a systems approach to biology

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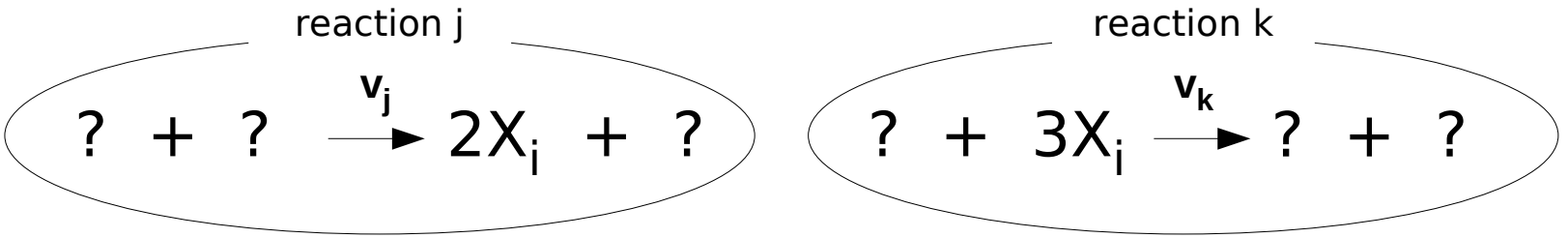
lecture 9

29 september 2011

4. metabolism, continued

flux balance analysis

rates of change of concentration are linear functions of reaction rates



$$\frac{dx_i}{dt} = \cdots + 2v_j + \cdots - 3v_k + \cdots$$

$$\frac{dx}{dt} = N.v(x;k)$$

irreversible reactions, so $v_j \geq 0$

stoichiometric matrix (of integers)
size: #species x #reactions

Berhard Palsson, **Systems Biology: Properties of Reconstructed Networks**, Cambridge University Press, 2006

flux balance analysis

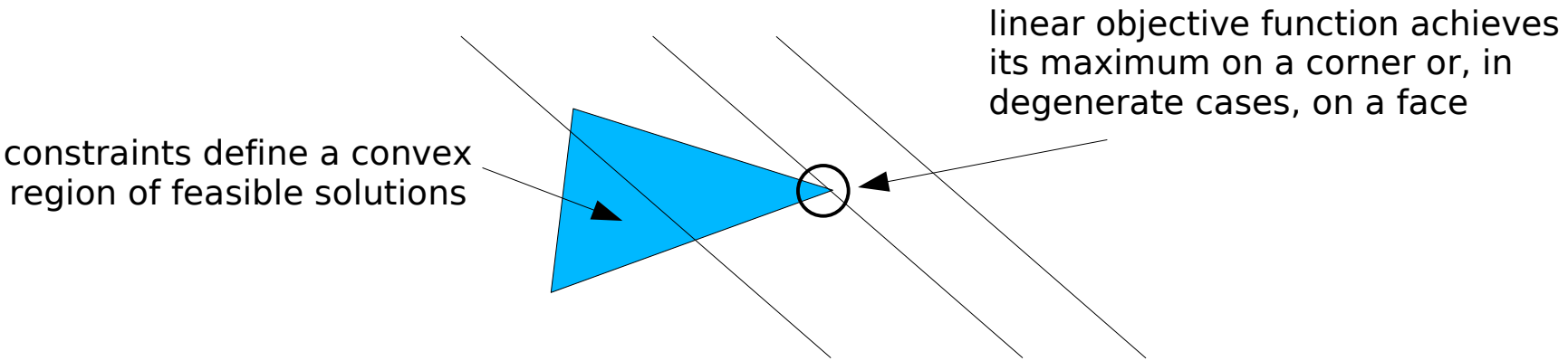
steady-state reaction fluxes satisfy a linear constraint

$$N.v(x;k) = 0 \quad v_j \geq 0$$ constraints

if it is assumed that, at steady state, some linear optimality criterion is achieved

$$\max \sum_j \alpha_j v_j$$ ← objective function

this defines a linear programming problem, for which solutions can be calculated for large networks of reactions



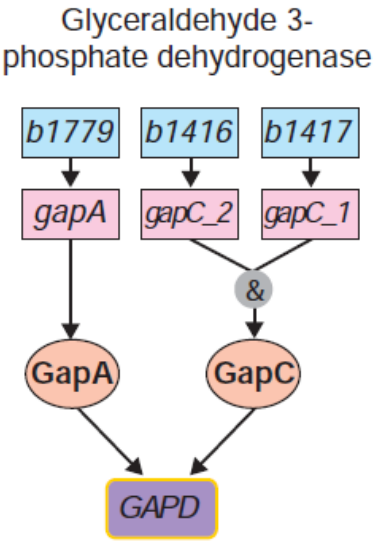
whole-genome models

increasingly complete models of (mostly microbial) metabolism

Table 1 | **Genome-scale networks reconstructed to date**

Organism/organelle	Number of genes	Number of metabolites	Number of reactions	Year
Haemophilus influenzae	296	343	488	1999
Escherichia coli	660 904	436 625	720 931	2000 2003
Helicobacter pylori	291	340	388	2002
Saccharomyces cerevisiae	708 750	584 646	842 1,149	2003 2004
Geobacter sulfurreducens	588	541	523	2004
Mitochondria	N/A	230	189	2004

* →



(*) genes, proteins & reactions linked

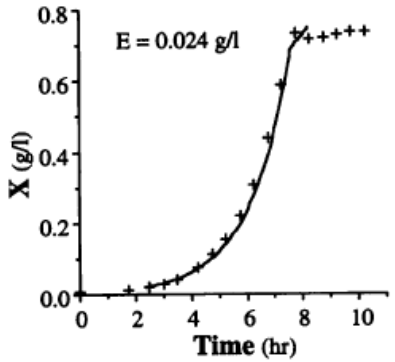
with ionic (including proton) balancing

Price, Reed, Palsson, *"Genome-scale models of microbial cells: evaluating the consequences of constraints"*, Nature Rev Microbio, **2**:886-97 2004

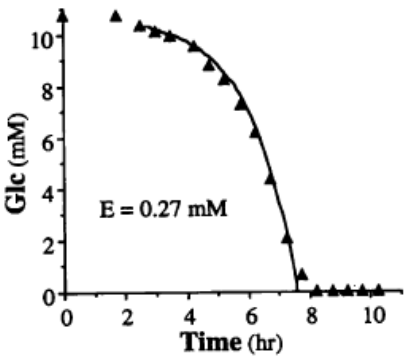
pros

for *E coli* growth on some substrates (glucose), biomass maximisation predicts overall growth rate and exchange rates between cells and the culture medium

