nature chemical biology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41589-019-0364-9

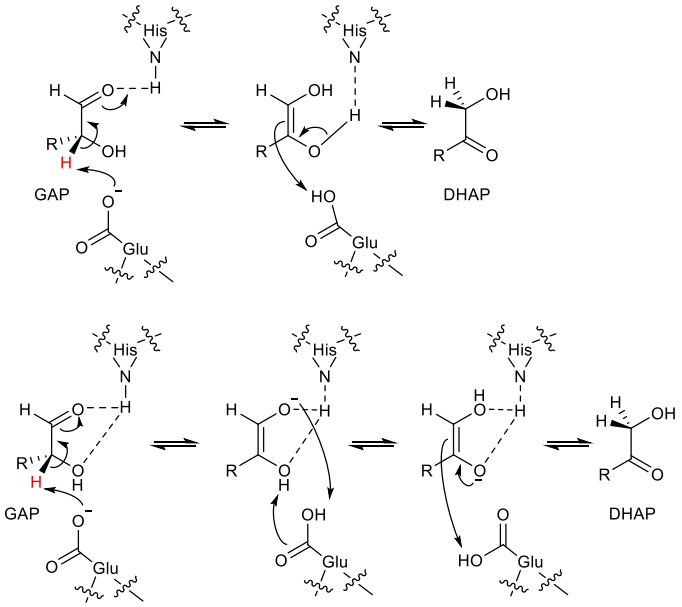
In the format provided by the authors and unedited.

Near-equilibrium glycolysis supports metabolic homeostasis and energy yield

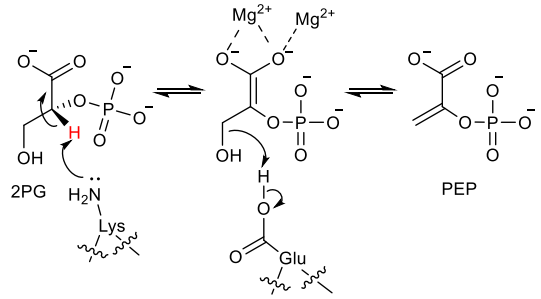
Junyoung O. Park[1,2,3], Lukas B. Tanner[2], Monica H. Wei[4], Daven B. Khana[5,6], Tyler B. Jacobson[5,6], Zheyun Zhang[2], Sara A. Rubin[4], Sophia Hsin-Jung Li[7], Meytal B. Higgins[2,8], David M. Stevenson[5,6], Daniel Amador-Noguez[5,6]* and Joshua D. Rabinowitz[2,4]*

[1]Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, Los Angeles, CA, USA. [2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA. [3]Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ, USA. [4]Department of Chemistry, Princeton University, Princeton, NJ, USA. [5]Department of Bacteriology, University of Wisconsin—Madison, Madison, WI, USA. [6]Great Lakes Bioenergy Research Center, University of Wisconsin—Madison, Madison, WI, USA. [7]Department of Molecular Biology, Princeton University, Princeton, NJ, USA. [8]Present address: Corporate Strategic Research, ExxonMobil Research and Engineering Company, Annandale, NJ, USA.
*e-mail: amadornoguez@wisc.edu; joshr@princeton.edu

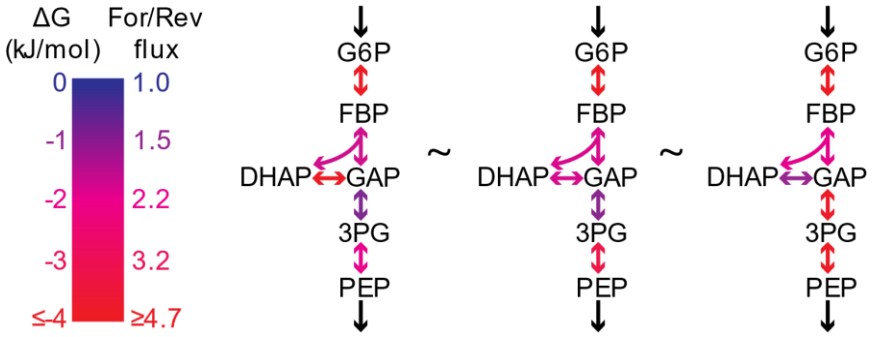
a Triosephosphate isomerase (TPI)



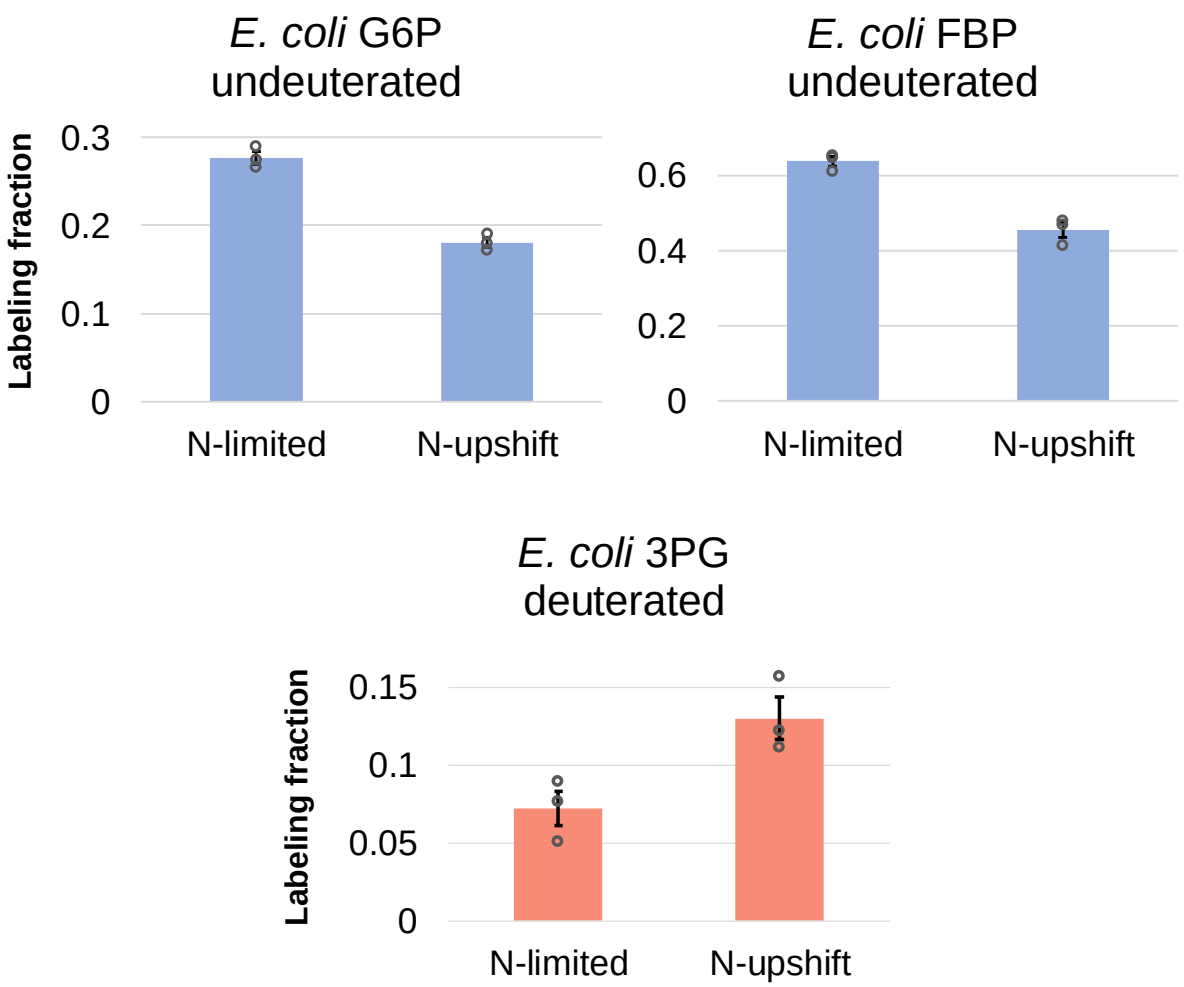
b Enolase (ENO)



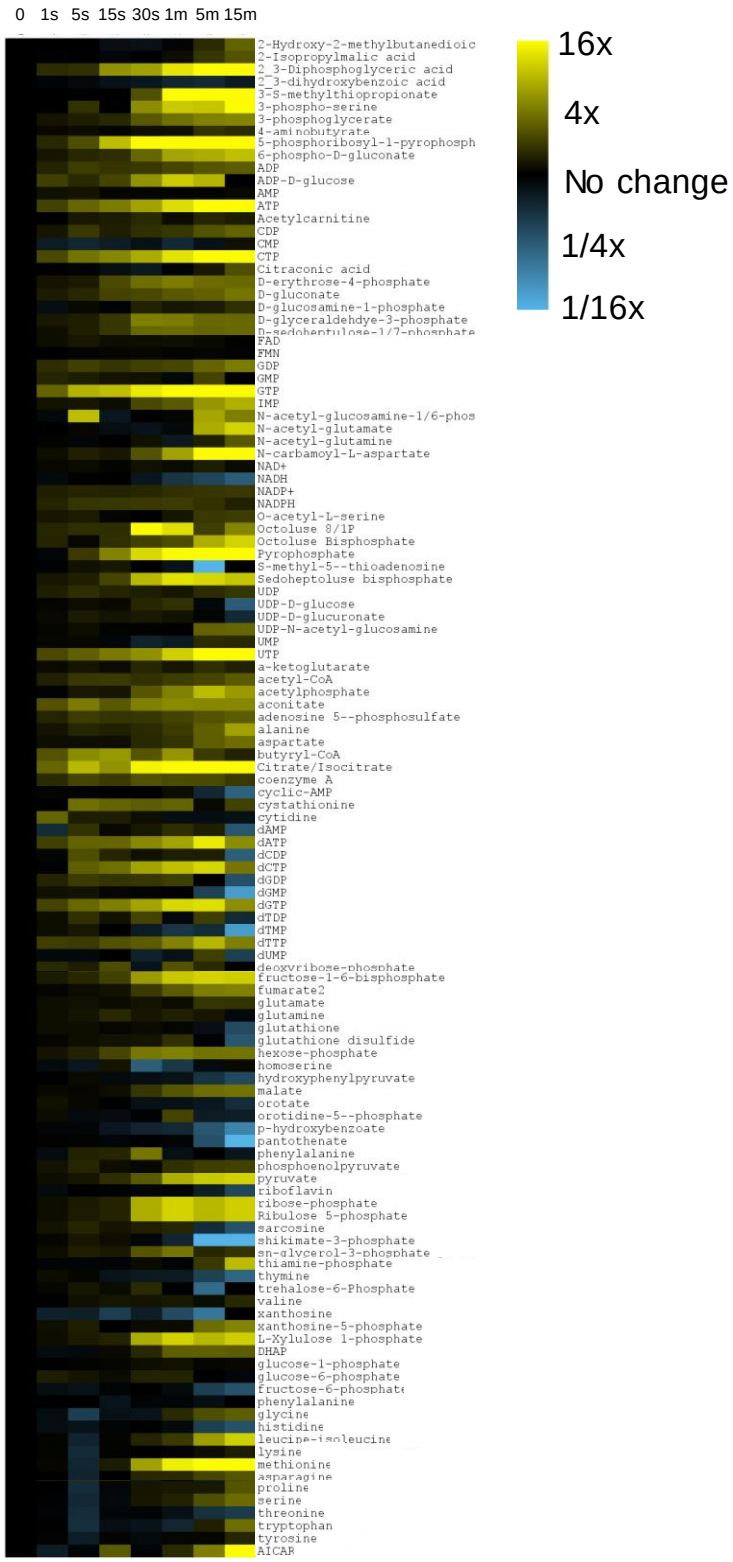
Supplementary Figure 1. Deuterium loss from [5-$^{2}H_{1}$]glucose in glycolysis. Deuterium is lost to solvent via (**a**) reverse triosephosphate isomerase reaction (GAP→DHAP) and (**b**) enolase reaction. Two proposed mechanisms of TPI are shown.



