similarly, we detected an increase in the median cell size in response to a pulse of (C) 800μM proline but not (D) 80μM proline.

Figure S6. GO term enrichment of response to growth rate in steady-state and dynamic conditions. GO categories were systematically tested for non-random clustering. Significant functional categories are indicated.

Figure S7. Acceleration of mRNA decay rates in response to a pulse of proline. A small number of genes show evidence of accelerated degradation when a pulse of proline is added to cells growing in an ammonium-limited chemostat.

2. Supplementary tables

Table S1. Identification of transcripts that are differentially expressed as a result of nitrogen-limitation. We analyzed data from Brauer et al (2008) comparing a model in which gene expression was modeled as a function of nutrient limitation and growth rate with one in which gene expression was modeled simply as a function of growth rate as in Brauer et al (2008). Column Headers: Avg = the intercept or the average expression across all samples, Growth Rate = slope of regression, Glucose, Leucine, Ammonium, Phosphorous, Sulfur, Uracil = nutrient specific shift in expression, R-squared = proportion of variance explained by the model, Fval = the FDR-corrected p-value of the F-statistic testing whether the model including a nutrient specific term and a growth rate term explain a greater portion of the variance than a model with only a growth rate term. p-val(Growth Rate) = significance of growth rate response, p-val(Glucose), p-val(Leucine), p-val(Ammonium), p-val(Phosphorous), p-val(Sulfur), p-val(Uracil) = significance of nutrient-specific effects. Membership in each of the nitrogen-responsive regulons (1 = member, 0 = non-member) as defined in Godard et al (2007) is indicated.

Table S2. Identification of transcripts differentially expressed as a function of variation in nitrogen source in nitrogen-limiting conditions. We compared a model in which gene expression was modeled as a function of nitrogen limitation and growth rate with one in which gene expression was modeled as a function of growth rate. Column Headers: Avg = the intercept or the average expression across all samples, Growth Rate = slope of regression, Allantoin, Arginine,Glutamate, Glutamine, Proline, Urea, = nitrogen specific shift in expression, R-squared = proportion of variance explained by the model, Fval = the FDR-corrected p-value of the F-statistic testing whether the model including a nutrient specific term and a growth rate term explain a greater portion of the variance than a model with only a growth rate term. p-val(Growth Rate) = significance of growth rate effect, p-val(Allantoin), p-val(Arginine), p-val(Glutamate), p-val(Glutamine), p-val(Proline), p-val(Urea) = significance of nutrient-specific effects. Membership in each of the nitrogen-responsive regulons (1 = member, 0 = non-member) as defined in Godard et al (2007) is indicated.

Table S3. ANCOVA analysis of gene expression contrasting transcript behavior in steady-state glutamine-limited chemostats with transcript behavior in ammonium-limited chemostats transiently perturbed by the addition of 40μM

glutamine or 80μM proline. Column headers: Glutamine q-val = significance of glutamine effect, Proline q-val = significance of proline, Dynamic q-val = significance of response to computationally predicted instantaneous growth rate, Glutamine coefficient = modeled effect of glutamine, Proline coefficient = modeled effect of proline, Dynamic coefficient = response to computationally predicted instantaneous growth rate, steady-state coefficient = response to experimentally controlled growth rate as reported in Table S2.

Table S4. Rates of mRNA decay estimated for each yeast transcript in response to alterations in environmental nitrogen. We calculated rates of mRNA degradation in response to transient relief of nitrogen-limitation via addition of 40μM glutamine to an ammonium-limited chemostat growing at D=0.12hr^{-1} and tested for evidence of accelerated degradation by comparison to steady-state mRNA degradation rates reported in Neymotin et al., (2014).

Table S5. Rates of mRNA decay estimated for each yeast transcript in response to a glutamine pulse in a proline batch culture. We calculated rates of mRNA decay in response to transient relief of nitrogen-limitation via addition of 400μM glutamine to a batch culture growing in the presence of 800μM proline and tested for evidence of accelerated degradation by comparison to steady-state mRNA degradation rates reported in Neymotin et al., (2014).

Table S6. Comparison of transcript abundance in NCR derepressed and NCR repressed conditions. FPKM values for each transcript from cells growing in starved for nitrogen (NCR derepressed) and FPKM values for each transcript for cells growing in YPD (NCR repressed).

Table S7. Complete DNA microarray data matrix. The data are log2-transformed ratios of mRNA abundance for each sample compared to a common reference assayed on two-color DNA microarrays.

3. Supplementary methods

In a chemostat the culture is continuously diluted through the addition of new media and removal of old media and cells from the vessel. The flow (f) has units mL/hr and the volume (V) of the chemostat is in mL. The dilution rate (D) is $D = f/V$ with units (hr^{-1}).

The fundamental equations of the chemostat, which model the rate of change of cell density (x) and limiting nutrient concentration (s) are:

$$\frac{dx}{dt} = \mu_{max}\frac{s}{K_s + s}x - Dx \tag{1}$$

$$\frac{ds}{dt} = DR - Ds - \frac{x}{Y}\mu_{max}\frac{s}{K_s + s} \tag{2}$$

In this model μ_{max} is the maximal growth rate supported by the media, K_s is the half-maximal growth rate constant, R is the concentration of the limiting nutrient in the feed media vessel and Y is the cell yield.

