Cellular Metabolic Models

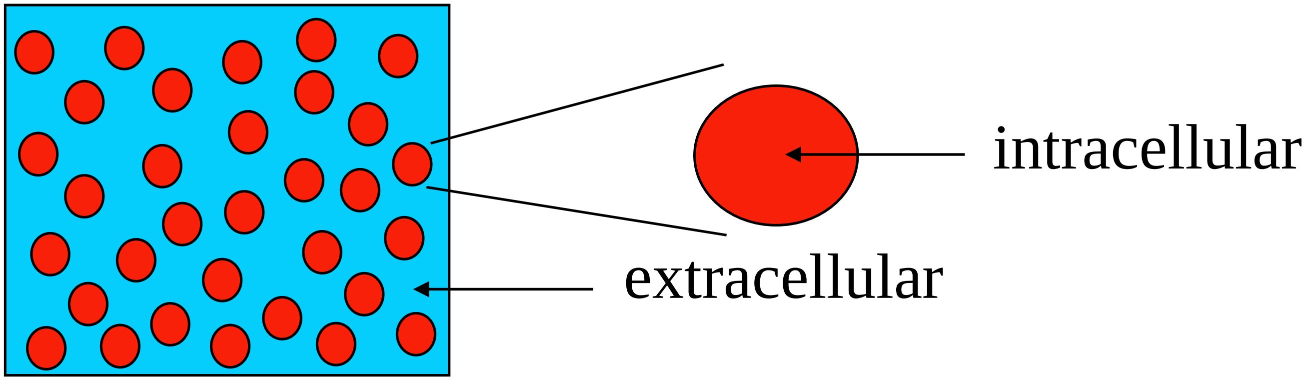
1. Cellular metabolism
2. Modeling cellular metabolism
3. Flux balance model of yeast glycolysis
4. Kinetic model of yeast glycolysis

