Supplementary Material

for

Mathematical analysis of the regulation of competing methyltransferases

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In these supplementary materials we give the full details of the mathematical model. Figure 1 shows a schematic diagram of the biochemical reactions in the model. Full substrate and enzyme names are given in the legend.

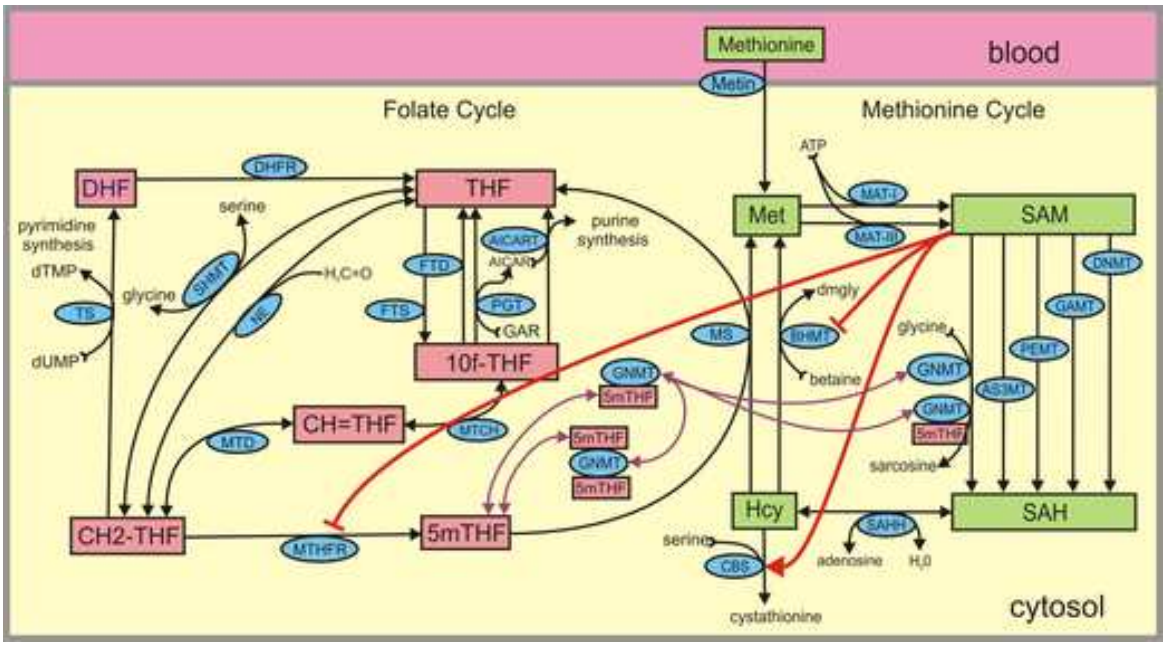

Figure S1. Folate and methionine metabolism with competing methyltransferases. Substrates are indicated by rectangular boxes, green in the methionine cycle and red in the folate cycle, except for GNMT which is both an enzyme and a substrate since it can bind to two molecules of 5mTHF. Each arrow represents a biochemical reaction and the blue ellipse on the arrow contains the acronym of the enzyme that catalyzes the reaction. Substrate abbreviations: Met, methionine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Hcy, homocysteine; 5mTHF, 5-methyltetrahydrofolate; THF, tetrahydrofolate; 10fTHF, 10-formyltetrahydrofolate; DHF, dihydrofolate; CH2-THF, 5,10-methylenetrahydrofolate; CH=THF, 5,10-methenyltetrahydrofolate. Enzyme abbreviations: AICAR(T), aminoimidazolecarboxamide ribonucleotide (transferase); FTD, 10-formyltetrahydrofolate dehydrogenase; FTS, 10-formyltetrahydrofolate synthase; MTCH, 5,10-methylenetetrahydrofolate cyclohydrolase; MTD, 5,10-methylenetetrahydrofolate dehydrogenase; MTHFR, 5,10-methylenetetrahydrofolate reductase; TS, thymidylate synthase; SHMT, serine hydroxymethyltransferase; PGT, phosphoribosyl glycinamidetransformalase; DHFR, dihydrofolate reductase; NE, nonenzymatic interconversion of THF and 5,10-CH2-THF; MAT-I,methionine adenosyl transferase I; MAT-III, methionine adenosyl transferase III; GNMT, glycine N-methyltransferase; AS3MT, arsenic methyltransferase; PEMT, phosphotidylethanolamine methyltransferase; GAMT, gunadino-acetate methyltransferase; DNMT, DNA-methyltransferase; SAHH, S-adenosylhomocysteine hydrolase; CBS, cystathionine β-synthase; MS, methionine synthase; BHMT, betaine-homocysteine methyltransferase.