Estimating K_s using steady-state chemostats

At steady-state, the rate of change in cell density is equal to zero (i.e. $\frac{dx}{dt} = 0$) as is the rate of change in the concentration of the limiting nutrient (i.e. $\frac{ds}{dt} = 0$) . Therefore, equation 1 reduces to:

$$\mu_{max}\frac{s}{K_s + s} = D \tag{3}$$

Substituting this equality into equation 2 yields:

$$0 = DR - Ds - \frac{x}{Y}D \tag{4}$$

and therefore the residual concentration of the limiting nutrient, s, can be estimated by:

$$s = R - \frac{x}{Y} \tag{5}$$

Rearrangement of equation 3 yields:

$$s = \frac{DK_S}{\mu_{max} - D} \tag{6}$$

K_s can then be estimated using non linear least squares regression using empirically measured values of D and μ_{max} and the estimated values of s determined using equation 5.

Modeling growth kinetics in chemostat perturbation experiments

All perturbation experiments were performed using steady-state cultures that were limited for ammonium sulfate. By varying the dilution rate of ammonium limited cultures and determining the cell density we estimated the residual concentration of ammonium. Using the methods described above we estimated that for ammonium sulfate $K_s = 103\mu M$ nitrogen (or $51.5\mu M$ ammonium sulfate).

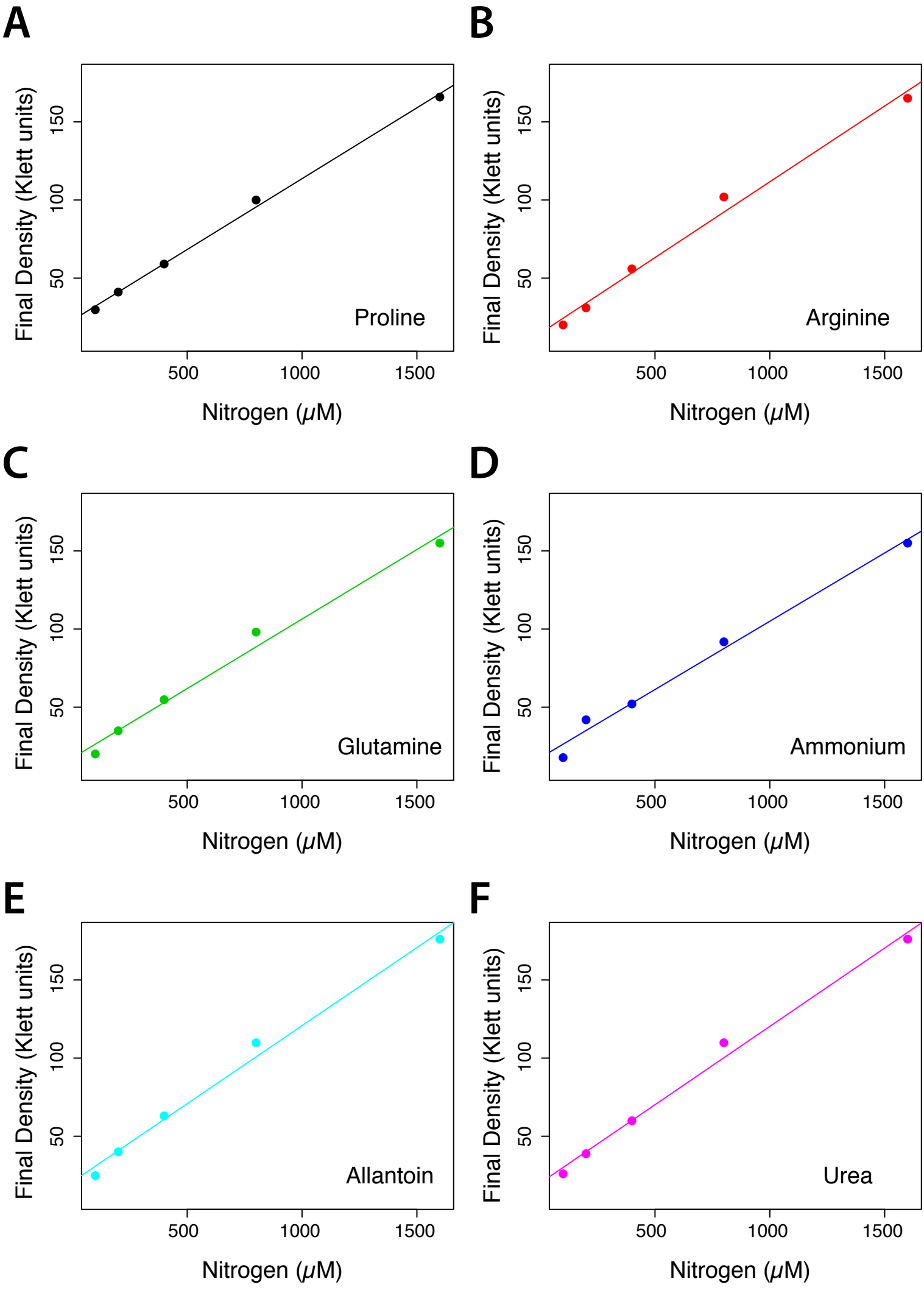
We modeled the effect of an instantaneous shift in nitrogen concentration using an alternate source of nitrogen using the following system of equations to model the change in cell density with time ($\frac{dx}{dt}$; equation 7), the change in the concentration of the continuously added limiting nitrogen source ($\frac{ds}{dt}$; equation 8), and the "pulsed" nitrogen source ($\frac{dp}{dt}$; equation 9), which is added in a single bolus with no further addition. ν_max is the maximal growth rate supported by the "pulsed" nitrogen source and K_p is the half-maximal growth rate constant of the perturbing nitrogen source :