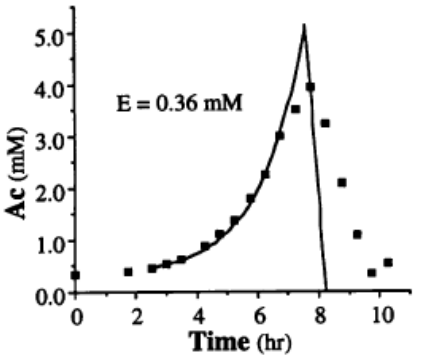
growth rate



glucose intake



acetate secretion



sub-optimal growth on other substrates (glycerol) can be improved to the predicted maximum by in-vitro selection

enzyme mechanisms, rate constants, etc are not needed

Varma, Palsson, *"Stoichiometric flux balance models quantitatively predict growh and metabolic by-product secretion in wild-type Escherchia coli W3110"*, Appl Env Microbiol **60**:3724-31 1994

Ibarra, Edwards, Palsson, *"Escherchia coli K12 undergoes adaptive evolution to achieve in-silico predicted optimal growth"*, Nature **420**:186-9 2002

and cons

internal fluxes cannot be accurately predicted

the direction of flux depends on thermodynamics, not stoichiometry; optimal solutions may have key reactions going backwards unless additonal constraints are imposed to force reactions in the right directions

NO FREE LUNCH !

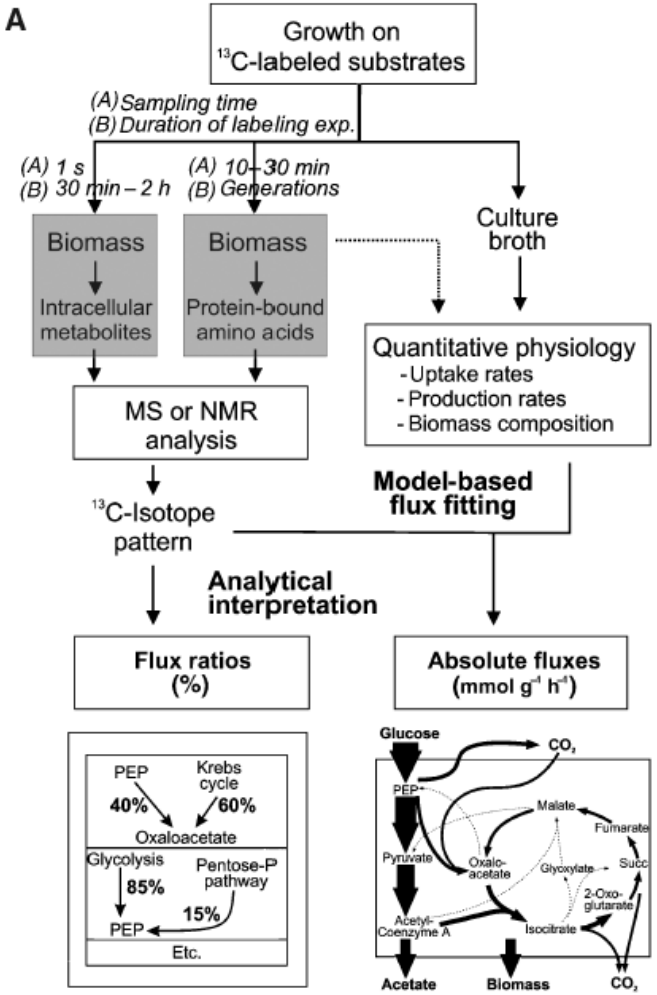
nonlinear objective functions (ATP yield per flux unit) yield better predictions of intracellular fluxes (*)

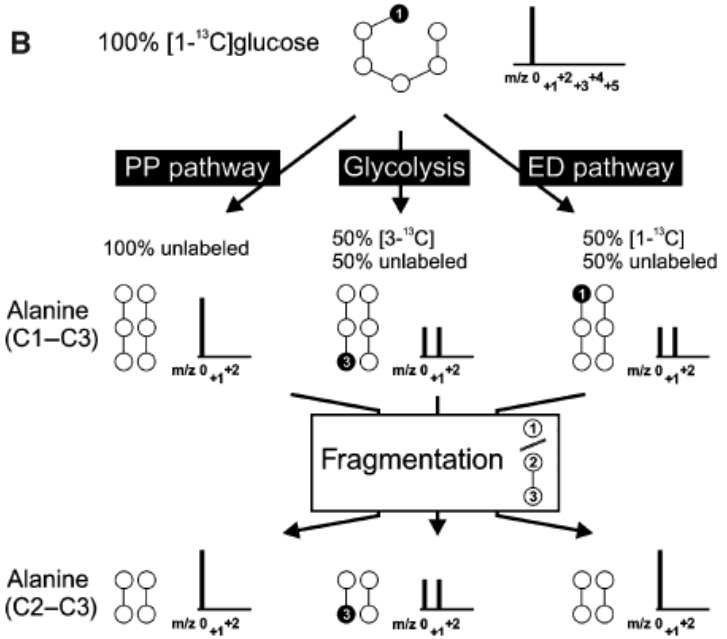
regulatory changes cannot be modelled and are not predicted

OK for microbial growth but for multicellular systems, objective functions have not been found

(*) Shuetz, Kuepfer, Sauer, *"Systematic evaluation of objective functions for predicting intracellular fluxes in Escherichia coli"*, Mol Sys Biol **3**:119 2007

measuring fluxes



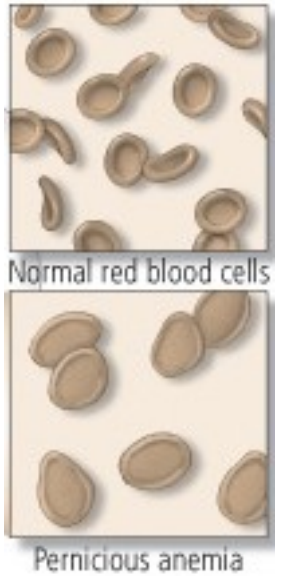


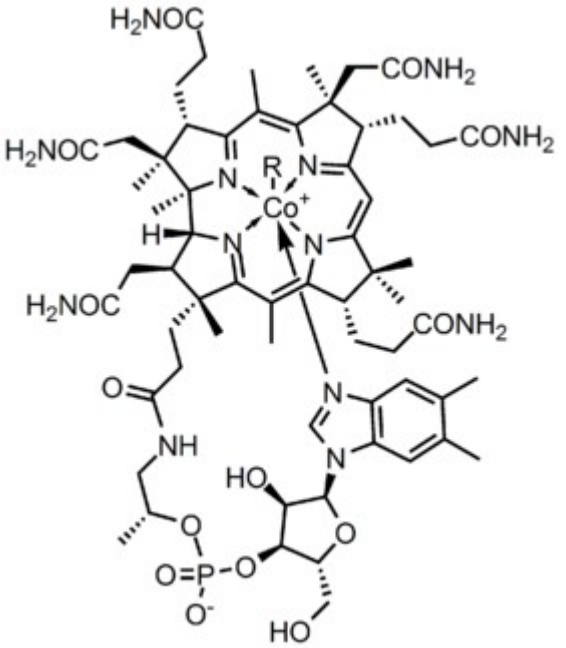
Uwe Sauer, *"Metabolic networks in motion: 13C-based flux analysis"*, Mol Sys Biol **2**:62 2006

vitamin hunting

“Pernicious anemia is a disease of unknown origin, characterized by its chronic and intermittent progressive weakness to death; hypertrophy of the red bone marrow, and blood showing low hemoglobin, high color index and the presence of megaloblasts and other immature blood cell forms.”