In specifying the differential equations, we use lower case letters and simple abbreviations for the substrates; these abbreviations are indicated in Table 1, below. Velocities are always indicated by V_X where the subscript X gives the name of the enzyme that catalyzes that

particular velocity. Each velocity depends, of course, on the current values of various of the substrates.

Table S1, Variable names and usual acronyms

variable	usual acronym
met	MET
sam	SAM
sah	SAH
hcy	HCY
dhf	DHF
thf	THF
fthf	10f-THF
ch	CH=THF
ch2	CH2-THF
mthf	5mTHF
gnmt	GNMT
gnmtf	GNMT-5mTHF
fgnmtf	5mTHF-GNMT-5mTHF

The 13 differential equations are simply mass balance equations that say that the rate of change of the concentration of a substrate is the sum of the velocities of the reactions that make the substrate minus the sum of the reactions that use the substrate. The differential equations follow:

$$
\begin{aligned}
\frac{d}{dt}(met) &= metin(t) + V_{\mathrm{BHMT}}(hcy, bet, sam, sah) + V_{\mathrm{MS}}(mthf, hcy) - V_{\mathrm{MATI}}(met, sam) \\
&\quad -V_{\mathrm{MATIII}}(met, sam) \\
\frac{d}{dt}(sam) &= V_{\mathrm{MATI}}(met, sam) + V_{\mathrm{MATIII}}(met, sam) - V_{\mathrm{GNMT}}(sam, sah, gly, gnmt, gnmtf) \\
&\quad -V_{\mathrm{AS3MT}}(sam, sah, ias) - V_{\mathrm{PEMT}}(sam, sah, pe) - V_{\mathrm{GAMT}}(sam, sah, gaa) - V_{\mathrm{DNMT}}(sam) \\
\frac{d}{dt}(sah) &= V_{\mathrm{GNMT}}(sam, sah, gly, gnmt, gnmtf) + V_{\mathrm{AS3MT}}(sam, sah, ias) + V_{\mathrm{PEMT}}(sam, sah, pe) \\
&\quad +V_{\mathrm{GAMT}}(sam, sah, gaa) + V_{\mathrm{DNMT}}(sam) - V_{\mathrm{SAAH}}(sah, hcy) \\
\frac{d}{dt}(hcy) &= V_{\mathrm{SAAH}}(sah, hcy) - V_{\mathrm{BHMT}}(hcy, bet, sam, sah) - V_{\mathrm{MS}}(mthf, hcy) - V_{\mathrm{CBS}}(hcy, sam, sah, ser) \\
\frac{d}{dt}(dhf) &= V_{\mathrm{TS}}(dump, ch2) - V_{\mathrm{DHFR}}(dhf, nadph) \\
\frac{d}{dt}(thf) &= V_{\mathrm{DHFR}}(dhf, nadph) + V_{\mathrm{MS}}(mthf, hcy) + V_{\mathrm{FTD}}(fthf) + V_{\mathrm{PGT}}(fthf, gar) + V_{\mathrm{AICART}}(fthf, aic) \\
&\quad -V_{\mathrm{FTS}}(thf, hcooh, fthf) - V_{\mathrm{SHMT}}(ser, thf, gly, ch2) - V_{\mathrm{NE}}(thf, hcoh, ch2) \\
\frac{d}{dt}(fthf) &= V_{\mathrm{MTCH}}(ch, fthf) + V_{\mathrm{FTS}}(thf, hcooh, fthf) - V_{\mathrm{PGT}}(fthf, gar) \\
&\quad -V_{\mathrm{AICART}}(fthf, aic) - V_{\mathrm{FTD}}(fthf) \\
\frac{d}{dt}(ch) &= V_{\mathrm{MTD}}(ch2, ch) - V_{\mathrm{MTCH}}(ch, fthf) \\
\frac{d}{dt}(ch2) &= V_{\mathrm{SHMT}}(ser, thf, gly, ch2) + V_{\mathrm{NE}}(thf, hcoh, ch2) - V_{\mathrm{TS}}(dump, ch2) \\
&\quad -V_{\mathrm{MTD}}(ch2, ch) - V_{\mathrm{MTHFR}}(ch2, nadph, sam, sah) \\
\frac{d}{dt}(mthf) &= V_{\mathrm{MTHFR}}(ch2, nadph, sam, sah) - V_{\mathrm{MS}}(mthf, hcy) + k_2(gnmtf) \\
&\quad -2k_1(mthf)(gnmt) + k_4(fgnmtf) - k_3(mthf)(gnmtf) \\
\frac{d}{dt}(gnmt) &= k_2(gnmtf) - 2k_1(mthf)(gnmt) \\
\frac{d}{dt}(gnmtf) &= -k_2(gnmtf) + 2k_1(mthf)(gnmt) - k_3(mthf)(gnmtf) + k_4(fgnmtf) \\
\frac{d}{dt}(fgnmtf) &= k_3(mthf)(gnmtf) - k_4(fgnmtf)
\end{aligned}
$$

Some of the reactions depend on the concentrations of other substrates that are not variable (in the model) and are assumed to be constant. These are give in Table S2.

