

## Metabolite concentrations, fluxes, and free energies imply efficient enzyme usage

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## Supplementary Results

**Supplementary Table 1. Metabolic flux distributions determined using <sup>13</sup>C-tracers.**

Reaction		Substrates	Products	Mammalian iBMK cells			Yeast			<i>E. coli</i>		
				flux	L.B.	U.B.	flux	L.B.	U.B.	flux	L.B.	U.B.
Glc Uptake	net	GLC	G6P	2.30	2.29	2.30	69.0	63.7	69.1	76.0	75.3	77.2
PGI	net	G6P	F6P	2.21	2.21	2.22	64.0	59.0	64.2	59.5	58.5	60.9
	xch			44.82	4.88	157.81	542.4	238.5	5024.6	67.9	45.8	87.9
	for			47.04	7.10	160.03	606.4	210.1	5083.5	127.4	105.2	147.6
	rev			44.82	4.88	157.81	542.4	147.6	5024.6	67.9	45.8	87.9
PFK	net	F6P	FBP	2.22	2.22	2.23	63.5	58.2	64.4	62.9	62.1	64.4
	xch			0.24	-	0.24	0.9	-	0.9	3.1	-	5.2
	for			2.46	-	2.47	64.3	-	64.4	66.0	-	68.3
	rev			0.24	-	0.24	0.9	-	0.9	3.1	-	5.2
FBA	net	FBP	DHAP + GAP	2.22	2.22	2.23	63.5	58.2	64.4	62.9	62.7	64.3
	xch			0.38	0.35	0.43	158.4	136.4	160.8	54.6	50.1	58.8
	for			2.61	2.58	2.66	221.9	194.7	224.3	117.5	112.3	122.8
	rev			0.38	0.35	0.43	158.4	136.4	160.8	54.6	50.1	58.8
TPI	net	DHAP	GAP	2.18	2.18	2.19	54.2	49.1	54.3	62.3	61.4	63.8
	xch			5.95	5.90	6.48	185.7	147.5	185.7	174.4	160.6	187.4
	for			8.13	8.08	8.66	239.9	196.4	239.9	236.7	222.5	249.8
	rev			5.95	5.90	6.48	185.7	147.5	185.7	174.4	160.6	187.4
GAPD	net	GAP	13BPG	4.41	4.40	4.42	117.9	107.7	118.1	130.3	128.9	133.0
PGK	net	13BPG	3PG	4.41	4.40	4.42	117.9	107.7	118.1	130.3	128.9	133.0
PGM	net	3PG	2PG	4.16	4.15	4.16	106.3	106.3	106.3	122.2	121.7	123.7
ENO	net	2PG	PEP	4.16	4.15	4.16	106.3	106.3	106.3	122.2	121.7	123.7
PYK	net	PEP	Pyr	4.16	4.15	4.16	105.4	105.4	106.4	98.9	98.8	100.0
G6PDH	net	G6P	6PG	0.05	0.03	0.05	2.0	1.9	2.1	15.4	15.3	15.9
GND	net	6PG	Ru5P + CO2	0.05	0.03	0.05	2.0	1.9	2.1	11.6	11.1	12.0
	xch			0.00	0.00	0.00	0.0	0.0	0.0	0.1	0.0	0.3
	for			0.05	0.03	0.05	2.0	1.9	2.1	11.7	11.3	12.3
	rev			0.00	0.00	0.00	0.0	0.0	0.0	0.1	0.0	0.3
EDD, EDA	net	6PG	Pyr + GAP	-	-	-	-	-	-	3.8	3.5	4.2
RPI	net	Ru5P	R5P	0.04	0.03	0.04	1.2	1.2	1.2	7.8	7.7	7.9
	xch			0.02	0.00	0.26	1.0	0.2	1736.3	5030.9	26.2	6134.7
	for			0.06	0.04	0.29	2.1	1.4	1737.5	5038.7	34.0	6142.5
	rev			0.02	0.00	0.26	1.0	0.2	1736.3	5030.9	26.2	6134.7
RPE	net	Ru5P	X5P	0.01	0.00	0.01	0.8	0.8	0.8	3.8	3.6	4.0
	xch			9.47	0.47	39.76	9.9	9.3	12.6	120.9	102.5	167.6
	for			9.48	0.47	39.76	10.7	10.1	13.5	124.7	106.3	171.4
	rev			9.47	0.47	39.76	9.9	9.3	12.6	120.9	102.5	167.6
TKT1	net	R5P + X5P	S7P + GAP	0.01	0.00	0.01	0.6	0.6	0.6	2.9	2.7	3.1
	xch			0.11	0.08	0.12	8.0	7.0	8.3	17.1	16.2	17.9
	for			0.12	0.08	0.12	8.6	7.6	8.9	19.9	19.1	21.0
	rev			0.11	0.08	0.12	8.0	7.0	8.3	17.1	16.2	17.9
TAL	net	S7P + GAP	E4P + F6P	0.01	0.00	0.01	0.6	0.6	0.7	2.9	2.9	3.0
	xch			0.00	0.00	0.00	0.1	0.0	0.1	0.4	0.3	0.4
	for			0.01	0.00	0.01	0.7	0.6	0.8	3.3	3.1	3.5
	rev			0.00	0.00	0.00	0.1	0.0	0.1	0.4	0.3	0.4
TKT2	net	E4P + X5P	F6P + GAP	0.01	0.00	0.01	0.2	0.2	0.2	0.9	0.8	1.0
	xch			0.02	0.01	0.02	0.4	0.4	0.4	1.1	0.9	1.1
	for			0.02	0.01	0.02	0.6	0.5	0.7	2.0	1.8	2.2
	rev			0.02	0.01	0.02	0.4	0.4	0.4	1.1	0.9	1.1
PDH	net	Pyr	AcCoA + CO2	0.53	0.52	0.54	2.8	2.8	3.2	87.7	87.2	88.5
	xch			0.000	0.000	0.002	0.0	0.0	0.4	0.0	0.0	0.6
	for			0.532	0.522	0.535	2.8	2.8	3.2	87.7	87.2	88.5
	rev			0.000	0.000	0.002	0.0	0.0	0.4	0.0	0.0	0.6
PDC	net	Pyr	AcetAld + CO2	-	-	-	96.1	96.1	96.1	-	-	-
	xch			-	-	-	0.0	0.0	1.1	-	-	-
	for			-	-	-	96.1	96.1	97.2	-	-	-
	rev			-	-	-	0.0	0.0	1.1	-	-	-
CS	net	AcCoA + OAA	Cit/Icit	0.53	0.53	0.54	1.8	1.8	1.8	24.0	23.3	25.0
IDH	net	Cit/Icit	OGA + CO2	0.35	0.35	0.36	1.8	1.8	1.8	23.3	22.7	24.6
	xch			0.04	0.04	0.05	-	-	-	-	-	-
	for			0.40	0.39	0.40	-	-	-	-	-	-
	rev			0.04	0.04	0.05	-	-	-	-	-	-
AKGDH	net	OGA	SuccCoA + CO2	1.08	0.70	1.08	0.0	0.0	0.0	17.6	17.4	18.0
	xch			0.00	0.00	0.00	-	-	-	1.8	0.2	2.6

	for			1.08	0.70	1.08	-	-	-	19.5	17.6	20.2
	rev			0.00	0.00	0.00	-	-	-	1.8	0.2	2.6
SUCOAS	net	SuccCoA	Succ	1.08	0.71	1.08	0.0	0.0	0.0	17.6	17.5	18.0
SUCD	net	Succ	Fum	1.08	0.71	1.10	0.0	0.0	0.0	18.3	18.1	19.1
FUM	net	Fum	Mal	1.10	1.09	1.11	0.0	0.0	0.0	18.3	18.1	19.1
	xch			32.57	5.32	239.13	685.4	0.7	2011.6	5597.4	2050.6	6133.1
	for			33.67	6.42	240.23	685.4	0.7	2011.6	5615.6	2068.7	6151.2
	rev			32.57	5.32	239.13	685.4	0.7	2011.6	5597.4	2050.6	6133.1
MDH	net	Mal	OAA	0.56	0.55	0.57	-3.2	-12.6	0.0	18.9	18.1	20.0
	xch			2.44	2.07	3.27	1.7	1.0	3381.0	86.3	67.0	100.2
	for			3.01	2.63	3.83	1.7	1.0	3381.0	105.2	85.5	119.1
	rev			2.44	2.07	3.27	4.8	1.3	3388.6	86.3	67.0	100.2
ME	net	Mal	Pyr + CO2	0.71	0.71	0.72	3.2	0.0	12.6	0.0	0.0	0.4
PC	net	Pyr + CO2	OAA	0.09	0.09	0.10	6.6	3.4	16.5	-	-	-
PPC	net	PEP + CO2	OAA	-	-	-	-	-	-	19.5	19.3	20.1
PPCK	net	OAA	PEP + CO2	-	-	-	0.0	0.0	1.1	-	-	-
ACITL	net	Cit	AcCoA + OAA	0.18	0.18	0.19	-	-	-	-	-	-
Glyoxylate shunt	net	Icit + AcCoA	Succ + Mal	-	-	-	-	-	-	0.6	0.4	0.6
Gln uptake	net		Glutamine		0.88		-	-	-	-	-	-
Ser uptake	net		Serine		0.13		-	-	-	-	-	-
Gly uptake	net		Glycine		0.08		-	-	-	-	-	-
EX_Lac	net	Lactate			4.13		-	-	-	-	-	-
EX_ACcoA	net	AcCoA for FA			0.18		-	-	-	-	-	-
EX_AC_EtOH	net	EtOH + Acetate + AcCoA for FA		-	-	-		96.1		-	-	-
EX_AC	net	Acetate + AcCoA for FA		-	-	-	-	-	-		65.0	
EX_G6P	net	G6P			0.04			3.0			1.1	
EX_F6P	net	F6P			0.0002			1.4			0.4	
EX_DHAP_GLYC3P	net	Glyc3P			0.04			9.3			0.7	
EX_Asn	net	Asn			0.04			0.2			1.3	
EX_Asp	net	Asp			0.05			0.5			1.3	
EX_Gly	net	Gly			0.08			0.5			3.2	
EX_Ile	net	Ile			0.04			0.3			1.5	
EX_Lys	net	Lys			0.08			0.5			1.8	
EX_Met	net	Met			0.02			0.1			0.8	
EX_Thr	net	Thr			0.04			0.3			1.3	
EX_Pyrimidine	net	Pyrimidine - except for dTMP			0.01			0.2			1.6	
EX_dTMP	net	dTMP			0.002			0.0			0.1	
EX_Arg	net	Arg			0.05			0.3			1.6	
EX_Gln	net	Gln			0.05			0.2			1.4	
EX_Glu	net	Glu			0.05			0.5			1.6	
EX_Pro	net	Pro			0.06			0.3			1.2	
EX_Phe	net	Phe			0.04			0.2			0.9	
EX_Trp	net	Trp			0.002			0.0			0.3	
EX_Tyr	net	Tyr			0.02			0.2			0.7	
EX_Cys	net	Cys			0.01			0.0			0.5	
EX_Ser	net	Ser			0.06			0.3			1.8	
EX_Ala	net	Ala			0.12			0.8			2.7	
EX_Leu	net	Leu			0.08			0.5			2.4	
EX_Val	net	Val			0.05			0.5			2.2	
EX_ATP	net	Purine			0.02			0.2			2.3	
EX_His	net	His			0.02			0.1			0.5	