(Louisa Burns, **Cells of the Blood**, Volume 4, Montfort & Co, 1911)

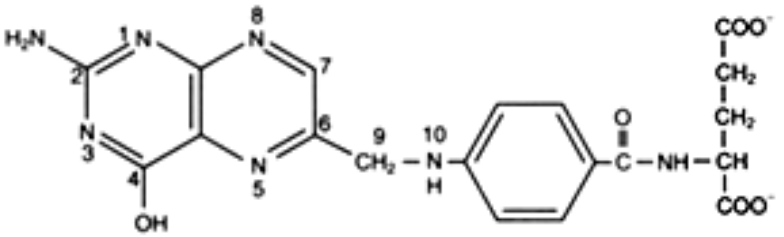




vitamin B12, cobalamin

anemia of pregnancy

1888-1964



vitamin B9, folic acid

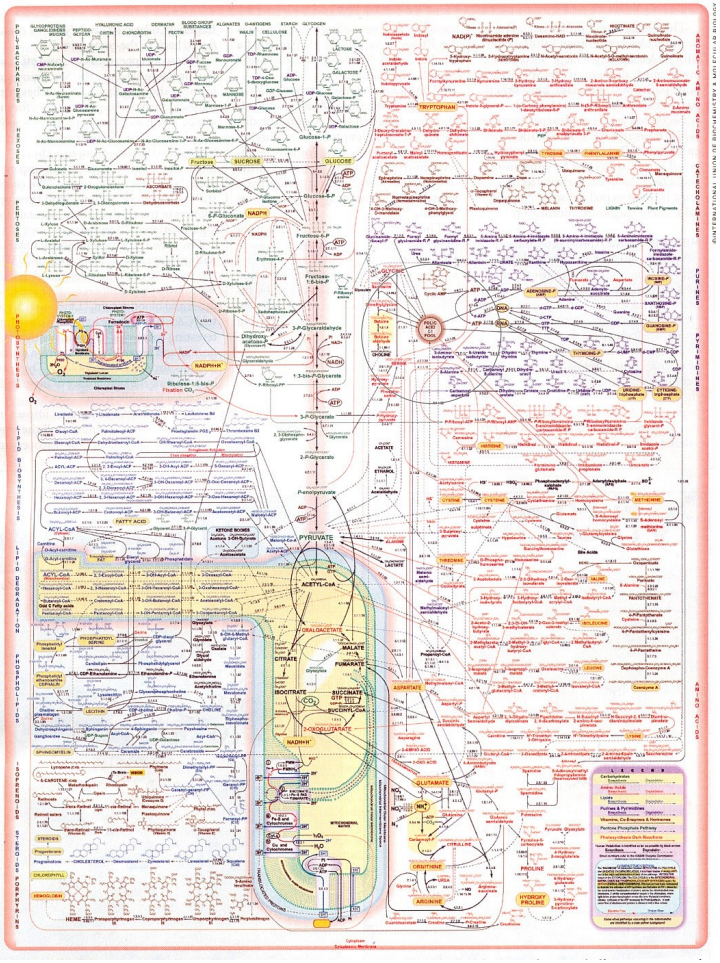
folate-mediated one-carbon metabolism (FOCM)

biochemically, FOCM links synthesis of thymidylate (a pyrmidine), purines, methyl-groups for DNA and protein methylation and amino-acid metabolism

clinically, FOCM malfunction is implicated in

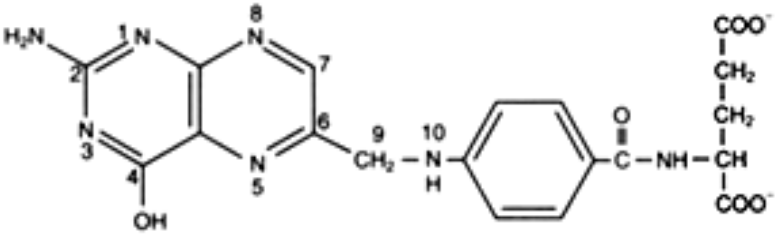
- neural-tube defects in pregnancy
- colorectal and other epithelial cancers
- cardiovascular disease
- neurodegenerative disease

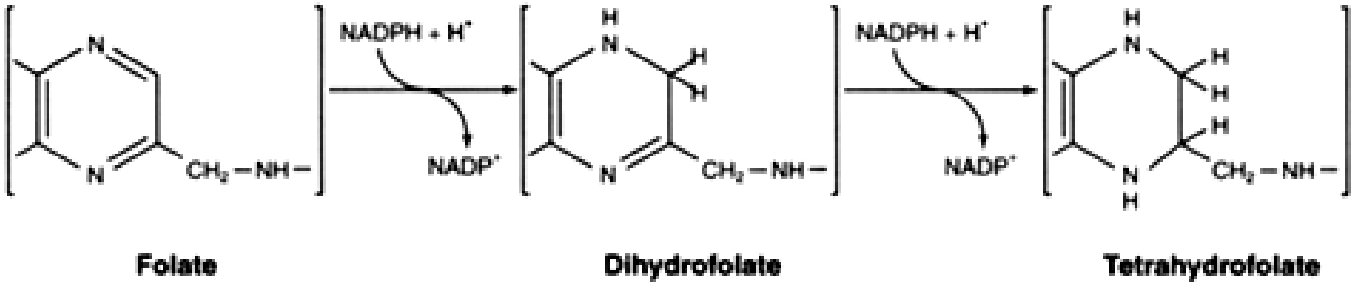
FOCM is targetted in cancer chemotherapy (methotrexate, fluorouracil)



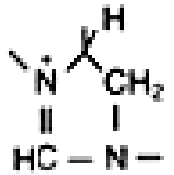
folate chemistry

folate has multiple redox states

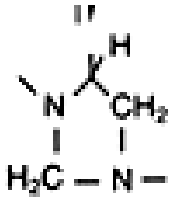




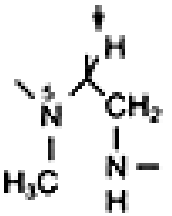
and THF has multiple redox states while carrying a single carbon