Cellular Metabolic Models

Cellular Metabolism

Basic Concepts

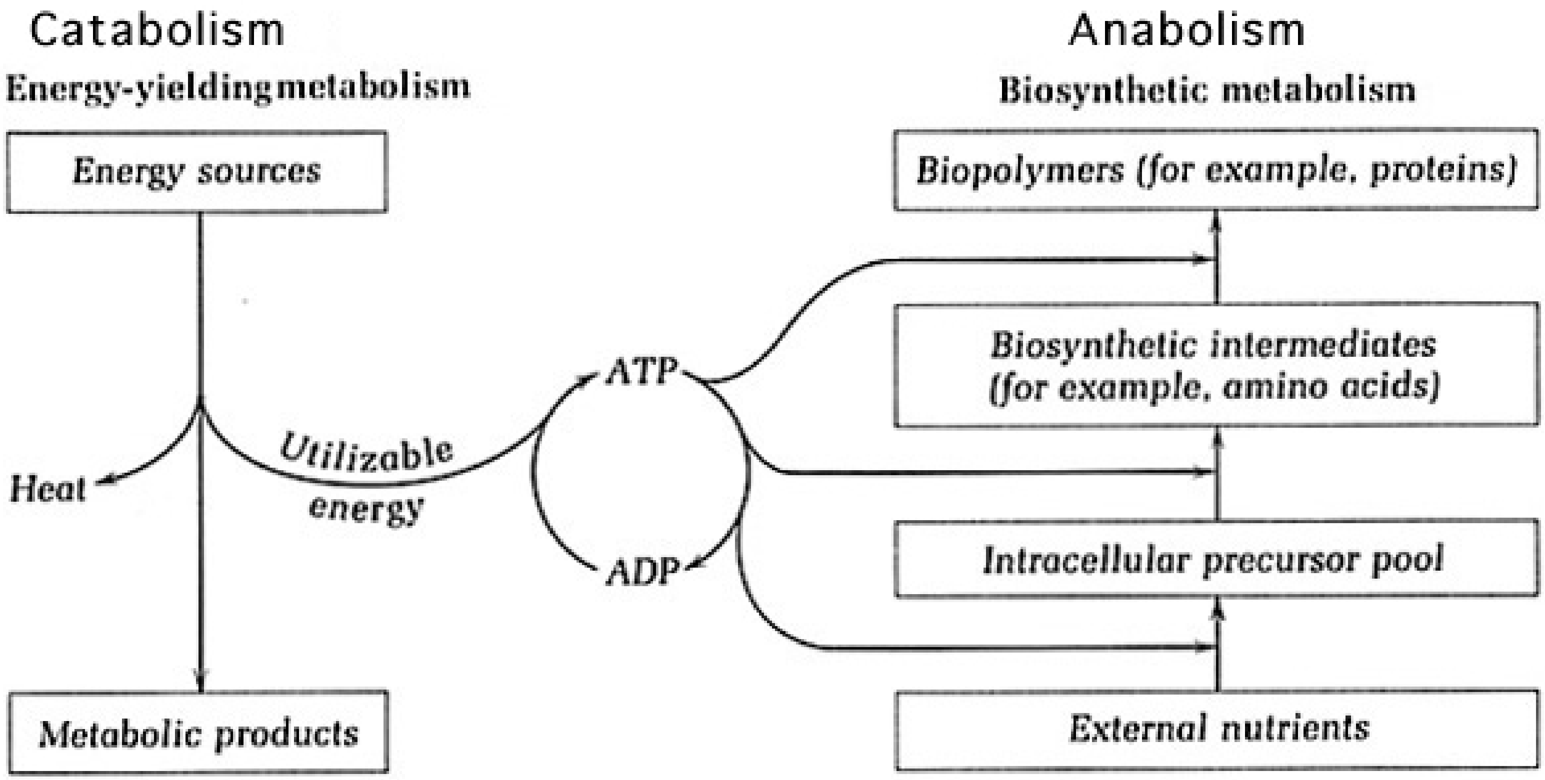
- Intracellular & extracellular phases



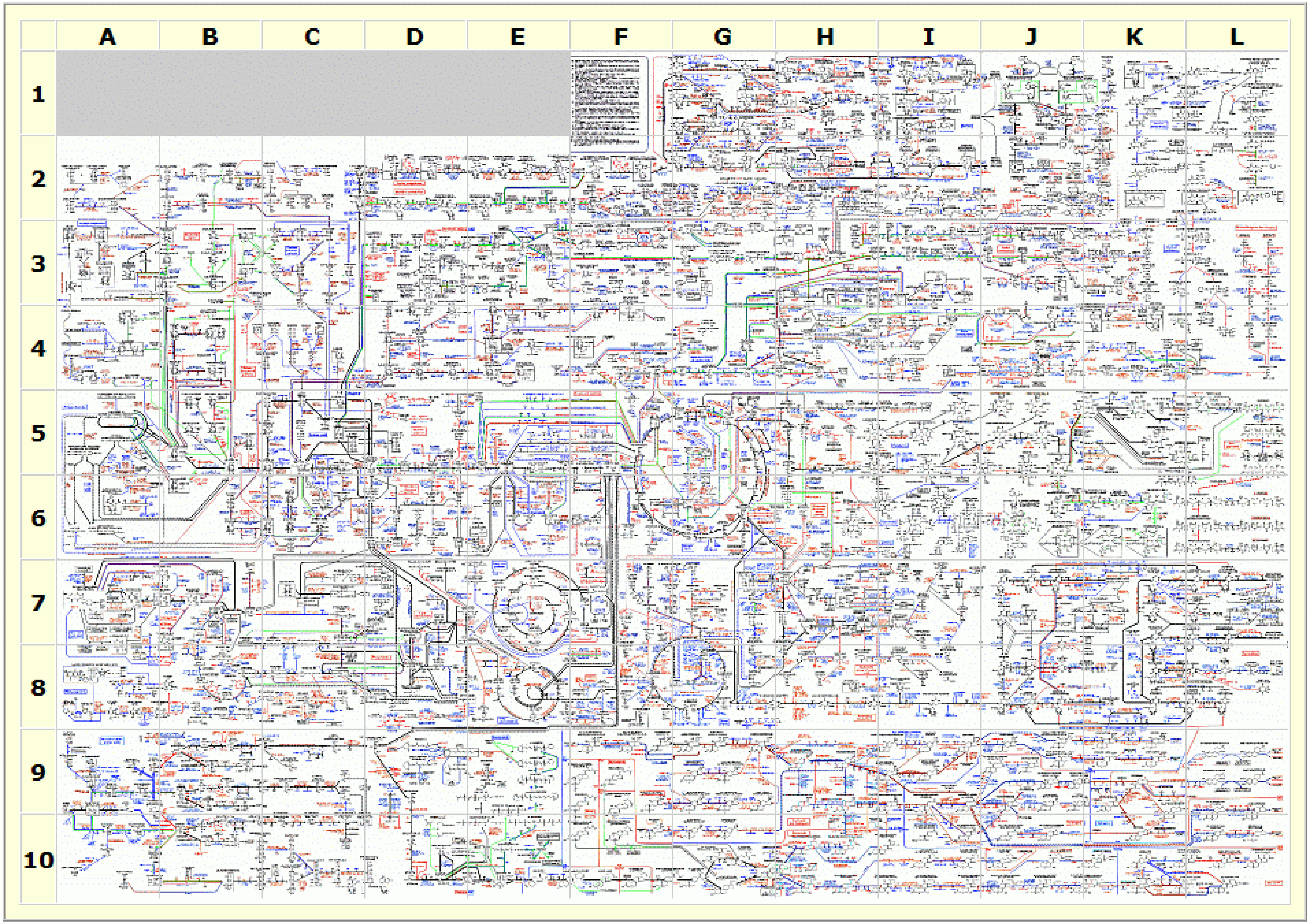
  - » Intracellular – inside a cell
  - » Extracellular – outside the cells
- Cellular growth
  - » Growing cells consume nutrients from and secrete products to the extracellular environment
  - » Division: one cell → two cells
  - » cells + substrates → more cells + products

