Supplementary Material – Model Details

for

In Silico Experimentation with a Model of Hepatic Mitochondrial Folate Metabolism

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The model consists of 23 differential equations that express the rate of change of each of the substrates in the rectangular boxes in Figure 1. Each of the differential equations is a mass balance equation that says that the time rate of change of the particular metabolite is the sum of the rates at which it is being made minus the rates it is being consumed in biochemical reactions plus or minus net transport rates from other compartments. In order to display the differential equations in a coherent and understandable way, we have chosen notation for the variables and reaction rates that is both more uniform and more spare than some notation commonly in use, for example the concentration of 5-methyltetrahydrofolate is denoted 5mf instead of the usual [5mTHF]. All of this notation is described in Part A, below. In Part B we give the differential equations, which are written in terms of reaction and transport rates. In Part C the kinetic formulas and constants for these reaction and transport rates are given with some justifications. Part D describes how the model works, what is given and what is computed, in particular experiments.

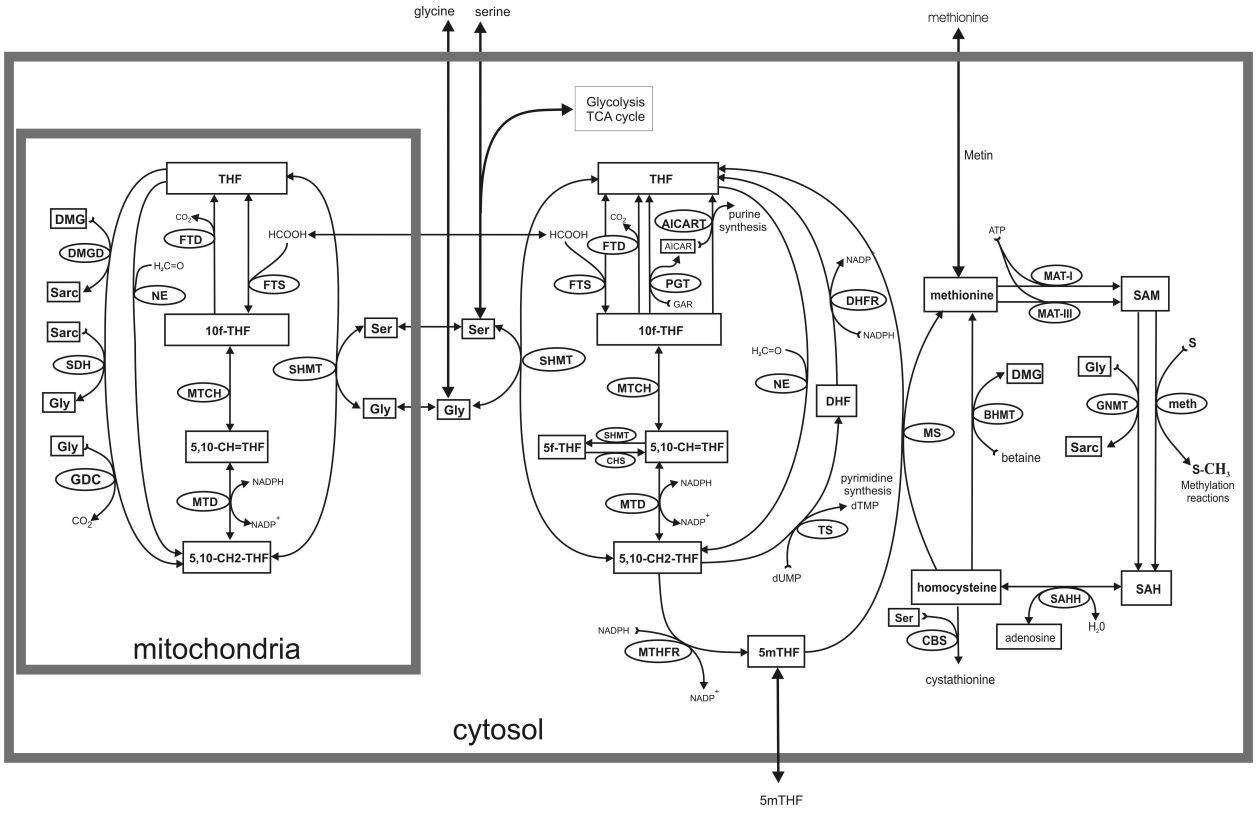

Figure 1: **Folate and Methionine Metabolism.**

Part A: Notation.