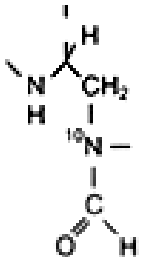
5,10-methenyl-THF



5,10-methylene-THF



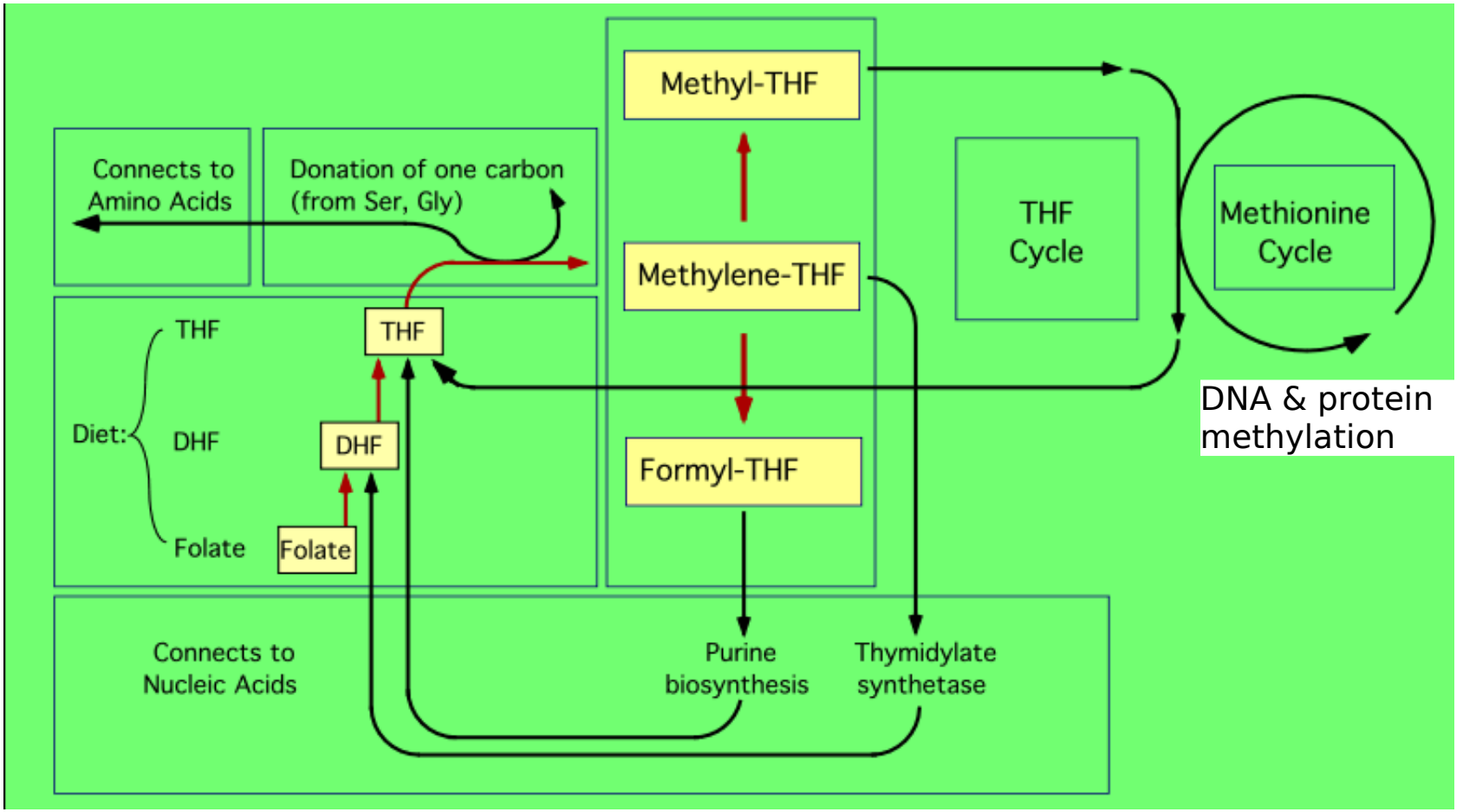
5-methyl-THF



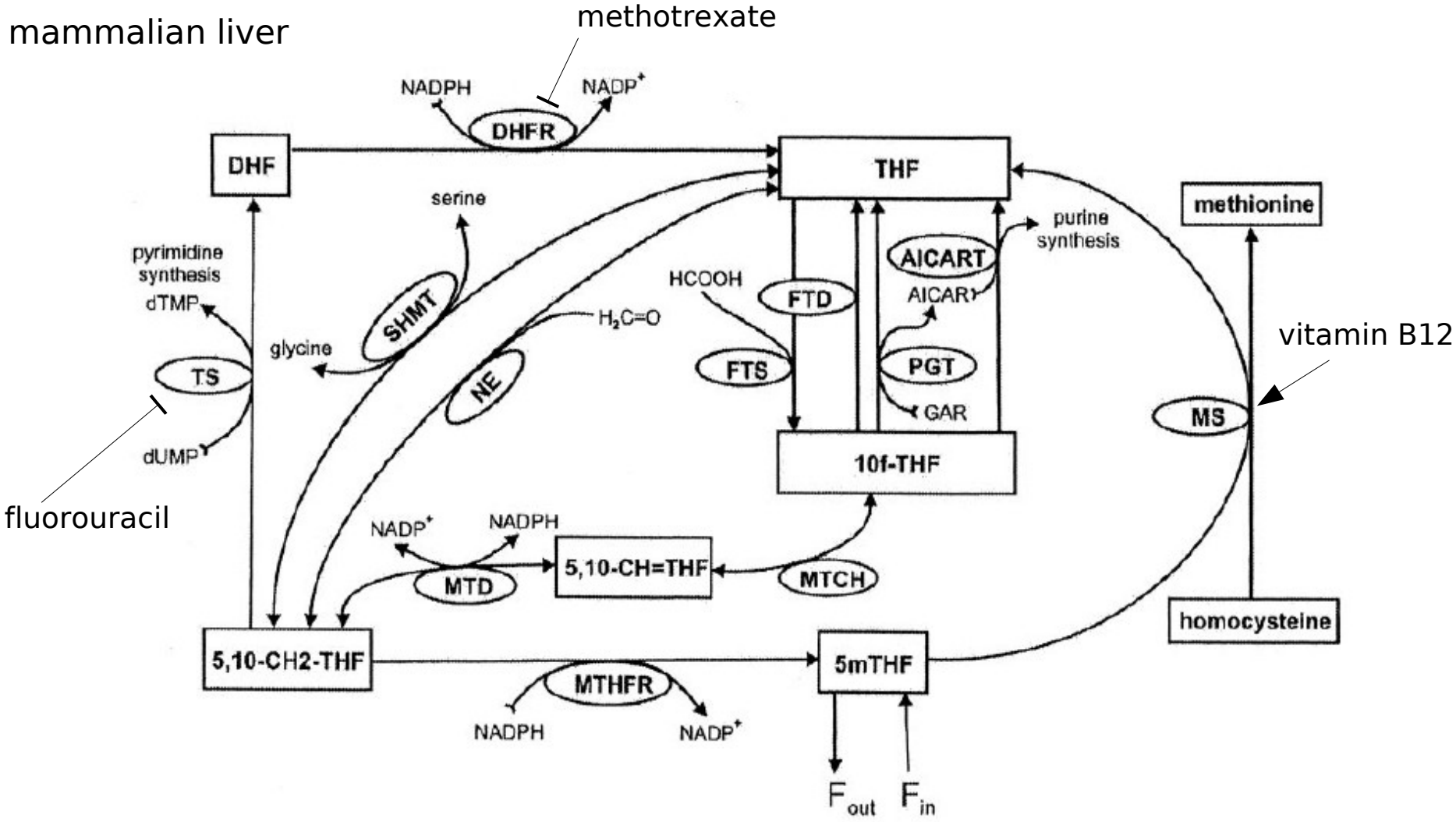
10-formyl-THF

folate cycle

interlinked cycles of one-carbon transfers



biochemistry



Fred Nijhout, Michael Reed, Paula Budu, Cornelia Ulrich, *"A mathematical model of the folate cycle"*, J Biol Chem **279**:55008-16 2004

differential equations

metabolite rates are a balance between production and consumption fluxes

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{DHF}] = V_{\mathrm{TS}} - V_{\mathrm{DHFR}}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[\mathrm{THF}] = V_{\mathrm{MS}} - V_{\mathrm{FTS}} + V_{\mathrm{PGT}} + V_{\mathrm{AICART}} + V_{DHFR} - V_{\mathrm{SHMT}} - V_{\mathrm{NE}} + V_{\mathrm{FTD}}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[5\mathrm{mTHF}] = V_{\mathrm{MTHFR}} - V_{\mathrm{MS}} + F_{\mathrm{in}} - F_{\mathrm{out}}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[5,10\text{-}\mathrm{CH_2}\text{-}\mathrm{THF}] = V_{\mathrm{SHMT}} + V_{\mathrm{NE}} - V_{\mathrm{TS}} - V_{\mathrm{MTD}} - V_{\mathrm{MTHFR}}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[5,10\text{-}\mathrm{CH}{=}\mathrm{THF}] = V_{\mathrm{MTD}} - V_{\mathrm{MTCH}}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}[10\mathrm{f}\text{-}\mathrm{THF}] = V_{\mathrm{MTCH}} + V_{\mathrm{FTS}} - V_{\mathrm{FGT}} - V_{\mathrm{AICART}} - V_{\mathrm{FTD}}$$

enzyme kinetics

random-order bi-bi reaction with quasi-steady state (Michaelis-Menten) assumptions

$$V = \frac{V_{max}\frac{[S]}{K_{m,S}}\frac{[F]}{K_{m,F}}}{1 + \frac{[S]}{K_{m,S}} + \frac{[F]}{K_{m,F}} + c\frac{[S]}{K_{m,S}}\frac{[F]}{K_{m,F}}}$$

independent substrates (c = 1) gives a product form

$$V = V_{\max} \cdot \frac{[\mathrm{S}]}{K_{m,\mathrm{S}} + [\mathrm{S}]} \cdot \frac{[\mathrm{F}]}{K_{m,\mathrm{F}} + [\mathrm{F}]}$$

and for a reversible reaction

