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

flux balance analysis

steady-state reaction fluxes satisfy a linear constraint

\[ N \cdot v(x; k) = 0 \quad v_j \geq 0 \]

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

\[ \max \sum_{j} \alpha_j v_j \]

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

(* genes, proteins & reactions linked

with ionic (including proton) balancing

**pros**

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

![Graphs showing growth rate, glucose intake, and acetate secretion](image)

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


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 additional 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

measuring fluxes

A

1. Growth on $^{13}$C-labeled substrates
   - (A) Sampling time
   - (B) Duration of labeling exp.

2. Biomass
   - Intracellular metabolites
   - Protein-bound amino acids

3. Culture broth

4. Quantitative physiology
   - Uptake rates
   - Production rates
   - Biomass composition

5. MS or NMR analysis

6. $^{13}$C-Isotope pattern

7. Analytical interpretation

8. Flux ratios (%)

9. Absolute fluxes (mmol g$^{-1}$ h$^{-1}$)

10. Metabolism pathway table:
    - PEP: 40%
    - Krebs cycle: 60%
    - Oxaloacetate: 60%
    - Glycolysis: 85%
    - Pentose-P pathway: 15%
    - PEP: Etc.

11. Metabolism diagram:
    - Glucose → CO$_2$
    - PEP
    - Krebs cycle
    - Oxaloacetate
    - Glycolysis
    - Pentose-P pathway
    - Acetate
    - Biomass
    - CO$_2$

B

1. 100% [1-$^{13}$C]glucose

2. 100% unlabeled

3. 50% [3-$^{13}$C]

4. 50% [1-$^{13}$C]

5. 50% unlabeled

6. Flux ratios (%)

7. Absolute fluxes (mmol g$^{-1}$ h$^{-1}$)

8. Metabolism diagram:
    - PP pathway
    - Glycolysis
    - ED pathway

9. Fragmentation

“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)
anemia of pregnancy

1888-1964

vitamin B9, folic acid
**Folate-mediated one-carbon metabolism (FOCM)**

Biochemically, FOCM links synthesis of thymidylate (a pyrimidine), 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 targeted 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
differential equations

metabolite rates are a balance between production and consumption fluxes

\[
\frac{d}{dt} [\text{DHF}] = V_{TS} - V_{DHFR} \\
\frac{d}{dt} [\text{THF}] = V_{MS} - V_{FTS} + V_{PGT} + V_{AICART} + V_{DHFR} - V_{SHMT} - V_{NE} + V_{FTD} \\
\frac{d}{dt} [5\text{inTHF}]=V_{MTHFR} - V_{MS} + F_{in} - F_{out} \\
\frac{d}{dt} [5,10\text{-CH}_2\text{-THF}] = V_{SHMT} + V_{NE} - V_{TS} - V_{MTD} - V_{MTHFR} \\
\frac{d}{dt} [5,10\text{-CH}\text{-THF}] = V_{MTD} - V_{MTCH} \\
\frac{d}{dt} [10f\text{-THF}] = V_{MTCH} + V_{FTS} - V_{FGT} - V_{AICART} - V_{FTD}
\]
enzyme kinetics

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

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

independent substrates (\(c = 1\)) gives a product form

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

and for a reversible reaction

\[
V = V_{\text{max}}^f \cdot \frac{[S_f]}{K_{m,S_f} + [S_f]} \cdot \frac{[F_f]}{K_{m,F_f} + [F_f]} - V_{\text{max}}^r \cdot \frac{[S_r]}{K_{m,S_r} + [S_r]} \cdot \frac{[F_r]}{K_{m,F_r} + [F_r]}
\]
parameters