The complete names of the enzymes indicated by acronyms in Figure 1 are as follows.

Table S1: Enzyme names and acronyms.

mitochondrial folate cycle enzymes	
mSHMT	serinehydroxymethyltransferase
mFTD	10-formyltetrahydrofolate dehydrogenase
mFTS	10-formyltetrahydrofolate synthase
mMTCH	5,10-methenyltetrahydrofolate cyclohydrolase
mMTD	5,10-methylenetetrahydrofolate dehydrogenase
mNE	non-enzymatic conversion
DMGD	dimethylglycine dehydrogenase
SDH	sarcosine dehydrogenase
GDC	glycine decarboxylase (glycine cleavage system)
cytosolic folate cycle enzymes	
cSHMT	serinehydroxymethyltransferase
DHFR	dihydrofolate reductase
cFTD	10-formyltetrahydrofolate dehydrogenase
cFTS	10-formyltetrahydrofolate synthase
cMTCH	5,10-methenyltetrahydrofolate cyclohydrolase
cMTD	5,10-methylenetetrahydrofolate dehydrogenase
MTHFR	5,10-methylenetetrahydrofolate reductase
AICART	aminoimidazolecarboxamide ribonucleotide transferase
PGT	Phosphoribosyl glycinamidetransformalase
cNE	non-enzymatic conversion
TS	thymidylate synthase
methionine cycle enzymes	
MAT-I	methionine adenosyl transferase I
MAT-III	methionine adenosyl transferase III
GNMT	glycine N-methyltransferase
DNMT	DNA-methyltransferase
SAAH	S-adenosylhomocysteine hydrolase
MS	methionine synthase
BHMT	betaine-homocysteine methyltransferase
CBS	cystathionine β-synthase

We will use lower case three letter abbreviations for the concentrations of metabolites (μM). A prefix of m, c, or b, for mitochondria, cytosol, or blood, indicates the compartment. Metabolites occuring in only one compartment (like met), or metabolites whose concentrations are assumed equal in different compartments (like dmg and src) have no prefixes.

Table S2: Names of variables (μM).

mitochondrial folate cycle metabolites

mthf	tetrahydrofolate
m2cf	5-10-methylenetetrahydrofolate
m1cf	5-10-methenyltetrahydrofolate
m10f	10-formyltetrahydrofolate

cytosolic folate cycle metabolites

cthf	tetrahydrofolate
c2cf	5-10-methylenetetrahydrofolate
c1cf	5-10-methenyltetrahydrofolate
c10f	10-formyltetrahydrofolate
dhf	dihydrofolate
5mf	5-methyltetrahydrofolate
aic	P-ribosyl-5-amino-4-imidazole carboxamide

methionine cycle metabolites

met	methionine
sam	S-adenosylmethionine
sah	S-adenosylhomocysteine
hcy	homocysteine

other metabolites

mgly	mitochondrial glycine
mser	mitochondrial serine
mCOO	mitochondrial formate
cgly	cytosolic glycine
cser	cytosolic serine
cCOO	cytosolic formate
dmg	dimethylglycine
src	sarcosine

Table S3: Names of constants (μM).

GAR	10	glycinamide ribonucleotide
NADPH	50	nicotinamide adenine dinucleotide phosphate
BET	50	betaine
HCHO	500	formaldehyde
DUMP	20	deoxyuridine monophosphate
bgly	300	blood glycine (varies in some experiments)
bser	150	blood serine (varies in some experiments)
bmet	30	blood methionine (varies in some experiments)
FOL	20	total cellular folate (varies in some experiments)

It is assumed that total cellular folate is equally divided between the mitochondria and the cytosol [1] and that the mitochondria occupy one quarter of the volume of the cell. Thus the total normal folate concentration in the mitochondria is 40 μM, and in the cytosol is 13.3 μM.

Part B: The Equations.