$$V = V^{f}_{\max} \cdot \frac{[\mathrm{S}_f]}{K_{m,\mathrm{S}f} + [\mathrm{S}_f]} \cdot \frac{[\mathrm{F}_f]}{K_{m,\mathrm{F}_f} + [\mathrm{F}_f]} - V^{r}_{\max} \cdot \frac{[\mathrm{S}_r]}{K_{m,\mathrm{S}_r} + [\mathrm{S}_r]} \cdot \frac{[\mathrm{F}_r]}{K_{m,\mathrm{F}_r} + [\mathrm{F}_r]}$$

parameters

one advantage of assuming quasi-steady state is that aggregated parameters (V_{max}, K_M) have been measured for many metabolic enzymes, in contrast to the underlying mass-action rate constants, which have not

Kinetic parameter values used in the model (times in h, concentrations in μM)

Parameter	Literature	Model	References
DHFR			
$K_{m,DHF}$	0.12–1.9	0.5	15, 21, 24, 25
$K_{m,NADPH}$	0.3–5.6	4.0	15, 21, 24, 25
V_{max}	350–23,000	50	15, 21, 24
TS			
$K_{m,dUMP}$	5–37	6.3	15, 21, 36, 37
$K_{m,5,10\text{-}CH2\text{-}THF}$	10–45	14	15, 21, 36, 37
V_{max}	30–4200	50	24, 37
MTD (positive direction is from 5,10-CH_2-THF to 5,10-CH=THF)			
$K_{m,5,10\text{-}CH2\text{-}THF}$	2–5	2	21, 28
V_{max}	520–594,000	200,000	15, 21, 28
$K_{m,5,10\text{-}CH=THF}$	1–10	10	13, 28
V_{max}	594,000	594,000	38

BRENDA – http://www.brenda-enzymes.org/ gives K_M, k_{cat}, K_i, specific activity, etc

concentrations

Concentrations of substrates (μM)

	Substrate	Literature	Model	References
initial conditions	[5mTHF]	4.6–8	5.16[a]	26, 27
	[THF]	1.8–6.8	6.73[a]	21, 24, 26, 28
	[DHF]	0.023–0.12	0.027[a]	21, 24
	[5,10-CH_2-THF]	1–2.5	0.94[a]	24
	[5,10-CH=THF]	2.7–11.2	1.15[a]	21, 24
	[10f-THF]	1–16	5.99[a]	21, 24, 26, 27
assumed constant	[Ser]	120–470	468	15, 23, 24
	[Gly]	1600–2700	1850	15, 23, 24
	[dUMP]	6.2–24.8	20	15, 21, 24
	[GAR]	10	10	24
	[AICAR]	1.6–2.1	2.1	15, 21, 24
	[HCOOH]	500–900	900	21, 23
	[NADPH]	50–200	50	24, 29
	[Hcy]	0.3–7	1	6, 30

parameter values and concentrations are measured in different cell types under different conditions and can span many orders of magnitude

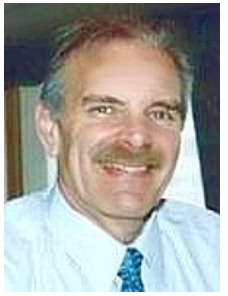
values can sometimes be picked to satisfy other experimentally derived constraints