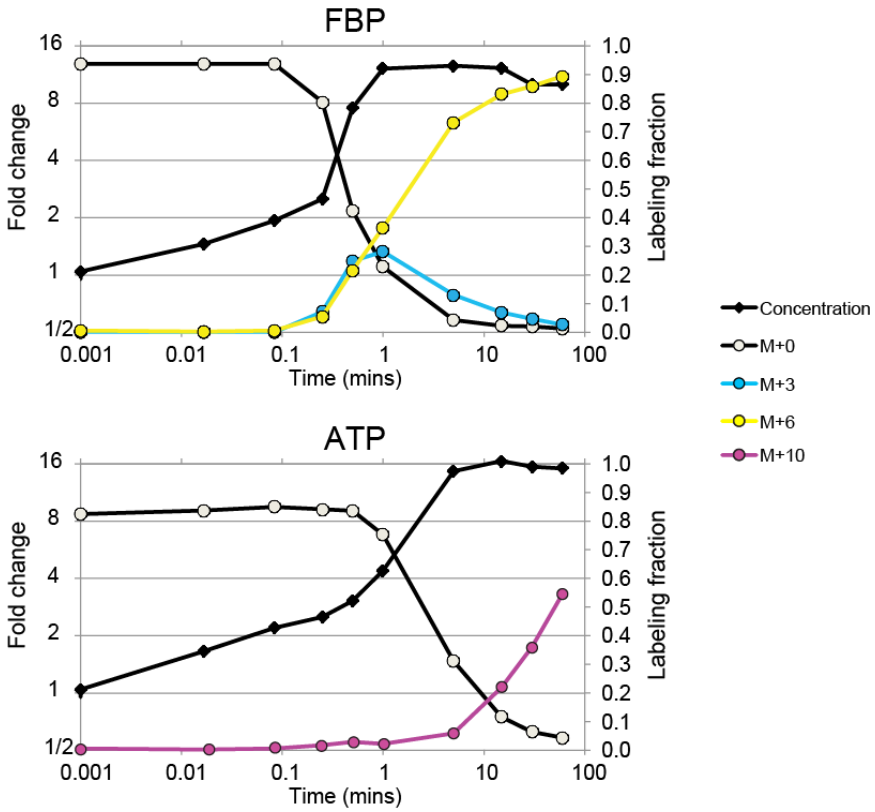
Supplementary Figure 2. Tracing [5-$^{2}H_{1}$]glucose alone is insufficient for ΔG determination. Simulation of ^{2}H labeling using a glycolytic flux model revealed that each ^{2}H labeling gradient across glycolysis may be due to a combination of greatly forward-driven TPI and highly reversible lower glycolytic reactions (left) or of highly reversible TPI and greatly forward-driven lower glycolytic reactions (right), or various combinations in-between (center). G6P: glucose-6-phosphate, F6P: fructose-6-phosphate, FBP: fructose-1,6-bisphosphate, DHAP: dihydroxyacetone phosphate, GAP: glyceraldehyde-3-phosphate, 3PG: 3-phosphoglycerate, 23BPG: 2,3-bisphosphoglycerate, 2PG: 2-phosphoglycerate, and PEP: phosphoenolpyruvate



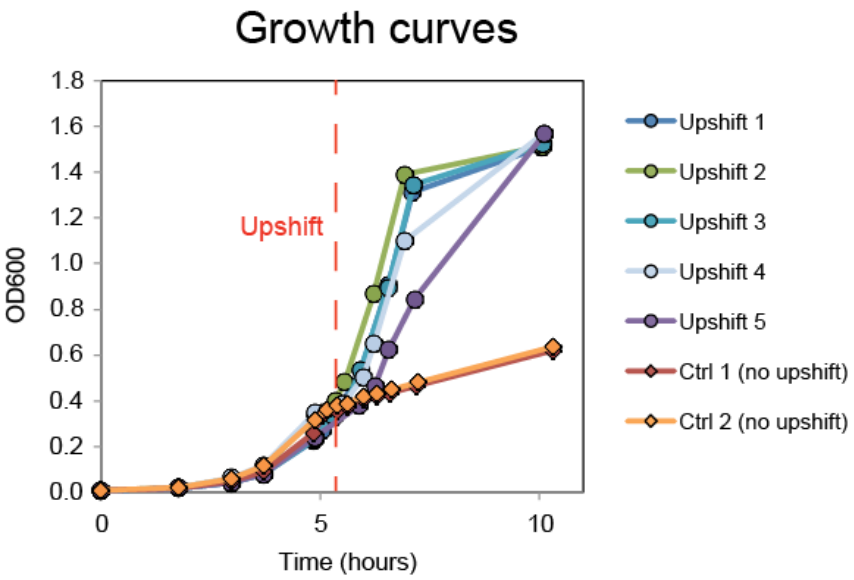
Supplementary Figure 3. Statistically significant changes in deuterium labeling in *E. coli* N-upshift. The centre and error bars represent the mean ± s.e.m. (n=3 biologically independent samples). G6P $p<0.001$, FBP $p<0.01$, and 3PG $p<0.05$ by two-tailed t-tests.



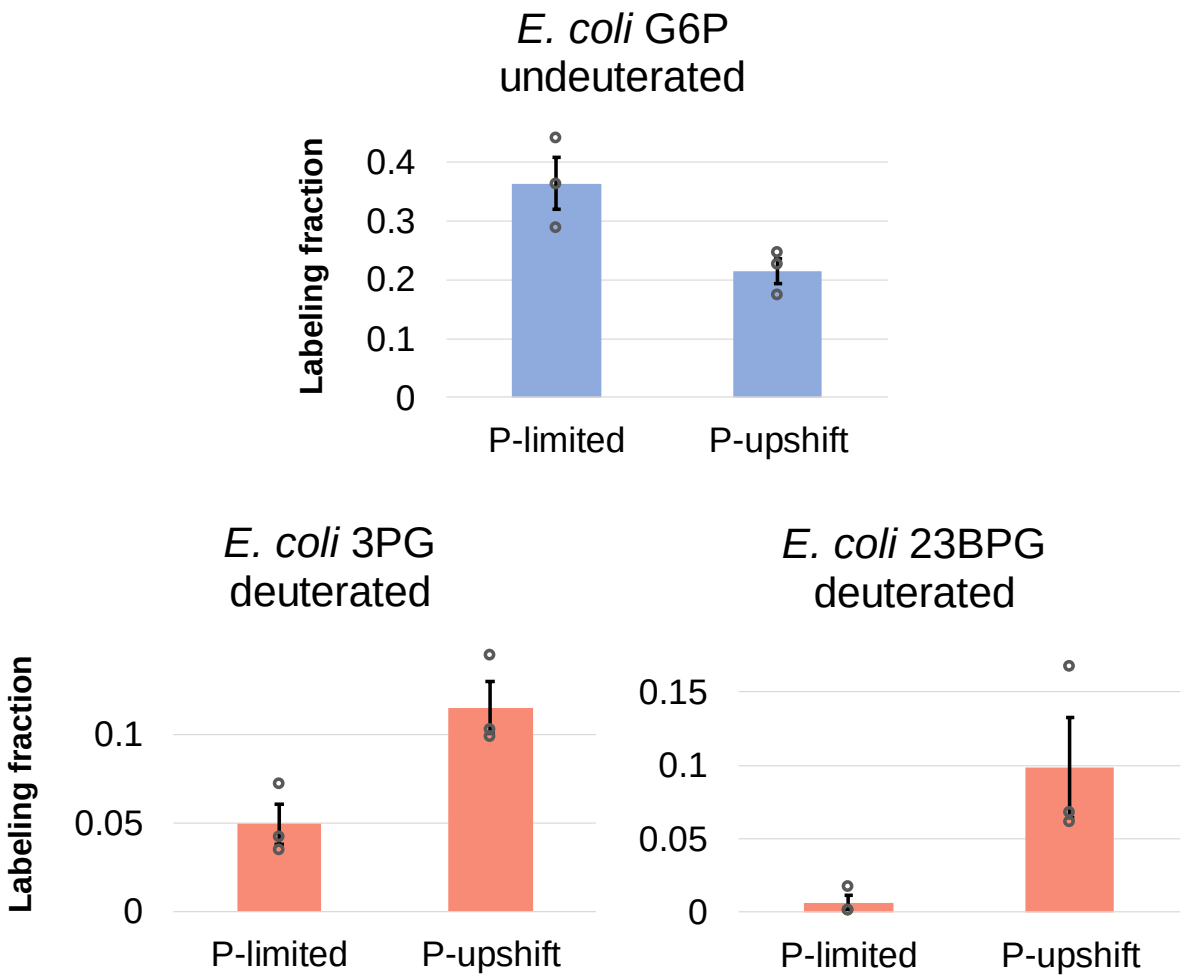
Supplementary Figure 4. Metabolite level changes upon phosphorus upshift. Rapid changes in metabolite levels over 15 minutes are shown in yellow (increase) and blue (decrease). Data were collected using filter cultures to enable sampling and quenching on the 1s timescale.



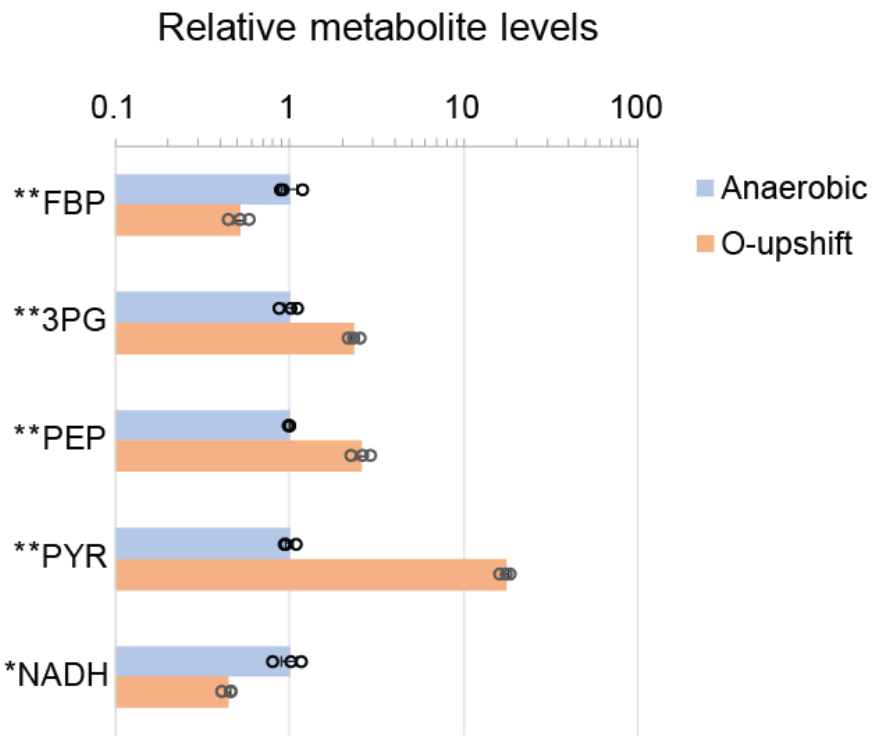
Supplementary Figure 5. FBP and ATP levels and labeling upon phosphorus upshift.
Phosphorus-limited *E. coli* growing on filter culture were subjected to phosphate upshift simultaneously with switching from unlabeled to [U-$^{13}C_6$]glucose. Fructose-1,6-bisphosphate rapidly increases by incorporating new carbons from [U-$^{13}C_6$]glucose while ATP concentration increases prior to substantial ^{13}C assimilation.