For each of the biochemical reactions indicated by a reaction arrow in Figure 1, we denote the velocity of the reaction (in μM/hr) by a capital V whose subscript is the acronym for the enzyme that catalyzes the reaction. Thus, for example, the velocity of the methionine synthase reaction is denoted by V_{MS}. Each of these velocities depends on the current values one or more of the variables (metabolite concentrations) and possibly also on one or more of the constants. Velocities of reactions that occur in both the mitochondria and cytosol wiil be distinguished by $_{\rm c}$ and $_{\rm m}$, for example, $V_{{\rm c}SHMT}$ and $V_{{\rm m}SHMT}$.

$$\begin{aligned}
\left(\frac{d}{dt}\right)_{\rm m}{\rm thf} &= V_{\rm mFTD}({\rm m10f}) - V_{\rm mSHMT}({\rm mser}, {\rm mthf}, {\rm mgly}, {\rm m2cf}) - V_{\rm mFTS}({\rm mthf}, {\rm mcoo}, {\rm m10f}) \\
&\quad -V_{\rm mNE}({\rm mthf}, {\rm HCHO}, {\rm m2cf}) - V_{\rm GDC}({\rm mgly}, {\rm mthf}) - V_{\rm SDH}({\rm msrc}, {\rm mthf}) \\
&\quad -V_{\rm DMGD}({\rm mdmg}, {\rm mthf}) \\
\left(\frac{d}{dt}\right)_{\rm m}{\rm 2cf} &= V_{\rm mSHMT}({\rm mser}, {\rm mthf}, {\rm mgly}, {\rm m2cf}) + V_{\rm mNE}({\rm mthf}, {\rm HCHO}, {\rm m2cf}) + V_{\rm GDC}({\rm mgly}, {\rm mthf}) \\
&\quad -V_{\rm mMTD}({\rm m2cf}, {\rm m1cf}) + V_{\rm SDH}({\rm msrc}, {\rm mthf}) + V_{\rm DMGD}({\rm mdmg}, {\rm mthf}) \\
\left(\frac{d}{dt}\right)_{\rm m}{\rm 1cf} &= V_{\rm mMTD}({\rm m2cf}, {\rm m1cf}) - V_{\rm mMTCH}({\rm m1cf}, {\rm m10f}) \\
\left(\frac{d}{dt}\right)_{\rm m}{\rm 10f} &= V_{\rm mFTS}({\rm mthf}, {\rm mcoo}, {\rm m10f}) + V_{\rm mMTCH}({\rm m1cf}, {\rm m10f}) - V_{\rm mFTD}({\rm m10f}) \\
\left(\frac{d}{dt}\right)_{\rm c}{\rm thf} &= V_{\rm MS}({\rm hcy}, {\rm 5mf}) + V_{\rm DHFR}({\rm dhf}, {\rm cNADPH}) + V_{\rm cFTD}({\rm c10f}) + V_{\rm PGT}({\rm c10f}, {\rm GARP}) \\
&\quad +V_{\rm AICART}({\rm c10f}, {\rm aic}) - V_{\rm cFTS}({\rm cthf}, {\rm ccoo}, {\rm c10f}) - V_{\rm cSHMT}({\rm cser}, {\rm cthf}, {\rm cgly}, {\rm c2cf}) \\
&\quad -V_{\rm mNE}({\rm mthf}, {\rm HCHO}, {\rm m2cf}) \\
\left(\frac{d}{dt}\right)_{\rm c}{\rm 2cf} &= V_{\rm cSHMT}({\rm cser}, {\rm cthf}, {\rm cgly}, {\rm c2cf}) + V_{\rm mNE}({\rm mthf}, {\rm HCHO}, {\rm m2cf}) - V_{\rm TS}({\rm DUMP}, {\rm c2cf}) \\
&\quad -V_{\rm MTHFR}({\rm c2cf}, {\rm cNADPH}, {\rm sam}, {\rm sah}) - V_{\rm cMTD}({\rm c2cf}, {\rm c1cf}) \\
\left(\frac{d}{dt}\right)_{\rm c}{\rm 1cf} &= V_{\rm cMTD}({\rm c2cf}, {\rm c1cf}) - V_{\rm cMTCH}({\rm c1cf}, {\rm c10f})
\end{aligned}$$