Fred Nijhout, Michael Reed, Paula Budu, Cornelia Ulrich, *"A mathematical model of the folate cycle"*, J Biol Chem **279**:55008-16 2004; see the Methods section

perspective

Ulrich
nutritional
molecular
epidemiology

Nijhout
evolutionary
developmental
biology



Reed
mathematical
biology

http://metabolism.math.duke.edu/

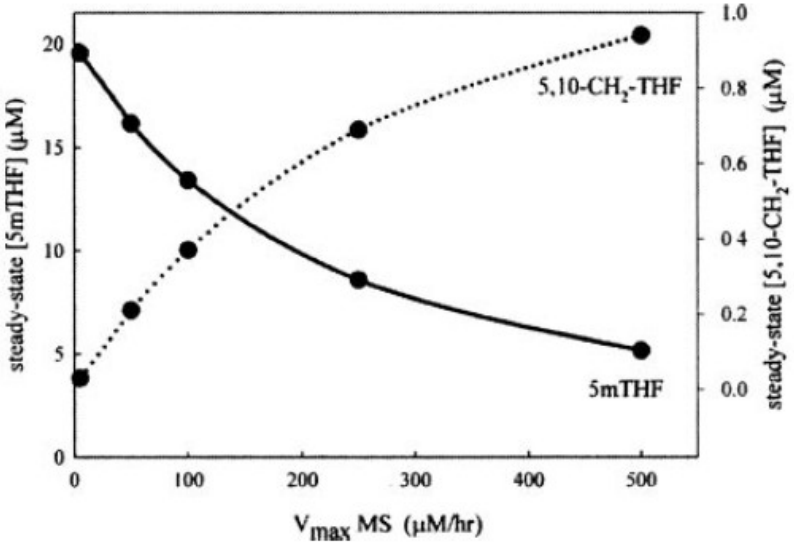
clarify whether knowledge of folate biochemistry can rigorously account for physiological and clinical observations

determine the relationship between folate cycle architecture and function

predict effect of genetic polymorphisms and interactions with diet

methyl trap

The Methyl Trap Hypothesis—It is well known that vitamin B_{12} deficiency results in a secondary folate deficiency. This observation is explained by the "methyl trap" hypothesis, which proposes that B_{12} deficiency reduces the activity of MS and this leads to the accumulation of 5mTHF at the expense of other folate forms (50–52).

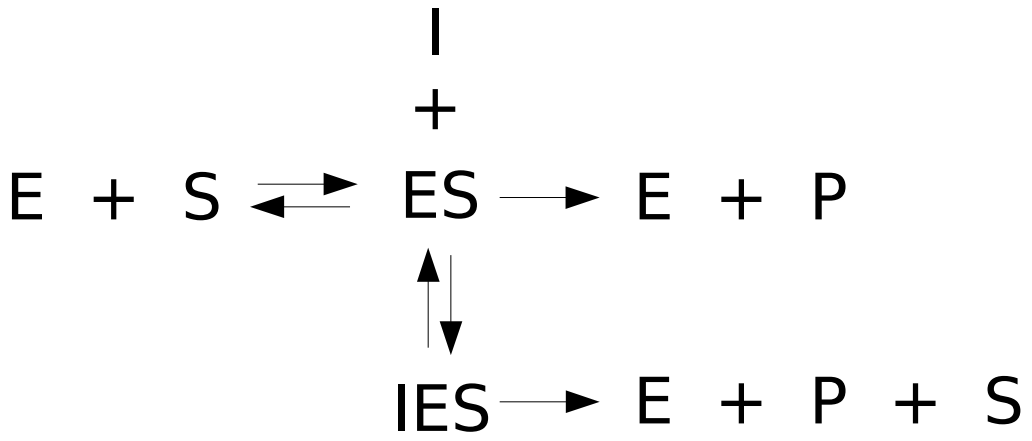


Shane, Stokstad, *"Vitamin B12-folate relationships"*, Annu Rev Nutr **5**:115-41 1985

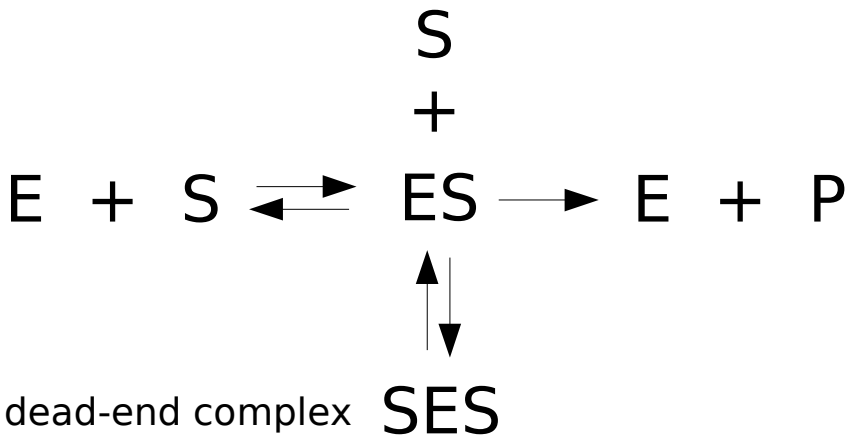
folate buffering

It has been known for some time that in mammalian liver folates are tightly bound to a number of specific folate-binding proteins (66–70). Interestingly, these folate-binding proteins have turned out to be the enzymes involved in the folate cycle (26, 42, 71–74). The total concentration of folate binding sites on these proteins exceeds the total concentration of the folate pools, and they bind folates with dissociation constants in the 100 nM range. This binding not only reduces pools of free folates but also inhibits the activities of the enzymes.