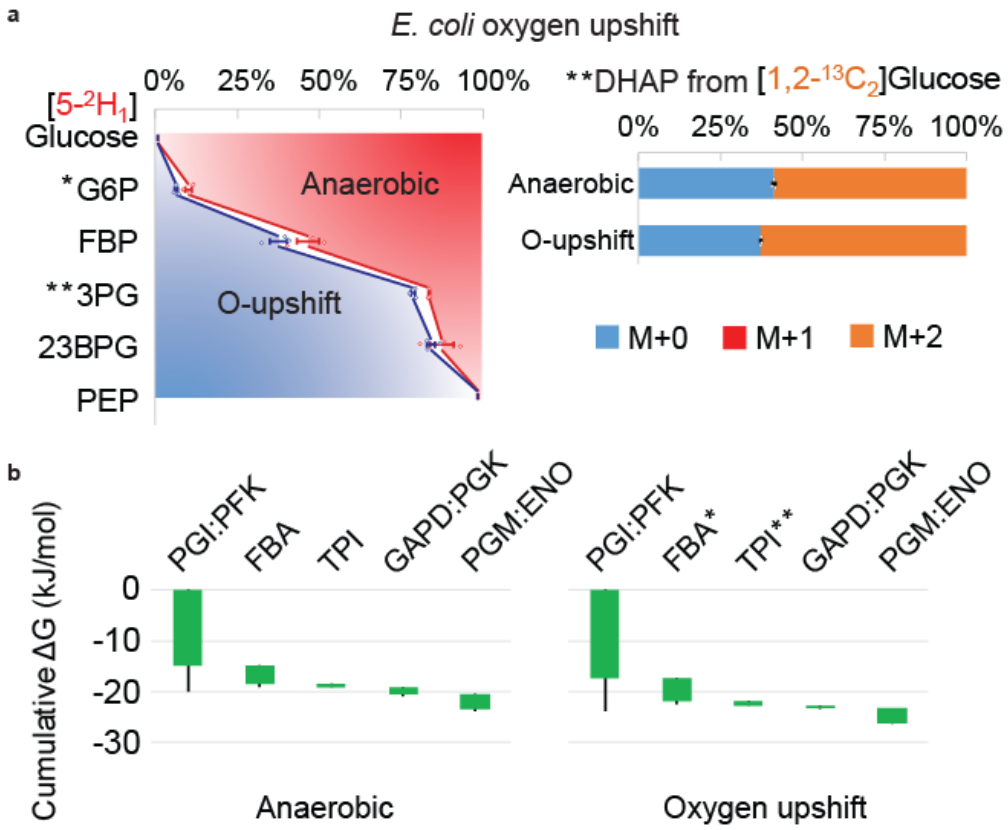
Supplementary Figure 6. Growth rate increases upon phosphorus upshift. Initially, seven cultures started on 0.15 mM phosphate medium. Five cultures were provided with 1.32 mM phosphate when cell growth was slowing down from phosphorus limitation (dashed red line). Two cultures were not provided with additional phosphate (control).



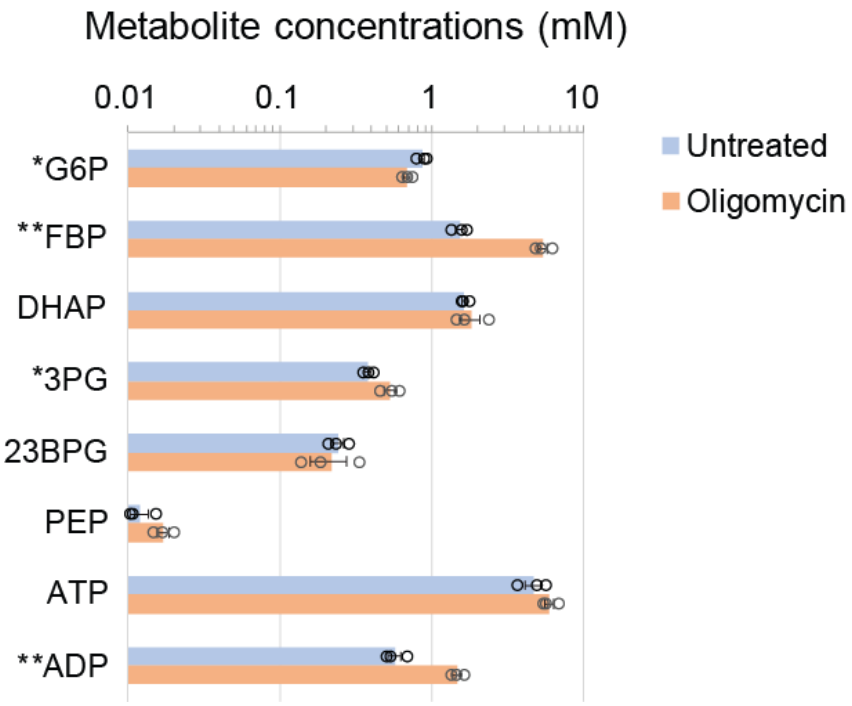
Supplementary Figure 7. Statistically significant changes in deuterium labeling in *E. coli* phosphorus upshift. The centre and error bars represent the mean ± s.e.m. (n=3 biologically independent samples). G6P $p<0.05$, 3PG $p<0.05$, and 23BPG $p<0.05$ by two-tailed t-tests.



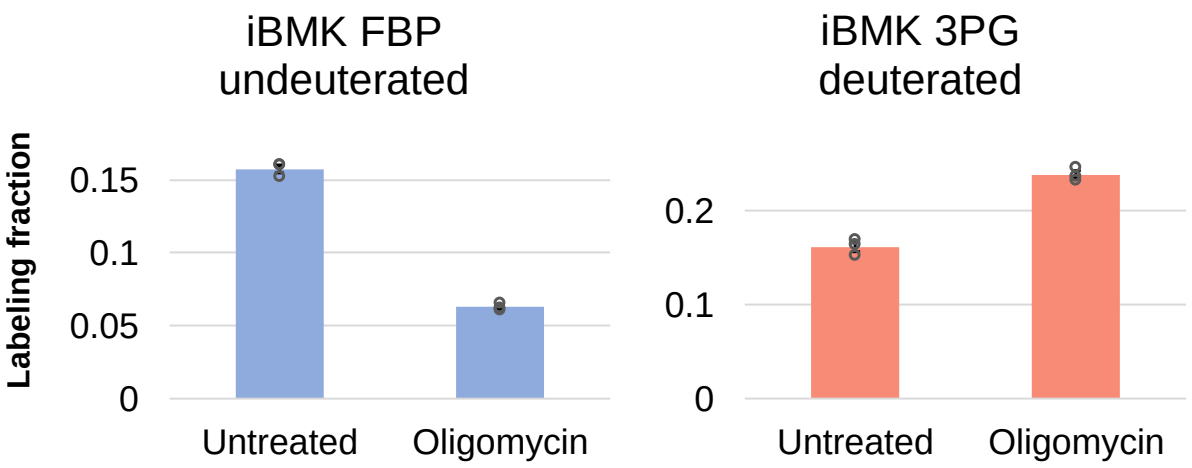
Supplementary Figure 8. Changes in metabolite levels upon oxygen upshift in *E. coli*. The centre and error bars represent the mean ± s.e.m. (n=3 biologically independent samples). Metabolites were measured by LC-MS in anaerobic culture condition and 5 minutes after shifting to aerobic environment. * and ** represent $p<0.05$ and $p<0.01$ by two-tailed t-tests.



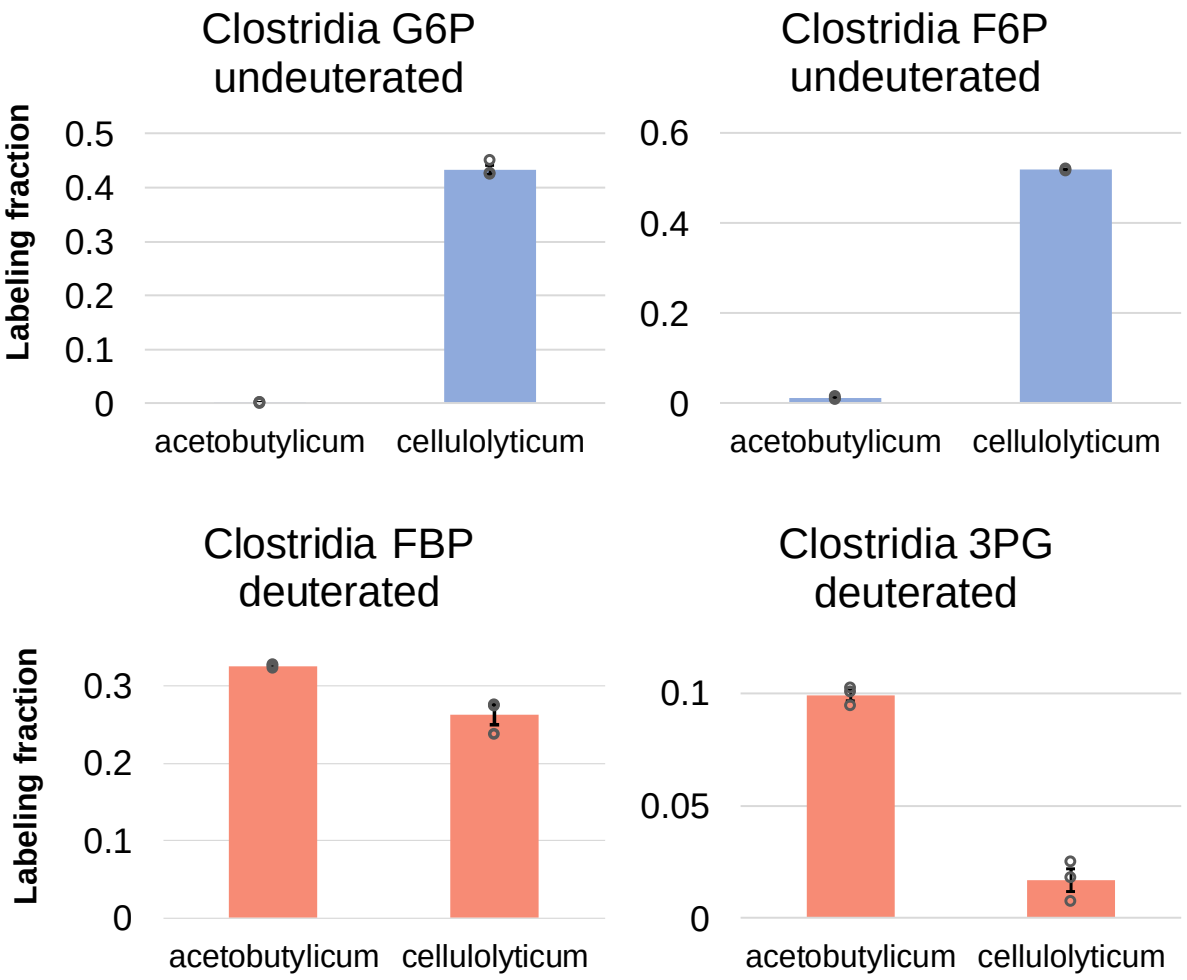
Supplementary Figure 9. [5-$^{2}H_1$] and [1,2-$^{13}C_2$] glucose labeling and ΔG during oxygen upshift. (**a**) *E. coli* cultured on [5-$^{2}H_1$]- or [1,2-$^{13}C_2$]-glucose in anaerobic environment and five minutes after shifting to aerobic environment. G6P and 3PG gained significant deuterium labeling while the DHAP also gained ^{13}C labeling. The centre and error bars represent the mean ± s.e.m. (n=3 biologically independent samples). (**b**) Corresponding ΔG were inferred from the flux modeling results that best fit the observed ^{2}H and ^{13}C labeling. Each of the reaction(s) ΔG (<0) is represented by the height of the green bar below. The bottom edges indicate the cumulative ΔG up to the corresponding step in glycolysis. Whiskers show s.e.m.; * and ** represent $p<0.05$ and $p<0.01$ by two-tailed t-tests or bootstrapping (**Online Methods**).