Table S2. Constant concentrations (μM) in the model

abbreviation	value	name
aic	2.1	AICARP
bet	50	betaine
dump	20	DUMP
gaa	10	guanadinoacetate
gar	10	GAR
gly	1850	glycine
hcoh	500	HCOH (formaldehyde)
hcooh	900	HCOOH (formate)
ias	1	inorganic arsenic
nadph	50	NADPH
pe	10	phosphotidylethanolamine
ser	468	serine

The details of the biochemistry and the biology are in the functional forms that show how each of the velocities depends on the current values of the variables that influence it. Many reactions have Michaelis-Menten kinetics in one of the following standard forms:

$$V = \frac{V_{max}[S]}{K_m + [S]}, \qquad V = \frac{V_{max}[S_1][S_2]}{(K_{S_1} + [S_1])(K_{S_2} + [S_2])}$$

$$V = \frac{V^f_{max}[S_1][S_2]}{(K_{S_1} + [S_1])(K_{S_2} + [S_2])} - \frac{V^b_{max}[P_1][P_2]}{(K_{P_1} + [P_1])(K_{P_2} + [P_2])}$$

for unidirectional, one substrate, unidirectional, two substrates, and bidirectional, two substrates, two products, respectively. For these reactions, Table S3 lists the K_m and V_{max} values. In general, we take K_m values from the literature. V_{max} values are extremely variable because they depend on enzyme expressions levels that vary in time and therefore experimental measurements *in vivo* are difficult and unreliable. We usually adjust the V_{max} values so as to obtain the typical substrate concentration values that we find in the literature. Parameters have sometimes been chosen by comparing model outputs in various circumstances to qualitative and quantitative experimental data.

Table S3. Model kinetic parameters (time in hrs, concentration in μM)

parameter	literature	model	reference
AICART			
$K_{m,fthf}$	5.9-50	5.9	[1][2][3][4]
$K_{m,aicar}$	10-100	100	[1][2][4]
V_{max}		55000	
DHFR			
$K_{m,dhf}$	0.12-1.9	0.5	[2][4][5][6]
$K_{m,nadph}$	0.3-5.6	4.0	[2][4][5][6]
V_{max}	350-23000	2000	[2][4][5]
FTD			
$K_{m,fthf}$	0.9-20	20	[7][8]
V_{max}		500	
FTS(forward direction from thf to fthf)			
$K_{m,thf}$	0.1-600	3	[3][4]
$K_{m,hcooh}$	8-1000	43	[3][4]
V_{max}	100-468000	3900	[3][4]
MS			
$K_{m,mthf}$	22-34	25	[9][10]
$K_{m,thf}$	0.1-6	1	[11]
V_{max}		244	[11]
MTCH(forward direction from ch to fthf)			
$K_{m,ch}$	4-250	250	[2][3][4]
V_{max}	880-1380000	500000	[2][3]
$K_{m,fthf}$	20-450	100	[2][3][4]
V_{max}	10.5-1380000	20000	[2][3]
MTD(positive direction from ch2 to ch)			
$K_{m,ch2}$	2-5	2	[3][4]
V_{max}	520-594000	80000	[5][3][4]
$K_{m,ch}$	1-10	10	[3][12]
V_{max}	594000	600000	[3]
PGT			

$K_{m,fthf}$	4.9-58	4.9	[4][2][13][14]
$K_{m,gar}$	520	520	[4][2][13][14]
V_{max}	6600-16200	24300	[4][2][13][14]
SAHH			
$K_{m,sah}$	0.75-15.2	6.5	[15][16][17][18]
V_{max}		320	
$K_{m,hcy}$	56.6-200	150	[16][17][19]
SHMT(positive direction is from thf to ch2)			
$K_{m,ser}$	350-1300	600	[2][3][4][20]
$K_{m,thf}$	45-300	50	[2][3][4][21]
V_{max}	500-162000	5200	[2][3][21]
$K_{m,gly}$	3000-10000	10000	[2][3][4][20][5]
$K_{m,ch2}$	3000-10000	3200	[2][3][5][21]
V_{max}	12600-120000000	15000000	[2][3][5]

Now we discuss in detail the methylation reactions, the more difficult modeling issues, and reactions with non-standard kinetics.