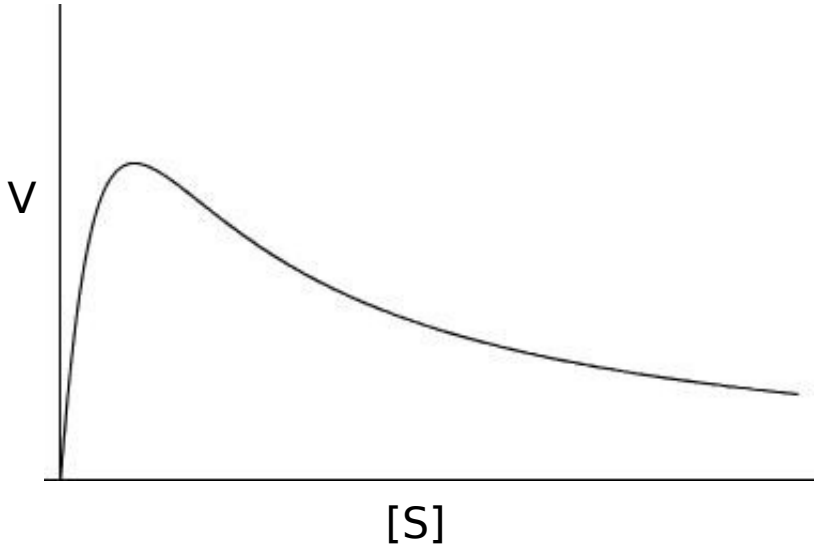
non-competitive inhibition



substrate inhibition



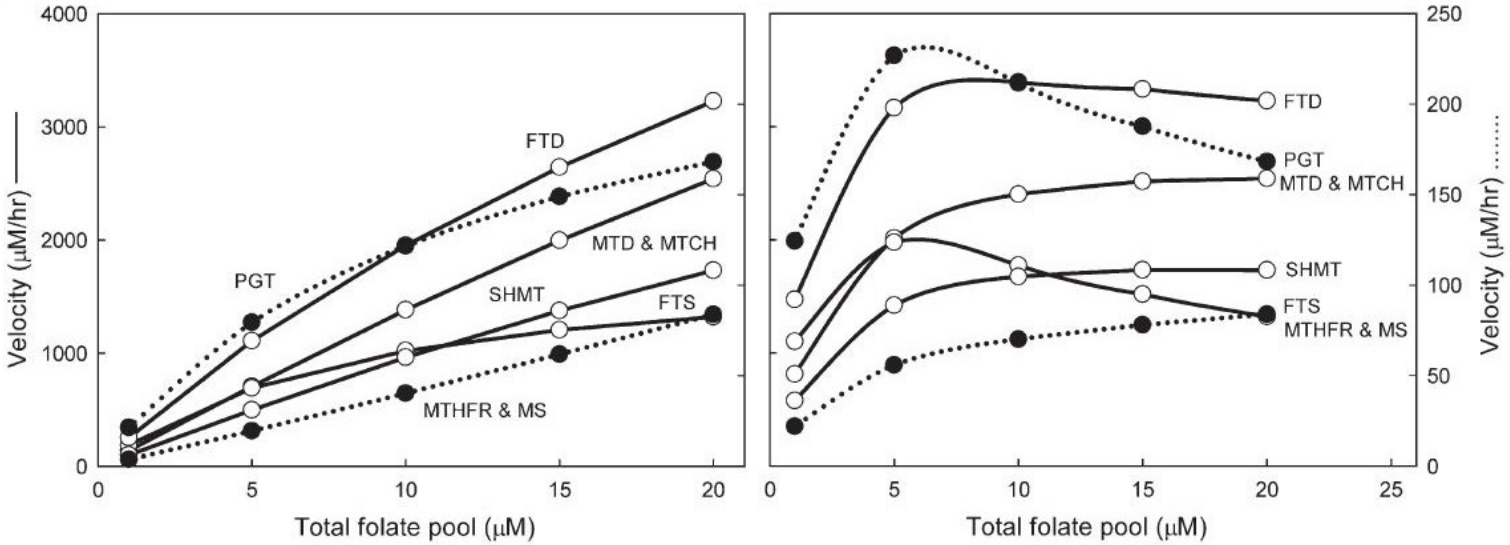
$$V = \frac{V_{max}[S]}{K_M + [S] + \frac{[S]^2}{K_I}}$$



Reed, Lieb, Nijhout, *"The biological significance of substrate inhibition: a mechanism with diverse functions"*, Bioessays **32**:422-9 2010

rate robustness

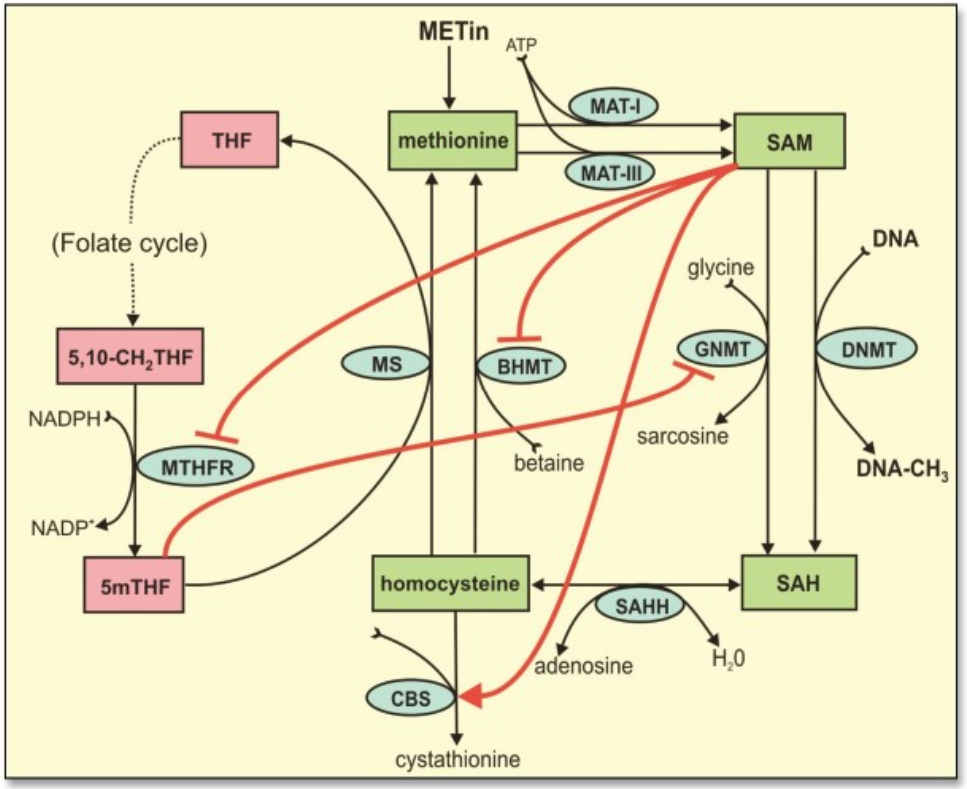
THF is assumed to non-competitively inhibit all enzymes



depleted. Our ancestors had diets that likely varied seasonally in their content of folate and other B vitamins. Thus, substrate inhibition in the folate cycle is probably an evolutionary mechanism to protect us against large seasonal swings in folate availability [51].