Supplementary Figure 10. Metabolite levels in untreated and oligomycin-treated conditions. The metabolite levels were measured 30 minutes after the addition of oligomycin or DMSO vehicle (untreated) in mammalian iBMK cells. The centre and error bars represent the mean ± s.e.m. (n=3 biologically independent samples). * and ** represent $p<0.05$ and $p<0.01$ by two-tailed t-tests.

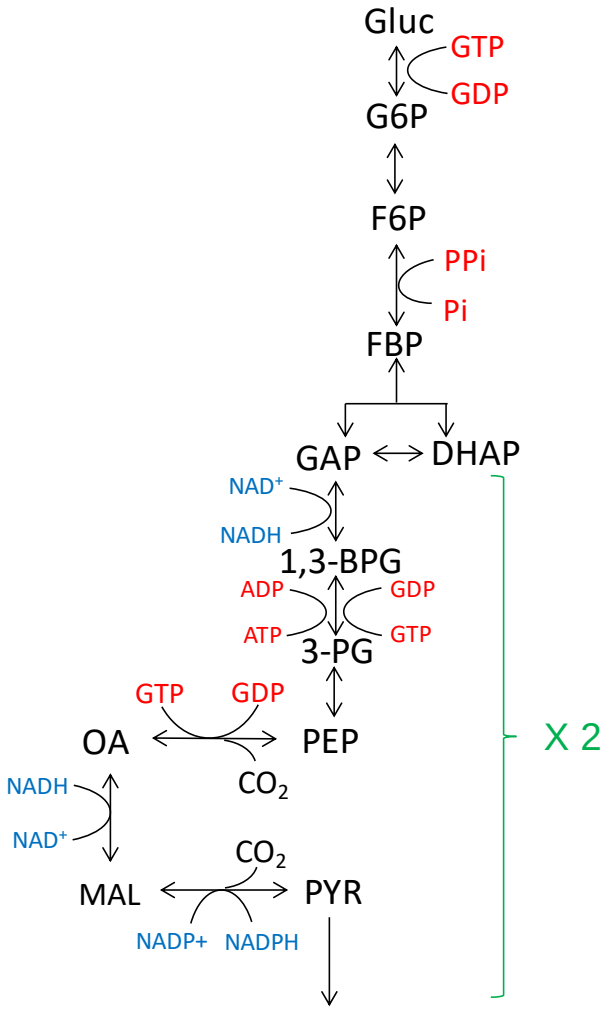


Supplementary Figure 11. Statistically significant changes in deuterium labeling in mammalian cells treated with oligomycin. The centre and error bars represent the mean ± s.e.m. (n=3 biologically independent samples). FBP $p<10^{-5}$ and 3PG $p<0.001$ by two-tailed t-tests.

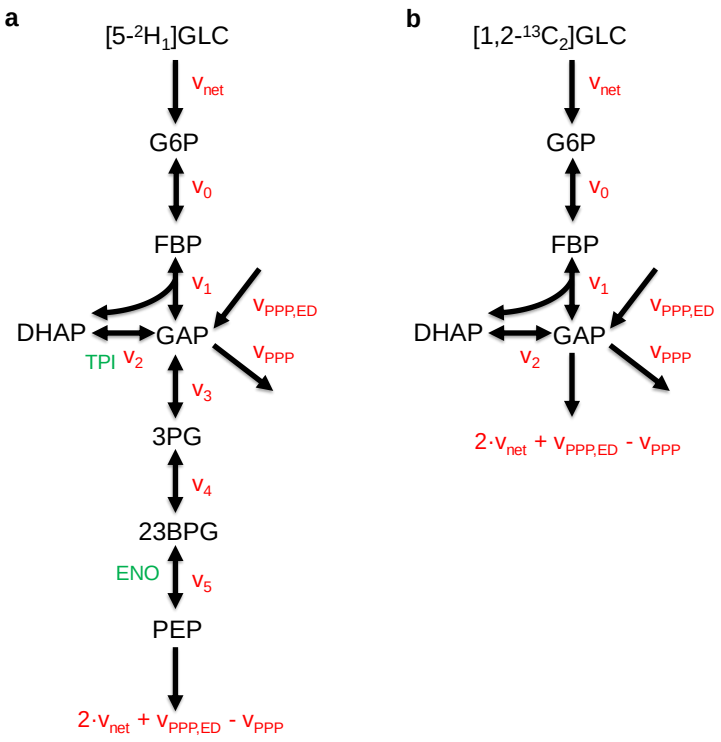


Supplementary Figure 12. Statistically significant differences in deuterium labeling between *C. acetobutylicum* and *C. cellulolyticum*. The centre and error bars represent the mean ± s.e.m. (n=3 biologically independent samples). G6P $p<0.01$, F6P $p<0.01$, FBP $p<0.05$, and 3PG $p<0.05$ by two-tailed t-tests.

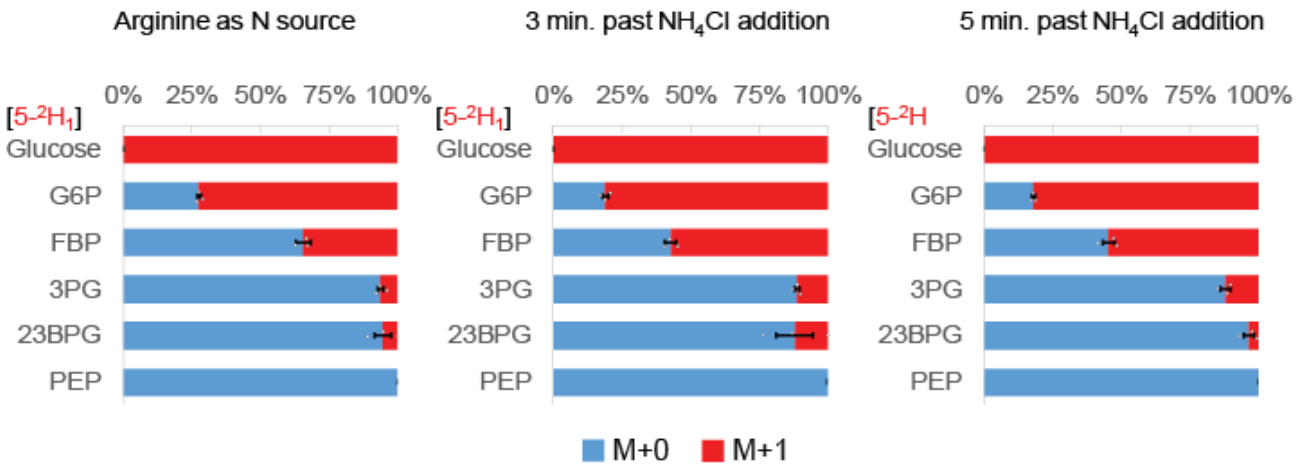
C. cellulolyticum glycolysis



Supplementary Figure 13. Reversible glycolysis of *C. cellulolyticum* yields three ATP-equivalents per glucose due to the PPi-dependent PFK. Because PPi is a byproduct not substrate of biomass synthesis, the use of PPi instead of ATP in the PFK step effectively results in generation of an extra ATP-equivalent in glycolysis.



Supplementary Figure 14. Model of glycolysis for reversibility and ΔG calculation. (**a**) The set of reactions to solve for fluxes from [5-$^{2}H_{1}$]glucose tracing. (**b**) The set of reactions to solve for fluxes from [1,2-$^{13}C_{2}$]glucose tracing. $v_{0,b}$ denotes the backward flux through lumped PGI-PFK reactions; $v_{1,b}$, FBA; $v_{2,b}$, TPI; $v_{3,b}$, GAPD-PGK; $v_{4,b}$, first half of PGM (3PG<=>23BPG); $v_{5,b}$, the latter half of PGM and ENO; $v_{PPP,ED}$, lumped PPP and EDP fluxes that result in GAP; and v_{PPP} lumped non-oxidative PPP backward fluxes.



Supplementary Figure 15. Deuterium labeling fractions of glycolytic intermediates in *E. coli* before and after nitrogen upshift. The labeling patterns three minutes and five minutes after NH_4Cl addition are comparable, suggesting a quasi-steady state. The centre and error bars represent the mean ± s.e.m. (n=3 biologically independent samples).

Supplementary Table 1. *E. coli* metabolite labeling with [5-$^{2}H_{1}$]glucose

		N-replete			N-limited			N-upshift (5 mins)		
Metabolite	**Labeling**	**Measured**	**s.e.**	**Simulated**	**Measured**	**s.e.**	**Simulated**	**Measured**	**s.e.**	**Simulated**
G6P	M+0	4.4%	0.4%	3.5%	27.7%	0.7%	27.5%	18.1%	0.5%	18.1%
	M+1	95.6%	0.4%	96.5%	72.3%	0.7%	72.5%	81.9%	0.5%	81.9%
FBP	M+0	34.9%	0.7%	32.4%	63.8%	1.3%	62.8%	45.4%	1.7%	39.7%
	M+1	65.1%	0.7%	67.5%	36.2%	1.3%	37.2%	54.6%	1.7%	60.3%
DHAP	M+0	98.8%	0.7%	99.7%			99.7%			100.0%
	M+1	1.2%	0.7%	0.3%			0.3%			0.0%
3PG	M+0	73.8%	0.5%	74.7%	92.8%	1.1%	92.9%	87.0%	1.4%	88.5%
	M+1	26.2%	0.5%	25.3%	7.2%	1.1%	7.1%	13.0%	1.4%	11.5%
23BPG	M+0	82.1%	0.7%	82.0%	94.7%	1.7%	94.6%	92.7%	1.7%	92.9%
	M+1	17.9%	0.7%	18.0%	5.3%	1.7%	5.4%	7.3%	1.7%	7.1%
PEP	M+0	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%
	M+1	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
6PG	M+0	3.9%	0.3%	4.5%	33.0%	1.0%	33.1%	16.7%	1.7%	18.1%
	M+1	96.1%	0.3%	95.5%	67.0%	1.0%	66.8%	83.3%	1.7%	81.9%

G6P denotes glucose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; 23BPG, 2,3-bisphosphoglycerate; PEP, phosphoenolpyruvate; and 6PG, 6-phosphogluconate. The “Measured” column represents the mean of three or four biologically independent samples measured by LC-MS. The “s.e.” column represents the standard error of the mean. The “Simulated” column represents the simulation values that best fit the measured labeling.