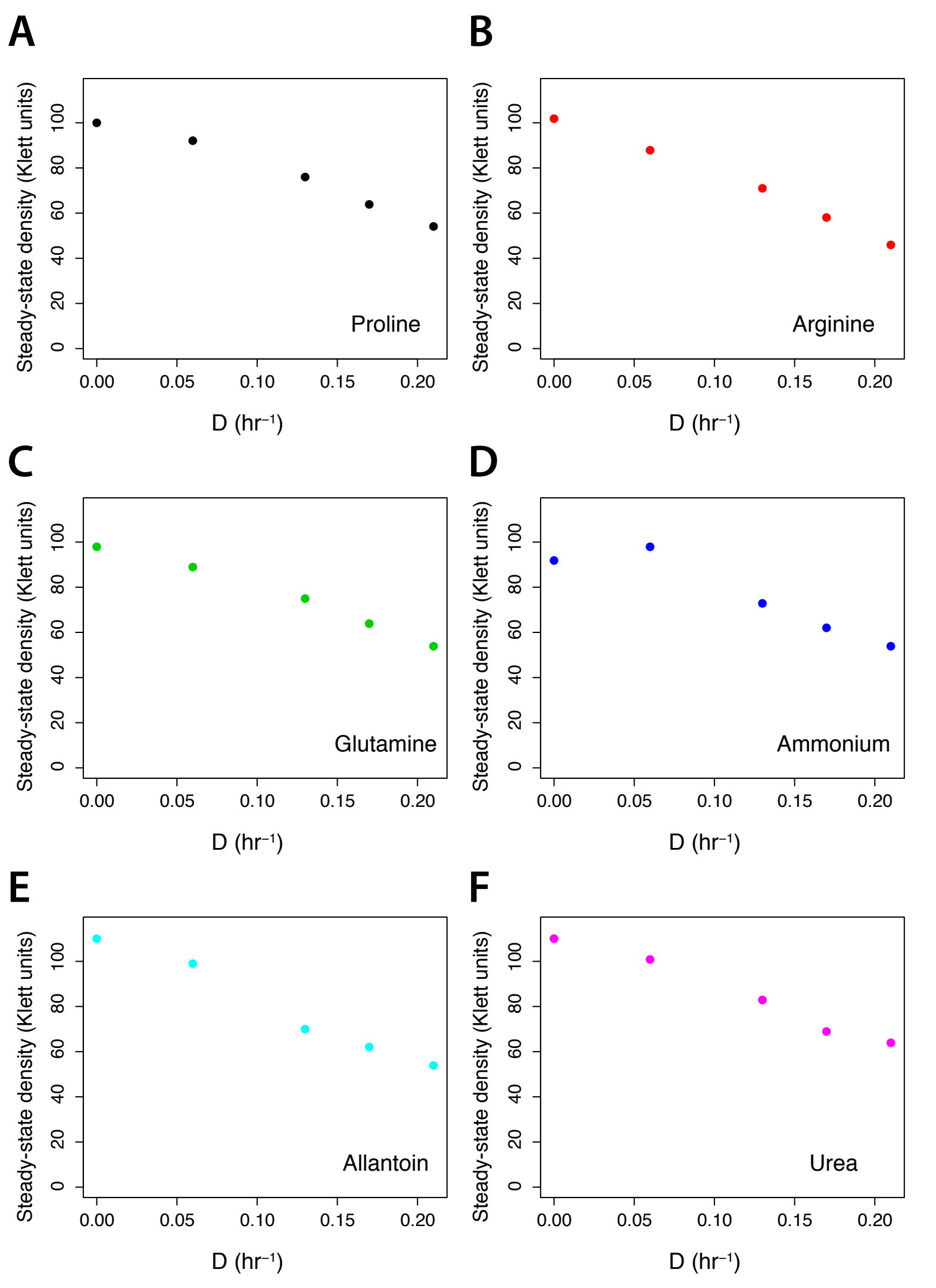
$$\frac{dx}{dt} = \mu_{max}\frac{s}{K_s + s}x + \nu_{max}\frac{p}{K_p + p}x - Dx \tag{7}$$

