Supporting Information for

"Differentiating metabolites formed from de novo synthesis versus macromolecule decomposition"

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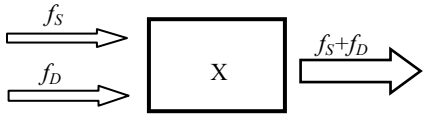
Derivation of equations and definition of half-time for convergence

Consider the simple model in Scheme 1: Metabolite X, whose total concentration X^T is (at least approximately) at steady state, is generated in the labeled form by *de novo* synthesis and in the unlabeled form (X^U) by protein degradation, with the influx from the two sources being f_S and f_D, respectively. (Note that as X is at steady state, the total efflux = the total influx = f_S+f_D; the nature of the effluxes, e.g., protein synthesis versus amino acids catabolism, does not affect the observed labeling patterns.)



Scheme 1

X^U changes with respect to time as follows:

$$\frac{dX^U}{dt} = f_D - \left(f_D + f_S\right)\frac{X^U}{X^T} \quad (1)$$

Assuming steady-state for metabolite fluxes also (i.e. f_D, f_S constant), Equation (1) has the analytical solution:

$$\frac{X^U}{X^T} = \left(1 - \frac{f_D}{f_D + f_S}\right)\exp\left(-\frac{f_D + f_S}{X^T}t\right) + \frac{f_D}{f_D + f_S} \quad (2)$$

and I reflects the initial fraction of unlabeled X (i.e., $X^U/X^T=I$ when t=0). This solution suggests that for cells grown under identical conditions but with different initial fraction of unlabeled free metabolite, say, I_1 and I_2, the unlabeled fraction will eventually converge to $f_D/(f_D+f_S)$, with a half-time for convergence ($t^c_{1/2}$) defined as the time needed for half of the initial difference (i.e., $|I_1-I_2|$) to disappear given by

$$t^c_{1/2} = \ln 2 \cdot X^T \cdot (f_D+f_S)^{-1} \quad (3)$$

Note that $t^c_{1/2}$ is proportional to the pool size X^T divided by the total flux through the pool with the initial fraction labeling irrelevant.

For the unstarved case (Fig. 1a), I_1=100% and $I_2=f_D/(f_D+f_S)$=8%, thus $t^c_{1/2}$ will be the time needed for ~ 46% of the metabolite to become labeled. As shown in Fig. 1a, $t^c_{1/2}$ ~ 3 min. For the C-starved cells, I_1 = 100% (for the starve-switch; Fig. 1b) and I_2 = 10% (for the switch-starve; Fig. 1c). At ~ 50 min after the N-switch, the unlabeled fraction became ~70% for the starve-switch and 25% for the switch starve, leaving a "gap" of 45% which is half of the original "gap". Therefore the $t^c_{1/2}$ for the starved case is ~ 50 min. As C-removal had only a small effect on the pool sizes, the ~ 17 fold increase of $t^c_{1/2}$ in C-starved cases is largely attributable to a decrease in the total nitrogen flux.

The above analysis assumes flux steady state throughout the C-starvation period (i.e., an immediate transition to fixed, reduced fluxes upon initiation of C-starvation). While this assumption is clearly an oversimplification, it is possible that fluxes drop quickly following C-starvation to a new, reduced steady state (as occurs for the concentrations of glycolytic compounds and cAMP). If this is indeed the case, the above treatment will provide a reasonable estimation of the changes in flux.

Construction of the model including changing fluxes during C-starvation.

To explicitly consider flux changes upon C-removal, an ODE model was constructed as above but with f_s and f_D described by functions, not constants. Matlab code for the model is provided at the end of the Supporting Material. In the model, X^T was defined to be 1 for simplicity and C-starvation starts at t = 5min. N-switch happens at 0 min (i.e., 5 min prior to carbon starvation) for simulating the switch-starve experiment and at 15 min for the starve-switch experiment (i.e., 10 min post carbon starvation).