Supplementary Table 2. *E. coli* metabolite labeling with [1,2-$^{13}C_2$]glucose

		N-replete			N-limited			N-upshift (5 mins)		
Metabolite	**Labeling**	**Measured**	**s.e.**	**Simulated**	**Measured**	**s.e.**	**Simulated**	**Measured**	**s.e.**	**Simulated**
G6P	M+0	0.2%	0.2%	0.2%	1.5%	0.6%	2.4%	1.1%	0.4%	0.2%
	M+1	0.2%	0.2%	0.4%	1.7%	0.7%	1.0%	0.9%	0.4%	1.2%
	M+2	98.5%	0.3%	98.7%	83.2%	1.5%	83.1%	90.3%	0.4%	90.4%
	M+3	0.0%	0.2%	0.2%	2.7%	0.6%	2.6%	1.2%	0.4%	1.9%
	M+4	1.0%	0.2%	0.6%	9.2%	0.8%	9.2%	5.7%	0.4%	5.5%
	M+5	0.1%	0.2%	0.0%	1.7%	0.6%	1.8%	0.8%	0.4%	0.8%
	M+6	0.0%	0.6%	0.0%	0.0%	0.6%	0.1%	0.0%	0.5%	0.0%
F6P	M+0	0.0%	0.6%	1.2%	2.2%	0.6%	2.6%	0.6%	0.4%	0.2%
	M+1	2.8%	0.7%	2.2%	1.7%	0.6%	1.1%	0.8%	0.5%	1.3%
	M+2	92.3%	0.5%	91.9%	80.3%	1.5%	81.4%	90.8%	1.1%	89.4%
	M+3	0.9%	0.7%	0.9%	2.2%	0.7%	2.8%	2.2%	0.4%	2.0%
	M+4	4.1%	0.3%	3.5%	12.1%	1.5%	10.2%	4.5%	0.5%	6.1%
	M+5	0.0%	0.6%	0.3%	1.5%	0.6%	1.9%	1.0%	0.4%	0.9%
	M+6	0.0%	0.6%	0.0%	0.0%	0.6%	0.1%	0.0%	0.5%	0.0%
FBP	M+0	4.8%	0.6%	7.6%	10.4%	1.4%	14.5%	4.5%	0.4%	7.2%
	M+1	2.1%	0.3%	1.9%	1.4%	0.6%	1.0%	1.2%	0.6%	1.3%
	M+2	79.5%	0.5%	77.3%	66.9%	1.5%	63.4%	77.5%	1.5%	77.3%
	M+3	1.2%	0.5%	1.3%	3.2%	0.8%	2.8%	3.2%	1.4%	2.1%
	M+4	12.1%	0.8%	11.6%	16.8%	1.1%	16.5%	13.4%	0.9%	11.2%
	M+5	0.2%	0.2%	0.3%	0.8%	0.6%	1.8%	0.2%	0.4%	0.9%
	M+6	0.0%	0.6%	0.0%	0.4%	0.6%	0.0%	0.0%	0.5%	0.0%
DHAP	M+0	37.3%	0.3%	37.2%	49.0%	1.2%	48.1%	47.7%	1.0%	45.8%
	M+1	1.9%	0.2%	1.7%	1.3%	0.7%	1.0%	1.6%	0.6%	1.5%
	M+2	60.6%	0.2%	60.7%	49.3%	1.5%	49.1%	49.4%	1.1%	51.6%
	M+3	0.2%	0.2%	0.4%	0.4%	0.6%	1.9%	1.3%	0.6%	1.1%
3PG	M+0	52.7%	0.7%	53.3%	53.8%	1.0%	52.3%	54.6%	1.5%	52.4%
	M+1	1.1%	0.2%	1.3%	0.7%	0.6%	0.9%	2.2%	0.5%	1.3%
	M+2	46.0%	0.8%	45.1%	45.5%	0.9%	45.0%	43.1%	1.5%	45.4%
	M+3	0.2%	0.2%	0.3%	0.0%	0.6%	1.7%	0.0%	0.5%	0.9%
S7P	M+0	0.3%	0.3%	0.1%	1.4%	0.9%	2.7%	0.0%	0.5%	0.3%
	M+1	2.8%	0.7%	3.2%	3.5%	0.9%	4.2%	4.5%	0.8%	7.4%
	M+2	58.4%	0.8%	58.9%	45.9%	1.5%	48.1%	46.3%	1.3%	47.5%
	M+3	14.4%	0.8%	13.6%	14.8%	1.0%	16.2%	19.3%	1.5%	20.2%
	M+4	17.9%	0.8%	17.8%	21.9%	1.2%	13.4%	23.0%	1.5%	10.7%
	M+5	6.2%	0.8%	5.8%	9.1%	1.5%	11.5%	6.8%	1.5%	12.3%
	M+6	0.0%	0.2%	0.5%	3.4%	1.1%	3.8%	0.1%	0.4%	1.6%
	M+7	0.0%	0.6%	0.0%	0.0%	0.6%	0.1%	0.0%	0.5%	0.0%
6PG	M+0	0.0%	0.2%	0.2%						
	M+1	3.1%	0.4%	2.1%						
	M+2	96.3%	0.5%	96.3%						
	M+3	0.0%	0.6%	0.7%						
	M+4	0.5%	0.2%	0.7%						
	M+5	0.0%	0.2%	0.0%						
	M+6	0.0%	0.6%	0.0%						

G6P denotes glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; S7P, sedoheptulose-7-phosphate; and 6PG, 6-phosphogluconate. The “Measured” column represents the mean of three or four biologically independent samples measured by LC-MS. The “s.e.” column represents the standard error of the mean. The “Simulated” column represents the simulation values that best fit the measured labeling.

Supplementary Table 3. *E. coli* ΔG (kJ/mol) measured with [5-$^{2}H_{1}$]- and [1,2-$^{13}C_{2}$]-glucose

Reaction	Substrates	Products	N-replete ΔG	N-replete s.e.*	N-limited ΔG	N-limited s.e.*	N-upshift (5 mins) ΔG	N-upshift (5 mins) s.e.*	N-upshift P-value**
PGI:PFK	G6P	FBP	-9.11	0.68	-4.45	0.49	-15.15	4.91	0.04
FBA	FBP	DHAP + GAP	-2.48	0.08	-1.41	0.10	-3.09	0.16	0.00
TPI	DHAP	GAP	-0.92	0.02	-0.24	0.02	-0.36	0.06	0.01
GAPD:PGK	GAP	3PG	-4.33	1.94	-4.13	1.84	-4.61	2.08	0.77
PGM:ENO	3PG	PEP	-11.32	2.62	-7.88	3.25	-11.66	4.79	0.20
PGI:ENO	G6P	PEP	-27.60	4.51	-14.15	3.32	-27.28	6.04	0.00

* Standard error was obtained by dividing the 95% confidence intervals by 4

** P-values were estimated by the fraction of overlapped areas between two ΔG distributions

Supplementary Table 4. *E. coli* metabolite labeling with [5-$^{2}H_{1}$]glucose upon phosphorus and oxygen upshift

Metabolite	Labeling	P-limited Measured	P-limited s.e.	P-limited Simulated	P-upshift (5 mins) Measured	P-upshift (5 mins) s.e.	P-upshift (5 mins) Simulated	Anaerobic Measured	Anaerobic s.e.	Anaerobic Simulated	O-upshift (5 mins) Measured	O-upshift (5 mins) s.e.	O-upshift (5 mins) Simulated
G6P	M+0	36.3%	1.9%	39.2%	21.5%	2.0%	24.1%	9.5%	0.7%	9.9%	5.8%	0.5%	6.7%
	M+1	63.7%	1.9%	60.8%	78.5%	2.0%	75.9%	90.5%	0.7%	90.1%	94.2%	0.5%	93.3%
FBP	M+0	52.3%	1.9%	53.8%	42.0%	2.0%	43.7%	46.8%	0.7%	46.2%	37.7%	0.5%	36.3%
	M+1	47.7%	1.9%	46.2%	58.0%	2.0%	56.2%	53.2%	0.7%	53.8%	62.3%	0.5%	63.7%
DHAP	M+0			100.0%			99.7%			99.7%			100.0%
	M+1			0.0%			0.3%			0.3%			0.0%
3PG	M+0	95.1%	1.1%	95.2%	88.5%	1.5%	88.7%	84.6%	0.2%	84.6%	79.8%	0.5%	80.2%
	M+1	4.9%	1.1%	4.8%	11.5%	1.5%	11.3%	15.4%	0.2%	15.4%	20.2%	0.5%	19.8%
23BPG	M+0	99.4%	0.6%	98.4%	90.1%	2.0%	89.8%	88.5%	0.7%	88.5%	85.4%	0.5%	85.4%
	M+1	0.6%	0.6%	1.6%	9.9%	2.0%	10.2%	11.5%	0.7%	11.5%	14.6%	0.5%	14.6%
PEP	M+0	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%
	M+1	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

G6P denotes glucose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; 23BPG, 2,3-bisphosphoglycerate; and PEP, phosphoenolpyruvate. The “Measured” column represents the mean of three biologically independent samples measured by LC-MS. The “s.e.” column represents the standard error of the mean. The “Simulated” column represents the simulation values that best fit the measured labeling.