**Supplementary Table 2. Isotope-tracer signatures of reaction reversibility and associated measured  $\Delta G$ .**

Reaction	Substrates	Products	[1,2-13C2] Glc	[3-13C1] Glc	[U-13C6] Glc*	50% [U-13C6] Glc	[U-13C5] Gln*	Mammalian iBMK cells			Yeast			<i>E. coli</i>		
								L.B.	$\Delta G$	U.B.	L.B.	$\Delta G$	U.B.	L.B.	$\Delta G$	U.B.
PGI	G6P	F6P	1-labeled G6P	2-labeled G6P		3-labeled G6P		-0.97	-0.12	-0.04	-0.89	-0.28	-0.03	-2.14	-1.59	-1.34
PFK	F6P	FBP	0- or 4-labeled F6P	0- or 2- labeled F6P		3-labeled F6P		-	-	-6.01	-	-	-10.63	-	-	-6.64
FBA	FBP	DHAP + GAP	0- or 4-labeled FBP	0- or 2- labeled FBP		3-labeled FBP		-5.11	-4.83	-4.67	-0.89	-0.85	-0.78	-2.08	-1.93	-1.90
TPI	DHAP	GAP	0-labeled DHAP	0-labeled DHAP				-0.81	-0.79	-0.75	-0.72	-0.64	-0.60	-0.84	-0.77	-0.74
GND	6PG	Ru5P + CO2	1-labeled 6PG		5-labeled 6PG	5-labeled 6PG		-	-11.15	-10.23	-	-22.86	-13.07	-	-11.05	-9.67
EDD, EDA	6PG	Pyr + GAP	0- or 4-labeled 6PG	0- or 2-labeled 6PG		3-labeled 6PG		-	-	-	-	-	-	-	-28.32	-13.56
RPI	Ru5P	R5P	1-labeled R5P	1-labeled R5P		0- or 5-labeled R5P		-5.61	-2.73	-0.31	-4.98	-2.02	-0.002	-0.668	-0.004	-0.003
RPE	Ru5P	X5P	1-labeled X5P, 3-labeled Ru5P	1-labeled X5P, 2-labeled Ru5P		0- or 5-labeled X5P, 2- or 3- labeled Ru5P		-0.021	-0.003	0.000	-0.21	-0.20	-0.16	-0.09	-0.08	-0.06
TKT1	R5P + X5P	S7P + GAP	3-labeled X5P	2-labeled X5P		2- or 3-labeled X5P		-0.12	-0.12	-0.01	-0.21	-0.19	-0.18	-0.42	-0.39	-0.39
TAL	S7P + GAP	E4P + F6P	1- or 3-labeled S7P	1-labeled S7P		3- or 4-labeled S7P		-2.47	-2.41	-0.45	-10.77	-5.99	-5.62	-5.91	-5.25	-5.21
TKT2	E4P + X5P	F6P + GAP	2- or 4-labeled X5P	1-labeled X5P		2- or 3-labeled X5P		-0.74	-0.72	-0.10	-1.06	-0.93	-0.77	-1.79	-1.58	-1.44
PDH	Pyr	AcCoA + CO2		0-labeled Pyr	2-labeled Pyr	2-labeled Pyr		-	-27.25	-14.34	-	-13.39	-5.05	-	-19.81	-13.05
PDC	Pyr	AcetAld + CO2			2-labeled Pyr	2-labeled Pyr		-	-	-	-	-20.91	-11.27	-	-	-
IDH	Cit/Icit	OGA + CO2		0-labeled Cit/Icit	0-labeled Cit/Icit	2- or 4-labeled Cit/Icit	3- or 5-labeled Cit/Icit	-5.97	-5.60	-5.33	-	-	-	-	-	-
AKGDH	OGA	SuccCoA + CO2	1-labeled OGA	1-labeled OGA	1- or 3-labeled OGA	1- or 4-labeled OGA	2- or 4-labeled OGA	-	-30.95	-17.79	-	-	-	-	-	-5.29
FUM	Fum	Mal	0-labeled Fum	1-labeled Fum	3-labeled Fum	0- or 3-labeled Fum	0- or 3-labeled Fum	-0.48	-0.08	-0.01	-0.0002	0.0000	0.0262	-0.02	-0.01	-0.01
MDH	Mal	OAA	0-labeled Mal	1-labeled Mal	3-labeled Mal	0- or 3-labeled Mal	0- or 1-labeled Mal	-0.62	-0.52	-0.41	0.00	2.68	2.68	-0.63	-0.50	-0.45
SHMT	Ser	Gly + 5,10-CH2-THF	1-labeled Ser	0-labeled Ser	1- or 2-labeled Ser	1- or 2-labeled Ser		-1.85	-1.68	-1.60	-4.04	-3.28	-3.25	-	-8.36	-7.16

\* Used for mammalian cells only

### Supplementary Table 3. Absolute metabolite concentrations that were constrained by thermodynamics.

Metabolite[compartment] \ Concentration(M)	BIGG ID	KEGG ID	Brenda ID	Mammalian iBMK	L.B.	U.B.	Yeast	L.B.	U.B.	<i>E. coli</i>	L.B.	U.B.
1,3-bisphosphoglycerate[c]*	13dpg[c]	C00236	18032	2.24E-6	6.77E-7	3.28E-6	4.91E-6	3.26E-7	6.47E-5	1.65E-5	1.07E-5	2.97E-5
2-phosphoglycerate[c]*	2pg[c]	C00631	17976	9.49E-6	1.37E-6	9.18E-5	2.38E-5	4.39E-6	1.60E-4	9.18E-5	3.81E-5	3.22E-4
carbon dioxide[c]**	co2[c]	C00011	28651	7.63E-3	6.37E-3	9.55E-3	8.16E-5	6.01E-5	9.01E-5	7.52E-5	5.02E-5	7.54E-5
carbon dioxide[m]**	co2[m]	C00011	28651	6.53E-3	6.37E-3	9.55E-3	7.71E-5	6.01E-5	9.01E-5	-	-	-
coenzyme-A[m]**	coa[m]	C00010	11741	4.04E-3	4.00E-3	6.00E-3	4.90E-3	4.00E-3	6.00E-3	-	-	-
dihydroxyacetonephosphate [directly measured]	dhap	C00111	89172	-	-	-	8.07E-4	7.01E-4	9.13E-4	3.06E-3	2.90E-3	3.22E-3
dihydroxyacetonephosphate[c]*	dhap[c]	C00111	89172	1.63E-3	1.47E-3	1.75E-3	8.23E-4	7.46E-4	9.10E-4	-	-	-
erythrose-4-phosphate[c]*	e4p[c]	C00279	59851	1.03E-5	7.54E-6	1.58E-5	1.46E-5	6.92E-6	1.95E-5	4.90E-5	4.19E-5	5.64E-5
fructose-6-phosphate[c]*	f6p[c]	C00085	56501	9.69E-5	8.18E-5	1.27E-4	2.37E-3	1.53E-3	2.92E-3	2.52E-3	2.16E-3	2.89E-3
fumarate [directly measured]	fum	C00122	19266	3.87E-4	2.83E-4	4.90E-4	1.21E-4	8.05E-5	1.61E-4	1.15E-4	3.00E-6	4.42E-3
fumarate[c]*	fum[c]	C00122	19266	-	-	-	-	-	-	2.88E-4	2.85E-4	2.93E-4
fumarate[m]*	fum[m]	C00122	19266	-	-	-	1.24E-4	8.88E-5	1.54E-4	-	-	-
glucose-6-phosphate[c]*	g6p[c]	C00092	22626	6.75E-4	6.74E-4	1.05E-3	5.31E-3	4.36E-3	6.18E-3	7.88E-3	7.59E-3	8.17E-3
glyceraldehyde-3-phosphate[c]*	g3p[c]	C00661	59	1.41E-4	1.28E-4	1.52E-4	1.18E-4	1.07E-4	1.30E-4	2.71E-4	2.56E-4	2.89E-4
isocitrate[c]*	icit[c]	C00311	23698	-	-	-	-	-	-	3.67E-5	4.68E-6	4.29E-5
isocitrate[m]*	icit[m]	C00311	23698	3.21E-5	2.10E-5	3.38E-5	5.79E-6	1.90E-6	9.79E-6	-	-	-
oxaloacetate[c]*	oaa[c]	C00036	19271	-	-	-	-	-	-	4.87E-7	2.81E-7	8.55E-7
oxaloacetate[m]*	oaa[m]	C00036	19271	2.01E-6	1.18E-6	3.31E-6	5.01E-7	1.32E-7	9.86E-7	-	-	-
phosphate (orthophosphate)[c]**	pi[c]	C00009	92038	5.83E-3	4.00E-3	6.00E-3	4.93E-2	4.00E-2	6.00E-2	2.39E-2	1.60E-2	2.40E-2
ribose-5-phosphate [directly measured]	r5p	C00117	34575	7.83E-5	2.84E-5	1.28E-4	1.52E-4	1.14E-4	1.90E-4	-	-	-
ribose-5-phosphate[c]*	r5p[c]	C00117	34575	2.84E-5	2.84E-5	4.01E-5	-	-	-	7.87E-4	7.86E-4	8.36E-4
ribulose-5-phosphate[c]*	ru5p-D[c]	C00199	20587	5.27E-6	4.10E-6	5.85E-6	1.22E-4	7.73E-5	1.95E-4	1.12E-4	1.12E-4	1.27E-4
sedoheptulose-7-phosphate [directly measured]	s7p	C05382	24563	1.51E-5	1.21E-5	1.81E-5	3.61E-4	3.29E-4	3.92E-4	8.82E-4	8.40E-4	9.24E-4
sedoheptulose-7-phosphate[c]*	s7p[c]	C05382	24563	1.81E-5	1.28E-5	1.81E-5	-	-	-	-	-	-
succinyl-CoA [directly measured]	succoa	C00091	55439	-	-	-	-	-	-	2.33E-4	1.42E-4	3.83E-4
succinyl-CoA[m]*	succoa[m]	C00091	55439	6.80E-6	3.49E-7	3.16E-6	-	-	-	-	-	-
xylulose-5-phosphate [directly measured]	xu5p-D	C00231	29847	3.15E-5	2.33E-5	3.95E-5	-	-	-	-	-	-
xylulose-5-phosphate[c]*	xu5p-D[c]	C00231	29847	2.99E-5	2.34E-5	3.31E-5	2.46E-4	1.59E-4	3.93E-4	1.81E-4	1.80E-4	2.03E-4

\* Concentrations satisfy directly measured 95% confidence intervals of both  $\Delta G$  and concentrations. [c] and [m] denote values consistent with reaction free energies in cytosol and mitochondria, respectively.

\*\* CO<sub>2</sub> concentration was calculated using Henry's law at the respective culture conditions. Coenzyme A and phosphate concentrations were taken from literature.

### Supplementary Table 4. Reaction free energies ( $\Delta G$ ) consistent with directly measured metabolite concentrations.