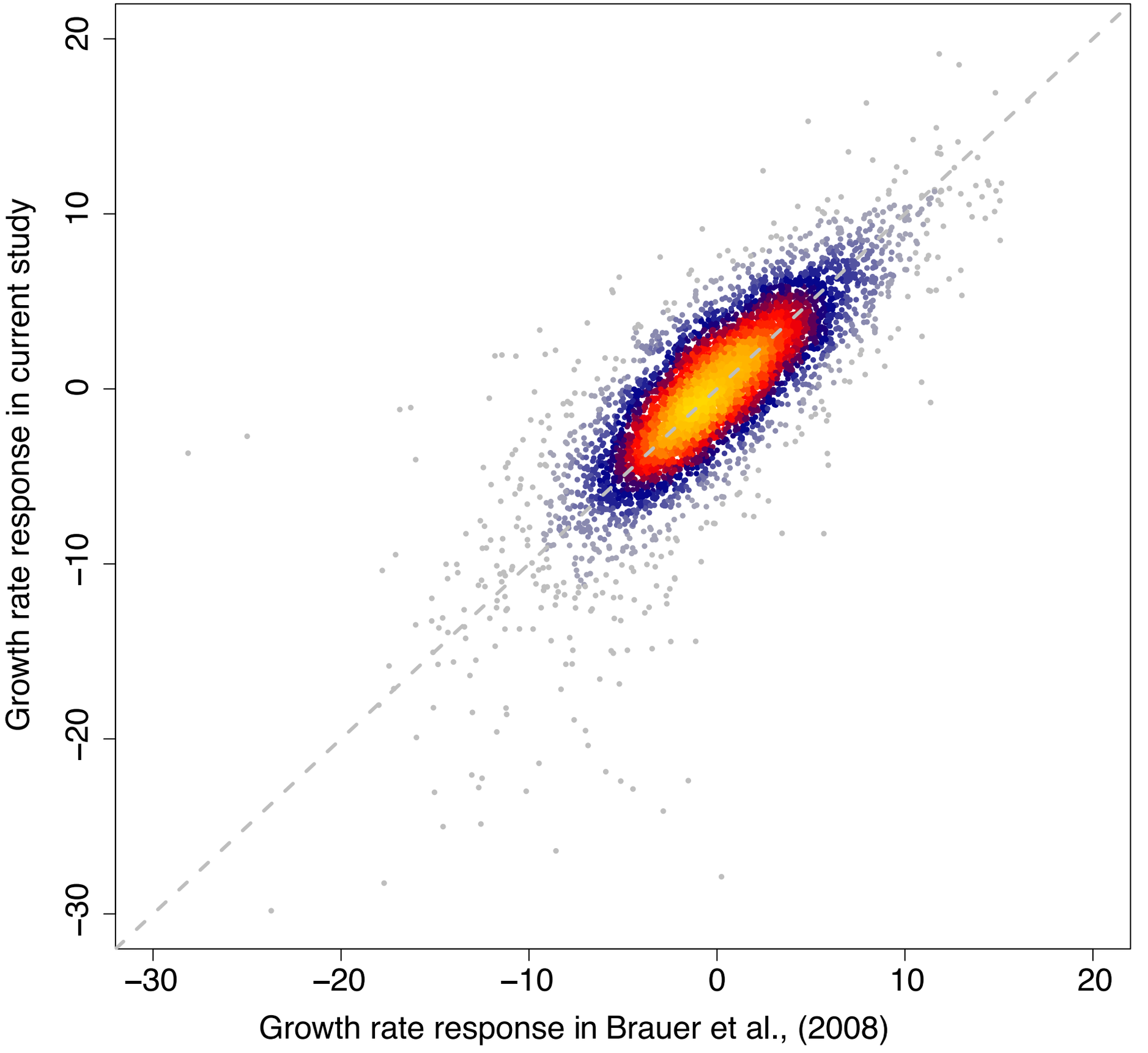
$$\frac{ds}{dt} = DR - Ds - \frac{x}{Y}\mu_{max}\frac{s}{K_s + s} \tag{8}$$

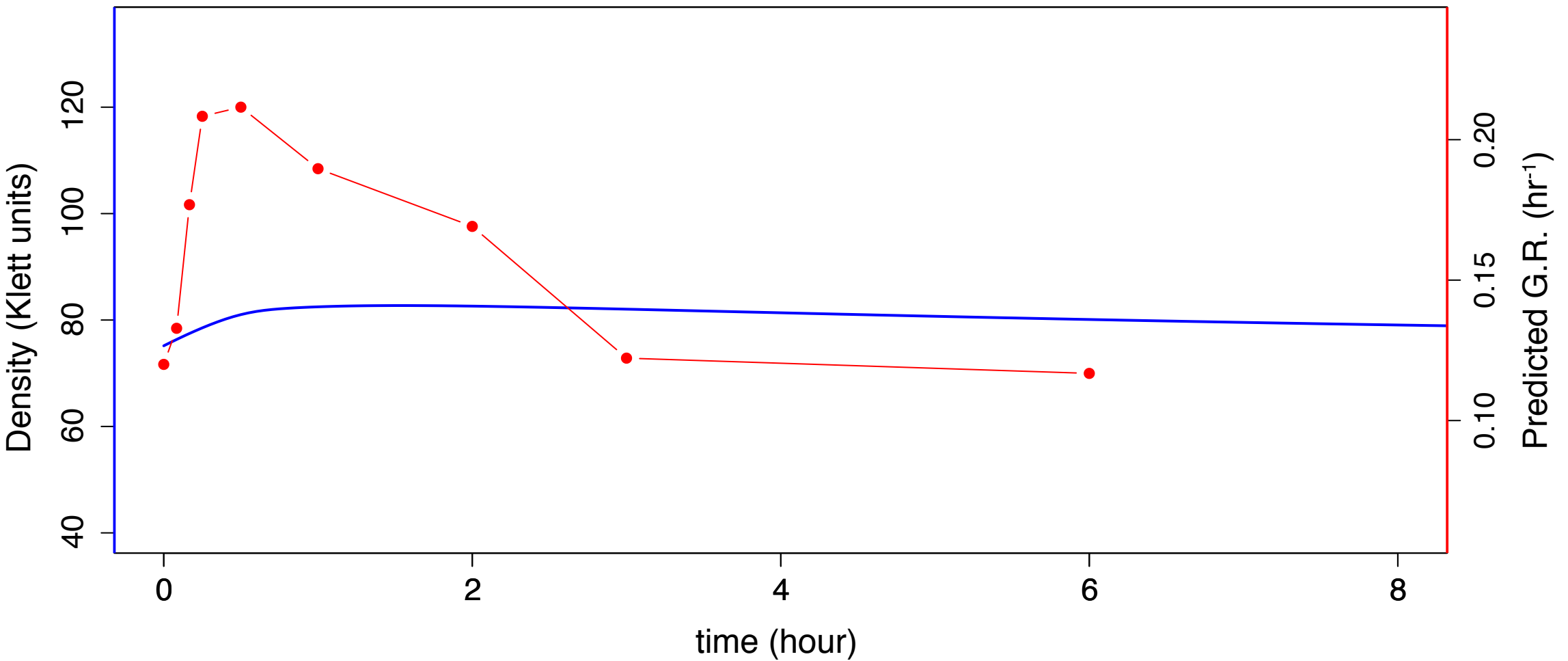
$$\frac{dp}{dt} = -Dp - \frac{x}{W}\nu_{max}\frac{p}{K_p + p} \tag{9}$$