Supplementary Table 5. *E. coli* metabolite labeling with [1,2-$^{13}C_2$]glucose upon phosphorus and oxygen upshift

		P-limited			P-upshift (5 mins)			Anaerobic			O-upshift (5 mins)		
Metabolite	Labeling	Measured	s.e.	Simulated	Measured	s.e.	Simulated	Measured	s.e.	Simulated	Measured	s.e.	Simulated
G6P	M+0	1.0%	0.4%	1.0%	0.6%	0.2%	0.5%	0.3%	0.2%	0.3%	0.4%	0.2%	0.2%
	M+1	1.3%	0.4%	1.8%	1.2%	0.2%	1.3%	0.3%	0.2%	0.4%	0.6%	0.2%	0.7%
	M+2	72.8%	1.9%	75.4%	83.6%	1.0%	84.4%	93.3%	0.7%	93.8%	94.3%	0.4%	95.2%
	M+3	4.1%	0.9%	3.1%	2.1%	0.3%	1.8%	1.0%	0.2%	0.7%	0.9%	0.2%	0.6%
	M+4	18.3%	0.8%	16.6%	11.7%	0.5%	11.4%	4.6%	0.4%	4.4%	3.6%	0.2%	3.2%
	M+5	2.2%	0.7%	2.1%	0.6%	0.2%	0.6%	0.4%	0.2%	0.4%	0.2%	0.2%	0.2%
	M+6	0.2%	0.4%	0.1%	0.2%	0.2%	0.0%	0.1%	0.2%	0.0%	0.1%	0.2%	0.0%
FBP	M+0	7.2%	1.9%	5.6%	6.5%	2.0%	5.4%	6.6%	0.6%	7.4%	4.9%	0.2%	5.3%
	M+1	1.7%	0.4%	2.0%	1.7%	0.2%	1.8%	0.9%	0.2%	0.7%	1.6%	0.2%	1.4%
	M+2	64.0%	1.9%	66.8%	67.9%	2.0%	72.3%	72.6%	0.7%	71.8%	76.4%	0.2%	75.8%
	M+3	3.9%	0.9%	3.5%	2.5%	0.3%	2.4%	1.5%	0.2%	1.2%	1.9%	0.2%	1.8%
	M+4	20.5%	1.3%	19.7%	20.3%	2.0%	17.4%	17.4%	0.6%	18.3%	14.6%	0.2%	15.1%
	M+5	2.3%	0.6%	2.3%	0.7%	0.2%	0.8%	0.6%	0.2%	0.6%	0.4%	0.2%	0.5%
	M+6	0.3%	0.4%	0.1%	0.4%	0.2%	0.0%	0.4%	0.2%	0.0%	0.3%	0.2%	0.0%
DHAP	M+0	47.6%	1.5%	47.6%	44.6%	1.0%	43.8%	40.8%	0.2%	40.8%	36.6%	0.2%	36.6%
	M+1	2.1%	0.4%	1.7%	1.7%	0.2%	1.8%	0.5%	0.3%	0.7%	1.6%	0.2%	1.6%
	M+2	48.9%	1.9%	48.8%	53.1%	1.1%	53.7%	58.3%	0.5%	57.9%	61.3%	0.2%	61.3%
	M+3	1.4%	0.7%	2.0%	0.6%	0.2%	0.7%	0.3%	0.2%	0.6%	0.6%	0.2%	0.6%
3PG	M+0	53.4%	0.5%	53.1%	52.5%	0.2%	52.5%	52.3%	0.2%	52.2%	52.7%	0.2%	52.6%
	M+1	1.8%	0.4%	1.5%	1.5%	0.2%	1.5%	0.4%	0.2%	0.6%	1.1%	0.2%	1.2%
	M+2	43.0%	1.1%	43.6%	45.3%	0.2%	45.3%	46.9%	0.3%	46.7%	45.9%	0.2%	45.7%
	M+3	1.9%	0.4%	1.8%	0.7%	0.2%	0.6%	0.5%	0.2%	0.5%	0.3%	0.2%	0.4%

G6P denotes glucose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; and 3PG, 3-phosphoglycerate. The “Measured” column represents the mean of three biologically independent samples measured by LC-MS. The “s.e.” column represents the standard error of the mean. The “Simulated” column represents the simulation values that best fit the measured labeling.

Supplementary Table 6. *E. coli* ΔG (kJ/mol) measured with [5-$^{2}H_1$]- and [1,2-$^{13}C_2$]-glucose upon phosphorus and oxygen upshift

			P-limited		P-upshift (5 mins)		P-upshift	Anaerobic		O-upshift (5 mins)		O-upshift
Reaction	Substrates	Products	ΔG	s.e.*	ΔG	s.e.*	P-value**	ΔG	s.e.*	ΔG	s.e.*	P-value**
PGI:PFK	G6P	FBP	-15.22	6.62	-14.46	6.47	0.85	-14.92	5.17	-17.43	6.35	0.55
FBA	FBP	DHAP + GAP	-3.78	0.48	-8.01	2.62	0.62	-3.64	0.65	-4.42	0.62	0.02
TPI	DHAP	GAP	-0.33	0.06	-0.47	0.07	0.00	-0.63	0.02	-0.93	0.01	0.00
GAPD:PGK	GAP	3PG	-12.73	4.52	-12.40	5.89	0.81	-1.38	0.47	-0.59	0.09	0.23
PGM:ENO	3PG	PEP	-2.12	0.76	-6.67	2.30	0.01	-2.90	0.37	-2.81	0.23	0.53
PGI:ENO	G6P	PEP	-33.80	7.14	-41.11	8.49	0.85	-21.26	5.29	-26.20	6.41	0.46

* Standard error was obtained by dividing the 95% confidence intervals by 4

** P-values were estimated by the fraction of overlapped areas between two estimated ΔG distributions

Supplementary Table 7. Mammalian iBMK cell metabolite labeling with [5-$^{2}H_{1}$]glucose

		Untreated (DMSO)			Oligomycin (30 mins)		
Metabolite	**Labeling**	**Measured**	**s.e.**	**Simulated**	**Measured**	**s.e.**	**Simulated**
G6P*	M+0	8.0%	1.0%	9.8%	3.6%	0.9%	2.3%
	M+1	92.0%	1.0%	90.2%	96.4%	0.9%	97.7%
FBP	M+0	15.7%	0.3%	15.6%	6.3%	0.2%	6.4%
	M+1	84.3%	0.3%	84.4%	93.7%	0.2%	93.6%
DHAP	M+0			99.8%			99.7%
	M+1			0.2%			0.2%
3PG	M+0	83.8%	0.5%	83.8%	76.2%	0.4%	76.2%
	M+1	16.2%	0.5%	16.2%	23.8%	0.4%	23.8%
23BPG	M+0	86.8%	1.0%	86.8%	86.5%	0.9%	86.5%
	M+1	13.2%	1.0%	13.2%	13.5%	0.9%	13.5%
PEP	M+0	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%
	M+1	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

* G6P represents the measurement of 6PG as accurate hexose phosphate reading was interfered by glycogen degradation

G6P denotes glucose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; 23BPG, 2,3-bisphosphoglycerate; and PEP, phosphoenolpyruvate. The “Measured” column represents the mean of three biologically independent samples measured by LC-MS. The “s.e.” column represents the standard error of the mean. The “Simulated” column represents the simulation values that best fit the measured labeling.

Supplementary Table 8. Mammalian iBMK cell metabolite labeling with [1,2-$^{13}C_2$]glucose

		Untreated (DMSO)			Oligomycin (30 mins)		
Metabolite	**Labeling**	**Measured**	**s.e.**	**Simulated**	**Measured**	**s.e.**	**Simulated**
G6P*	M+0	1.9%	0.7%	0.4%	1.6%	0.9%	1.4%
	M+1	1.1%	0.6%	0.7%	0.3%	0.3%	0.2%
	M+2	94.0%	0.7%	95.3%	97.8%	0.9%	97.3%
	M+3	0.0%	0.2%	0.6%	0.0%	0.2%	0.2%
	M+4	2.9%	0.2%	2.5%	0.3%	0.3%	0.8%
	M+5	0.0%	0.2%	0.4%	0.0%	0.2%	0.1%
	M+6	0.0%	0.2%	0.0%	0.0%	0.2%	0.0%
FBP	M+0	0.8%	0.2%	2.5%	0.3%	0.2%	0.3%
	M+1	0.0%	0.2%	0.1%	0.0%	0.2%	0.0%
	M+2	93.1%	0.2%	92.6%	95.9%	0.2%	95.7%
	M+3	0.0%	0.2%	0.0%	0.0%	0.2%	0.2%
	M+4	6.0%	0.2%	4.8%	3.8%	0.2%	3.5%
	M+5	0.1%	0.2%	0.0%	0.0%	0.2%	0.2%
	M+6	0.0%	0.2%	0.0%	0.0%	0.2%	0.0%
DHAP	M+0	36.1%	1.0%	34.1%	32.5%	0.9%	32.3%
	M+1	0.0%	0.2%	0.1%	0.0%	0.2%	0.0%
	M+2	63.8%	1.0%	65.8%	67.5%	0.9%	67.4%
	M+3	0.1%	0.2%	0.0%	0.0%	0.2%	0.3%
3PG	M+0	49.4%	0.3%	50.3%	49.2%	0.9%	50.3%
	M+1	0.3%	0.3%	0.1%	0.0%	0.2%	0.0%
	M+2	50.2%	0.4%	49.6%	50.8%	0.9%	49.5%
	M+3	0.1%	0.2%	0.0%	0.0%	0.2%	0.2%
S7P	M+0	1.0%	1.0%	0.0%	0.6%	0.6%	1.4%
	M+1	1.3%	0.4%	1.7%	0.6%	0.6%	0.1%
	M+2	57.9%	1.0%	58.2%	78.9%	0.9%	80.7%
	M+3	13.8%	1.0%	15.2%	5.3%	0.9%	5.8%
	M+4	16.3%	1.0%	17.5%	9.4%	0.9%	4.8%
	M+5	7.3%	0.5%	6.7%	4.6%	0.5%	5.3%
	M+6	2.2%	1.0%	0.7%	0.5%	0.5%	1.7%
	M+7	0.0%	0.2%	0.0%	0.0%	0.2%	0.0%

* G6P represents the measurement of 6PG as accurate hexose phosphate reading was interfered by glycogen degradation

G6P denotes glucose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; and S7P, sedoheptulose-7-phosphate. The “Measured” column represents the mean of three biologically independent samples measured by LC-MS. The “s.e.” column represents the standard error of the mean. The “Simulated” column represents the simulation values that best fit the measured labeling.

Supplementary Table 9. Mammalian iBMK cell ΔG (kJ/mol) measured with [5-$^{2}H_{1}$]- and [1,2-$^{13}C_{2}$]-glucose

			Untreated (DMSO)		Oligomycin (30 mins)		
Reaction	**Substrates**	**Products**	**ΔG**	**s.e.***	**ΔG**	**s.e.***	**P-value****
PGI:PFK	G6P	FBP	-13.48	5.02	-11.97	4.85	0.26
FBA	FBP	DHAP + GAP	-5.09	0.13	-10.82	1.40	0.00
TPI	DHAP	GAP	-1.00	0.05	-1.13	0.08	0.01
GAPD:PGK	GAP	3PG	-1.39	0.45	-10.03	4.77	0.03
PGM:ENO	3PG	PEP	-2.13	0.17	-5.83	1.14	0.00
PGI:ENO	G6P	PEP	-22.10	5.00	-34.36	8.30	0.00

* Standard error was obtained by dividing the 95% confidence intervals by 4

** P-values were estimated by the fraction of overlapped areas between two estimated ΔG distributions

Supplementary Table 10. Clostridia metabolite labeling with [5-$^{2}H_{1}$]glucose

		C. acetobutylicum			*C. cellulolyticum*		
Metabolite	**Labeling**	**Measured**	**s.e.**	**Simulated**	**Measured**	**s.e.**	**Simulated**
G6P	M+0	0.1%	0.2%	0.7%	43.3%	0.8%	37.9%
	M+1	99.9%	0.2%	99.3%	56.7%	0.8%	62.1%
F6P	M+0	1.1%	0.2%	0.7%	51.9%	0.2%	51.7%
	M+1	98.9%	0.2%	99.2%	48.1%	0.2%	48.3%
FBP	M+0	67.4%	0.2%	67.8%	73.7%	0.8%	71.0%
	M+1	32.6%	0.2%	32.2%	26.3%	0.8%	29.0%
DHAP	M+0	100.0%	0.0%	99.7%	100.0%	0.0%	100.0%
	M+1	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%
3PG	M+0	90.1%	0.2%	90.1%	98.3%	0.5%	97.8%
	M+1	9.9%	0.2%	9.9%	1.7%	0.5%	2.2%
PEP	M+0	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%
	M+1	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
S7P	M+0	49.0%	0.4%	48.1%	59.7%	0.6%	61.0%
	M+1	51.0%	0.4%	51.7%	40.3%	0.6%	39.0%

G6P denotes glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; and S7P, sedoheptulose-7-phosphate. The “Measured” column represents the mean of three biologically independent samples measured by LC-MS. The “s.e.” column represents the standard error of the mean. The “Simulated” column represents the simulation values that best fit the measured labeling.