Reaction	Mammalian iBMK	L.B.	U.B.	Yeast	L.B.	U.B.	<i>E. coli</i>	L.B.	U.B.
G6P => F6P	-0.53	-0.97	-0.04	-0.18	-0.78	-0.03	-1.60	-2.09	-1.34
F6P + ATP => FBP + ADP	-13.37	-15.78	-10.47	-21.23	-22.83	-19.05	-24.71	-26.28	-23.07
FBP -> DHAP + GAP	-4.87	-5.11	-4.68	-0.84	-0.90	-0.78	-1.98	-2.08	-1.90
DHAP => GAP	-0.79	-0.81	-0.75	-0.65	-0.71	-0.59	-0.79	-0.84	-0.74
GAP + NAD + Pi -> 13BPG + NADH	-2.03	-4.07	0.00	-6.67	-13.34	0.00	-1.32	-2.63	0.00
13BPG + ADP => 3PG + ATP	-2.20	-4.07	0.00	-6.87	-13.34	0.00	-1.42	-2.63	0.00
3PG => 2PG	-5.30	-10.84	0.00	-4.53	-9.06	0.00	-3.17	-5.51	0.00
2PG => PEP	-5.42	-10.84	0.00	-4.67	-9.06	0.00	-2.75	-5.51	0.00
PEP + ADP => Pyr + ATP	-3.76	-7.31	-0.07	-9.60	-11.03	-8.10	-7.09	-9.14	-5.09
Pyr + NAD + CoA => AcCoA + NADH + CO <sub>2</sub>	-28.35	-30.92	-27.05	-56.96	-59.40	-54.88	-28.04	-39.31	-20.04
6PG + NADP => Ru5P + NADPH + CO <sub>2</sub>	-9.84	-12.15	-7.57	-10.95	-14.11	-8.07	-15.08	-23.34	-8.81
Ru5P => Xu5P	-0.02	-0.02	0.00	-0.20	-0.21	-0.16	-0.08	-0.09	-0.06
Ru5P => R5P	-1.91	-2.17	-0.37	-1.83	-3.72	-0.08	-0.004	-0.316	-0.003
Xu5P + R5P => GAP + S7P	-0.06	-0.12	-0.01	-0.21	-0.22	-0.19	-0.40	-0.42	-0.39
S7P + GAP => E4P + F6P	-1.31	-2.47	-0.45	-6.14	-8.81	-5.62	-5.43	-5.91	-5.21
Xu5P + E4P => GAP + F6P	-0.39	-0.74	-0.10	-0.91	-0.97	-0.77	-1.61	-1.79	-1.44
AcCoA + OAA => Cit + CoA	-41.19	-43.14	-38.04	-13.13	-16.11	-8.25	-36.62	-45.80	-25.12
Cit => Icit *	-0.02	-1.23	0.00	-3.51	-6.00	-1.87	-2.12	-6.00	-0.28
Icit + NAD => aKG + NADH + CO <sub>2</sub> *	-5.70	-5.97	-5.33	-5.88	-6.00	-1.87	-	-	-
Icit + NADP => aKG + NADPH + CO <sub>2</sub>	-	-	-	-	-	-	-5.94	-15.44	0.00
aKG + NAD + CoA => SuccCoA + NADH + CO <sub>2</sub>	-34.10	-29.32	-25.20	-60.16	-85.06	-29.42	-11.78	-24.58	-5.29
SuccCoA + ADP + Pi => Succ + CoA + ATP	-	-	-	-	-	-	-21.50	-31.02	-10.37
Fum => Mal	-0.15	-0.48	-0.01	0.0009	-0.0002	0.0300	-0.01	-0.02	-0.01
Mal + NAD => OAA + NADH	-0.52	-0.62	-0.41	2.26	0.00	2.90	-0.52	-0.63	-0.45
Mal + NAD => Pyr + NADH + CO <sub>2</sub>	-	-	-	-9.17	-11.12	-8.95	-9.74	-12.56	-8.06
Ser + thf => Gly + mlthf	-1.72	-1.85	-1.60	-3.30	-4.04	-3.25	-8.68	-28.74	-7.16

\* The lower bounds for aconitase (yeast and *E. coli*) and isocitrate dehydrogenase (yeast) were set to -6 kJ/mol

**Supplementary Table 5. Comprehensive absolute cellular metabolite concentrations.**

Metabolite[compartment] \ Concentration(M)	BiGG ID	KEGG ID	Brenda ID	Mammalian IBMK	L.B.	U.B.	Yeast	L.B.	U.B.	<i>E. coli</i>	L.B.	U.B.
1,3-bisphosphoglycerate[c]*	13dpg[c]	C00236	18032	2.24E-6	6.77E-7	3.28E-6	4.91E-6	3.26E-7	6.47E-5	1.65E-5	1.07E-5	2.97E-5
2,3-bisphosphoglycerate	23dpg	C01159	18034	2.37E-4	1.76E-4	3.16E-4	3.61E-5	2.58E-5	5.07E-5	8.29E-5	6.08E-5	1.05E-4
2,3-dihydroxybenzoic acid	23dhb	C00196	24972	-	-	-	-	-	-	1.38E-4	1.17E-4	1.64E-4
2-dehydro-D-gluconate	2dhgln	C06473	-	2.77E-6	2.05E-6	3.48E-6	-	-	-	-	-	-
2-phosphoglycerate[c]*	2pg[c]	C00631	17976	9.49E-6	1.37E-6	9.18E-5	2.38E-5	4.39E-6	1.60E-4	9.18E-5	3.81E-5	3.22E-4
3-phosphoglycerate	3pg	C00197	17981	3.75E-4	2.88E-4	4.63E-4	5.78E-4	3.45E-4	8.10E-4	1.54E-3	1.51E-3	1.58E-3
3-phospho-serine	pser-L	C01005	36327	4.40E-4	3.45E-4	5.35E-4	-	-	-	-	-	-
4-hydroxybenzoate	4hbz	C00156	-	-	-	-	-	-	-	5.22E-5	4.43E-5	6.15E-5
6-phospho-D-gluconate	6pgc	C00345	22563	1.65E-5	1.23E-5	2.20E-5	2.43E-4	1.77E-4	3.08E-4	3.77E-3	3.69E-3	3.85E-3
acetoacetyl-CoA	aacoa	C00332	13538	-	-	-	-	-	-	2.18E-5	1.37E-5	3.47E-5
acetyl-CoA	accoa	C00024	36329	2.88E-5	2.25E-5	3.50E-5	4.36E-5	3.62E-5	5.22E-5	6.06E-4	5.29E-4	6.94E-4
acetylphosphate	actp	C00227	-	-	-	-	-	-	-	1.07E-3	1.02E-3	1.13E-3
aconitate	acon-C	C00417	23413	1.10E-5	8.72E-6	1.34E-5	-	-	-	1.61E-5	1.38E-5	1.88E-5
ac-serine	acser	C00979	-	5.72E-5	7.28E-6	1.07E-4	-	-	-	-	-	-
adenine	ade	C00147	20886	-	-	-	-	-	-	1.47E-6	1.26E-6	1.71E-6
adenosine	adn	C00212	850	-	-	-	-	-	-	1.31E-7	9.44E-8	1.82E-7
adenosine-phosphosulfate	aps	C00224	1024	-	-	-	-	-	-	6.63E-6	5.10E-6	8.62E-6
ADP	adp	C00008	1228	5.69E-4	4.52E-4	7.15E-4	4.88E-4	4.29E-4	5.52E-4	5.55E-4	4.37E-4	7.04E-4
ADP-glucose	adpglc	C00498	7320	-	-	-	-	-	-	4.27E-6	2.83E-6	6.44E-6
a-ketoglutarate	akg	C00026	21003	7.97E-4	7.86E-4	8.08E-4	8.48E-4	7.91E-4	9.04E-4	4.43E-4	3.12E-4	6.31E-4
alanine	ala-L	C00041	17908	6.98E-3	6.19E-3	7.77E-3	2.23E-2	1.96E-2	2.51E-2	2.55E-3	2.32E-3	2.80E-3
AMP	amp	C00020	147	4.23E-5	3.57E-5	5.02E-5	8.12E-5	6.38E-5	1.03E-4	2.81E-4	2.32E-4	3.41E-4
anthranilate	anth	C00108	-	-	-	-	-	-	-	3.48E-6	3.34E-6	3.62E-6
arginine	arg-L	C00062	36213	2.55E-4	1.75E-4	3.35E-4	2.18E-2	1.76E-2	2.61E-2	5.69E-4	4.79E-4	6.75E-4
asparagine	asn-L	C00152	19609	2.15E-4	1.56E-4	2.74E-4	5.69E-3	5.23E-3	6.14E-3	5.11E-4	4.42E-4	5.92E-4
aspartate	asp-L	C00049	19556	1.49E-2	1.38E-2	1.60E-2	6.29E-3	5.79E-3	6.79E-3	4.23E-3	3.56E-3	5.04E-3
ATP	atp	C00002	1395	4.67E-3	2.40E-3	6.94E-3	1.93E-3	1.66E-3	2.21E-3	9.63E-3	8.13E-3	1.14E-2
carbamoyl-aspartate	cbasp	C00438	21131	-	-	-	-	-	-	5.90E-4	3.64E-4	9.55E-4
carbon dioxide[c]**	co2[c]	C00011	28651	7.63E-3	6.37E-3	9.55E-3	8.16E-5	6.01E-5	9.01E-5	7.52E-5	5.02E-5	7.54E-5
carbon dioxide[m]**	co2[m]	C00011	28651	6.53E-3	6.37E-3	9.55E-3	7.71E-5	6.01E-5	9.01E-5	-	-	-
citrate	cit	C00158	23703	5.84E-4	5.59E-4	6.09E-4	1.49E-3	1.31E-3	1.67E-3	1.96E-3	1.10E-3	3.48E-3
citrulline	citr-L	C00327	22426	-	-	-	2.70E-2	2.41E-2	3.00E-2	1.35E-3	1.23E-3	1.48E-3
CMP	cmp	C00055	36522	1.18E-5	9.13E-6	1.44E-5	5.18E-6	2.95E-6	7.40E-6	3.60E-4	1.87E-4	6.94E-4
coenzyme-A	coa	C00010	11741	-	-	-	-	-	-	1.37E-3	8.83E-5	2.12E-2
coenzyme-A(m)**	coa[m]	C00010	11741	4.04E-3	4.00E-3	6.00E-3	4.90E-3	4.00E-3	6.00E-3	-	-	-
CTP	ctp	C00063	27764	8.97E-4	7.55E-4	1.04E-3	2.49E-4	2.02E-4	2.95E-4	2.73E-3	2.27E-3	3.27E-3
cyclic-AMP	camp	C00575	664	1.30E-7	6.29E-8	1.97E-7	4.00E-7	3.19E-7	4.82E-7	3.52E-5	2.82E-5	4.39E-5
cysteine	cys-L	C00097	17926	8.40E-5	6.19E-5	1.06E-4	-	-	-	-	-	-
cytidine	cytd	C00475	27472	-	-	-	-	-	-	2.59E-6	1.18E-6	5.67E-6
cytosine	csn	C00380	19308	-	-	-	-	-	-	1.41E-5	8.65E-6	2.29E-5
dAMP	damp	C00360	1037	1.68E-5	5.76E-6	2.79E-5	-	-	-	8.84E-6	2.99E-6	2.62E-5
dATP	datp	C00131	1369	9.74E-7	7.52E-7	1.20E-6	9.78E-6	6.55E-6	1.30E-5	1.55E-5	8.13E-6	2.96E-5
dCDP	dcdp	C00705	27662	1.82E-6	1.62E-6	2.02E-6	-	-	-	-	-	-
dCMP	dcmp	C00239	27589	3.71E-5	2.55E-5	4.88E-5	-	-	-	-	-	-
dCTP	dctp	C00458	27759	-	-	-	4.46E-6	1.96E-6	6.96E-6	3.45E-5	2.60E-5	4.57E-5
deoxyadenosine	dad-2	C00559	836	-	-	-	-	-	-	2.82E-6	1.80E-6	4.41E-6
deoxyguanosine	dgsn	C00330	842	-	-	-	-	-	-	5.22E-7	4.11E-7	6.62E-7
deoxyribose-5-phosphate	2dr5p	C00673	34541	-	-	-	-	-	-	3.03E-4	4.82E-5	1.90E-3
dGMP	dgmpp	C00362	1047	-	-	-	-	-	-	5.07E-5	3.91E-5	6.58E-5
dihydroorotate	dhor-S	C00337	20954	7.35E-4	6.30E-4	8.40E-4	-	-	-	1.19E-5	1.16E-5	1.23E-5
dihydroxyacetonephosphate	dhap	C00111	89172	-	-	-	8.07E-4	7.01E-4	9.13E-4	3.06E-3	2.90E-3	3.22E-3
dihydroxyacetonephosphate[c]*	dhap[c]	C00111	89172	1.63E-3	1.47E-3	1.75E-3	8.23E-4	7.46E-4	9.10E-4	-	-	-
dTDP	dtdp	C00363	1308	-	-	-	-	-	-	3.78E-4	3.37E-4	4.26E-4
dTMP	dtmp	C00364	1170	1.18E-5	7.20E-6	1.65E-5	-	-	-	-	-	-
dTTP	dtpp	C00459	1479	-	-	-	-	-	-	4.62E-3	4.21E-3	5.08E-3
erythrose-4-phosphate[c]*	e4p[c]	C00279	59851	1.03E-5	7.54E-6	1.58E-5	1.46E-5	6.92E-6	1.95E-5	4.90E-5	4.19E-5	5.64E-5
FAD	fad	C00016	33700	5.60E-6	3.17E-6	8.03E-6	3.53E-5	2.93E-5	4.13E-5	1.73E-4	9.33E-5	3.19E-4
flavin mononucleotide	fmn	C00061	36301	-	-	-	-	-	-	5.37E-5	3.84E-5	7.51E-5
fructose-1,6-bisphosphate	f6p	C00354	22767	1.52E-3	1.40E-3	1.63E-3	4.00E-3	3.41E-3	4.59E-3	1.52E-2	1.40E-2	1.64E-2
fructose-6-phosphate[c]*	f6p[c]	C00085	56501	9.69E-5	8.18E-5	1.27E-4	2.37E-3	1.53E-3	2.92E-3	2.52E-3	2.16E-3	2.89E-3
fumarate	fum	C00122	19266	3.87E-4	2.83E-4	4.90E-4	1.21E-4	8.05E-5	1.61E-4	1.15E-4	3.00E-6	4.42E-3
fumarate[c]*	fum[c]	C00122	19266	-	-	-	-	-	-	2.88E-4	2.85E-4	2.93E-4
fumarate[m]*	fum[m]	C00122	19266	4.85E-4	4.80E-4	4.90E-4	1.24E-4	8.88E-5	1.54E-4	-	-	-
GDP	gdp	C00035	36200	3.02E-5	1.96E-5	4.08E-5	4.21E-5	2.63E-5	5.79E-5	6.76E-4	4.99E-4	9.16E-4
gluconate	glcn	C00257	22387	2.11E-4	1.24E-4	2.98E-4	9.51E-5	7.75E-5	1.13E-4	4.16E-5	5.74E-6	3.02E-4
gluconolactone	glc	C00198	-	-	-	-	-	-	-	1.04E-3	6.47E-4	1.68E-3
glucosamine-6-phosphate	gam6p	C00352	22727	-	-	-	-	-	-	1.15E-3	9.59E-4	1.39E-3
glucose-6-phosphate[c]*	g6p[c]	C00092	22626	6.75E-4	6.74E-4	1.05E-3	5.31E-3	4.36E-3	6.18E-3	7.88E-3	7.59E-3	8.17E-3
glutamate	glu-L	C00025	21361	6.38E-2	4.36E-2	8.39E-2	3.91E-2	3.66E-2	4.17E-2	9.60E-2	9.24E-2	9.98E-2
glutamine	gln-L	C00064	20287	1.72E-2	1.62E-2	1.82E-2	3.55E-2	3.23E-2	3.86E-2	3.81E-3	3.50E-3	4.15E-3
glutathione	gthrd	C00051	1508	3.09E-3	2.95E-3	3.22E-3	4.30E-3	4.12E-3	4.48E-3	1.66E-2	1.53E-2	1.79E-2
glutathione disulfide	gthox	C00127	47962	1.80E-5	1.57E-5	2.03E-5	9.73E-5	8.71E-5	1.07E-4	2.37E-3	1.94E-3	2.90E-3
glyceraldehyde-3-phosphate[c]*	g3p[c]	C00661	59	1.41E-4	1.28E-4	1.52E-4	1.18E-4	1.07E-4	1.30E-4	2.71E-4	2.56E-4	2.89E-4
glycerate	glyc-R	C00258	28971	-	-	-	-	-	-	1.41E-3	6.44E-4	3.08E-3
glycine	gly	C00037	15491	3.71E-3	3.61E-3	3.81E-3	-	-	-	-	-	-
GMP	gmp	C00144	1070	1.81E-5	1.12E-5	2.49E-5	1.02E-5	6.30E-6	1.41E-5	2.37E-5	1.66E-5	3.38E-5
GTP	gtp	C00044	32483	6.77E-4	6.52E-4	7.03E-4	2.47E-4	1.72E-4	3.22E-4	4.87E-3	1.57E-3	1.51E-2
guanine	gua	C00242	20891	-	-	-	-	-	-	1.88E-4	1.32E-4	2.69E-4
guanosine	gsn	C00387	85516	1.35E-6	1.19E-6	1.51E-6	-	-	-	1.62E-6	1.22E-6	2.17E-6
hexose-phosphate***				1.07E-3	7.55E-4	1.39E-3	5.86E-3	4.87E-3	6.84E-3	8.75E-3	8.43E-3	9.08E-3