We modeled the pulse experiments by initiating numerical integration with the steady-state values for ammonium limited cultures growing at a dilution rate of $0.12hr^{-1}$ (Figure 2) and an instantaneous increase of either $40\mu M$ glutamine, $400\mu M$ glutamine, $80\mu M$ proline, $800\mu M$ proline or a mixture of $20\mu M$ glutamine and $40\mu M$ proline.

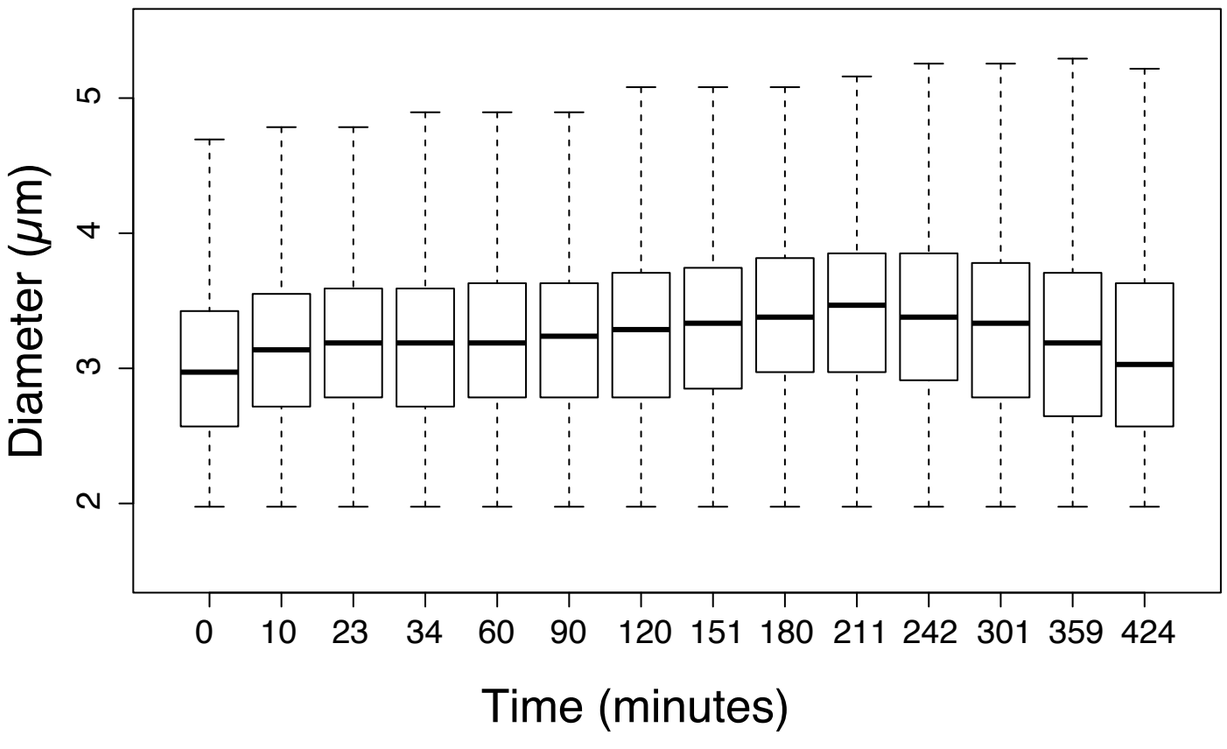

A
Final Density (Klett units)
50
100
150
500
1000
1500
Nitrogen (μM)
Proline
B
Final Density (Klett units)
50
100
150
500
1000
1500
Nitrogen (μM)
Arginine
C
Final Density (Klett units)
50
100
150
500
1000
1500
Nitrogen (μM)
Glutamine
D
Final Density (Klett units)
50
100
150
500
1000
1500
Nitrogen (μM)
Ammonium
E
Final Density (Klett units)
50
100
150
500
1000
1500
Nitrogen (μM)
Allantoin
F
Final Density (Klett units)
50
100
150
500
1000
1500
Nitrogen (μM)
Urea