Supplementary Table 11. Clostridia metabolite labeling with [1,2-$^{13}C_2$]glucose

		C. acetobutylicum			*C. cellulolyticum*		
Metabolite	**Labeling**	**Measured**	**s.e.**	**Simulated**	**Measured**	**s.e.**	**Simulated**
G6P	M+0	0.8%	0.4%	0.2%	8.1%	0.2%	8.5%
	M+1	0.0%	0.2%	0.0%	0.0%	0.2%	0.4%
	M+2	99.1%	0.4%	99.6%	79.8%	0.2%	78.9%
	M+3	0.1%	0.2%	0.0%	1.4%	0.2%	1.0%
	M+4	0.0%	0.2%	0.2%	9.6%	0.2%	10.6%
	M+5	0.0%	0.2%	0.0%	1.1%	0.2%	0.6%
	M+6	0.0%	0.2%	0.0%	0.0%	0.2%	0.0%
FBP	M+0	17.8%	0.4%	16.8%	12.9%	0.8%	15.9%
	M+1	0.2%	0.2%	0.0%	0.1%	0.2%	0.6%
	M+2	58.3%	0.4%	61.0%	68.6%	0.8%	61.6%
	M+3	0.1%	0.2%	0.1%	1.0%	0.5%	1.3%
	M+4	23.0%	0.2%	22.0%	15.9%	0.8%	19.9%
	M+5	0.1%	0.2%	0.0%	1.5%	0.3%	0.8%
	M+6	0.6%	0.2%	0.0%	0.0%	0.2%	0.0%
DHAP	M+0	43.5%	0.2%	43.1%	42.1%	0.8%	42.7%
	M+1	0.0%	0.2%	0.0%	0.0%	0.2%	0.6%
	M+2	56.5%	0.2%	56.8%	57.6%	0.8%	55.8%
	M+3	0.0%	0.2%	0.0%	0.3%	0.2%	0.8%
3PG	M+0			50.2%	50.6%	0.2%	50.3%
	M+1			0.0%	0.0%	0.2%	0.5%
	M+2			49.8%	47.8%	0.2%	48.5%
	M+3			0.0%	1.5%	0.2%	0.7%
S7P	M+0	20.2%	0.4%	19.4%	22.7%	0.2%	22.9%
	M+1	0.0%	0.2%	0.4%	1.6%	0.2%	2.5%
	M+2	40.1%	0.4%	38.6%	45.6%	0.2%	45.7%
	M+3	0.7%	0.4%	1.0%	6.2%	0.3%	5.9%
	M+4	24.4%	0.3%	25.1%	16.8%	0.2%	17.3%
	M+5	0.0%	0.2%	0.6%	3.2%	0.2%	3.2%
	M+6	14.6%	0.4%	14.9%	3.7%	0.3%	2.4%
	M+7	0.0%	0.2%	0.0%	0.3%	0.2%	0.0%

G6P denotes glucose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; and S7P, sedoheptulose-7-phosphate. The “Measured” column represents the mean of three biologically independent samples measured by LC-MS. The “s.e.” column represents the standard error of the mean. The “Simulated” column represents the simulation values that best fit the measured labeling.

Supplementary Table 12. Clostridia ΔG (kJ/mol) measured with [5-$^{2}H_{1}$]- and [1,2-$^{13}C_{2}$]-glucose

Reaction	Substrates	Products	*C. acetobutylicum* ΔG	*C. acetobutylicum* s.e.*	*C. cellulolyticum* ΔG	*C. cellulolyticum* s.e.*	P-value**
PGI	G6P	F6P	-2.79	1.37	-0.79	0.04	0.01
PFK	F6P	FBP	-12.23	1.16	-0.92	0.10	0.00
FBA	FBP	DHAP + GAP	-0.64	0.01	-0.87	0.07	0.00
TPI	DHAP	GAP	-0.38	0.01	-0.39	0.04	0.18
GAPD:PGK	GAP	3PG	-14.18	6.33	-0.12	0.01	0.00
PGM:ENO	3PG	PEP	-3.78	0.49	-0.14	0.01	0.00
PGI:ENO	G6P	PEP	-31.70	5.83	-3.24	0.15	0.00

* Standard error was obtained by dividing the 95% confidence intervals by 4

** P-values were estimated by the fraction of overlapped areas between two estimated ΔG distributions

Supplementary Table 13. Carbon and hydrogen mapping in glycolysis, ED pathway, and pentose phosphate pathway

Reaction	Substrates (CCChhh*)	Products (CCChhh*)
GLC Uptake	GLC (ABCDEFabcdefg)	G6P (ABCDEFabcdefg)
PGI	G6P (ABCDEFab†cdefg)	F6P (ABCDEFb†acdefg)
PFK	F6P (ABCDEFbacdefg)	FBP (ABCDEFbacdefg)
FBA	FBP (ABCDEFbacdefg)	DHAP (CBAhcab) + GAP (DEFdefg) + H (h)
TPI	DHAP (ABChcab) + H (i)	GAP (ABChiab) + H (c)
GAPD	GAP (ABChiab)	13BPG (ABCiab) + NADH (h)
PGK	13BPG (ABCiab)	3PG (ABCiab)
PGM1	3PG (ABCiab)	23BPG (ABCiab)
PGM2	23BPG (ABCiab)	2PG (ABCiab)
ENO	2PG (ABCiab)	PEP (ABCab) + H (i)
PYK	PEP (ABCab) + H (h)	PYR (ABCabh)
G6PDH	G6P (ABCDEFabcdefg)	6PG (ABCDEFbcdefg) + NADPH (a)
GND	6PG (ABCDEFbcdefg) + H (h)	RU5P (BCDEFhbdefg) + CO2 (A) + NADPH (c)
RPI	RU5P (ABCDEh†bdefg)	R5P (ABCDEbh†defg)
RPE	RU5P (ABCDEhbdefg) + H (a)	XU5P (ABCDEhbaefg) + H (d)
TKT1	XU5P (ABCDEabcdef) + R5P (FGHIJuwxyz)	GAP (CDEcdef) + S7P (ABFGHIJabuwxyz)
TAL	GAP (CDEcdef) + S7P (ABFGHIJabuwxyz)	F6P (ABFCDEabucdef) + E4P (GHIJwxyz)
TKT2	XU5P (ABCDEabcdef) + E4P (FGHIwxyz)	GAP (CDEcdef) + F6P (ABFGHIabwxyz)
SBA	DHAP (CBAhcab) + E4P (DEFGdefgi)	SBP (ABCDEFGbacdefgi) + H (h)
SBPase	SBP (ABCDEFGbacdefgi)	S7P (ABCDEFGbacdefgi)
EDD	6PG (ABCDEFbcdefg) + H (h)	DDG6P (ABCDEFhcdefg) + H (b)
EDA	DDG6P (ABCDEFhcdefg) + H (i)	PYR (ABCihc) + GAP (DEFdefg)

* Carbon atoms are denoted by capital letters and Hydrogen atoms by lowercase letters;
for each reaction, the changes in letter positions indicate molecular transitions between substrates and products

† Hydrogen atom (e.g., h†) denotes probabilistic loss to solvent

Supplementary Notes

Analytical solution to Gibbs free energies of glycolytic reactions

The unlabeled and labeled metabolites (subscript U and L denote their fractions) from [5-$^2H_1$]glucose were mass balanced, where forward and backward fluxes of reaction n are denoted by $v_{n,f}$ and $v_{n,b}$ (**Supplementary Fig. 14a**). $v_{PPP,ED}$ denotes the one-way flux from six-carbon molecules to three-carbon molecules via the PPP and, if applicable, the EDP (the lumped PPP and EDP fluxes that result in GAP). v_{PPP} denotes the one-way flux accounting for all GAP-consuming non-oxidative pentose phosphate pathway reactions: the reverse transketolase (R5P + Xu5P ⇔ S7P + GAP and E4P + Xu5P ⇔ F6P + GAP) and the forward transaldolase (GAP + S7P ⇔ F6P + E4P). 6PG is 6-phosphogluconate, whose labeling can be directly measured or inferred from G6P labeling as G6P is the direct precursor of 6PG.

$$G6P_U \times v_{0,f} = GLC_U \times v_{net} + FBP_U \times v_{0,b}$$

$$FBP_U \times (v_{0,b} + v_{1,f}) = DHAP_U \times GAP_U \times v_{1,b} + G6P_U \times v_{0,f}$$

$$DHAP_U \times (v_{1,b} + v_{2,f}) = (FBP_U + FBP_L) \times v_{1,f} + (GAP_U + GAP_L) \times v_{2,b}$$

$$GAP_U \times (v_{1,b} + v_{2,b} + v_{3,f} + v_{PPP}) = FBP_U \times v_{1,f} + DHAP_U \times v_{2,f} + 3PG_U \times v_{3,b} + 6PG_U \times v_{PPP,ED}$$

$$3PG_U \times (v_{3,b} + v_{4,f}) = GAP_U \times v_{3,f} + 23BPG_U \times v_{4,b}$$

$$23BPG_U \times (v_{4,b} + v_{5,f}) = 3PG_U \times v_{4,f} + PEP_U \times v_{5,b}$$

$$PEP_U \times (v_{5,b} + 2 \times v_{net} + v_{PPP,ED} - v_{PPP}) = (23BPG_U + 23BPG_L) \times v_{5,f}$$

These equations were simplified using the following equalities.

$$GLC_U = 0, DHAP_U = 1,\ PEP_U = 1,$$

$$G6P_U + G6P_L = FBP_U + FBP_L = GAP_U + GAP_L = 3PG_U + 3PG_L = 23BPG_U + 23BPG_L = 1,$$

$$v_{0,f} - v_{0,b} = v_{1,f} - v_{1,b} = v_{2,f} - v_{2,b} = v_{net}\ , v_{3,f} - v_{3,b} = v_{4,f} - v_{4,b} = v_{5,f} - v_{5,b} = 2 \times v_{net} + v_{PPP,ED} - v_{PPP}$$

All fluxes were normalized to net flux through upper glycolysis: $v_{net} = 1$

$$G6P_U \times (1 + v_{0,b}) = FBP_U \times v_{0,b}$$

$$FBP_U \times (v_{0,b} + 1 + v_{1,b}) = GAP_U \times v_{1,b} + G6P_U \times (1 + v_{0,b})$$

$$GAP_U \times (v_{1,b} + v_{2,b} + 2 + v_{3,b} + v_{PPP,ED}) = FBP_U \times (1 + v_{1,b}) + 1 + v_{2,b} + 3PG_U \times v_{3,b} + 6PG_U \times v_{PPP,ED}$$

$$3PG_U \times (v_{3,b} + 2 + v_{4,b} + v_{PPP,ED} - v_{PPP}) = GAP_U \times (2 + v_{3,b} + v_{PPP,ED} - v_{PPP}) + 23BPG_U \times v_{4,b}$$

$$23BPG_U \times (v_{4,b} + 2 + v_{5,b} + v_{PPP,ED} - v_{PPP}) = 3PG_U \times (2 + v_{4,b} + v_{PPP,ED} - v_{PPP}) + v_{5,b}$$