$$
\begin{aligned}
\left(\frac{d}{dt}\right)\text{c10f} &= V_{\text{cFTS}}(\text{cthf}, \text{ccoo}, \text{c10f}) + V_{\text{cMTCH}}(\text{c1cf}, \text{c10f}) - V_{\text{cFTD}}(\text{c10f}) \\
&\quad -V_{\text{ART}}(\text{c10f}, \text{aic}) - V_{\text{PGT}}(\text{c10f}, \text{GARP}) \\
\left(\frac{d}{dt}\right)\text{dhf} &= V_{\text{TS}}(\text{DUMP}, \text{c2cf}) - V_{\text{DHFR}}(\text{dhf}, \text{cNADPH}) \\
\left(\frac{d}{dt}\right)\text{5mf} &= V_{MTHFR}(\text{c2cf}, \text{NADPH}, \text{sam}, \text{sah}) - V_{\text{MS}}(\text{hcy}, \text{5mf}) \\
\left(\frac{d}{dt}\right)\text{aic} &= V_{\text{PGT}}(\text{c10f}, \text{GARP}) - V_{\text{AICART}}(\text{c10f}, \text{aic}) \\
\left(\frac{d}{dt}\right)\text{met} &= V_{\text{BHMT}}(\text{hcy}, \text{BET}, \text{sam}, \text{sah}) + V_{\text{MS}}(\text{hcy}, \text{5mf}) + V_{\text{bGLYc}}(\text{bmet}, \text{met}) \\
&\quad -V_{\text{MATI}}(\text{met}, \text{sam}) - V_{\text{MATIII}}(\text{met}, \text{sam}) \\
\left(\frac{d}{dt}\right)\text{sam} &= V_{\text{MATI}}(\text{met}, \text{sam}) + V_{\text{MATIII}}(\text{met}, \text{sam}) \\
&\quad -V_{\text{GNMT}}(\text{sam}, \text{sah}, \text{5mf}, \text{cgly}) - V_{\text{DNMT}}(\text{sam}, \text{sah}) \\
\left(\frac{d}{dt}\right)\text{sah} &= V_{\text{GNMT}}(\text{sam}, \text{sah}, \text{5mf}, \text{cgly}) + V_{\text{DNMT}}(\text{sam}, \text{sah}) - V_{\text{SAAH}}(\text{sah}, \text{hcy}) \\
\left(\frac{d}{dt}\right)\text{hcy} &= V_{\text{SAAH}}(\text{sah}, \text{hcy}) - V_{\text{CBS}}(\text{hcy}, \text{sam}, \text{sam}, \text{cser}) - V_{\text{BHMT}}(\text{hcy}, \text{BET}, \text{sam}, \text{sah}) \\
&\quad -V_{\text{MS}}(\text{hcy}, \text{5mf}) \\
\left(\frac{d}{dt}\right)\text{mgly} &= V_{\text{mSHMT}}(\text{mser}, \text{mthf}, \text{mgly}, \text{m2cf}) - 3V_{\text{mGLYc}}(\text{cgly}, \text{mgly}) - V_{\text{GDC}}(\text{mgly}, \text{mthf}) \\
&\quad +V_{\text{SDH}}(\text{msrc}, \text{mthf}) \\
\left(\frac{d}{dt}\right)\text{mser} &= -3V_{\text{mSERc}}(\text{cser}, \text{mser}) - V_{\text{mSHMT}}(\text{mser}, \text{mthf}, \text{mgly}, \text{m2cf}) \\
\left(\frac{d}{dt}\right)\text{mcoo} &= -3V_{\text{mHCOOHc}}(\text{ccoo}, \text{mcoo}) - V_{\text{mFTS}}(\text{mthf}, \text{mcoo}, \text{m10f}) \\
\left(\frac{d}{dt}\right)\text{cgly} &= V_{\text{bGLYc}}(\text{bgly}, \text{cgly}) + V_{\text{cSHMT}}(\text{cser}, \text{cthf}, \text{cgly}, \text{c2cf}) + V_{\text{mGLYc}}(\text{cgly}, \text{mgly}) \\
&\quad -V_{\text{GNMT}}(\text{sam}, \text{sah}, \text{5mf}, \text{cgly})
\end{aligned}
$$