histidine	his-L	C00135	23731	4.10E-4	3.86E-4	4.34E-4	-	-	-	6.76E-5	4.58E-5	9.97E-5
histidinol	histd	C00860		-	-	-	-	-	-	1.28E-5	1.26E-5	1.30E-5
homocysteine	hcys-L	C00155	84645	-	-	-	-	-	-	3.70E-4	3.65E-4	3.75E-4
hydroxyisocaproic acid		C03467		-	-	-	2.68E-5	2.23E-5	3.12E-5	-	-	-
IDP	idp	C00104	1009	-	-	-	-	-	-	2.38E-5	1.76E-5	3.22E-5
IMP	imp	C00130	1203	1.23E-5	9.06E-6	1.55E-5	3.80E-5	2.32E-5	5.28E-5	2.72E-4	1.73E-4	4.26E-4
inosine	ins	C00294	645	1.33E-6	5.38E-7	2.11E-6	6.12E-6	4.94E-6	7.31E-6	-	-	-
isocitrate[c]*	icit[c]	C00311	23698	-	-	-	-	-	-	3.67E-5	4.68E-6	4.29E-5
isocitrate[m]*	icit[m]	C00311	23698	3.21E-5	2.10E-5	3.38E-5	5.79E-6	1.90E-6	9.79E-6	-	-	-
isoleucine (assumed 1/2 ile+leu)	ile-L	C00407	22459	1.76E-3	1.66E-3	1.86E-3	3.53E-4	3.00E-4	4.05E-4	1.52E-4	1.49E-4	1.54E-4
isoleucine+leucine				3.52E-3	3.31E-3	3.72E-3	7.05E-4	6.00E-4	8.10E-4	3.03E-4	2.98E-4	3.08E-4
ITP	itp	C00081	1200	-	-	-	-	-	-	2.05E-4	1.38E-4	3.03E-4
leucine (assumed 1/2 ile+leu)	leu-L	C00123	22465	1.76E-3	1.66E-3	1.86E-3	3.53E-4	3.00E-4	4.05E-4	1.52E-4	1.49E-4	1.54E-4
lysine	lys-L	C00047	22685	5.06E-4	4.58E-4	5.54E-4	5.16E-3	2.89E-3	7.44E-3	4.05E-4	3.27E-4	5.02E-4
malate	mal-L	C00149	19469	1.39E-3	1.06E-3	1.72E-3	9.25E-4	6.82E-4	1.17E-3	1.68E-3	1.66E-3	1.70E-3
malonyl-CoA	malcoa	C00083	13046	4.95E-6	4.76E-6	5.13E-6	-	-	-	3.54E-5	4.05E-7	3.09E-3
methionine	met-L	C00073	20519	6.39E-4	6.19E-4	6.59E-4	1.91E-4	1.49E-4	2.33E-4	1.45E-4	1.31E-4	1.61E-4
methyilmalonic acid		C02170		7.26E-5	3.76E-5	1.08E-4	-	-	-	-	-	-
myo-inositol	inost	C00137	82786	-	-	-	-	-	-	5.72E-6	4.22E-6	7.75E-6
N-acetyl-glucosamine-1/6-phosphate	acgam1p	C04256	26142	7.47E-6	4.38E-6	1.06E-5	1.50E-4	1.36E-4	1.65E-4	8.19E-5	7.25E-5	9.26E-5
N-acetyl-glutamine			24082	6.24E-6	5.60E-6	6.87E-6	6.49E-5	5.14E-5	7.84E-5	-	-	-
N-Acetyl-L-alanine			21296	1.03E-5	8.77E-6	1.19E-5	8.08E-6	6.38E-6	9.77E-6	-	-	-
N-Acetyl-L-aspartic acid	Nacasp	C01042	23785	2.90E-3	2.77E-3	3.03E-3	-	-	-	-	-	-
N-acetyl-ornithine	acorn	C00437	24348	-	-	-	2.29E-4	1.93E-4	2.65E-4	4.33E-5	2.71E-5	6.94E-5
NAD+	nad	C00003	11259	5.02E-4	4.72E-4	5.32E-4	2.44E-3	2.29E-3	2.60E-3	2.55E-3	2.32E-3	2.80E-3
NADH	nadh	C00004	11384	7.50E-5	6.36E-5	8.82E-5	1.07E-4	9.32E-5	1.22E-4	8.36E-5	5.45E-5	1.27E-4
NADP+	nadp	C00006	48210	2.84E-5	2.55E-5	3.14E-5	1.83E-4	1.61E-4	2.04E-4	2.08E-6	1.40E-7	3.11E-5
NADPH	nadph	C00005	48213	6.54E-5	5.87E-5	7.21E-5	2.21E-4	1.93E-4	2.50E-4	1.21E-4	1.10E-4	1.34E-4
ornithine	orn	C00077	20623	-	-	-	4.49E-3	4.04E-3	4.94E-3	1.01E-5	6.81E-6	1.51E-5
orotate	orot	C00295	20827	8.41E-6	2.19E-6	1.46E-5	-	-	-	-	-	-
oxaloacetate[c]*	oaa[c]	C00036	19271	-	-	-	-	-	-	4.87E-7	2.81E-7	8.55E-7
oxaloacetate[m]*	oaa[m]	C00036	19271	2.01E-6	1.18E-6	3.31E-6	5.01E-7	1.32E-7	9.86E-7	-	-	-
pentose-phosphate***				-	-	-	-	-	-	1.32E-3	9.83E-4	1.77E-3
phenylalanine	phe-L	C00079	27187	8.40E-4	7.97E-4	8.84E-4	2.73E-4	2.15E-4	3.31E-4	1.82E-5	1.77E-5	1.87E-5
phenylpyruvate	phpyr	C00166	28296	1.77E-3	1.71E-3	1.82E-3	-	-	-	8.98E-5	5.01E-5	1.61E-4
phosphate (orthophosphate)[c]**	pi[c]	C00009	92038	5.83E-3	4.00E-3	6.00E-3	4.93E-2	4.00E-2	6.00E-2	2.39E-2	1.60E-2	2.40E-2
phosphoenolpyruvate	pep	C00074	17779	1.16E-5	6.86E-6	1.98E-5	2.91E-5	2.33E-5	3.48E-5	1.84E-4	1.46E-4	2.31E-4
proline	pro-L	C00148	21286	1.23E-3	1.13E-3	1.34E-3	1.36E-3	8.38E-4	1.87E-3	3.85E-4	3.72E-4	3.99E-4
propionyl-CoA	ppcoa	C00100	36287	-	-	-	-	-	-	5.32E-6	3.88E-6	7.29E-6
PRPP	prpp	C00119	20749	-	-	-	4.70E-5	3.67E-5	5.73E-5	2.58E-4	1.36E-4	4.92E-4
pyruvate	pyr	C00022	17694	5.88E-3	5.40E-3	6.35E-3	9.40E-3	8.51E-3	1.04E-2	3.66E-3	3.13E-3	4.20E-3
quinolinate	quln	C03722	24851	-	-	-	-	-	-	1.15E-5	2.41E-6	5.49E-5
riboflavin	ribflv	C00255	7793	-	-	-	-	-	-	1.90E-5	1.72E-5	2.11E-5
ribose-5-phosphate	r5p	C00117	34575	7.83E-5	2.84E-5	1.28E-4	1.52E-4	1.14E-4	1.90E-4	-	-	-
ribose-5-phosphate[c]*	r5p[c]	C00117	34575	2.84E-5	2.84E-5	4.01E-5	-	-	-	7.87E-4	7.86E-4	8.36E-4
ribulose-5-phosphate[c]*	ru5p-D[c]	C00199	20587	5.27E-6	4.10E-6	5.85E-6	1.22E-4	7.73E-5	1.95E-4	1.12E-4	1.12E-4	1.27E-4
S-adenosyl-L-homocysteine	ahcys	C00021	5433	5.71E-7	-1.61E-7	1.30E-6	-	-	-	-	-	-
S-adenosyl-L-methionine	amet	C00019	6347	-	-	-	-	-	-	1.84E-4	1.19E-4	2.84E-4
sedoheptulose-7-phosphate	s7p	C05382	24563	1.51E-5	1.21E-5	1.81E-5	3.61E-4	3.29E-4	3.92E-4	8.82E-4	8.40E-4	9.24E-4
sedoheptulose-7-phosphate[c]*	s7p[c]	C05382	24563	1.81E-5	1.28E-5	1.81E-5	-	-	-	-	-	-
serine	ser-L	C00065	17936	4.86E-3	4.75E-3	4.97E-3	3.87E-3	2.35E-3	5.38E-3	1.13E-3	1.06E-3	1.20E-3
shikimate	skm	C00493	23975	-	-	-	-	-	-	1.41E-5	7.02E-6	2.81E-5
sn-glycerol 3-phosphate	glyc3p	C00093	18111	-	-	-	2.81E-4	2.54E-4	3.08E-4	4.90E-5	1.29E-5	1.87E-4
succinate	succ	C00042	19270	3.52E-4	2.87E-4	4.30E-4	6.35E-4	5.66E-4	7.05E-4	5.69E-4	3.41E-4	9.49E-4
succinyl-CoA	succoa	C00091	55439	-	-	-	-	-	-	2.33E-4	1.42E-4	3.83E-4
succinyl-CoA[m]*	succoa[m]	C00091	55439	6.80E-6	3.49E-7	3.16E-3	-	-	-	-	-	-
taurine	taur	C00245	15566	-	-	-	5.64E-6	5.21E-6	6.06E-6	-	-	-
threonine	thr-L	C00188	19816	6.69E-3	6.44E-3	6.94E-3	6.69E-3	4.52E-3	8.86E-3	1.26E-3	1.22E-3	1.29E-3
thymidine	thymd	C00214	991	2.64E-6	1.88E-6	3.40E-6	3.45E-6	2.65E-6	4.24E-6	-	-	-
trehalose	tre	C01083	39582	-	-	-	8.40E-3	6.53E-3	1.03E-2	-	-	-
tryptophan	trp-L	C00078	2198	1.80E-4	1.72E-4	1.89E-4	5.55E-5	4.63E-5	6.46E-5	1.21E-5	1.14E-5	1.29E-5
tyrosine	tyr-L	C00082	27212	9.38E-4	8.88E-4	9.88E-4	2.48E-4	2.29E-4	2.68E-4	2.89E-5	1.87E-5	4.47E-5
UDP	udp	C00015	27564	1.33E-4	1.25E-4	1.42E-4	3.68E-5	2.90E-5	4.47E-5	1.79E-3	1.18E-3	2.72E-3
UDP-glucose	udpg	C00029	6433	1.53E-3	1.28E-3	1.77E-3	2.68E-4	2.50E-4	2.86E-4	2.50E-3	1.23E-3	5.11E-3
UDP-glucuronate	udpglcur	C00167	36564	9.75E-5	8.45E-5	1.11E-4	-	-	-	5.66E-4	1.20E-4	2.67E-3
UDP-N-acetyl-glucosamine	uacgam	C00043	8030	8.97E-3	8.80E-3	9.14E-3	1.02E-3	9.28E-4	1.11E-3	9.24E-3	6.79E-3	1.26E-2
UMP	ump	C00105	27452	1.45E-5	1.33E-5	1.57E-5	1.45E-5	8.69E-6	2.04E-5	-	-	-
uridine	uri	C00299	27316	-	-	-	-	-	-	2.09E-3	1.96E-3	2.24E-3
UTP	utp	C00075	27658	1.76E-3	1.55E-3	1.98E-3	4.94E-4	4.28E-4	5.59E-4	8.29E-3	7.76E-3	8.86E-3
valine	val-L	C00183	20495	1.51E-3	1.44E-3	1.58E-3	2.50E-3	2.37E-3	2.63E-3	4.02E-3	3.53E-3	4.58E-3
xylulose-5-phosphate	xu5p-D	C00231	29847	3.15E-5	2.33E-5	3.95E-5	-	-	-	-	-	-
xylulose-5-phosphate[c]*	xu5p-D[c]	C00231	29847	2.99E-5	2.34E-5	3.31E-5	2.46E-4	1.59E-4	3.93E-4	1.81E-4	1.80E-4	2.03E-4