The functions $f_S(t)$ and $f_D(t)$ were constrained by the following: $f_S(0)$ and $f_D(0)$ must match experimental values for the unstarved cells; $f_S(\infty)$ and $f_D(\infty)$ must both be small (the sum $f_S(\infty) + f_D(\infty)$ must decay to ~ 5% of the initial flux); and the ratio f_D:f_S must increase during C-starvation. A simple pair of functions meeting these requirements was exponential decay functions with different final steady-state values:

$$f(t) = f_0*((1-\alpha)\exp(-kt)+ \alpha) \quad (4)$$

For simplicity, we fit the data with the time-constant k fixed for both f_S and f_D at 0.17 min^{-1} and with $\alpha = 0$ for f_S and 0.2 for f_D. With f_S and f_D defined as above, the total flux reduced >20 fold after 20 min of C-removal. The simulated fluxes are shown in Supplementary Figure 3. Description of the fluxes using these simple decay functions results in a model which produces the experimental results well (Fig. 1). Alternative flux functions which conform to the above-stated constraints are also feasible, however.

Supporting Discussion

Factors influencing details of labeling kinetics

Although similar, the labeling kinetics for different amino acids are not identical as shown in Figure 1. These subtle differences may partly reflect differing extents of residual biosynthesis of different amino acids; in addition, these patterns are sensitive to the differences in the absolute magnitudes of amino acid pool sizes relative to fluxes. The similarity of the CMP and proline patterns likely reflects their having (by chance) similar pool sizes relative to fluxes; the precise pattern for other nucleotides is likely to be different. Due to the limited ability of cold methanol to extract nucleotides from *E. coli,*[1] more complete nucleotide information was not obtained in the present experiment.

Relationship to prior literature

The observation of decreased biosynthetic flux in response to carbon starvation was expected. The relatively slow protein degradation flux in the starved cells was less so, given prior literature[2,3] reporting increased protein degradation in starved *E. coli*. The modest protein degradation flux measured here would, nevertheless, result in substantial proteome degradation over long starvation intervals (e.g. > 4 hours) as observed previously. Much of the protein degradation flux in growing cells likely arises from fast turnover of a small pool of very short lived proteins.[4] Turnover of these proteins likely slows during carbon starvation (resulting in a decrease in protein degradation flux), while the fraction of the proteome subject to degradation likely in turn increases. One particular case of a normally short-lived protein being stabilized by starvation involves the key transcriptional regulator RPOS.[5] Evolving proteomic technologies, when paired with isotopic labeling techniques such as those described here, are poised to enable determination of the lifetimes of the full spectrum of proteins in both starved and unstarved conditions in the near future.[6,7] Combining proteomic and metabolomic approaches should provide a yet more complete picture of *E. coil's* starvation behavior.