one advantage of assuming quasi-steady state is that aggregated parameters \( (V_{\text{max}}, K_M) \) 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 \( \mu \text{M} \)) |
|---------------------------------|----------|---------|----------|
| Parameter                       | Literature | Model   | References |
| DHFR                            |           |         |           |
| \( K_m^\text{DHFR} \)           | 0.12–1.9  | 0.5     | 15, 21, 24, 25 |
| \( K_m^\text{NADPH} \)          | 0.3–5.6   | 4.0     | 15, 21, 24, 25 |
| \( V_{\text{max}} \)            | 350–28,000| 50      | 15, 21, 24  |
| TS                              |           |         |           |
| \( K_m^\text{dUMP} \)           | 5–37      | 6.3     | 15, 21, 36, 37 |
| \( K_m^\text{5,10-CH}_2\text{-THF} \) | 10–45     | 14      | 15, 21, 36, 37 |
| \( V_{\text{max}} \)            | 30–4200   | 50      | 24, 37     |
| MTD (positive direction is from 5,10-CH\(_2\)-THF to 5,10-CH=THF) | | | |
| \( K_m^\text{5,10-CH}_2\text{-THF} \) | 2–5       | 2       | 21, 28     |
| \( V_{\text{max}} \)            | 520–594,000| 200,000| 15, 21, 28  |
| \( K_m^\text{5,10-CH}=\text{THF} \) | 1–10      | 10      | 13, 28     |
| \( V_{\text{max}} \)            | 594,000   | 594,000 | 38         |

BRENDA – [http://www.brenda-enzymes.org/](http://www.brenda-enzymes.org/) gives \( K_M, k_{\text{cat}}, K_i, \) specific activity, etc
concentrations

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Literature (µM)</th>
<th>Model (µM)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>[5mTHF]</td>
<td>4.6–8</td>
<td>5.16*</td>
<td>26, 27</td>
</tr>
<tr>
<td>[THF]</td>
<td>1.9–6.8</td>
<td>6.73*</td>
<td>21, 24, 26, 28</td>
</tr>
<tr>
<td>[DHF]</td>
<td>0.023–0.12</td>
<td>0.027*</td>
<td>21, 24</td>
</tr>
<tr>
<td>[5,10-CH2-THF]</td>
<td>1–2.5</td>
<td>0.94*</td>
<td>24</td>
</tr>
<tr>
<td>[5,10-CH=THF]</td>
<td>2.7–11.2</td>
<td>1.15*</td>
<td>21, 24</td>
</tr>
<tr>
<td>[10'-THF]</td>
<td>1–16</td>
<td>5.99*</td>
<td>21, 24, 26, 27</td>
</tr>
<tr>
<td>[Scr]</td>
<td>120–470</td>
<td>468</td>
<td>15, 23, 24</td>
</tr>
<tr>
<td>[Gly]</td>
<td>1600–2700</td>
<td>1850</td>
<td>15, 23, 24</td>
</tr>
<tr>
<td>[dUMP]</td>
<td>6.2–24.8</td>
<td>20</td>
<td>15, 21, 24</td>
</tr>
<tr>
<td>[GAR]</td>
<td>10</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>[AICAR]</td>
<td>1.6–2.1</td>
<td>2.1</td>
<td>15, 21, 24</td>
</tr>
<tr>
<td>[HCOOH]</td>
<td>500–900</td>
<td>900</td>
<td>21, 23</td>
</tr>
<tr>
<td>[NADPH]</td>
<td>50–200</td>
<td>50</td>
<td>24, 29</td>
</tr>
<tr>
<td>[Hcy]</td>
<td>0.3–7</td>
<td>1</td>
<td>6, 30</td>
</tr>
</tbody>
</table>

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.

perspective

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<sub>12</sub> deficiency results in a secondary folate deficiency. This observation is explained by the “methyl trap” hypothesis, which proposes that B<sub>12</sub> 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

\[ \text{I} \]
\[ \text{E} \quad + \quad \text{S} \quad \leftrightarrow \quad \text{ES} \quad \rightarrow \quad \text{E} \quad + \quad \text{P} \]
\[ \text{IES} \quad \rightarrow \quad \text{E} \quad + \quad \text{P} \quad + \quad \text{S} \]
substrate inhibition

\[ E + S \rightleftharpoons ES \rightarrow ES + P \]

dead-end complex SES

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

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].

folute, 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

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

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
It has been proposed by Wagner et al.\cite{Wagner}, 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)

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

<table>
<thead>
<tr>
<th></th>
<th>Regulated</th>
<th>Unregulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNMT</td>
<td>0.0072</td>
<td>0.088</td>
</tr>
<tr>
<td>No GNMT</td>
<td>0.057</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**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”