$$\left(\frac{d}{dt}\right)_{\text{c}}\text{ser} = V_{\text{bSERc}}(_{\text{b}}\text{ser}, _{\text{c}}\text{ser}) + V_{\text{mSERc}}(_{\text{c}}\text{ser}, _{\text{m}}\text{ser}) - V_{\text{cSHMT}}(_{\text{c}}\text{ser}, _{\text{c}}\text{thf}, _{\text{c}}\text{gly}, _{\text{c}}\text{2cf}) - V_{\text{CBS}}(\text{hcy}, \text{sam}, \text{sam}, _{\text{c}}\text{ser}) - (1.2)_{\text{c}}\text{ser}$$

$$\left(\frac{d}{dt}\right)_{\text{c}}\text{coo} = V_{\text{mHCOOHc}}(_{\text{c}}\text{coo}, _{\text{m}}\text{coo}) - V_{\text{cFTS}}(_{\text{c}}\text{thf}, _{\text{c}}\text{coo}, _{\text{c}}\text{10f})$$

$$\left(\frac{d}{dt}\right)\text{dmg} = V_{\text{BHMT}}(\text{hcy}, _{\text{BET}}, \text{sam}, \text{sah}) - V_{\text{DMGD}}(_{\text{m}}\text{dmg}, _{\text{m}}\text{thf})$$

$$\left(\frac{d}{dt}\right)\text{src} = V_{\text{GNMT}}(\text{sam}, \text{sah}, \text{5mf}, _{\text{c}}\text{gly}) + V_{\text{DMGD}}(_{\text{m}}\text{dmg}, _{\text{m}}\text{thf}) - V_{\text{SDH}}(_{\text{m}}\text{src}, _{\text{m}}\text{thf})$$

The velocities of transport from blood to cytosol or mitochondria to cytosol are given by the transparent notation V_{bSERc} and V_{mSERc}, respectively. The units are in μM/hr increase *in the cytosol*. Since the cytosol is assumed to have three times the volume of the mitochondria, one μM increase in the cytosol due to transport from the mitochondria means a 3 μM decrease in the mitochondria. This is the reason for the 3's in the equations involving transport into and out of the mitochondria and in the transport kinetics below.

Part C: Kinetics.

For many of the velocities, we assume that their dependence on substrates has Michaelis-Menten form with one substrate

$$V = \frac{V_{max}[S]}{K_m + [S]},$$

or random order Michaelis-Menten form with two substrates:

$$V = \frac{V_{max}[S_1][S_2]}{(K_{m,1} + [S_1])(K_{m,2} + [S_2])}.$$

Some reactions, for example V_{cSHMT}, are assumed to have reversible random order Michaelis-Menten kinetics with two substrates in each direction. For all these velocities, the form of the kinetics is clear. The K_m and V_{max} values appear in Table S4 (modified from [2]), along with references. Enzymes that occur in both the mitochondria and the cytosol are assumed to have the same kinetics in both compartments. If the kinetic constants differ in the cytosol and the mitochondria that is indicated by the prefixes m and c, respectively. Reactions that do not have one of these simple forms and transport velocities are discussed individually after Table S4.

Table S4. Model kinetic parameter values (time in hrs., concentrations in μM).

