Supporting information

The results of all reported Maud runs can be found at https://github.com/biosustain/Methionine_model/blob/main/results.

Maud's kinetic model

Parameters

Table 1 shows all of Maud's unknown parameters along with their dimensions

Note that Maud's metabolic model includes some quantities that are not treated as parameters in its statistical model, including temperatures, compartment volumes and the formation energy of water. Maud treats these quantities as if they were known precisely: they can be configured by the user or default values can be used. Although in practice there can be considerable uncertainty regarding these quantities, we chose to disregard this uncertainty in the interest of simplicity.

Table 1: Parameters of Maud's statistical model

Parameter	Modelled quantity	Dimensions
$\Delta_f G$	Formation energy	metabolites
k_M	Michaelis Menten constants	Substrates of all enzyme/reactions and products of reversible enzyme/reactions
k_I	Inhibition constants	Inhibiting metabolite/compartments of enzyme/reactions exhibiting competitive inhibition
k_{cat}	Rate constants	Enzyme/reactions
L_0	Transfer constants	Allosteric interactions

Parameter	Modelled quantity	Dimensions
e_T	T dissociation constants	Modifying metabolites of allosteric inhibitions
e_R	R dissociation constants	Modifying metabolites of allosteric activations
$k_{cat\,pme}$	Rate constants of phosphorylation modifying enzymes	Phosphorylation modifying enzymes
v_{drain}	Drain fluxes	Drains, experiments
$Enzyme$	Enzyme concentrations	Enzymes, experiments
$C_{unbalanced}$	Unbalanced metabolite/compartment concentrations	Unbalanced metabolite/compartments, experiments
C_{pme}	Phosphorylation modifying enzyme concentrations	Phosphorylation modifying enzymes, experiments
ψ	Membrane potentials	Experiments

Solving the steady state problem for a given set of parameters in an experiment yields a vector $C_{balanced}$ of balanced metabolite concentrations. These are combined with the balanced metabolite concentrations $C_{unbalanced}$ to produce a vector C_{mic} with a concentration for each metabolite/compartment combination.

$\Delta_f G$ parameters can optionally be fixed; this can be useful for computational purposes, as for example to avoid estimating the formation energy of a metabolite about which there is no available information due to it only participating in irreversible reactions.

Rate equations

As discussed in the main text, Maud's kinetic model decomposes into factors contributing to the flux in a metabolic network in an experiment as shown in equation (1). For succinctness, and since Maud's model assumes that there are no interactions between experiments, we omit any notation referring to experiments below. We also omit any reference to the network's drain reactions: these are modelled as being exactly determined by the values of the parameter vector v_{drain}.

$$F(C;\theta) = Enzyme \cdot k_{cat} \cdot Reversibility \cdot Saturation \cdot Allostery \tag{1}$$

The term $Enzyme$ in equation (1) is a vector of non-negative real numbers representing the concentration of the enzyme catalysing each reaction.

The term k_{cat} in equation (1) is a vector of non-negative real numbers representing the amount of flux carried per unit of saturated enzyme.

The term *Reversibility* in equation (1) is a vector of real numbers capturing the impact of thermodynamic effects on the reaction's flux, as shown in equation (2).

$$Reversibility = 1 - \exp(\frac{\Delta_r G + RT \cdot S^T \ln(C_{mic})}{RT}) \tag{2}$$
$$\Delta_r G = S^T \Delta_f G + nF\psi$$

The terms in (2) have the following meanings:

- T is the temperature in Kelvin (a number),
- R is the gas constant (a number),
- $\Delta_r G$ is a vector representing the Gibbs free energy change of each reaction in standard conditions,
- $\Delta_f G$ is a vector representing the standard condition Gibbs free energy change of each metabolite's formation reaction, or in other words each metabolite's 'formation energy'.
- n is a vector representing the number of charges transported by each reaction.
- F is the Faraday constant (a number)
- ψ is a vector representing each reaction's membrane potential (these numbers only matter for reactions that transport non-zero charge)

Note that, for reactions with zero transported charge, the thermodynamic effect on each reaction is derived from metabolite formation energies. This formulation is helpful because, provided that all reactions' rates are calculated from the same formation energies, they are guaranteed to be thermodynamically consistent.

The term n accounts for both the charge and the directionality. For instance, a reaction that exports 2 protons to the extracellular space in the forward direction would have -2 charge. If a negatively charged molecule like acetate is exported in the forward direction, n would be 1.

Note that this way of modelling the effect of transported charge does not take into account that the concentration gradient used by the transport is that of the dissociated molecules. Thus, this expression is only correct for ions whose concentration can be expressed in the model only in the charged form; e.g., protons, K^+, Na^+, Cl^-, etc.