A
Steady-state density (Klett units)
0
20
40
60
80
100
0.00
0.05
0.10
0.15
0.20
D (hr⁻¹)
Proline
B
Steady-state density (Klett units)
0
20
40
60
80
100
0.00
0.05
0.10
0.15
0.20
D (hr⁻¹)
Arginine
C
Steady-state density (Klett units)
0
20
40
60
80
100
0.00
0.05
0.10
0.15
0.20
D (hr⁻¹)
Glutamine
D
Steady-state density (Klett units)
0
20
40
60
80
100
0.00
0.05
0.10
0.15
0.20
D (hr⁻¹)
Ammonium
E
Steady-state density (Klett units)
0
20
40
60
80
100
0.00
0.05
0.10
0.15
0.20
D (hr⁻¹)
Allantoin
F
Steady-state density (Klett units)
0
20
40
60
80
100
0.00
0.05
0.10
0.15
0.20
D (hr⁻¹)
Urea

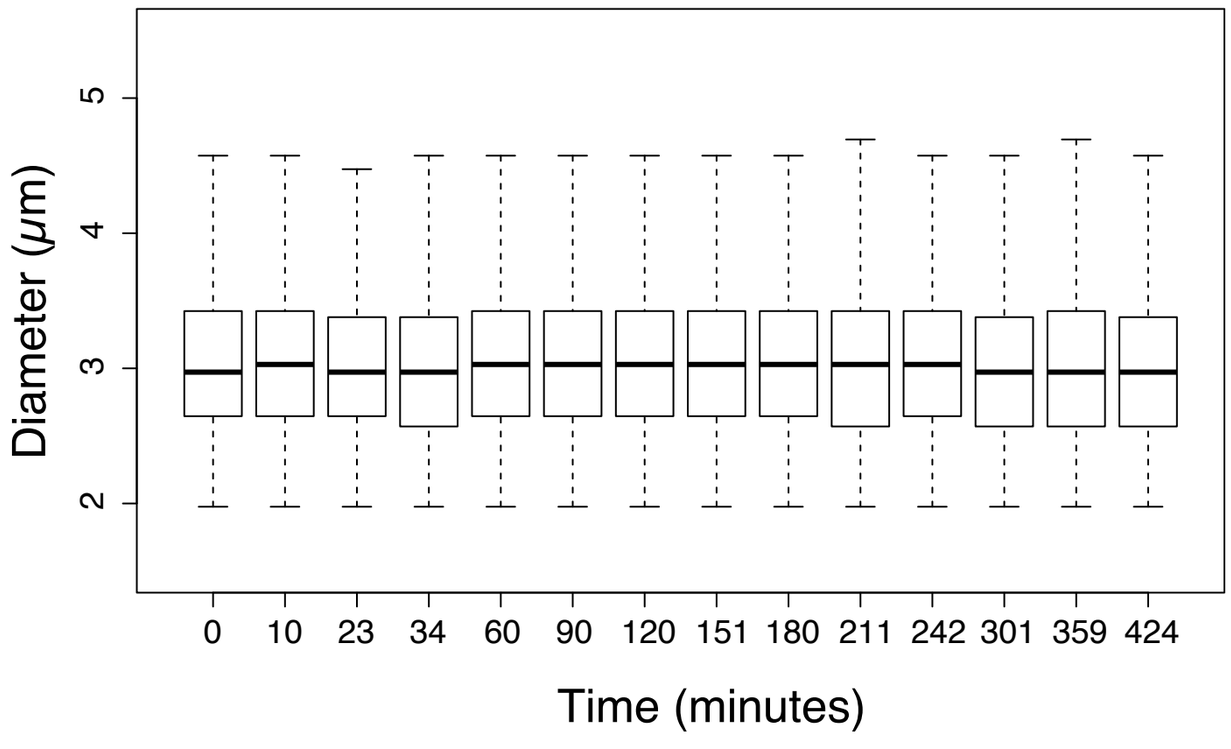

Growth rate response in current study
20
10
0
−10
−20
−30
−30
−20
−10
0
10
20
Growth rate response in Brauer et al., (2008)


Density (Klett units)
40
60
80
100
120
0
2
4
6
8
time (hour)
0.10
0.15
0.20
Predicted G.R. (hr-1)