\* Concentrations satisfy both measured 95% confidence interval and thermodynamic constraints. [c] and [m] denote values consistent with reaction free energies in cytosol and mitochondria, respectively.

\*\* CO2 concentration was calculated using Henry's law at the respective culture conditions. Coenzyme A and phosphate concentrations were taken from literature.

\*\*\* Hexose-phosphate includes glucose-6-phosphate and fructose-6-phosphate. Pentose-phosphate includes ribose-5-phosphate, ribulose-6-phosphate, and xylulose-5-phosphate.

**Supplementary Table 6. Reactions included in <sup>13</sup>C-metabolic flux analysis.**

EC Number	Reaction	Abbreviation	Reactants	Products	Mammalian iBMK cells	Yeast	<i>E. coli</i>	Irreversible constraint
2.7.1.1	hexokinase (PTS in <i>E. coli</i> )	hex	GLC	G6P	✓	✓	✓	✓
5.3.1.9	phosphoglucose isomerase	pgi	G6P	F6P	✓	✓	✓	
2.7.1.11	phosphofructokinase	pfk	F6P	FBP	✓	✓	✓	
4.1.2.13	fructose-bisphosphate aldolase	fba	FBP	DHAP + GAP	✓	✓	✓	
5.3.1.1	triose-phosphate isomerase	tpi	DHAP	GAP	✓	✓	✓	
1.2.1.12	glyceraldehyde-3-phosphate dehydrogenase	gapd	GAP	BPG	✓	✓	✓	
2.7.2.3	phosphoglycerate kinase	pgk	BPG	3PG	✓	✓	✓	
4.2.1.11	enolase	eno	3PG	PEP	✓	✓	✓	
2.7.1.40	pyruvate kinase	pyk	PEP	PYR	✓	✓	✓	
4.1.1.31	phosphoenolpyruvate carboxylase	ppc	PEP + CO <sub>2</sub>	OAA			✓	✓
4.1.1.32	phosphoenolpyruvate carboxykinase	ppck	OAA	PEP + CO <sub>2</sub>	✓	✓		✓
1.1.1.38; 1.1.1.39;								
1.1.1.40	malic enzyme	me	Mal	PYR + CO <sub>2</sub>	✓	✓	✓	✓
6.4.1.1	pyruvate carboxylase	pc	PYR + CO <sub>2</sub>	OAA	✓	✓		✓
4.1.1.3	oxaloacetate decarboxylase	oaadc	OAA	PYR + CO <sub>2</sub>			✓	✓
1.1.1.49 and 3.1.1.31	glucose-6-phosphate dehydrogenase and 6-phosphogluconolactonase	g6pdh	G6P	6PG	✓	✓	✓	✓
4.2.1.12 and 4.1.2.14	6-phosphogluconate dehydratase and 2-dehydro-3-deoxy-phosphogluconate aldolase	eda	m6PG	PYR + GAP			✓	
1.1.1.44	phosphogluconate dehydrogenase	gnd	6PG	Ru5P + CO <sub>2</sub>	✓	✓	✓	
5.3.1.6	ribose-5-phosphate isomerase	rpi	Ru5P	R5P	✓	✓	✓	
5.1.3.1	ribulose-5-phosphate 3-epimerase	rpe	Ru5P	X5P	✓	✓	✓	
2.2.1.1	transketolase	tkt1	X5P + R5P	S7P + GAP	✓	✓	✓	
2.2.1.1	transketolase	tkt2	X5P + E4P	GAP + F6P	✓	✓	✓	
2.2.1.2	transaldolase	tal	GAP + S7P	E4P + F6P	✓	✓	✓	
2.3.1.12	pyruvate dehydrogenase	pdh	PYR	AcCoA + CO <sub>2</sub>	✓	✓	✓	
4.1.1.1	pyruvate decarboxylase	cdc	PYR	AcetAld + CO <sub>2</sub>		✓		
4.1.3.7	citrate synthase	cs	OAA + AcCoA	CitCit	✓	✓	✓	
1.1.1.41 or 1.1.1.42	Isocitrate dehydrogenase	icdh	CitCit	aKG + CO <sub>2</sub>	✓	✓	✓	
2.3.1.61	2-oxoglutarate dehydrogenase	akgdh	aKG	SuccCoA + CO <sub>2</sub>	✓	✓	✓	
6.2.1.4 or 6.2.1.5	succinyl-CoA synthetase	sucoasA	SuccCoA	Succ	✓	✓	✓	
1.3.99.1	succinate dehydrogenase	sudc	Succ	Fum	✓	✓	✓	
4.2.1.2	fumarase	fumA	Fum	Mal	✓	✓	✓	
1.1.1.37	malate dehydrogenase	mdh	Mal	OAA	✓	✓	✓	
2.3.3.8	ATP-citrate lyase	acitl	CitCit	Mal + AcCoA_cytosol	✓			✓
4.1.3.1	isocitrate lyase	icl	CitCit	Glx + Succ		✓	✓	
2.3.3.9	malate synthase	mals	Glx + AcCoA	Mal		✓	✓	✓
1.1.1.95 and 2.6.1.52	phosphoglycerate dehydrogenase and phosphoserine transaminase		3PG	PSer	✓	✓	✓	
3.1.3.3	phosphoserine phosphatase		PSer	Ser	✓	✓	✓	
4.3.1.17	L-serine deaminase		Ser	PYR	✓	✓	✓	✓
2.1.2.1	serine hydroxymethyltransferase		Ser	Gly +1C-THFa	✓	✓	✓	
	glycine cleavage system		Gly	1C-THFa + CO <sub>2</sub>	✓	✓	✓	✓
	cysteine synthesis		Ser	Cys	✓	✓	✓	✓
2.6.1.2	L-alanine transaminase		PYR	Ala	✓	✓	✓	
4.1.3.18 and 1.1.1.86 and 4.2.1.9	acetolactate synthase and ketol-acid reductoisomerase and dihydroxy-acid dehydratase (2,3-dihydroxy-3-methylbutanoate)		2 PYR	AKV + CO <sub>2</sub>		✓	✓	
2.6.1.42	valine transaminase		AKV	Val		✓	✓	
4.1.3.12 and 4.2.1.33	2-isopropylmalate synthase and 2-isopropylmalate hydratase		AKV + AcCoA	IPPM		✓	✓	✓
4.2.1.33 and 1.1.1.85 and 2.6.1.42	leucine synthesis		IPPM	Leu + CO <sub>2</sub>		✓	✓	
	shikimate synthesis		PEP + E4P	SKM		✓	✓	
	chorismate synthesis		PEP + SKM	Chor		✓	✓	
	phenylpyruvate synthesis		Chor	PHPYR + CO <sub>2</sub>		✓	✓	
	phenylalanine synthesis		PHPYR	Phe		✓	✓	
	tyrosine synthesis		Chor	Tyr + CO <sub>2</sub>		✓	✓	
	tryptophan synthesis		Chor + PRPP + Ser	Trp + GAP + PYR + CO <sub>2</sub>		✓	✓	
	proline synthesis		Glu	Pro	✓	✓	✓	✓
	aKG - Glu interconversion		aKG	Glu	✓	✓	✓	
	Glu - Gln interconversion		Glu	Gln	✓	✓	✓	
2.3.1.1	N-acteylglutamate synthase		Glu + AcCoA	AcGlu		✓	✓	✓
	ornithine synthesis		AcGlu	Orn + AcCoA		✓	✓	✓
	citrulline synthesis (urea cycle)		Orn + CO <sub>2</sub>	CITRL	✓	✓	✓	
	arginine synthesis (urea cycle)		CITRL	Arg	✓	✓	✓	
	threonine to glycine		Thr	Gly + AcCoA	✓	✓	✓	
	OAA - asp interconversion		OAA	Asp	✓	✓	✓	
	homoserine synthesis		Asp	HomoSer		✓	✓	
	threonine synthesis		HomoSer	Thr		✓	✓	
	asparagine synthesis		Asp	Asn	✓	✓	✓	
	isoleucine synthesis		Thr + PYR	Ile + CO <sub>2</sub>		✓	✓	
	methionine synthesis		HomoSer + 1C-THFa	Met		✓	✓	✓
	lysine synthesis		aKG + AcCoA	Lys + CO <sub>2</sub>		✓	✓	