References

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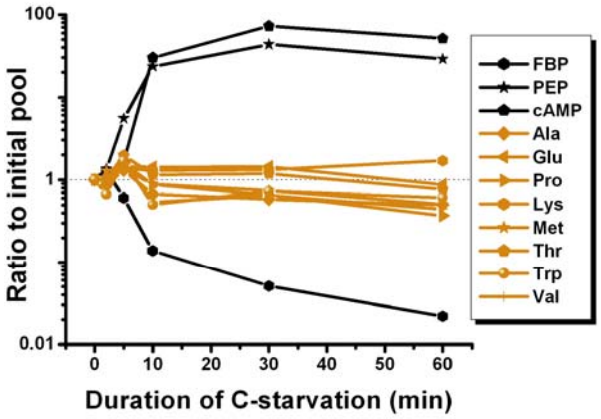
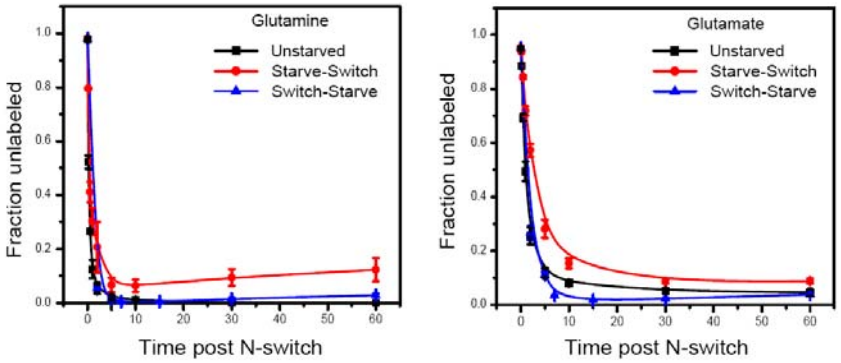
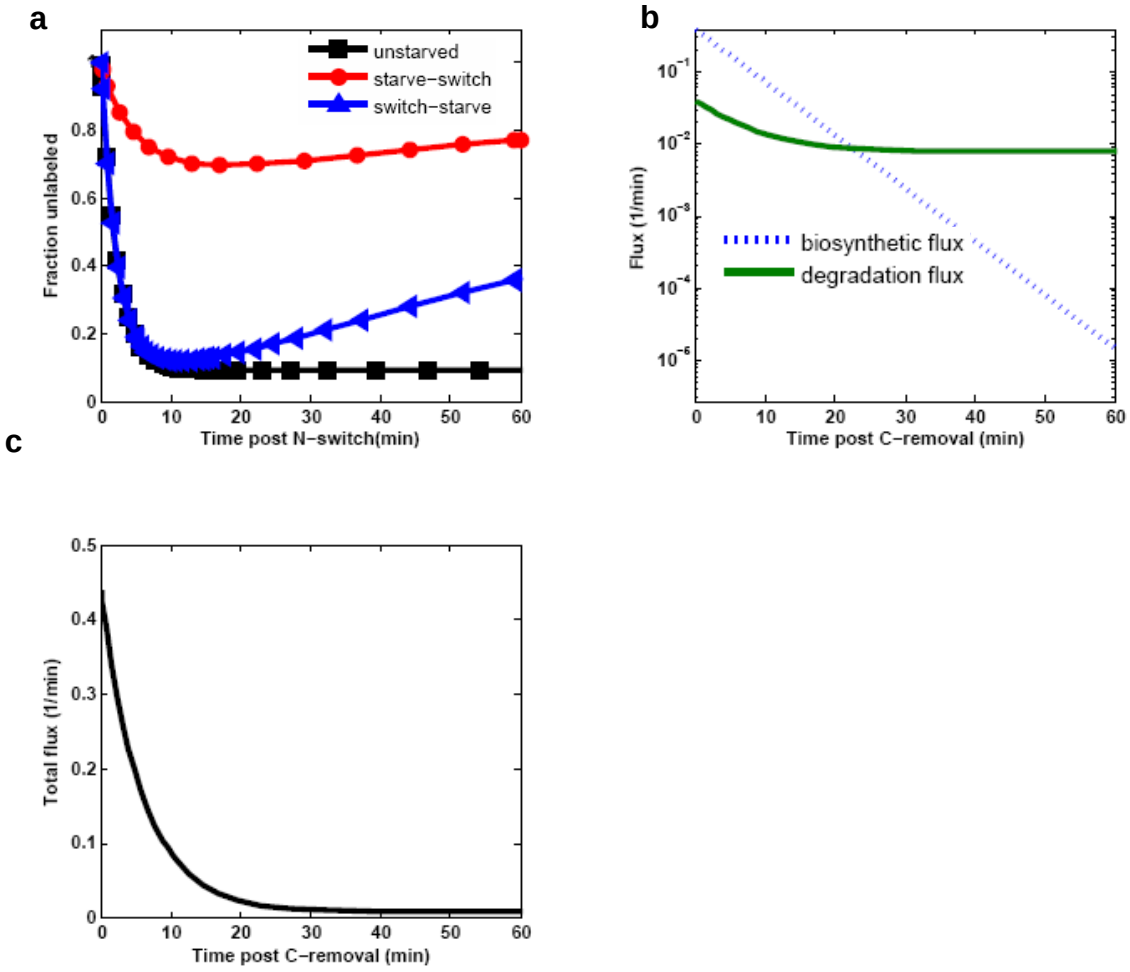


Supporting Figure 1. Response of metabolite pools to carbon starvation in *E.coli*. *E. coli* was transferred from glucose-containing minimal media to minimal media containing no carbon source at t=0. The X-axis represents minutes following glucose-removal and the Y-axis represents the pool size of the metabolite relative to the pool size prior to glucose removal. As shown in this figure, glucose withdrawal quickly resulted in profound concentration changes for glycolytic compounds and cAMP. In contrast, amino acid levels changed only modestly and slowly. Data shown are averages of n = 3 independent experiments.