Reed, Lieb, Nijhout, *"The biological significance of substrate inhibition: a mechanism with diverse functions"*, Bioessays **32**:422-9 2010

folate, methionine interactions

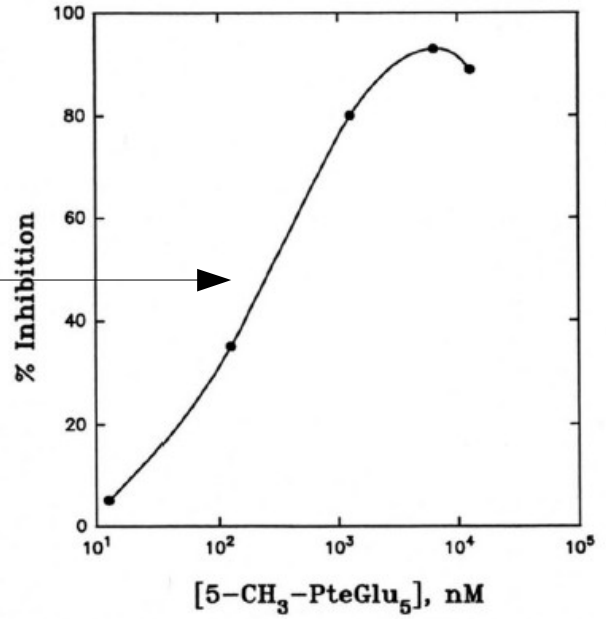


Nijhout, Reed, Anderson, Mattingly, James, Ulrich, *"Long-range allosteric interactions between the folate and methionine cycles stabilize DNA methylation reaction rate"*, Epigenetics **1**:81-87 2006

pragmatic allostery ...

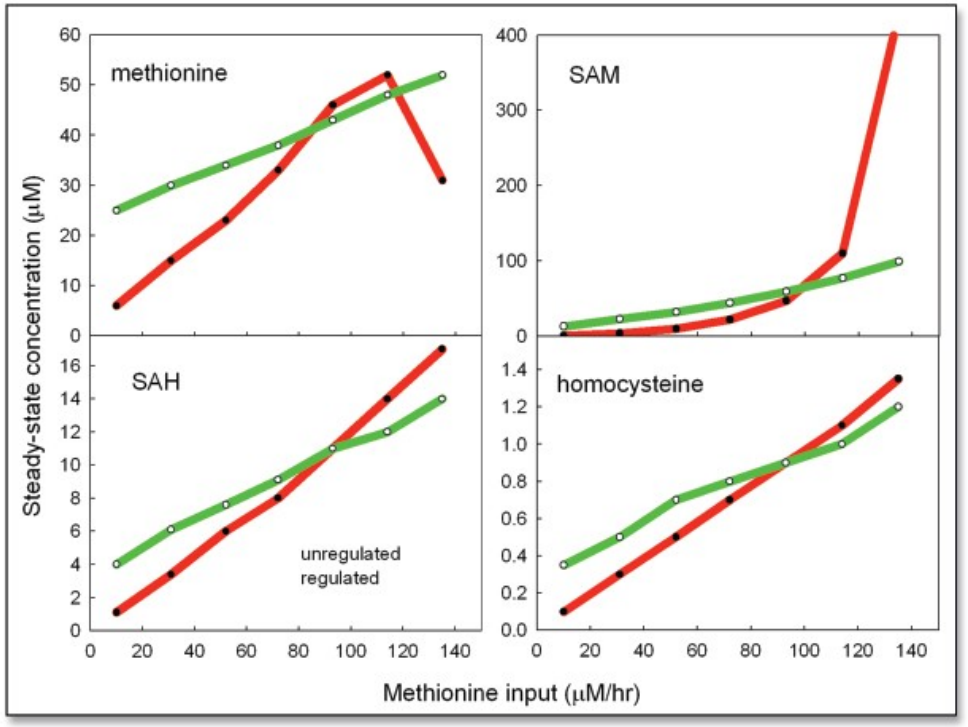
GNMT rate function is partially derived by fitting to experimental data

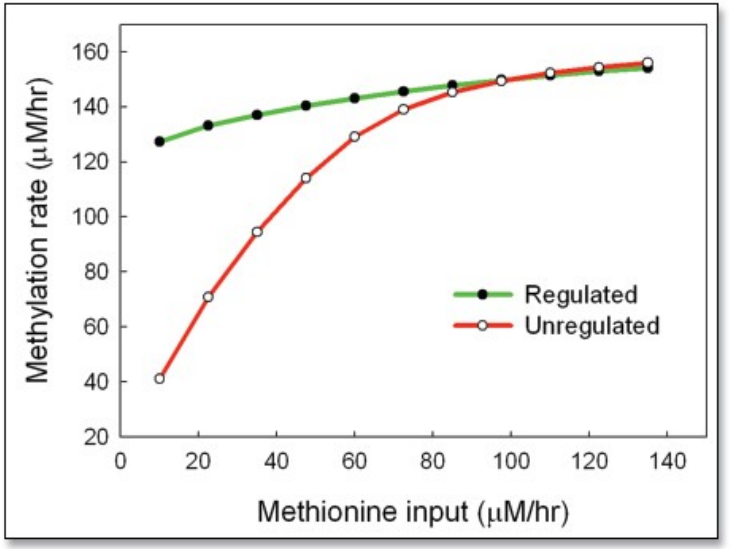
$$V_{GNMT} = \left(\frac{V_{max}[SAM]}{K_m + [SAM]}\right)\left(\frac{1}{1+\frac{[SAH]}{K_i}}\right)\left(\frac{4.38}{0.35+[5mTHF]}\right)$$



Yeo, Wagner, *"Purification and properties of pancreatic glycine N-methyl transferase"*, J Biol Chem **267**:24669-74 1992 (Figure 3)

allostery yields robustness and ... linearity?





Nijhout, Reed, Anderson, Mattingly, James, Ulrich, *"Long-range allosteric interactions between the folate and methionine cycles stabilize DNA methylation reaction rate"*, Epigenetics **1**:81-87 2006

redundant GNMT buffers methylation

It has been proposed by Wagner et al.[4,44] that the purpose of the GNMT reaction (in parallel to DNA methylation) is to buffer the DNA methylation rate against large swings in methionine input and [SAM].

methionine input rate is driven using a Markov process (Ornstein-Uhlenbeck)

$$r = \frac{\text{variance of DNA methylation rate}}{\text{variance of methionine input}}$$

	Regulated	Unregulated
GNMT	0.0072	0.088
No GNMT	0.057	0.15

Wagner, Briggs, Cook, *"Inhibition of glycine N-methyltransferase activity by folate derivatives: implications for regulation of methyl group metabolism"*, Biochem Biophys Res Comm **127**:746-52 1985

summing up

we know a great deal about the individual enzymes involved in metabolism

we know very little about how the metabolic system is regulated or how the metabolic paradox is implemented

metabolic systems balance supply and demand; they resemble economies, rather than engineering or physiological control systems

models can help relate biochemistry to physiology and clinical observations

experimental data on metabolite concentrations and fluxes is hard to obtain, making it difficult to develop an experimental “systems biochemistry”