Cellular Metabolism

- The sum of all chemical changes that take place in a cell through which energy and basic components are provided for essential processes, including the synthesis of new molecules and the breakdown and removal of others (National Cancer Institute).



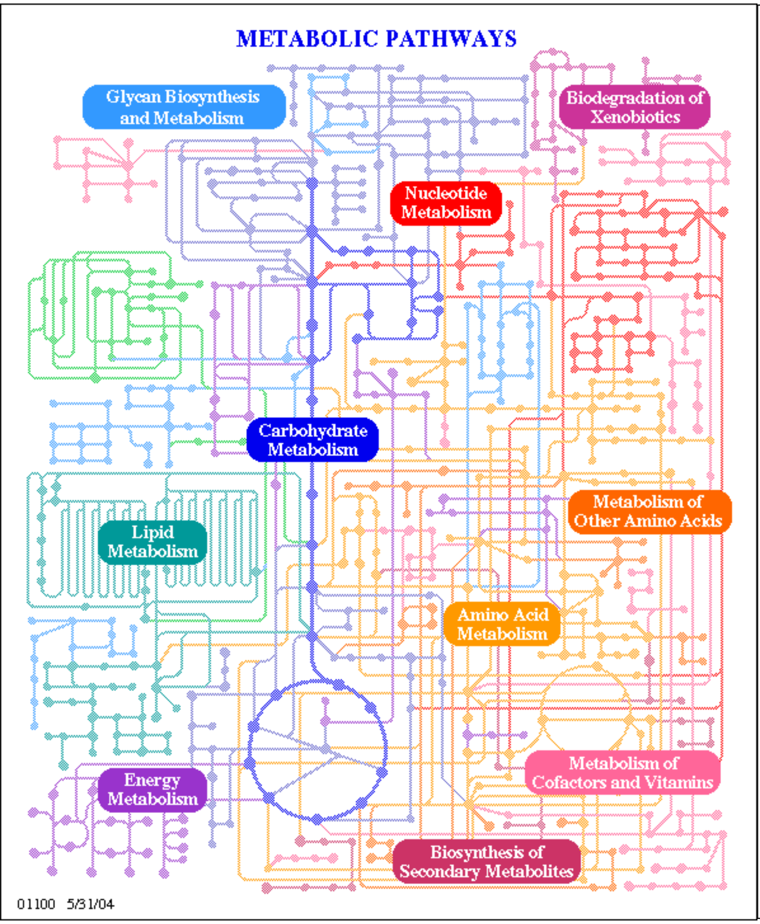
Cellular Metabolism is Complex

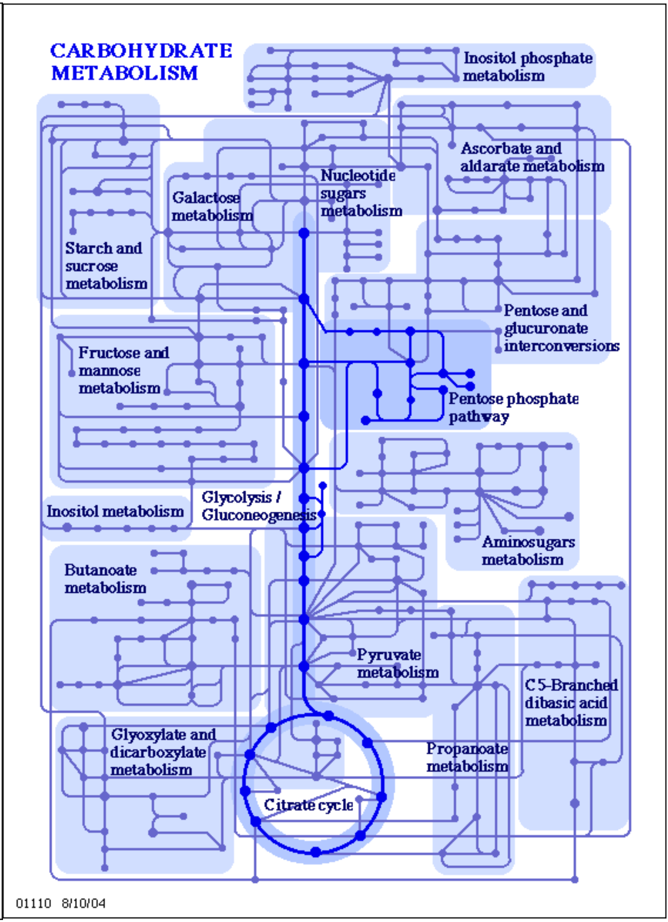


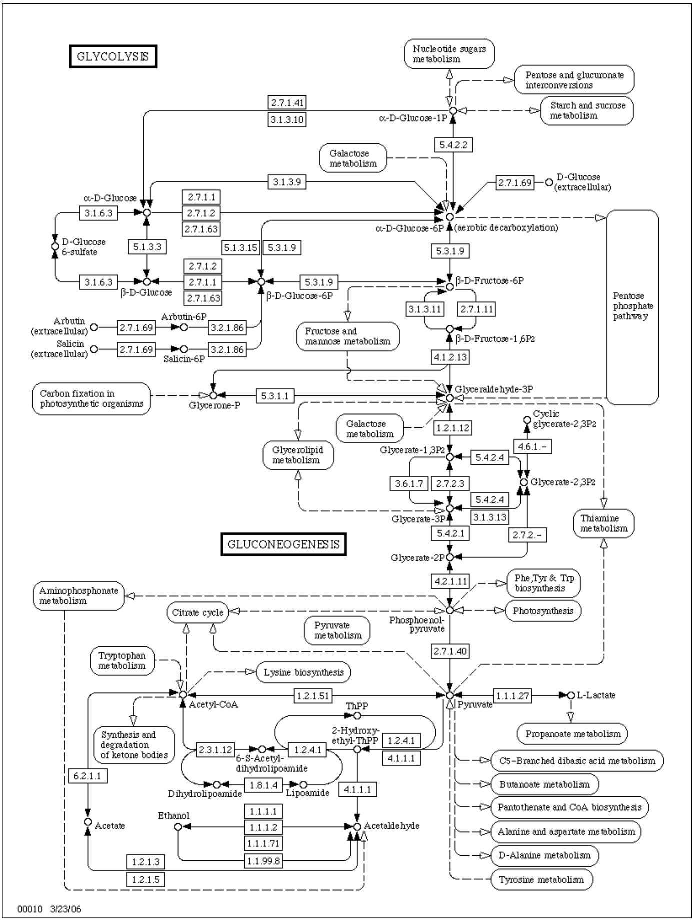
Metabolic Pathways

- Cellular metabolism is accomplished through an interconnected series of metabolic pathways
- Characteristics
  - » Initial substrates are products of other pathways or are externally supplied and transported across the cell membrane
  - » These substrates are converted to multiple metabolites through series of reactions
  - » Final products are used as substrates for other pathways, accumulate inside the cell, or are secreted across the cell membrane