AS3MT. Inorganic arsenic is metabolized in two methylation steps catalyzed by AS3MT. The first step uses utilizes a methyl group from SAM and is followed by a reduction step to produce methylarsonic acid (MMA). The second step uses utilizes a methyl group from SAM and is followed by a reduction step to produce dimethyarsinic acid (DMA), which is readily exported from the liver and cleared in the urine. We have recently studied the biochemistry of these methylation steps that are quite complicated [22]. For, example the first step shows substrate inhibition by inorganic arsenic and product inhibition by MMA and glutathione (GSH) both sequesters the arsenic compounds and activates AS3MT. In our study here, we are mainly interested in studying the availability of methyl groups from SAM, so we take the arsenic concentrations and the GSH concentration to be constant, and model just the first methylation step. SAM shows substrate inhibition for AS3MT [23], but the effect is small and occurs only at very high SAM concentrations, so we ignore it. Thus, the velocity of methylation is taken to be:

$$V_{\text{AS3MT}}(sam, sah, ias) \;=\; \frac{V_{max}(sam)}{(K_m(1+\frac{sah}{K_i})+sam)} \cdot \frac{iAs}{4.6+iAs}.$$

We take the K_m of AS3MT for SAM to be 50μM as determined in [24] and the K_m for iAs to be 4.6μM [25]. It is known that SAH inhibits AS3MT [26, 27], but the nature of the inhibition and the K_i are not known. We'll assume the inhibition is competitive and take $K_i = 10\mu$M, which is typical of other methyltransferases. A high, but realistic arsenic load

is 1 μM in liver [28] and a typical flux would be the order of magnitude of 1 μM/hr. So, we choose $V_{max} = 28\mu$/hr, which accomplishes this given that a typical SAM concentration is 24 μM.

BHMT. The kinetics of BHMT are Michaelis-Menten with the parameters $K_{m,hcy} = 12, K_{m,bet} = 100$, and $V_{max} = 502$ [29][30]. The form of the inhibition of BHMT by SAM and SAH was derived by non-linear regression on the data of [31] and scaled so that it equals 1 at the normal methionine input of 50μM/hr.

$$V_{\mathrm{BHMT}}(hcy, bet, sam, sah) \;=\; \frac{V_{max}(hcy)(bet)}{(K_{m,hcy} + hcy)(K_{m,bet} + bet)} \cdot e^{-.0021(sam+sah)} e^{.0021(28)}$$

Binding of 5mTHF to GNMT. In a series of papers, Wagner, Luka, and colleagues have studied the inhibitory effect of 5mTHF on the activity of GNMT [32, 23, 33, 34, 35]. GNMT has two binding sites for 5mTHF, so we assume the simple reversible reactions:

$$5mTHF \;+\; GNMT \;\rightleftarrows\; 5mTHF\text{-}GNMT$$

$$5mTHF\text{-}GNMT \;+\; 5mTHF \;\rightleftarrows\; 5mTHF\text{-}GNMT\text{-}5mTHF$$

with forward and backward rate constants, k_1 and k_2, for the first reaction and k_3 and k_4, for the second reaction. We choose the rate constants $k_1 = 50, k_2 = 1, k_3 = 1, k_4 = 1.6$ so that the K_D values are those found in Table 2 of [33].

CBS The kinetics of CBS are standard Michaelis-Menten with $K_{m,1} = 1000$ for hcy taken from [36] and $K_{m,2} = 2000$ for ser taken from [37], with $V_{max} = 117{,}000$. The form of the activation of CBS by sam and sah was derived by non-linear regression on the data in [38] and [39] and scaled so that it equals 1 when the external methionine concentration is 30 μM.

$$V_{\mathrm{CBS}} \;=\; \left(\frac{V_{max}(hcy)(ser)}{(K_{m,1} + hcy)(K_{m,2} + ser)}\right)\left(\frac{(1.2)(sam + sah)^2}{((30)^2 + (sam + sah)^2}\right)\left(\frac{(1.2)(28)^2}{((30)^2 + (28)^2}\right)^{-1}.$$

DNMT. The velocity of the DNMT reaction is given by

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

The inhibition by SAH is competitive [40]. We choose $K_m = 1.4\mu$M for SAM and $K_i = 1.4\mu$M for SAH as indicated in [41]. The reaction depends on the cytosines available, but since we take their concentration to be constant we fold that dependence into the V_{max}. The value $V_{max} = 12.5\mu$M/hr was chosen so that the flux of the DNMT reaction is normally (when the cell is not dividing) much less than the fluxes of GNMT, PEMT, and GAMT.