2.1.3.2	aspartate carbamoyltransferase		Asp + CO2	CBASP	✓	✓	✓	
3.5.2.3 and 1.3.3.1	dihydroorotase and dihydroorotic acid dehydrogenase		CBASP	OROT	✓	✓	✓	
2.4.2.10 and 4.1.1.23	pyrimidine synthesis		OROT + PRPP	UMP + CO2	✓	✓	✓	✓
	dTTP synthesis		UMP + 1C-THFa	dTTP	✓	✓	✓	✓
2.6.1.16	glutamine-fructose-6-phosphate transaminase		F6P	GAM6P	✓	✓	✓	✓
2.3.1.4	N-acetylglucosamine-6-phosphate synthase		GAM6P + AcCoA	ACGAM1P	✓	✓	✓	✓
	glycerol-3-phosphate synthesis		DHAP	GLYC3P	✓	✓	✓	
2.7.6.1	phosphoribosylpyrophosphate synthetase		R5P	PRPP	✓	✓	✓	✓
	histidine synthesis		PRPP + ATP	His + AICAR		✓	✓	✓
	5-aminoimidazole-4-carboxamide ribonucleotide synthesis		PRPP + Gly + 1C-THFa + CO2	AICAR	✓	✓	✓	✓
	purine synthesis		AICAR + 1C-THFa	ATP	✓	✓	✓	✓
1.2.1.10	acetaldehyde dehydrogenase		AcCoA	AcetAld			✓	
4.1.1.15 and 2.6.1.19 and 1.2.1.24	GABA shunt		Glu	Succ + CO2	✓			✓
2.6.1.13	ornithine transaminase		Glu	Orn	✓			
	cysteine degradation (desulfhydrase in E. coli)		Cys	PYR	✓	✓	✓	✓
2.8.1.7	cysteine desulfurase		Cys	Ala	✓			✓
	Tyr degradation		Tyr	AcetoAc + Fum + CO2	✓			✓
	Trp degradation		Trp	Ala + 2 AcCoA + 3 CO2 + 1C-THFa	✓			✓
	Ile degradation		Ile + CO2	SuccCoA + AcCoA + CO2	✓			✓
	Met degradation		Met + Ser + CO2	SuccCoA + CO2 + Cys + 1C-THFa	✓			✓
	Val degradation		Val + CO2	SuccCoA + 2 CO2	✓			✓
	Lys degradation		Lys	2 AcCoA + 2 CO2	✓			✓
	Leu degradation		Leu + CO2	AcetoAc + AcCoA + CO2	✓			✓
	His degradation		His	Glu + 1C-THFa	✓			✓
2.8.3.5 and 2.3.1.9	ketolysis		AcetoAc	2 AcCoA	✓			✓
1.14.16.1	phenylalaninase		Phe	Tyr	✓			
	efflux		G6P		✓	✓	✓	✓
			ACGAM1P		✓	✓	✓	✓
			R5P		✓	✓	✓	✓
			GLYC3P		✓	✓	✓	✓
			AcCoA_cyt		✓	✓	✓	✓
			Ala		✓	✓	✓	✓
			Arg		✓	✓	✓	✓
			Asn		✓	✓	✓	✓
			Asp		✓	✓	✓	✓
			Cys		✓	✓	✓	✓
			Gln		✓	✓	✓	✓
			Glu		✓	✓	✓	✓
			Gly		✓	✓	✓	✓
			His		✓	✓	✓	✓
			Ile		✓	✓	✓	✓
			Leu		✓	✓	✓	✓
			Lys		✓	✓	✓	✓
			Met		✓	✓	✓	✓
			Phe		✓	✓	✓	✓
			Pro		✓	✓	✓	✓
			Ser		✓	✓	✓	✓
			Thr		✓	✓	✓	✓
			Trp		✓	✓	✓	✓
			Tyr		✓	✓	✓	✓
			Val		✓	✓	✓	✓
			UMP		✓	✓	✓	✓
			dTTP		✓	✓	✓	✓
			ATP		✓	✓	✓	✓
			1C-THFa		✓	✓	✓	✓
			AcetoAc		✓			✓
			Succ			✓	✓	✓
			EtOH (AcetAld -> EtOH)			✓	✓	✓
			CO2		✓	✓	✓	✓
	influx		Glc		✓	✓	✓	✓
			AcCoA		✓			✓
			Arg		✓			✓
			Cys		✓			✓
			Gln		✓			✓
			Gly		✓			✓
			His		✓			✓
			Ile		✓			✓
			Leu		✓			✓
			Lys		✓			✓
			Met		✓			✓
			Phe		✓			✓
			Ser		✓			✓
			Thr		✓			✓
			Trp		✓			✓
			Tyr		✓			✓
			Val		✓			✓
			CO2		✓	✓	✓	✓

**Supplementary Table 7. Standard Gibbs free energy of glycolytic reactions at various pH and ionic strengths.**

PGI (G6P=>F6P) $\Delta G^{\circ}$ (kJ/mol)		Ionic Strength (M)										
		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51
	6.9	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51	2.51
	7.1	2.51	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
	7.3	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.49	2.49	2.49	2.49
	7.5	2.49	2.49	2.49	2.49	2.49	2.49	2.48	2.48	2.48	2.48	2.48
	7.7	2.48	2.48	2.48	2.47	2.47	2.47	2.47	2.47	2.46	2.46	2.46
	7.9	2.46	2.46	2.45	2.45	2.45	2.44	2.44	2.44	2.43	2.43	2.43
	8.1	2.43	2.42	2.42	2.41	2.41	2.40	2.40	2.39	2.39	2.38	2.38
	8.3	2.38	2.37	2.36	2.36	2.35	2.34	2.33	2.33	2.32	2.31	2.31
	8.5	2.31	2.29	2.28	2.27	2.26	2.24	2.23	2.22	2.21	2.20	2.20
	8.7	2.20	2.17	2.16	2.14	2.12	2.10	2.09	2.07	2.05	2.04	2.03

PFK (F6P+ATP=>FBP+ADP+H) $\Delta G^{\circ}$ (kJ/mol)		Ionic Strength (M)										
		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	-15.77	-15.94	-16.08	-16.22	-16.34	-16.45	-16.56	-16.66	-16.75	-16.83	-16.89
	6.9	-16.84	-17.01	-17.15	-17.29	-17.41	-17.53	-17.63	-17.73	-17.82	-17.91	-17.97
	7.1	-17.93	-18.09	-18.24	-18.38	-18.51	-18.62	-18.73	-18.83	-18.92	-19.01	-19.08
	7.3	-19.04	-19.21	-19.36	-19.50	-19.63	-19.75	-19.86	-19.96	-20.06	-20.15	-20.22
	7.5	-20.18	-20.36	-20.51	-20.66	-20.79	-20.91	-21.03	-21.13	-21.23	-21.33	-21.40
	7.7	-21.35	-21.53	-21.69	-21.84	-21.98	-22.11	-22.23	-22.34	-22.44	-22.54	-22.61
	7.9	-22.55	-22.74	-22.91	-23.06	-23.21	-23.34	-23.47	-23.58	-23.69	-23.80	-23.88
	8.1	-23.78	-23.98	-24.16	-24.32	-24.47	-24.62	-24.75	-24.88	-25.00	-25.11	-25.19
	8.3	-25.05	-25.26	-25.45	-25.63	-25.79	-25.95	-26.09	-26.23	-26.36	-26.48	-26.57
	8.5	-26.37	-26.60	-26.80	-26.99	-27.17	-27.34	-27.50	-27.64	-27.78	-27.92	-28.02
	8.7	-27.75	-27.99	-28.21	-28.42	-28.61	-28.80	-28.97	-29.13	-29.28	-29.43	-29.53

