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-methylenetetrahydrofolate; 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, guanidino-acetate methyltransferase; DNMNT, 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:
\[
\frac{d}{dt}(\text{met}) = \text{metin}(t) + V_{\text{BHMT}}(\text{hcy}, \text{bet}, \text{sam}, \text{sah}) + V_{\text{MS}}(\text{mthf}, \text{hcy}) - V_{\text{MATI}}(\text{met}, \text{sam}) - V_{\text{MATIII}}(\text{met}, \text{sam})
\]

\[
\frac{d}{dt}(\text{sam}) = V_{\text{MATI}}(\text{met}, \text{sam}) + V_{\text{MATIII}}(\text{met}, \text{sam}) - V_{\text{GNMT}}(\text{sam}, \text{sah}) - V_{\text{AS3MT}}(\text{sam}, \text{ias}) - V_{\text{PEMT}}(\text{sam}, \text{pe}) - V_{\text{MS}}(\text{mthf}, \text{hcy})
\]

\[
\frac{d}{dt}(\text{sah}) = V_{\text{AS3MT}}(\text{sam}, \text{ias}) + V_{\text{PEMT}}(\text{sam}, \text{pe}) - V_{\text{GNMT}}(\text{sam}, \text{gly}) - V_{\text{AS3MT}}(\text{sam}, \text{ias}) - V_{\text{SAAH}}(\text{sah}, \text{hcy})
\]

\[
\frac{d}{dt}(\text{hcy}) = V_{\text{SAAH}}(\text{sah}, \text{hcy}) - V_{\text{BHMT}}(\text{hcy}, \text{bet}, \text{sam}, \text{sah}) - V_{\text{MS}}(\text{mthf}, \text{hcy}) - V_{\text{CBS}}(\text{hcy}, \text{sam}, \text{sah}, \text{ser})
\]

\[
\frac{d}{dt}(\text{dhf}) = V_{\text{TS}}(\text{dump}, \text{ch2}) - V_{\text{DHFR}}(\text{dhf}, \text{nadph})
\]

\[
\frac{d}{dt}(\text{thf}) = V_{\text{DHFR}}(\text{dhf}, \text{nadph}) + V_{\text{MS}}(\text{mthf}, \text{hcy}) + V_{\text{FTD}}(\text{fthf}) + V_{\text{PGT}}(\text{fthf}, \text{gar}) + V_{\text{AICART}}(\text{fthf}, \text{aic}) - V_{\text{FTS}}(\text{thf}, \text{hcooh}, \text{fthf}) - V_{\text{SHMT}}(\text{ser}, \text{thf}, \text{gly}) - V_{\text{NE}}(\text{thf}, \text{hcoh}, \text{ch2})
\]

\[
\frac{d}{dt}(\text{fthf}) = V_{\text{MTCH}}(\text{ch}, \text{fthf}) + V_{\text{FTS}}(\text{thf}, \text{hcooh}) - V_{\text{FTD}}(\text{fthf}) - V_{\text{AICART}}(\text{fthf}, \text{aic}) - V_{\text{FTS}}(\text{thf}, \text{hcooh}, \text{fthf}) - V_{\text{FTD}}(\text{fthf})
\]

\[
\frac{d}{dt}(\text{ch}) = V_{\text{MTD}}(\text{ch2}, \text{ch}) - V_{\text{MTHFR}}(\text{ch2}, \text{nadph}, \text{sam}, \text{sah})
\]

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 given 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:

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

\[
V = \frac{V_{\text{max}}^f[S_1][S_2]}{(K_{S_1} + [S_1])(K_{S_2} + [S_2])} - \frac{V_{\text{max}}^b[P_1][P_2]}{(K_{P_1} + [P_1])(K_{P_2} + [P_2])}
\]

for unidirectional, one substrate, unidirectional, two substrates, and bidirectional, two substrates, two products, respectively. For these reactions, Table S3 lists the \(K_m\) and \(V_{\text{max}}\) values. In general, we take \(K_m\) values from the literature. \(V_{\text{max}}\) 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 \(V_{\text{max}}\) 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.
| 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 | [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 | [3][4] |
| **MS** |            |       |           |
| $K_{m,mthf}$ | 22-34      | 25    | [9][10]  |
| $K_{m,thf}$ | 0.1-6      | 1     | [11]     |
| $V_{max}$ |            | 244   |           |
| **MTCH** (forward direction from ch to fthf) | | | |
| $K_{m,ch}$ | 4-250      | 250   | [2][3][4] |
| $V_{max}$ |            | 880-1380000 | [2][3] |
| $K_{m,fthf}$ | 20-450     | 100   | [2][3][4] |
| $V_{max}$ |            | 10.5-1380000 | [2][3] |
| **MTD** (positive direction from ch2 to ch) | | | |
| $K_{m,ch2}$ | 2-5        | 2     | [3][4]   |
| $V_{max}$ |            | 520-594000 | [5][3][4] |
| $K_{m,ch}$ | 1-10       | 10    | [3][12]  |
| $V_{max}$ |            | 594000 | 600000   |
| **PGT** |            |       |           |
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:

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

We take the \(K_m\) of AS3MT for SAM to be 50\(\mu\)M as determined in [24] and the \(K_m\) for \(iAs\) to be 4.6\(\mu\)M [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 $1 \mu M$ in liver [28] and a typical flux would be the order of magnitude of $1 \mu M/hr$. So, we choose $V_{\text{max}} = 28\mu M/hr$, which accomplishes this given that a typical SAM concentration is $24 \mu M$.

**BHMT.** The kinetics of BHMT are Michaelis-Menten with the parameters $K_{m,\text{hcy}} = 12, K_{m,\text{bet}} = 100$, and $V_{\text{max}} = 502$ [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 50\mu M/hr.

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

**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:

$$5mTHF + GNMT \rightleftharpoons 5mTHF-GNMT$$

$$5mTHF-GNMT + 5mTHF \rightleftharpoons 5mTHF-GNMT-5mTHF$$

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 $V_{\text{max}} = 117,000$. 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 \mu M.

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

**DNMT.** The velocity of the DNMT reaction is given by

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

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 $V_{\text{max}}$. The value $V_{\text{max}} = 12.5\mu M/hr$ 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

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

The inhibition by SAH is competitive [42, 43]. We choose $K_m = 49\,\mu\text{M}$ for SAM and $K_i = 16\,\mu\text{M}$ for SAH as indicated in [44] and take $K_m = 13.3\,\mu\text{M}$ for $\text{gaa}$ as in [45]. The value $V_{\text{max}} = 210\,\mu\text{M/hr}$ 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 $K_n = 100\,\mu\text{M}$ as indicated in [44]. The inhibition by SAH is competitive [46] and has $K_i = 35\,\mu\text{M}$ [44]. The reaction has glycine as a substrate and we take $K_m = 12.2\,\mu\text{M}$ of GNMT for glycine as found in [47]. Thus,

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

where

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

This formula for $V_{\text{max}}$ 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 $V_{\text{max}} = 260$ and $K_m = 41$. The inhibition by SAM was derived by non-linear regression on the data from [47], Figure 5.

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

**MAT-III.** The methionine dependence of the MAT-III kinetics is from [48], Figure 5, fitted to a Hill equation with $V_{\text{max}} = 220$, $K_m = 300$. 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 $V_{\text{max}}$, but, in fact, SAM lowers the $K_m$ for methionine; for a detailed discussion, see [49].

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

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

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

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

The factor 3 scales the inhibition so that it has value 1 when the external methionine input is 50 $\mu M/\text{hr}$.

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

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

with rate constants are $k_1 = 0.03$, and $k_2 = 22$. $\text{hcho}$ represents formaldehyde.

**PEMT.** The velocity of the PEMT reaction is given by

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

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 (phosphatidylethanolamine) and we take $K_m = 5000\mu M$ of PEMT for pe as found in [55]. The value $V_{\text{max}} = 49100\mu M/\text{hr}$ 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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