GAMT. The velocity of the GAMT reaction is given by

$$V_{\text{GAMT}}(sam, sah, gaa) \;=\; \frac{V_{max}(sam)(gaa)}{(K_m(1+\frac{sah}{K_i})+sam)(K_m+gaa)}.$$

The inhibition by SAH is competitive [42, 43]. We choose $K_m = 49\mu$M for SAM and $K_i = 16\mu$M for SAH as indicated in [44] and take $K_m = 13.3\mu$M for gaa as in [45]. The value $V_{max} = 210\mu$M/hr was chosen so that the flux of the GAMT reaction is comparable to the fluxes of the GNMT and PEMT reactions, the two other methyl transferases that carry much of the methylation flux.

GNMT. The kinetics of GNMT for SAM are cooperative and we take the Hill coefficient to be $n = 2$ as suggested in [23] and we use $K_m = 100\mu$M as indicated in [44]. The inhibition by SAH is competitive [46] and has $K_i = 35\mu$M [44]. The reaction has glycine as a substrate and we take $K_m = 12.2\mu$M of GNMT for glycine as found in [**?**]. Thus,

$$V_{\text{GNMT}}(sam, sah, gnmt, gnmtf) \;=\; \frac{V_{max}(sam)^2}{(K_m(1+\frac{sah}{K_i}))^2+(sam)^2])}\frac{gly}{(K_m+gly)}.$$

where

$$V_{max} \;=\; (4026)(gnmt+(.5)(gnmtf)).$$

This formula for V_{max} resulted from our *in silico* experiments described under Results 3.1. The concentration of free GNMT, gnmt, is a variable in our model. GNMT can be bound by one or two molecules of 5mTHF. Our simulations and the data in [33] suggest strongly that once bound GNMT, namely gnmtf, retains 50% of it's activity. The factor 4026 is chosen so that GNMT has a normal reaction velocity comparable to the reaction velocities of PEMT and GAMT, the two other methyl transferases that carry much of the methylation flux.

MAT-I. The MAT-I kinetics are from [47], 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 [47], Figure 5.

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

MAT-III. The methionine dependence of the MAT-III kinetics is from [48], Figure 5, fitted to a Hill equation with $V_{max} = 220$, $K_m = 300$. The activation by SAM is from [47], Figure 5, fitted to a Hill equation with K_a= 360. We model the activation of MATIII by SAM by effectively changing the V_{max}, but, in fact, SAM lowers the K_m for methionine; for a detailed discussion, see [49].

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

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

$$V_{\text{MTHFR}} \;=\; \left(\frac{V_{max}(met)(nadph)}{(K_{m,1}+ch2)(K_{m,2}+nadph)}\right)\left(3.*\frac{10}{10+(sam-sah)}\right)$$

is standard Michaelis-Menten with $K_{m,1} = 50$, $K_{m,2} = 16$, and $V_{max} = 5300$ taken from [50][51][52]. The inhibition of MTHFR by SAM, the second factor, was derived by non-linear regression on the data of [53][54] and has the form 10/(10+ sam). In addition, SAH competes with sam for binding to the regulatory domain of MTHFR. It neither activates nor inhibits the enzyme [54] but prevents inhibition by sam; thus, we take our inhibitory factor to be:

$$\frac{10}{10 + (sam - sah)}.$$

The factor 3 scales the inhibition so that it has value 1 when the external methionine input is 50 μM/hr.

NE. The kinetics of the non-enzymatic reversible reaction between thf and ch2 are taken to be mass action,

$$V_{\mathrm{NE}} \;=\; k_1(thf)(hcho) - k_2(ch2),$$

with rate constants are $k_1 = 0.03$, and $k_2 = 22$. *hcho* represents formaldehyde.

PEMT. The velocity of the PEMT reaction is given by

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

The inhibition by SAH is non-competitive [55]. We choose $K_m = 18.2\mu$M for SAM and $K_i = 3.8\mu$M for SAH as indicated in [44]. The reaction depends on *pe* (phophatidylethanolamine) and we take $K_m = 5000\mu$M of PEMT for *pe* as found in [55].The value $V_{max} = 49100\mu$M/hr was chosen so that the flux of the PEMT reaction is comparable to the fluxes of the GNMT and GAMT reactions, the two other methyl transferases that carry much of the methylation flux.

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Conflict of Interest Statement. The authors declare that they have no conflicts of interest.

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