Supporting Figure 2. KFP of glutamine and glutamate under conditions described in the main text. Mean data are shown by symbols with error bars of ± 1 SE (n=3 independent experiments). The curves show a smoothed fit to the experimental data generated using the B-spline function of Origin (version 6.0, OriginLab Corporation, Northampton, MA). Fraction unlabeled = [signal of unlabeled compound]/ [signal of all forms of compound]. The rate of incorporation of labeling into these two central intermediates did not show a change comparable to their downstream amino acid products when the carbon source was removed. The slow down of labeling kinetics observed for their downstream products is therefore not due to slow labeling of glutamine and glutamate. In addition, these two intermediates maintained ~90% labeling for the duration of the experiment, confirming that *de novo* biosynthesis would indeed produce labeled amino acids and the observed unlabeled amino acids are being released by protein degradation.



Supporting Figure 3. Model including changing fluxes during C-starvation. (a) Simulated experimental results. (b, c) Simulated flux changes during C-starvation. (b) Contributors to amino acid influx on a log Y-axis. (c) Total flux (influx = efflux) on a linear Y-axis.

Matlab code for the ODE model

```
function [t,z]=Yuanetal()
[t,z]=ode23(@simulator,[0 75], [1,1,1]);
UT=t-5; % align N-switch time  for plotting
TW=t-15;% align N-switch time for plotting
n=size(t,1);
FD=zeros(n,1);
FS=zeros(n,1);
DEGi=0.17; %k for the decay of both fluxes
DEGii=0.20; %alpha for the degradation flux
D=0.04; % fD(0)
S=0.4; % fS(0)
%re-calculating fluxes for plotting
for i=1:n,
    if t(i) < 5
    FD(i)=D;
    FS(i)=S;
else
    FD(i)=D*((1-DEGii)*exp(-DEGi*(t(i)-5))+DEGii);
    FS(i)=S*exp(-DEGi*(t(i)-5));
    end
end

figure,
subplot(2,2,1), plot(UT(:,1),z(:,1),TW(:,1),z(:,2),t(:,1),z(:,3)),axis([0 60 0
1.1]),xlabel('Time post N-switch(min)'),ylabel('Fraction unlabeled');
subplot(2,2,2), plot(UT(:,1),FS),axis([0 60 0 0.4]), xlabel('Time post C-removal
(min)'),ylabel('Flux from biosynthesis'),
subplot(2,2,3), plot(UT(:,1),FD),axis([0 60 0 0.04]), xlabel('Time post C-removal
(min)'),ylabel('Flux from degradation');
subplot(2,2,4), plot(UT(:,1),(FS+FD)),axis([0 60 0 0.5]),xlabel('Time post C-
removal (min)'),ylabel('Total flux');

%the simulation function
    function xcl = simulator(t,y)
%Variables
UNST=y(1);
STSW=y(2);
SWST=y(3);
DEGi=0.17;
DEGii=0.2;
D=0.04;
S=0.4;
%FS and FD as a funtion of time, C-removal happens at t=5
if t < 5
   FS=S;
   FD=D;
else %flux changes after C-removal
 FS=S*exp(-DEGi*(t-5));
 FD=D*((1-DEGii)*exp(-DEGi*(t-5))+DEGii);
end
%unstarved case, N-swich happens at t=5
if t < 5
    dUNSTdt=0;
else
```

```
dUNSTdt=D-(S+D)*UNST;
end

%starve-switch, N-switch happens at t=15 (10min after C-removal)
if t < 15
    dSTSWdt=0;
else
dSTSWdt=FD-(FS+FD)*STSW; %flux read out from FD & FS
end

%switch-starve, N-switch happens at t=0 (5min prior to C-removal)
if t<5
    dSWSTdt=D-(D+S)*SWST; %flux does not change during first 5min
else
dSWSTdt=FD-(FS+FD)*SWST; %flux read out from FD and FS
end

xcl=[dUNSTdt;dSTSWdt;dSWSTdt];
    end
end
```