FBA (FBP=>DHAP+GAP) $\Delta G^{\circ}$ (kJ/mol)		Ionic Strength (M)										
		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	20.50	20.75	20.97	21.17	21.35	21.52	21.68	21.82	21.96	22.09	22.18
	6.9	20.68	20.92	21.14	21.33	21.52	21.68	21.84	21.98	22.12	22.24	22.34
	7.1	20.80	21.04	21.26	21.45	21.63	21.80	21.95	22.10	22.23	22.36	22.45
	7.3	20.89	21.13	21.35	21.54	21.72	21.89	22.05	22.19	22.33	22.45	22.55
	7.5	20.96	21.21	21.42	21.62	21.81	21.97	22.13	22.28	22.42	22.55	22.64
	7.7	21.04	21.28	21.50	21.71	21.89	22.07	22.23	22.38	22.52	22.65	22.75
	7.9	21.12	21.37	21.60	21.81	22.00	22.18	22.35	22.51	22.66	22.80	22.90
	8.1	21.23	21.49	21.73	21.95	22.16	22.34	22.52	22.69	22.84	22.99	23.10
	8.3	21.39	21.67	21.92	22.15	22.37	22.57	22.76	22.94	23.11	23.27	23.39
	8.5	21.62	21.91	22.19	22.44	22.67	22.89	23.10	23.30	23.48	23.66	23.79
	8.7	21.94	22.26	22.56	22.83	23.09	23.33	23.56	23.78	23.98	24.17	24.31

TPI (DHAP=>GAP) $\Delta G^{\circ}$ (kJ/mol)		Ionic Strength (M)										
		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	5.31	5.31	5.32	5.32	5.32	5.33	5.33	5.33	5.34	5.34	5.34
	6.9	5.35	5.36	5.36	5.36	5.37	5.37	5.37	5.37	5.37	5.38	5.38
	7.1	5.39	5.39	5.39	5.39	5.39	5.40	5.40	5.40	5.40	5.40	5.40
	7.3	5.41	5.41	5.41	5.41	5.41	5.41	5.41	5.42	5.42	5.42	5.42
	7.5	5.42	5.42	5.42	5.42	5.42	5.43	5.43	5.43	5.43	5.43	5.43
	7.7	5.43	5.43	5.43	5.43	5.43	5.43	5.43	5.43	5.43	5.43	5.43
	7.9	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44
	8.1	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44
	8.3	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44
	8.5	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44
	8.7	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44	5.44

GAPD (GAP+NAD+Pi=>13BPG+NADH+H)		Ionic Strength (M)										
$\Delta G^{\circ}$ (kJ/mol)		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	6.95	6.57	6.23	5.91	5.63	5.36	5.12	4.89	4.68	4.48	4.33
	6.9	5.34	4.96	4.63	4.32	4.04	3.79	3.55	3.33	3.12	2.92	2.78
	7.1	3.84	3.48	3.15	2.85	2.58	2.33	2.09	1.88	1.67	1.48	1.35
	7.3	2.45	2.09	1.76	1.47	1.20	0.96	0.73	0.51	0.31	0.13	-0.01
	7.5	1.12	0.76	0.45	0.16	-0.11	-0.35	-0.58	-0.79	-0.99	-1.17	-1.31
	7.7	-0.16	-0.51	-0.83	-1.12	-1.38	-1.62	-1.85	-2.05	-2.25	-2.43	-2.57
	7.9	-1.41	-1.76	-2.07	-2.36	-2.62	-2.86	-3.08	-3.29	-3.49	-3.67	-3.80
	8.1	-2.63	-2.98	-3.30	-3.58	-3.84	-4.08	-4.31	-4.51	-4.71	-4.89	-5.02
	8.3	-3.85	-4.19	-4.51	-4.79	-5.05	-5.29	-5.52	-5.72	-5.92	-6.10	-6.23
	8.5	-5.05	-5.40	-5.71	-6.00	-6.26	-6.50	-6.72	-6.93	-7.13	-7.31	-7.44
	8.7	-6.25	-6.60	-6.91	-7.20	-7.46	-7.70	-7.93	-8.14	-8.33	-8.51	-8.65

PGK (3PG+ATP=>13BPG+ADP)		Ionic Strength (M)										
$\Delta G^{\circ}$ (kJ/mol)		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	18.27	18.28	18.29	18.29	18.30	18.31	18.32	18.32	18.33	18.34	18.34
	6.9	18.42	18.43	18.43	18.44	18.45	18.46	18.46	18.47	18.48	18.48	18.49
	7.1	18.54	18.55	18.56	18.56	18.57	18.58	18.58	18.59	18.59	18.60	18.60
	7.3	18.64	18.64	18.65	18.65	18.66	18.66	18.67	18.67	18.68	18.68	18.68
	7.5	18.71	18.71	18.71	18.72	18.72	18.73	18.73	18.73	18.73	18.74	18.74
	7.7	18.75	18.76	18.76	18.76	18.76	18.77	18.77	18.77	18.77	18.77	18.77
	7.9	18.79	18.79	18.79	18.79	18.79	18.79	18.79	18.79	18.80	18.80	18.80
	8.1	18.80	18.81	18.81	18.81	18.81	18.81	18.81	18.81	18.81	18.81	18.81
	8.3	18.82	18.82	18.82	18.82	18.81	18.81	18.81	18.81	18.81	18.81	18.81
	8.5	18.82	18.82	18.82	18.82	18.81	18.81	18.81	18.81	18.81	18.81	18.81
	8.7	18.82	18.81	18.81	18.81	18.81	18.81	18.80	18.80	18.80	18.80	18.79

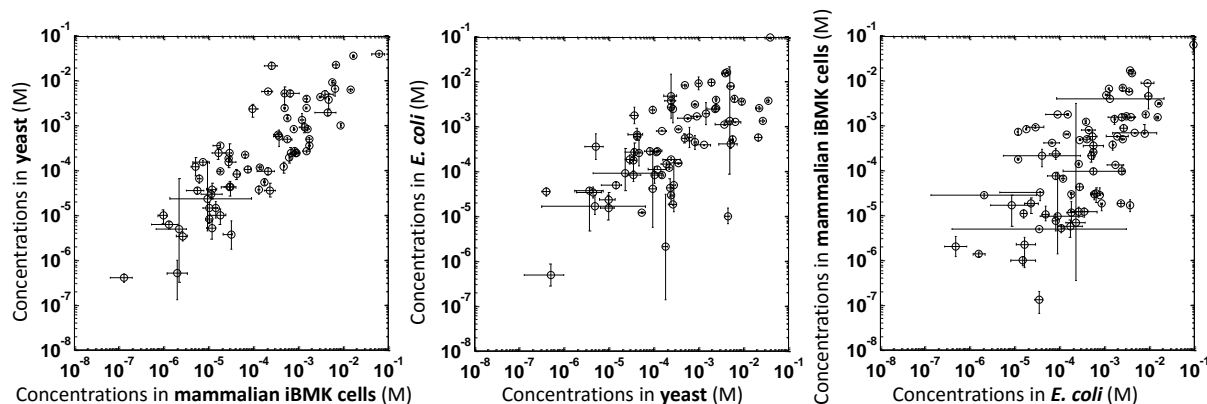
PGM (2PG=>3PG)		Ionic Strength (M)										
$\Delta G^{\circ}$ (kJ/mol)		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	-4.27	-4.26	-4.25	-4.24	-4.23	-4.23	-4.22	-4.22	-4.21	-4.21	-4.20
	6.9	-4.20	-4.20	-4.19	-4.19	-4.18	-4.18	-4.17	-4.17	-4.17	-4.17	-4.16
	7.1	-4.16	-4.16	-4.16	-4.15	-4.15	-4.15	-4.15	-4.14	-4.14	-4.14	-4.14
	7.3	-4.14	-4.14	-4.13	-4.13	-4.13	-4.13	-4.13	-4.12	-4.12	-4.12	-4.12
	7.5	-4.12	-4.12	-4.12	-4.12	-4.12	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	7.7	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.10	-4.10
	7.9	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10
	8.1	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10
	8.3	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10
	8.5	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10	-4.10
	8.7	-4.09	-4.09	-4.09	-4.09	-4.09	-4.09	-4.09	-4.09	-4.09	-4.09	-4.09

ENO (2PG=>PEP+H2O)		Ionic Strength (M)										
$\Delta G^{\circ}$ (kJ/mol)		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	6.9	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	7.1	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	7.3	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	7.5	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	7.7	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	7.9	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	8.1	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	8.3	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	8.5	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11
	8.7	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11	-4.11

PYK (PEP+ADP+H=>PYR+ATP)		Ionic Strength (M)										
$\Delta G^{\circ}$ (kJ/mol)		0.15	0.17	0.19	0.21	0.23	0.25	0.27	0.29	0.31	0.33	0.345
pH	6.7	-27.34	-27.30	-27.26	-27.22	-27.19	-27.16	-27.13	-27.11	-27.08	-27.06	-27.05
	6.9	-26.35	-26.30	-26.26	-26.22	-26.19	-26.16	-26.13	-26.11	-26.08	-26.06	-26.04
	7.1	-25.31	-25.27	-25.22	-25.19	-25.15	-25.12	-25.09	-25.06	-25.03	-25.01	-24.99
	7.3	-24.24	-24.19	-24.15	-24.11	-24.07	-24.04	-24.00	-23.98	-23.95	-23.92	-23.90
	7.5	-23.14	-23.09	-23.04	-23.00	-22.96	-22.92	-22.89	-22.86	-22.83	-22.80	-22.78
	7.7	-22.01	-21.95	-21.91	-21.86	-21.82	-21.79	-21.75	-21.72	-21.69	-21.66	-21.64
	7.9	-20.86	-20.80	-20.75	-20.71	-20.67	-20.63	-20.60	-20.56	-20.53	-20.50	-20.48
	8.1	-19.70	-19.64	-19.59	-19.55	-19.50	-19.47	-19.43	-19.40	-19.36	-19.34	-19.31
	8.3	-18.53	-18.47	-18.42	-18.37	-18.33	-18.29	-18.26	-18.22	-18.19	-18.16	-18.14
	8.5	-17.35	-17.29	-17.24	-17.19	-17.15	-17.11	-17.08	-17.04	-17.01	-16.98	-16.96
8.7	-16.17	-16.11	-16.06	-16.01	-15.97	-15.93	-15.90	-15.86	-15.83	-15.80	-15.78	