The term *Saturation* in equation (1) is a vector of non-negative real numbers representing, for each reaction, the fraction of enzyme that is saturated, i.e. bound to one of the reaction's substrates. To describe saturation we use equation (3), which is taken from Liebermeister, Uhlendorf, and Klipp (2010). Additionally, this term captures competitive inhibition: as competitive inhibitor concentration increases, the saturation denominator increases, effectively decreasing the saturation of the substrate on the total enzyme pool. Conversely, as the substrate concentration increases this term approaches 1.

$$Saturation_r = a \cdot \text{free enzyme ratio} \tag{3}$$

$$a = \prod_{\text{s substrate}} \frac{C^s_{mic}}{k^{rs}_M}$$

$$\text{free enzyme ratio} = \begin{cases} \prod_{\text{s sustrate}}(1 + \frac{C^s_{mic}}{k^{rs}_M})^{S_s r} + \sum_{\text{c inhibitor}} \frac{C^c_{mic}}{k^{rc}_I} & r \text{ irreversible} \\ -1 + \prod_{\text{s sustrate}}(1 + \frac{C^s_{mic}}{k^{rs}_M})^{S_s r} + \sum_{\text{c inhibitor}} \frac{C^c_{mic}}{k^{rc}_I} + \prod_{\text{p product}}(1 + \frac{C^p_{mic}}{k^{rp}_M})^{S_p r} & r \text{ reversible} \end{cases}$$

The term *Allostery* in equation (1) is a vector of non-negative numbers describing the effect of allosteric regulation on each reaction. Allosteric regulation happens when binding to a certain molecule changes an enzyme's shape in a way that changes its catalytic behaviour. We use equation (4) to describe this phenomenon, following the generalised MWC approach described in Monod, Wyman, and Changeux (1965), Changeux (2013), Popova and Sel'kov (1975) and Popova and Sel'kov (1979).

$$Allostery_r = \frac{1}{1 + L^r_0 \cdot (\text{free enzyme ratio}_r \cdot \frac{Qtense}{Qrelaxed})^{subunits}} \tag{4}$$

$$Qtense = 1 + \sum_{\text{i inhibitor}} \frac{C^i_{mic}}{e^{ri}_T}$$

$$Qrelaxed = 1 + \sum_{\text{a activator}} \frac{C^a_{mic}}{e^{ra}_R}$$

The parameter L_0 in equation (1) is called the transfer constant, and the parameter vectors e_T and e_R are called tense and relaxed dissociation constants respectively.

Finally, the term *Phosphorylation* in equation (1) captures the important effect whereby enzyme activity is altered due to a coupled process of phosphorylation and dephosphorylation. This description achieves a similar behaviour to the MWC formalism for describing allosteric regulation, but using the rates of phosphorylation and dephosphorylation rather than concentrations of metabolites.

$$Phosphorylation_r = (\frac{\alpha}{\alpha + \beta})^{subunits} \tag{5}$$

$$\alpha = \sum_{\text{p phosphoylator}} k^p_{cat\,pme} \cdot C^p_{pme}$$

$$\beta = \sum_{\text{d dephosphoylator}} k^d_{cat\,pme} \cdot C^d_{pme}$$

Methionine case study

Dataset generation

Starting with the model in Saa and Nielsen (2016), we extracted values for enzyme concentrations, boundary conditions and fluxes. We used these values to generate MCMC samples using Maud using the priors specified in section Section . When this was finished, we selected one sample with relatively high log probability to use as a ground truth in our case study. These parameter values are shown below in table Table 2. We manually inspected the parameter values to screen for any obviously implausible values; we did not find any of these.

Prior distributions compared with true parameter values

Table 2 shows the prior distributions we used for independent parameters.

Table 2: Parameter specification, marginal prior distributions and true parameter values used in our case study.

parameter name	1% prior quantile	99% prior quantile	true value	prior Z-score of true value
$e_R^{CBS1,ametc}$	3.430e-06	0.002480	9.3e-05	0.004
$e_R^{GNMT1,ametc}$	3.000e-05	0.002000	2.000e-05	-2.787
$e_R^{MAT3,ametc}$	1.000e-04	0.001000	3.170e-04	0.003
$e_R^{MAT3,met-Lc}$	4.500e-04	0.000800	6.000e-04	0.000
$e_R^{MTHFR1,ahcysc}$	1.120e-07	0.000081	2.000e-06	-0.101
$e_T^{GNMT1,mlthfc}$	1.120e-05	0.008050	2.290e-04	-0.136
$e_T^{MTHFR1,ametc}$	1.120e-07	0.000081	1.500e-05	0.549306
k_{cat}^{AHC1}	1.200e+02	400.000000	2.340e+02	0.179861
k_{cat}^{BHMT1}	6.000e+00	35.000000	1.380e+01	-0.135
k_{cat}^{CBS1}	1.000e+01	188.000000	7.020e+00	-2.887
k_{cat}^{GNMT1}	7.000e-01	60.000000	1.050e+01	0.352083
k_{cat}^{MAT1}	8.200e-02	59.100000	7.900e+00	0.44375
k_{cat}^{MAT3}	5.890e-01	424.000000	1.990e+01	0.080556
$k_{cat}^{METH-Gen}$	4.840e-01	349.000000	1.160e+00	-1.209
$k_{cat}^{MS1kcatMS1}$	1.000e+00	3.300000	1.770e+00	-0.091