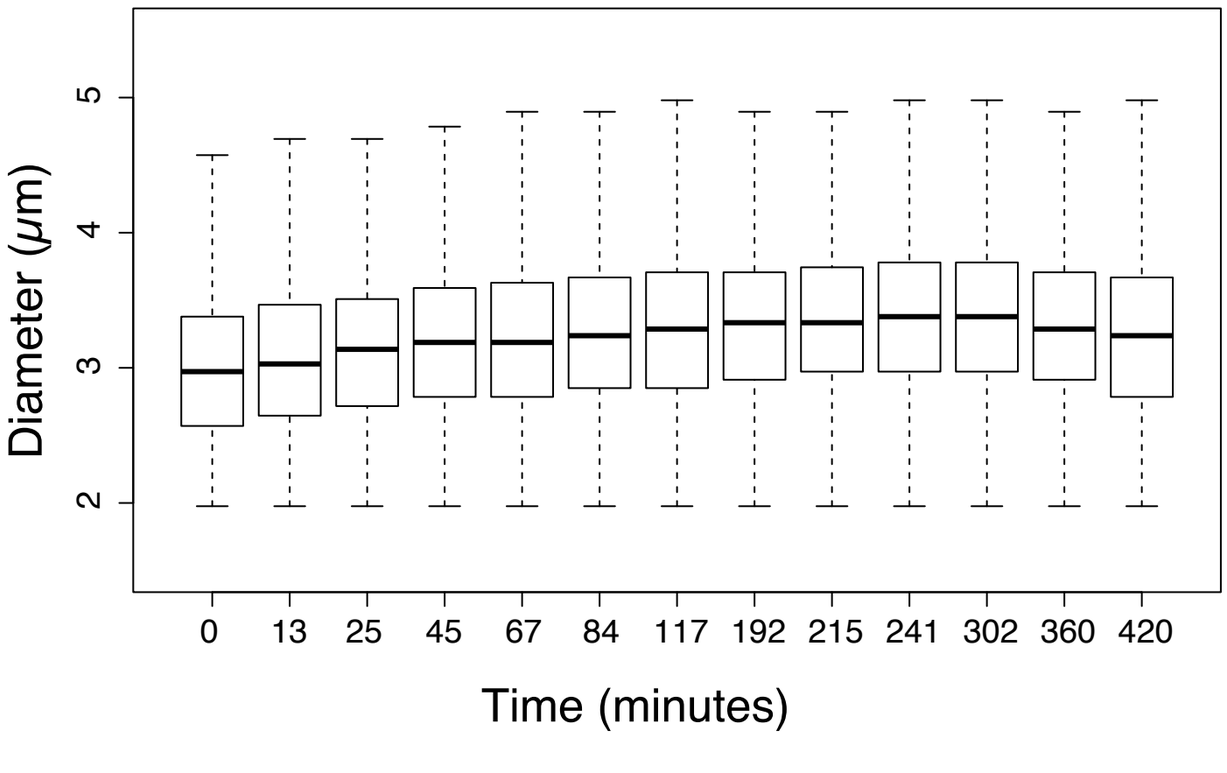
A


Diameter (μm)
2
3
4
5
0
10
23
34
60
90
120
151
180
211
242
301
359
424
Time (minutes)

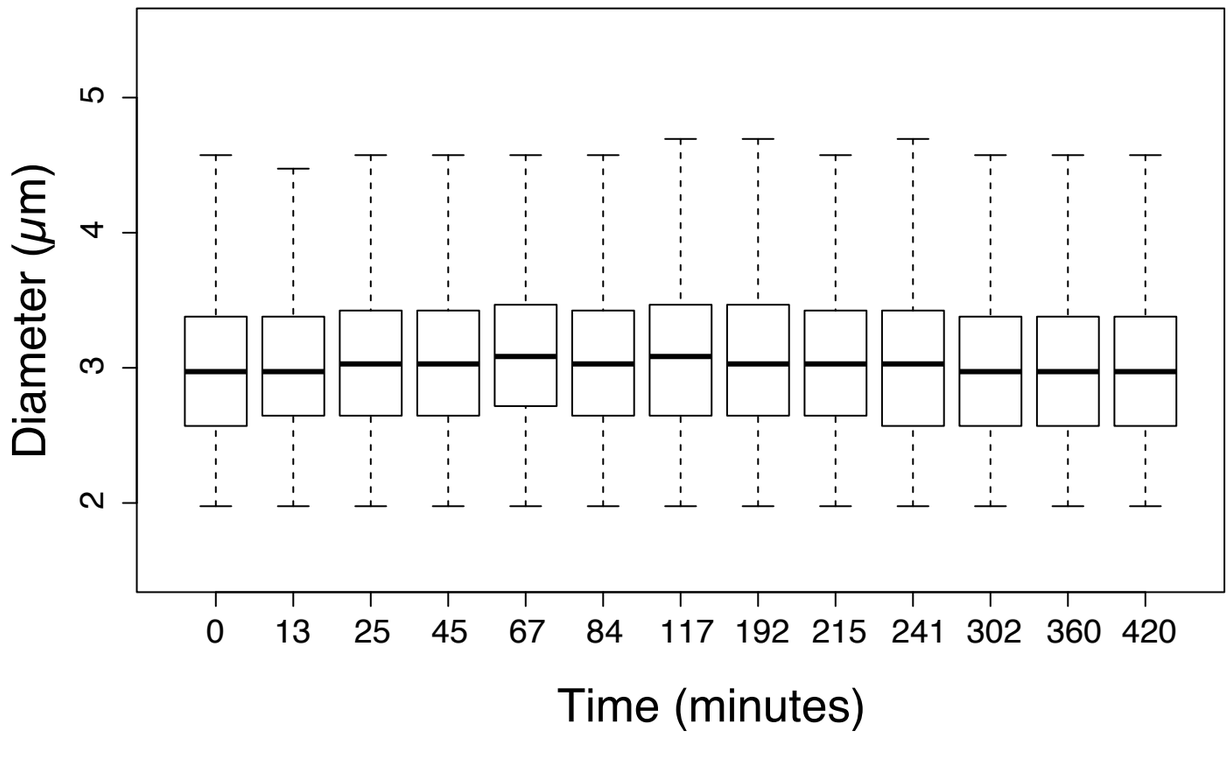
B


Diameter (μm)
2
3
4
5
0
10
23
34
60
90
120
151
180
211
242
301
359
424
Time (minutes)