Organization of Metabolic Pathways







www.genome.jp/kegg-bin/show_pathway?map01100

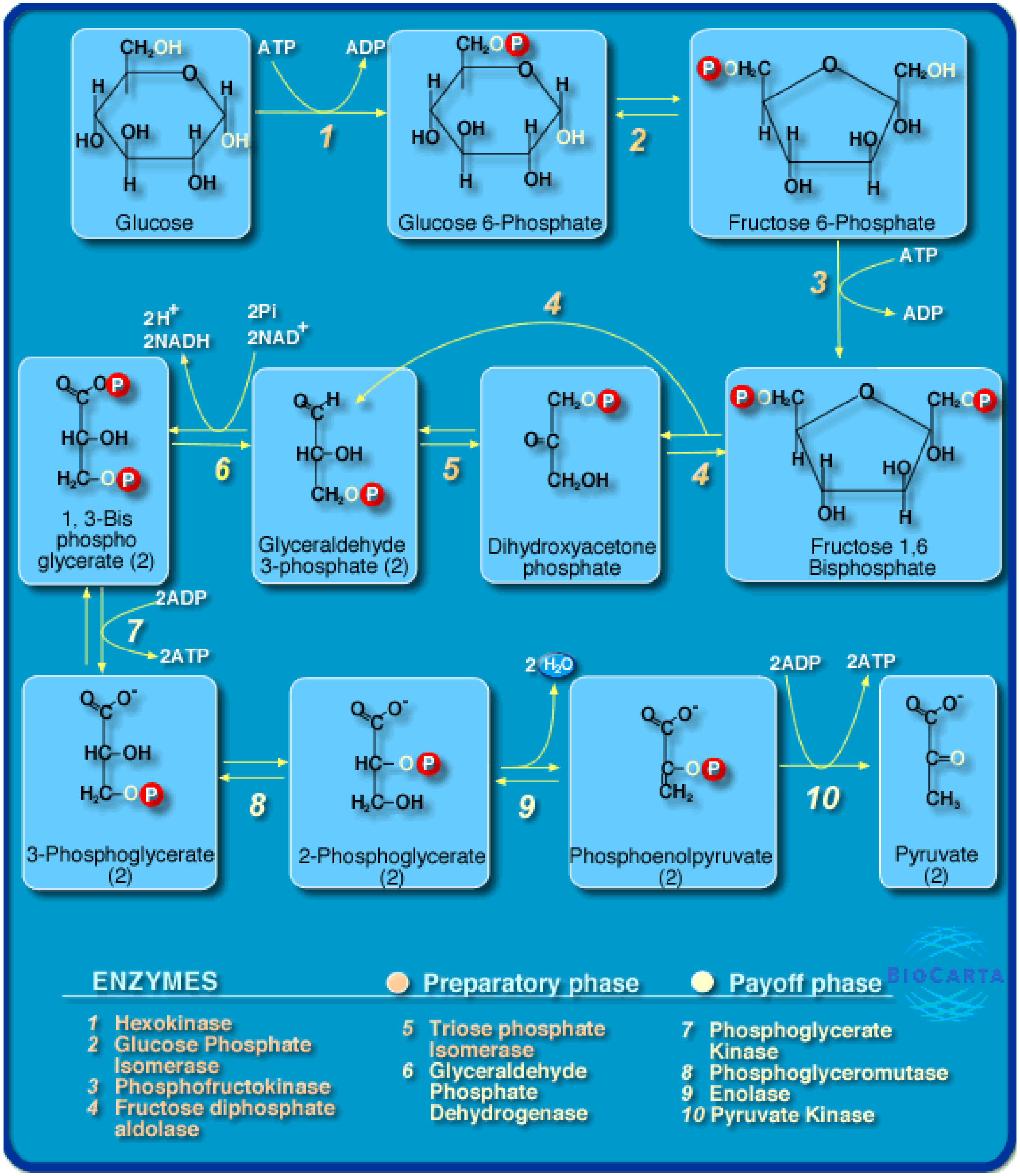
Biochemical Reactions

$$S \xrightarrow{E} P, \quad S \xleftrightarrow{E} P, \quad S_1 + S_2 \xrightarrow{E_1 \quad E_2} P_1 + P_2$$

$$S \xrightarrow{E_1} M_1 \xrightarrow{E_2} M_2 \xrightarrow{E_3} P$$

- Substrate (*S*): consumed by the reaction
- Product (*P*): produced by the reaction
- Metabolite (*M*): substrate or product of reaction
- Enzyme (*E*): protein that catalyzes the reaction
- Reaction may involve multiple substrates, enzymes, or products; can be irreversible or reversible

Glycolytic Pathway



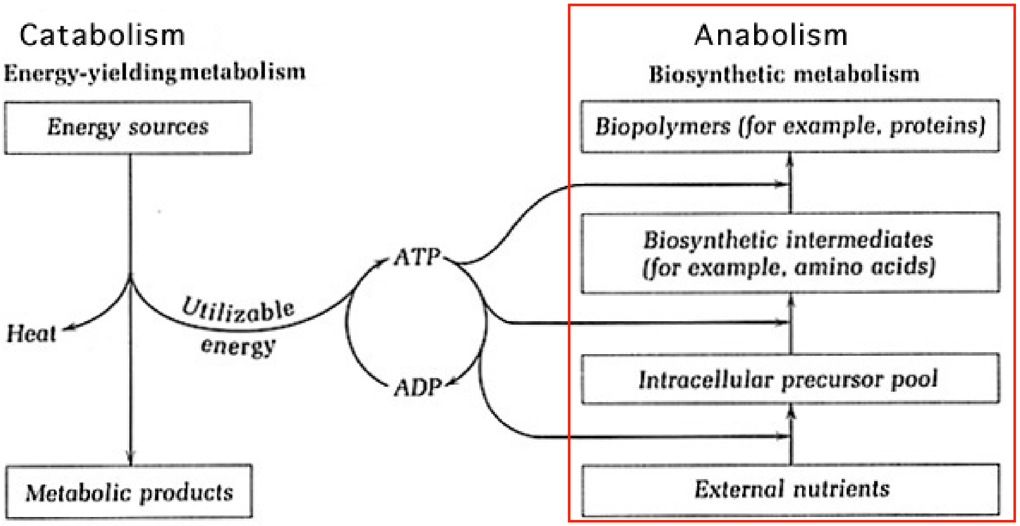
www.biocarta.com/pathfiles/h_glycolysisPathway.asp

- Converts glucose to pyruvate
- Generates ATP & NADH
- ATP drives energy consuming reactions
- NADH drives reduction reactions
- Glucose + 2 NAD$^+$ + 2 ADP + 2 Pi → 2 pyruvate + 2 NADH + 2 H$^+$ + 2 ATP + 2 H_2O