parameter name	1% prior quantile	99% prior quantile	true value	prior Z-score of true value
k_{cat}^{MTHFR1}	1.300e+00	4.200000	3.170e+00	0.183333
k_{cat}^{PROT1}	1.590e-01	0.222000	2.650e-01	0.41875
$k_I^{GNMT1,ahcysc}$	2.000e-06	0.001400	5.300e-05	0.010
$k_I^{MAT1,ametc}$	3.000e-04	0.000400	3.470e-04	0.014
$k_I^{METH-Gen,ahcysc}$	1.000e-06	0.000030	6.000e-06	0.021
$k_M^{AHC1,ahcysc}$	5.220e-05	0.037600	2.320e-05	-2.050
$k_M^{AHC1,adnc}$	1.670e-07	0.000120	5.660e-06	0.081944
$k_M^{AHC1,hcys-Lc}$	1.580e-07	0.000114	1.060e-05	0.318056
$k_M^{BHMT1,hcys-Lc}$	1.200e-05	0.000032	1.980e-05	0.049
$k_M^{BHMT1,glybc}$	4.720e-05	0.034000	8.460e-03	0.659028
$k_M^{CBS1,hcys-Lc}$	1.000e-06	0.000025	4.240e-05	3.090
$k_M^{CBS1,ser-Lc}$	2.000e-06	0.000004	2.830e-06	0.004
$k_M^{GNMT1,ametc}$	1.300e-05	0.009400	5.200e-04	0.1375
$k_M^{GNMT1,ahcysc}$	4.100e-07	0.000295	1.100e-05	0.000
$k_M^{GNMT1,glyc}$	5.480e-05	0.039500	2.540e-03	0.189583
$k_M^{GNMT1,sarcsc}$	3.730e-09	0.000003	1.000e-07	0.000
$k_M^{MAT1,met-Lc}$	1.400e-05	0.000720	1.070e-04	0.074
$k_M^{MAT1,atpc}$	5.270e-05	0.038000	2.030e-03	0.125694
$k_M^{MAT3,met-Lc}$	4.470e-05	0.032200	1.130e-03	-0.029
$k_M^{MAT3,atpc}$	5.270e-05	0.038000	2.370e-03	0.179167

parameter name	1% prior quantile	99% prior quantile	true value	prior Z-score of true value
$k_M^{METH-Gen,ametc}$	7.000e-06	0.000013	9.370e-06	-0.135
$k_M^{MS1,5mthfc}$	3.320e-06	0.002390	6.940e-05	-0.124
$k_M^{MS1,hcys-Lc}$	1.000e-06	0.000003	1.710e-06	-0.054
$k_M^{MTHFR1,mlthfc}$	7.500e-05	0.000088	8.080e-05	-0.158
$k_M^{MTHFR1,nadphc}$	1.600e-05	0.000028	2.090e-05	-0.105
$k_M^{PROT1,met-Lc}$	4.500e-05	0.000085	4.390e-05	-2.507
L_0^{CBS1}	3.730e-02	26.800000	1.030e+00	0.017
L_0^{GNMT1}	3.730e-02	26.800000	1.310e+02	0.3875
L_0^{MAT3}	3.730e-03	2.680000	1.080e-01	0.037
L_0^{MTHFR1}	1.120e-01	80.500000	3.920e-01	-1.018

$\Delta_f G$ parameters for most metabolites were fixed; those that were modelled as unknown had a multivariate normal prior distribution derived from eQuilibrator (Beber et al. 2021).

The values for $\Delta_f G$ parameters, as well as all other model parameters, can be found by inspecting the file `priors.toml` which is online at https://github.com/biosustain/Methionine_model/blob/main/data/methionine/priors.toml.

Computation

We conducted adaptive Hamiltonian Monte Carlo sampling for the full and missing- -data datasets. For the full dataset we obtained 1000 post-warmup samples each from 4 independent Markov chains after 1000 warm-up samples and "hot-starting" with a mass metric output by a previous model run.

For the missing-data dataset XXX.

Laplace approximation case study

To compare MCMC sampling with Laplace approximation we used a different model. The full Maud input folders can be found at https://github.com/biosustain/Methionine_model/tree/main/data/example_ode and https://github.com/biosustain/Methionine_model/tree/main/data/example_ode_laplace

To generate Laplace samples we used Maud's Laplace mode.

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