C


Diameter (μm)
2
3
4
5
0
13
25
45
67
84
117
192
215
241
302
360
420
Time (minutes)

D


Diameter (μm)
2
3
4
5
0
13
25
45
67
84
117
192
215
241
302
360
420
Time (minutes)

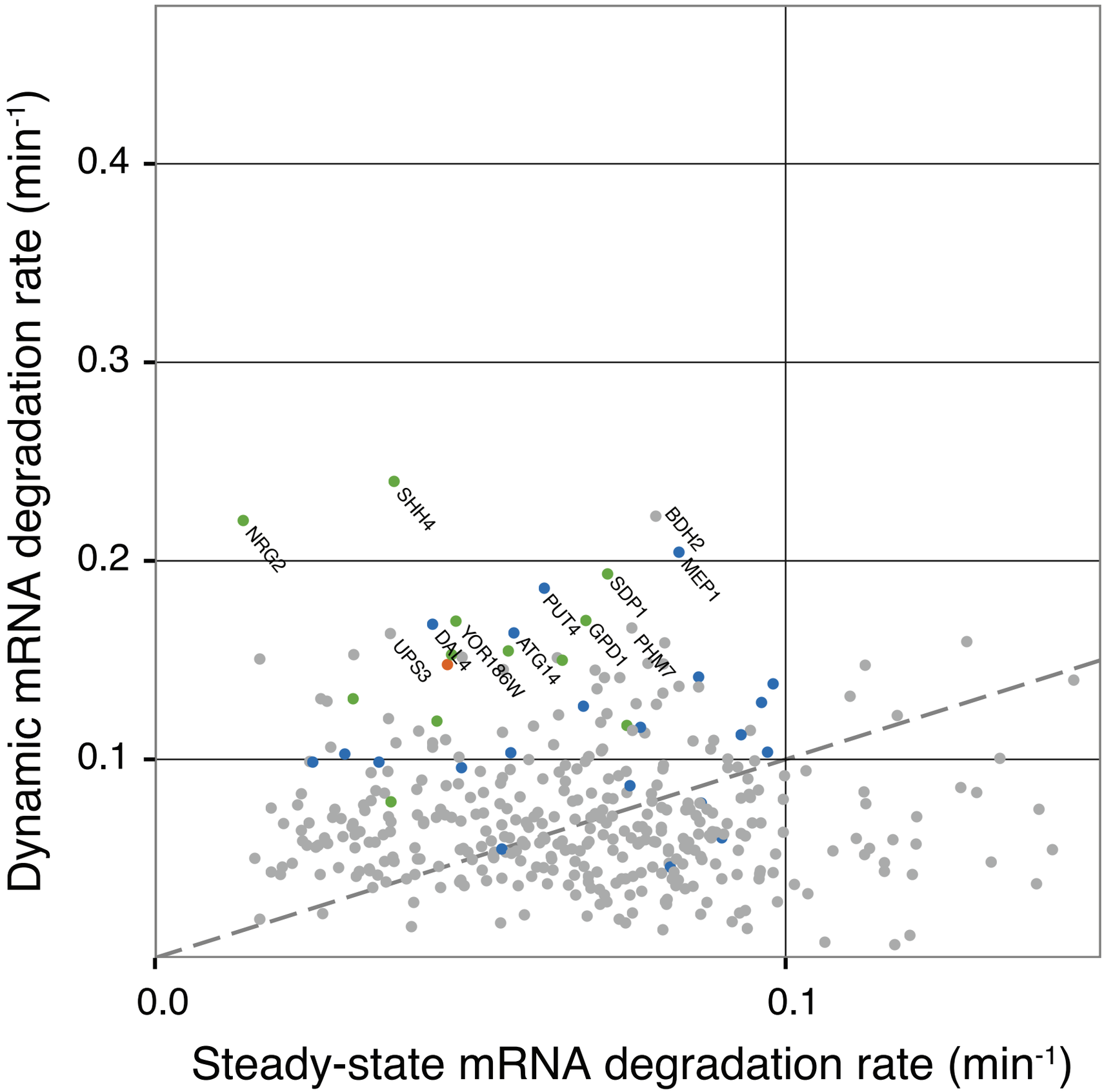
translation
regulation of transcription
rRNA processing
ribosome biogenesis
response to stress
ribosomal large subunit biogenesis

dynamic response

40
20
0
-20
-40

-30
-20
-10
0
10
20

steady-state response


Dynamic mRNA degradation rate (min-1)
0.4
0.3
0.2
0.1
0.0
0.1
Steady-state mRNA degradation rate (min-1)
NRG2
SHH4
BDH2
MEP1
SDP1
PUT4
GPD1
PHM7
YOR186W
ATG14
DAL4
UPS3