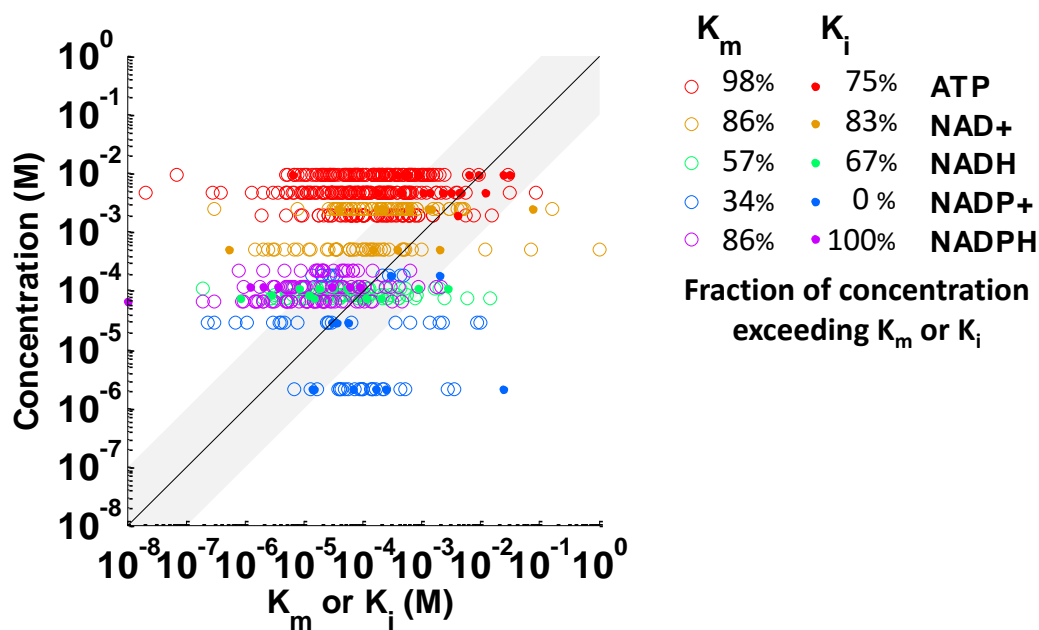
**Supplementary Table 8. Standard Gibbs free energy of reaction and standard error based on component contribution method.**

Reaction	Mammalian iBMK cells		Yeast		<i>E. coli</i>	
	$\Delta G^{\circ}$ (kJ/mol)	s.e.	$\Delta G^{\circ}$ (kJ/mol)	s.e.	$\Delta G^{\circ}$ (kJ/mol)	s.e.
G6P => F6P	2.50	0.59	2.50	0.58	2.47	0.55
F6P + ATP => FBP + ADP + H	-18.48	4.62	-17.27	4.59	-22.11	4.35
FBP -=> DHAP + GAP	20.85	0.81	20.52	0.81	22.07	0.77
DHAP => GAP	5.40	0.84	5.40	0.84	5.43	0.79
GAP + NAD + Pi -> 13BPG + NADH + H	3.14	4.55	4.59	4.52	-1.62	4.28
13BPG + ADP => 3PG + ATP	-18.59	0.68	-18.59	0.67	-18.77	0.64
3PG => 2PG	4.15	0.55	4.15	0.55	4.11	0.52
2PG => PEP + H2O	-4.11	0.47	-4.11	0.47	-4.11	0.44
PEP + ADP + H => Pyr + ATP	-24.78	4.55	-25.83	4.52	-21.78	4.28
Pyr + NAD + CoA => AcCoA + NADH + CO2	-35.19	4.95	-35.36	4.92	-35.50	4.66
6PG + NADP => Ru5P + NADPH + CO2	11.04	4.90	11.12	4.87	10.82	4.62
Ru5P => Xu5P	-3.52	1.77	-3.52	1.76	-3.52	1.67
Ru5P => R5P	-2.01	1.17	-2.01	1.17	-2.062	1.106
Xu5P + R5P => GAP + S7P	-3.98	2.95	-3.98	2.93	-4.15	2.78
S7P + GAP => E4P + F6P	-0.82	2.17	-0.82	2.16	-0.68	2.04
Xu5P + E4P => GAP + F6P	-10.25	2.95	-10.24	2.93	-10.35	2.78
AcCoA + OAA + H2O => Cit + CoA + H	-43.26	4.55	-38.79	4.52	-42.12	4.29
Cit => Icit	7.62	0.51	7.63	0.50	7.63	2.32
Icit + NAD => aKG + NADH + CO2	5.61	4.92	5.52	4.88	-	-
Icit + NADP => aKG + NADPH + CO2	-	-	-	-	5.92	4.63
aKG + NAD + CoA => SuccCoA + NADH + CO2	-28.41	5.97	-29.66	8.76	-29.42	5.62
SuccCoA + ADP + Pi => Succ + CoA + ATP	0.95	2.05	1.36	2.04	1.34	1.93
Fum + H2O => Mal	-3.45	0.43	-3.45	0.42	-3.45	0.40
Mal + NAD => OAA + NADH + H	22.28	4.53	26.42	4.50	23.59	4.26
Mal + NAD => Pyr + NADH + CO2	13.05	4.81	13.04	4.78	13.04	4.53
Ser + thf => Gly + mlthf + H2O	-6.64	1.91	-6.66	1.90	-6.58	1.80

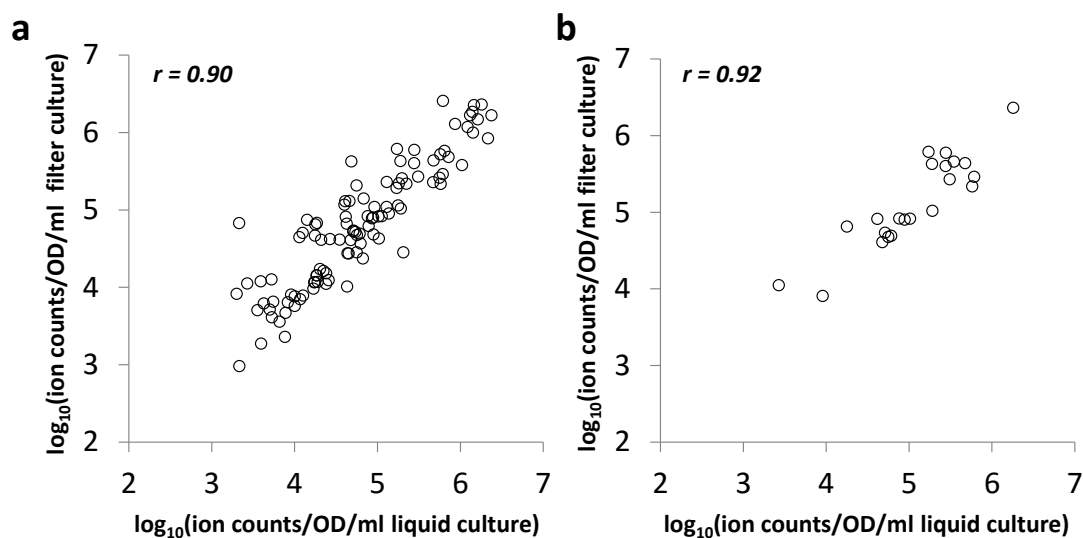


**Supplementary Figure 1. Conservation of absolute metabolite concentrations.**

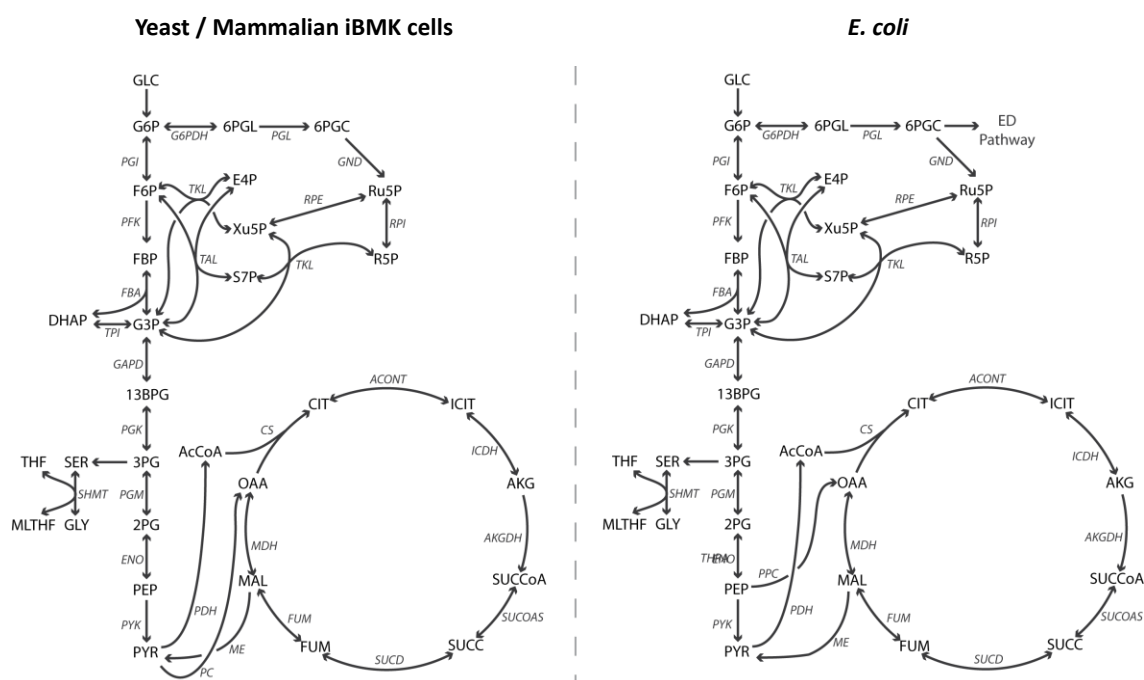
Comparison of absolute metabolite concentrations across organisms. Plotted data are for all measured metabolites. Error bars represent 95% confidence intervals.



**Supplementary Figure 2. ATP, NAD<sup>+</sup>, and NADPH but not NADP<sup>+</sup> saturate both active and regulatory binding sites.** Comparison of absolute metabolite concentrations (Y-axis) to enzyme binding site affinities (X-axis). Hollow circles show concentration- $K_m$  pairs and filled circles show concentration- $K_i$  pairs for *E. coli*, yeast, and mammalian iBMK cells.



**Supplementary Figure 3. Comparison of metabolite levels in *E. coli* liquid and filter cultures.** Ion counts of (a) all metabolites and (b) metabolites involved in central carbon metabolism. To determine filter culture density, the cells on the filter (47 mm diameter) were washed into 3 ml of sterile liquid media.



**Supplementary Figure 4. Central carbon metabolic network used for integrative computation of concentrations and free energies.** These reaction networks include several metabolites and reactions whose concentrations and free energy changes cannot be directly measured yet can be inferred using the direct measurements of other observed absolute metabolite concentrations and reaction free energies.

**Supplementary Data Set 1. Steady-state metabolite labeling from various  $^{13}\text{C}$ -tracers.**

Labeling of cellular metabolites in *E. coli* and yeast from [1,2- $^{13}\text{C}_2$ ]-glucose, [3- $^{13}\text{C}_1$ ]-glucose, and a 1:1 mix of unlabeled and [U- $^{13}\text{C}_6$ ]-glucose, and in mammalian iBMK cells from [1,2- $^{13}\text{C}_2$ ]-glucose, [U- $^{13}\text{C}_6$ ]-glucose, and [U- $^{13}\text{C}_5$ ]-glutamine.

**Supplementary Data Set 2. Comparison of absolute concentrations to enzyme binding site affinities for substrates.**  $K_m$  values in *E. coli*, *S. cerevisiae*, *Mus musculus*, and *Homo sapiens* were extracted from BRENDA. The parameters for mammalian cells are from enzymes in *Mus musculus* whenever possible, but otherwise in *Homo sapiens*. When multiple entries for the same enzyme-metabolite pair were available, its  $K_m$  was represented by their geometric mean.

**Supplementary Data Set 3. Comparison of absolute concentrations to enzyme binding site affinities for inhibitors.**  $K_i$  values in *E. coli*, *S. cerevisiae*, *Mus musculus*, and *Homo sapiens* were extracted from BRENDA. The parameters for mammalian cells are from enzymes in *Mus musculus* whenever possible, but otherwise in *Homo sapiens*. When multiple entries for the same enzyme-metabolite pair were available, its  $K_i$  was represented by their geometric mean.