These were rearranged to solve for the backward fluxes.

$$v_{0,b} = \frac{G6P_U}{FBP_U - G6P_U}$$

$$v_{1,b} = \frac{(FBP_U - G6P_U)(1 + v_{0,b})}{GAP_U - FBP_U} = \frac{FBP_U}{GAP_U - FBP_U}$$

$$v_{3,b} = \frac{2 \times GAP_U - G6P_U(1 + v_{0,b}) + v_{0,b}FBP_U - v_{2,b}(1 - GAP_U) + v_{PPP,ED}(GAP_U - 6PG_U) - 1}{3PG_U - GAP_U}$$

$$v_{4,b} = \frac{2 \times 3PG_U - G6P_U(1 + v_{0,b}) + v_{0,b}FBP_U - v_{2,b}(1 - GAP_U) + v_{PPP,ED}(3PG_U - 6PG_U) - v_{PPP}(3PG_U - GAP_U) - 1}{23BPG_U - 3PG_U}$$

$$v_{5,b} = \frac{2 \times 23BPG_U - G6P_U(1 + v_{0,b}) + v_{0,b}FBP_U - v_{2,b}(1 - GAP_U) + v_{PPP.ED}(23BPG_U - 6PG_U) - v_{PPP}(23BPG_U - GAP_U) - 1}{1 - 23BPG_U}$$

$v_{3,b}$, $v_{4,b}$ and $v_{5,b}$ can be further simplified by using

$$G6P_U(1 + v_{0,b}) = v_{0,b}FBP_U$$

To solve for $v_{n,b}$, where n∈{0,1,3,4,5}, measurements for the unlabeled fractions of glycolytic intermediates, $v_{2,b}$, $v_{PPP,ED}$, and v_{PPP} are required. All metabolites except for GAP_U were directly measured using LC-MS. GAP_U was solved using the above equation for $v_{1,b}$.

$$GAP_U = \frac{(FBP_U - G6P_U)(1 + v_{0,b})}{v_{1,b}} + FBP_U = \frac{FBP_U}{v_{1,b}} + FBP_U$$

Since $v_{2,b}$ was not solved above and $v_{1,b}$ was used for computing GAP_U, these missing fluxes, $v_{1,b}$ and $v_{2,b}$, were obtained using the metabolite labeling results from [1,2-$^{13}C_2$]glucose. The ^{13}C isotopomers of metabolites (single digit subscript m denotes the number of ^{13}C atoms while double digit subscript cd denotes c-th and d-th carbons ^{13}C labeled) were mass balanced (**Supplementary Fig. 14b**).

$$FBP_0 \times (v_{0,b} + v_{1,f}) = DHAP_0 \times GAP_0 \times v_{1,b} + G6P_0 \times v_{0,f}$$

$$DHAP_0 \times (v_{1,b} + v_{2,f}) = (FBP_0 + FBP_{56}) \times v_{1,f} + GAP_0 \times v_{2,b}$$

$$GAP_0 \times (v_{1,b} + v_{2,b} + 2 + v_{PPP,ED}) = (FBP_0 + FBP_{12}) \times v_{1,f} + DHAP_0 \times v_{2,f} + (1 - 6PG_4) \times v_{PPP,ED}$$

With $v_{n,f} = v_{n,b} + v_{net}$ for n=1,2 and fluxes normalized to net flux through upper glycolysis ($v_{net} = 1$),

$$FBP_0 \times (v_{0,b} + 1 + v_{1,b}) = DHAP_0 \times GAP_0 \times v_{1,b} + G6P_0 \times (v_{0,b} + 1)$$

$$DHAP_0 \times (v_{1,b} + 1 + v_{2,b}) = (FBP_0/GAP_0) \times (1 + v_{1,b}) + GAP_0 \times v_{2,b}$$

$$GAP_0 \times (v_{1,b} + v_{2,b} + 2 + v_{PPP,ED}) = (1 - FBP_4/DHAP_2) \times (1 + v_{1,b}) + DHAP_0 \times (1 + v_{2,b}) + (1 - 6PG_4) \times v_{PPP,ED}$$

Solving for $v_{1,b}$ and $v_{2,b}$,

$$v_{1,b} = \frac{(1 + v_{0,b})(FBP_0 - G6P_0)}{DHAP_0 \times GAP_0 - FBP_0}$$

$$v_{2,b} = \frac{(DHAP_0 - FBP_0/GAP_0) \times (1 + v_{1,b})}{GAP_0 - DHAP_0}$$

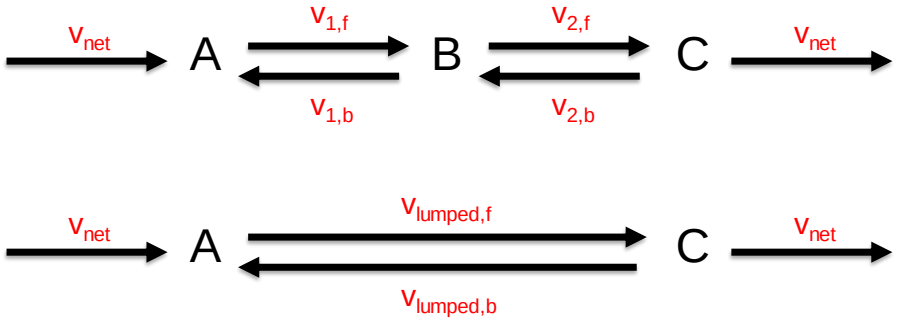
Using these to solve for $v_{PPP,ED}$,

$$v_{PPP,ED} = \frac{(1 - FBP_4/DHAP_2 - GAP_0) \times (1 + v_{1,b}) + (DHAP_0 - GAP_0) \times (1 + v_{2,b})}{GAP_0 + 6PG_4 - 1}$$

v_{PPP} can be solved since the difference between $v_{PPP,ED}$ and v_{PPP} corresponds to the approximate four and five carbon requirements for aromatic amino acids, histidine, and nucleotides, which can be estimated using the growth rate and biomass composition.

Gibbs free energy of lumped reactions

Consider a two-reaction system with a constant influx of A and an equal magnitude efflux of C at the rate of v_{net}. The first reaction is A ⇌ B and the second reaction is B ⇌ C. The lumped reaction is A ⇌ C.



The forward component of lumped reaction ($v_{lumped,f}$) represents the rate of A being converted to C. We can write $v_{lumped,f}$ as a fraction of A-to-B conversion ($v_{1,f}$) because B can convert either to C or back to A. That fraction can be expressed in terms of the rates of reactions whose starting substrate is B: $v_{1,b}$ and $v_{2,f}$.

$$v_{lumped,f} = v_{1,f}\frac{v_{2,f}}{v_{1,b} + v_{2,f}}$$

Similarly, the backward component of lumped reaction ($v_{lumped,b}$), which represents the rate of C being converted to A, can be expressed as follows:

$$v_{lumped,b} = v_{2,b}\frac{v_{1,b}}{v_{1,b} + v_{2,f}}$$

Using these two equalities, we find that the ΔG of the lumped reaction (ΔG_{lumped}) is the sum of the individual reaction ΔG values ($\Delta G_1 + \Delta G_2$) computed based on the individual reaction reversibilities – as we would expect for a state function – and that ΔG_{lumped} can be obtained by the ratio of forward to backward rates of one lumped reaction:

$$\Delta G_1 + \Delta G_2 = RT\ln\frac{v_{1,b}}{v_{1,f}} + RT\ln\frac{v_{2,b}}{v_{2,f}} = RT\ln\frac{v_{1,b}v_{2,b}}{v_{1,f}v_{2,f}}$$

$$= RT\ln\frac{\frac{v_{1,b}}{v_{1,b}+v_{2,f}}v_{2,b}}{v_{1,f}\frac{v_{2,f}}{v_{1,b}+v_{2,f}}} = RT\ln\frac{v_{lumped,b}}{v_{lumped,f}}$$

$$= \Delta G_{lumped}$$

Therefore, the flux-ratio-based computation of ΔG is valid for lumped reactions.

Gibbs free energy change upon oxygen upshift

The difference between the sum of the Gibbs energies of the final products and the sum of the Gibbs energies of the initial substrates of the full glycolysis net reaction inside the cell determines the overall glycolytic thermodynamics. Two factors that determine ΔG of the pathway are 1) the transport reactions that determine the concentrations of intracellular metabolites by bringing in and out the substrates and the products, and 2) the cofactor ratios that are controlled more globally rather than by a single pathway. The ΔG values of the intermediate steps are determined by the enzyme abundance and activities, which impact the relative concentrations of glycolytic intermediates.

For O_2 upshift, the anomaly of slower glycolytic flux but greater thermodynamic driving force may be explained in part by altered fate of pyruvate:

(anaerobic condition) G6P + 2 NADH + 3 ADP + 2 Pi → 2 Ethanol + 2 Formate + 2 NAD + 3 ATP + 2 H_2O

$\Delta G'^{\circ} \sim -115$ kJ/mol

(aerobic condition) G6P + 4 NAD + 5 ADP + 4 Pi → 2 Acetate + 2 CO_2 + 4 NADH + 5 ATP + 2 H_2O

$\Delta G'^{\circ} \sim -141$ kJ/mol

In addition, aerobic condition is more forward driven due to more NAD and less NADH. Flux is, however, gated by ATP now being made also by oxidative phosphorylation (and also because glycolysis now makes 5 instead of 3 ATP separate from oxidative phosphorylation, as shown above), leading to slower glycolysis to maintain flux balance for ATP. This is likely implemented in part by pyruvate accumulation that inhibits Enzyme I.

Limitations of the GibbsIT method

Two important limitations of the GibbsIT method are:

1) The method is based on measuring the relative forward and backward fluxes. When the reaction is carried by a single enzyme or a series of enzymes, the ratio of forward to backward flux reflects ΔG as described in the text. In certain cases, however, different enzymes may catalyze the "forward" versus "backward" reactions using different cofactors. In such cases, the ratio of forward to backward flux does not reflect thermodynamics, but rather the activities of the associated enzymes, with the reactions together forming an energy-burning futile cycle. In glycolysis, such cycling may occur at the phosphofructokinase step (fructose bisphosphatase mimics the reverse phosphofructokinase). Another example in the vicinity of glycolysis is pyruvate cycling. In *E. coli,* futile cycle reaction pairs include phosphoenolpyruvate carboxylase-phosphoenolpyruvate carboxykinase and pyruvate kinase-phosphoenolpyruvate synthase.

2) The method requires measurement of the ratio of forward to backward flux. When reversibility is very low, determination of the backward flux is difficult and ΔG cannot be determined accurately. In such cases, ΔG is better determined based on $\Delta G'^{\circ}$ and concentrations, whereas GibbsIT is preferred for reactions with readily measurable reverse flux (i.e., -10 kJ/mol $< \Delta G < 0$ kJ/mol).