Parameter	Literature	Model	Reference
AICART			
$K_{\mathrm{m,10f}}$	5.9-50	5.9	[3][4][5][6]
$K_{\mathrm{m,aic}}$	10-100	100	[3][4][6]
V_{max}	370-44400	55000	
DHFR.			
$K_{\mathrm{m,dhf}}$	0.12-1.9	0.5	[7][3][4][8]
$K_{\mathrm{m,NADPH}}$	0.3-5.6	4.0	[7][3][4][8]
V_{max}	350-23000	2000	[7][3][4]
DMGD			
$K_{\mathrm{m,dmg}}$	50	50	[9]
$K_{\mathrm{m,mthf}}$	-	50	
V_{max}	-	15000	
FTD.			
$K_{\mathrm{m,10f}}$	0.9-20	20	[10][11]
${}_{\mathrm{c}}V_{max}$		500	
${}_{\mathrm{m}}V_{max}$		1050	
cFTS.			
$K_{\mathrm{m,thf}}$	0.1 - 600	3	[3][5]
$K_{\mathrm{m,coo}}$	8 - 1000	43	[3][5]
V_{max}	100 - 486000	3900	[3][5]
mFTS.			
$K_{\mathrm{m,thf}}$	0.1 - 600	3	[3][5]
$K_{\mathrm{m,coo}}$	8 - 1000	43	[3][5]
V_{max}	100 - 486000	2000	[3][5]
$K_{\mathrm{m,10f}}$	-	22	
V_{max}	-	6300	
GDC			
$K_{\mathrm{m,mgly}}$	3400-40000	3400	[15][12][13][14]
$K_{\mathrm{m,mthf}}$	50	50	[16]
V_{max}	-	15000	
MS.			
$K_{\mathrm{m,5mf}}$	22-34	25	[17][18]
$K_{\mathrm{m,hcy}}$	0.1-6	1	[19]
V_{max}		500	[19]

Parameter	Literature	Model	Reference
MTCH. (positive direction is from 1cf to 10f)			
$K_{\mathrm{m,1cf}}$	4-250	250	[3][4][5]
cV_{max}	880-1380000	500000	[4][5]
mV_{max}	880-1380000	790000	[4][5]
$K_{\mathrm{m,10f}}$	20-450	100	[3][4][5]
V_{max}	10.5-1380000	20000	[4][5]
MTD. (positive direction is from 2cf to 1cf)			
$K_{\mathrm{m,2cf}}$	2-5	2	[3][5]
cV_{max}	520-594000	80000	[7][3][5]
mV_{max}	520-594000	180000	[7][3][5]
$K_{\mathrm{m,1cf}}$	1-10	10	[20][5]
V_{max}	594000	600000	[5]
PGT.			
$K_{m,\mathrm{10f}}$	4.9-58	4.9	[3][4][21][22]
$K_{\mathrm{m,GAR}}$	520	520	[3][4][21][22]
V_{max}	6600-16200	24300	[3][4][21][22]
SAHH. (positive direction is from sah to hcy)			
$K_{\mathrm{m,sah}}$		10	[23]
V_{max}		5000	[23]
$K_{\mathrm{m,hcy}}$		1	[23]
V_{max}		5000	[23]
SDH			
$K_{\mathrm{m,dmg}}$	320	320	[24]
$K_{\mathrm{m,mthf}}$	-	50	
V_{max}	-	15000	
SHMT. (positive direction is from thf to 2cf)			
$K_{\mathrm{m,ser}}$	350-1300	600	[3][4][5][25][26]
$K_{\mathrm{m,thf}}$	45-300	50	[3][4][5][27][28]
cV_{max}	500-162000	5200	[7][4][5][28]
mV_{max}	500-162000	11440	[7][4][5][28]
$K_{\mathrm{m,gly}}$	3000-10000	10000	[7][3][4][5][26]
$K_{\mathrm{m,2cf}}$	3200-10000	3200	[7][4][5][27]
cV_{max}	12600-120000000	15000000	[7][4][5]
mV_{max}	12600-120000000	30000000	[7][4][5]
TS.			
$K_{\mathrm{m,DUMP}}$	5-37	6.3	[7][3][29][30]
$K_{\mathrm{m,2cf}}$	10-45	14	[7][3][29][30]
V_{max}	30-4200	5000	[4][30]

We now discuss the reactions where the kinetics have a special form.

BHMT. The kinetics of BHMT are Michaelis-Menten with the parameters $K_{m,1} = 12$, $K_{m,2} = 100$, and $V_{max} = 2160$ [31],[32]. The form of the inhibition of BHMT by SAM was derived by non-linear regression on the data of [33] and scaled so that it equals 1 when the external methionine concentration is 30 μM.

$$V_{\mathrm{BHMT}} \;=\; e^{-.0021(\mathrm{sam}+\mathrm{sah})} e^{+.0021(76.7)} \frac{V_{max}(\mathrm{hcy})(\mathrm{BET})}{(K_{m,1}+\mathrm{hcy})(K_{m,2}+\mathrm{BET})}$$

The inhibition of BHMT is controversial because Bose et al. [34] found that sam has no effect on recombinant BHMT's ability to remethylate homocysteine. It is possible that sam affects the expression of BHMT rather than BHMT itself in which case the results of [33] and [34] would not be contradictory. In any case, the inhibition that we use has significant effects on the BHMT reaction only for sam concentrations well above normal. Thus, only the methionine and protein loading experiments would be affected by removing the inhibition of BHMT by hcy, in which case hcy does not rise as much during loading (simulations not shown). Similarly, we found in [23] that the presence or absence of this inhibition had little effect on the stabilization of DNA methylation.

CBS. The kinetics of CBS are standard Michaelis-Menten with $K_{m,1} = 1000$ for hcy taken from [35] and $K_{m,2} = 2000$ for cser taken from [36], with $V_{max} = 402,000$. The form of the activation of CBS by sam and sah was derived by non-linear regression on the data in [37] and [38] and scaled so that it equals 1 when the external methionine concentration is 30 μM.

$$V_{\mathrm{CBS}} \;=\; \left(\frac{V_{max}(\mathrm{hcy})(\mathrm{cser})}{(K_m+\mathrm{hcy})(K_m+\mathrm{cser})}\right)\left(\frac{(1.2)(\mathrm{sam}+\mathrm{sah})^2}{(30)^2+(\mathrm{sam}+\mathrm{sah})^2}\right).$$

DNMT. The DNA methylation reaction is given as a uni-reactant scheme with sam as substrate. That is, the substrates for methylation are assumed constant. Their variation can be modeled by varying the V_{max}. The kinetic constants, $V_{max} = 180$, $K_m = 1.4$, and $K_i = 1.4$ are from [39].

$$V_{\mathrm{DNMT}} \;=\; \frac{V_{max}(\mathrm{sam})}{K_m(1+\frac{\mathrm{sah}}{K_i})+\mathrm{sam}}.$$

GNMT. The first factor of the GNMT reaction is standard Michaelis-Menten with $V_{max} = 245$, and $K_{m,1} = 63$ for sam and $K_{m,2} = 130$ for cgly estimated from [41]. The second term is product inhibition by sah from [40] with $K_i = 18$. The third term, the long-range inhibition

of GNMT by 5mf, was derived by non-linear regression on the data of [42], Figure 3, and scaled so that it equals 1 when the external methionine concentration is 30 μM.

$$V_{\text{GNMT}} \;=\; \left(\frac{V_{max}(\text{sam})(\text{cgly})}{(K_{m,1}+\text{sam})(K_{m,2}+\text{cgly})}\right)\left(\frac{1}{1+\frac{\text{sah}}{K_i}}\right)\left(\frac{5.88}{0.35+5\text{mf}}\right)$$

MAT-I. The MAT-I kinetics are from [43], Table 1, and we take $V_{max} = 260$ and $K_m = 41$. The inhibition by sam was derived by non-linear regression on the data from [43], Figure 5.

$$V_{\text{MAT-I}} \;=\; \left(\frac{V_{max}(\text{met})}{K_m+\text{met}}\right)(0.23+(0.8)e^{-(0.0026)(\text{sam})})$$

MAT-III. The methionine dependence of the MAT-III kinetics is from [44], Figure 5, fitted to a Hill equation with $V_{max} = 220$, $K_m = 300$. The activation by sam is from [43], Figure 5, fitted to a Hill equation with $K_a = 360$.

$$V_{\text{MAT-III}} \;=\; \left(\frac{V_{max}(\text{met})^{1.21}}{K_m+(\text{met})^{1.21}}\right)\left(1+\frac{(7.2)(\text{sam})^2}{(K_a)^2+(\text{sam})^2}\right)$$

MTHFR. The first factor in the formula for the MTHFR reaction velocity

$$V_{\text{MTHFR}} \;=\; \frac{V_{max}(2\text{cf})(\text{NADPH})}{(K_{m,1}+2\text{cf})(K_{m,2}+\text{NADPH})}\cdot\frac{60}{10+\text{sam}-\text{sah}}$$

is standard Michaelis-Menten with $K_{m,1} = 50$, $K_{m,2} = 16$, and $V_{max} = 7000$ taken from [45][46][25].

The inhibition of MTHFR by sam, the second factor, was derived by non-linear regression on the data of [47][48] and has the form $10/(10+\text{sam})$. In addition, sah competes with sam for binding to the regulatory domain of MTHFR. It neither activates nor inhibits the enzyme [48] but prevents inhibition by sam; thus, we take our inhibitory factor to be:

$$I \;=\; \frac{10}{10+\text{sam}-\text{sah}}.$$

The factor 60 scales the inhibition so that it has value 1 when the external methionine concentration is 30 μM.

NE. The kinetics of the non-enzymatic reversible reaction between thf and 2cf are taken to be mass action,

$$V_{\rm NE} \;=\; k_1({\rm thf})({\rm HCHO}) - k_2({\rm 2cf}).$$

The rate constants are $k_1 = 0.03$, and $k_2 = 22$ in the cytosol and $k_2 = 20$ in the mitochondria.

We now discuss the kinetics of transport between the compartments. The general formula for the kinetics of transport between the blood and the cytosol is taken to be

$$V \;=\; \frac{V_{max}[bAA]}{K_m + [bAA]} \;-\; k_{\rm out}[cAA],$$

where AA stands for an amino acid and the prefixes b and c refer to the blood and cytosolic compartments, respectively. Thus the kinetics are Michaelis-Menten coming into the cell and linear going out. We take the kinetics of transport of serine and glycine between the cytosol and mitochondria to be Michaelis-Menten in both directions and the transport of formate between these two compartments to be linear in both directions.

Transport of amino acids into cells is accomplished by a relatively small number of transport systems each of which handles several amino acids. Each transporter is specialized to handle amino acids with particular ionic characteristics [49][50]. The transporters are saturable and the K_m values have been measured in many systems [51][52][53]. Relatively little is known about the kinetics by which amino acids leak out of cells so we take this process to be linear. This linear rate also includes the loss of cytosolic amino acids to other metabolic processes not in the model (see Figure 1), for example use in protein synthesis.

Table S5. Parameter values for transporters (hours, μM).

Parameter	Model value	Parameter	Model value
$V_{\rm bGLYc}$		$V_{\rm mGLYc}$	
K_m	150	$K_{m,{\rm c}{\rm gly}}$	5700
V_{max}	2000	V_{max}	10000
$k_{\rm out}$	1	$K_{m,{\rm m}{\rm gly}}$	5700
		V_{max}	10000/3
$V_{\rm bSERc}$		$V_{\rm mSERc}$	
K_m	150	$K_{m,{\rm c}{\rm ser}}$	5700
V_{max}	2700	V_{max}	10000
$k_{\rm out}$	1	$K_{m,{\rm m}{\rm ser}}$	5700
		V_{max}	10000/3
$V_{\rm bMETc}$		$V_{\rm mHCOOHc}$	
K_m	150	$k_{\rm in}$	100/3
V_{max}	910	$k_{\rm out}$	100
$k_{\rm out}$	1		

Part C: *In silico* experimentation.

If one starts with any initial values for the variables and solves the differential equations when the velocities have the formulas given in Part C, one finds that the variables all approach steady concentrations after a few hours. The concentrations of the variables and the velocities of reactions at this "normal" steady state are given in Table 1 in the main body of the paper. Most of the *in silico* experiments reported in the paper were done by starting the system at this steady state, changing one or more parameters, and letting the system relax to a new steady state. For example, in Section A of Results, the external glycine concentration, bgly, was varied systematically from 50 to 900 μM, and for each such concentration the resulting values of various velocities and metabolite concentrations were computed at steady state. In Section B of Results, the total cellular folate, FOL, was changed from 20 μM to 10 μM. By solving the differential equations we could report the effect of this change on velocities and metabolite concentrations. Similarly, the effect of changing the expression of cSHMT was computed by changing the V_{max} of the SHMT reaction and computing the new steady state. In other experiments, reactions, or whole groups of reactions, were removed entirely. Finally, in the methionine loading and protein loading experiemnts, the blood concentrations of glycine, serine, and methionine were allowed to vary in time. By solving the differential equations we saw how the various velocities and metabolite concentrations varied in time.

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