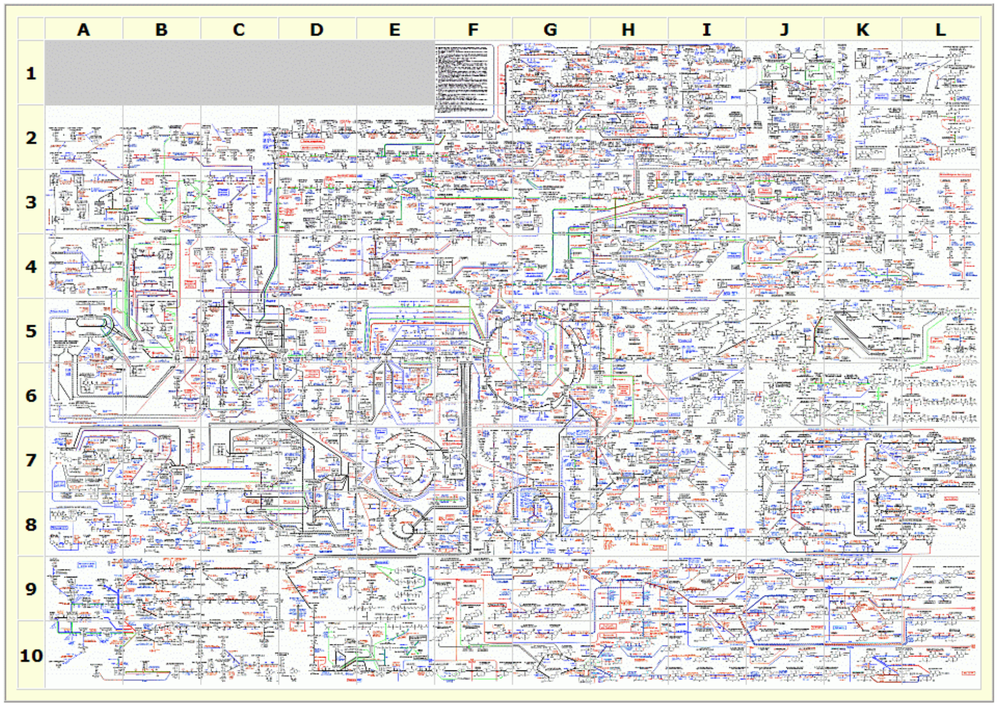
Cellular Metabolic Models

Modeling Cellular Metabolism

Level of Modeling Detail



Biomass



Lumped description (unstructured)

Detailed description (structured)

Increasing metabolic detail

Structured Metabolic Models

- General characteristics
  - Mechanistic description of cell growth and product formation rates
  - Detailed modeling of intracellular reactions
- Advantages
  - Sound theoretical basis
  - Superior predictive capabilities
  - Extensible to new environmental conditions
- Model types
  - Flux balance models – require reaction stoichiometry
  - Kinetic models – also require enzyme kinetics

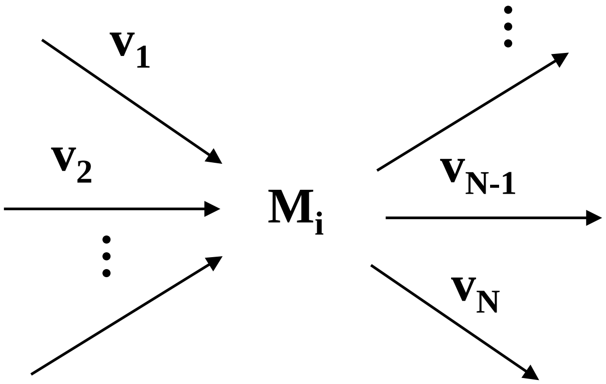
Kinetic Models

$$S \xrightarrow{v_1} M \xrightarrow{v_2} P$$

$$\frac{d[M]}{dt} = a_1 v_1 - a_2 v_2 - \mu [M]$$

- Stoichiometric coefficients (a_1,a_2)
  - » Determines number of product molecules produced per molecule of substrate consumed
  - » Often known from biochemistry literature
- Reaction rates (v_1,v_2)
  - » Requires knowledge of enzyme kinetics (i.e. how reaction rates depend on metabolite concentrations)
  - » Also called reaction flux
- Growth rate (μ)
  - » Cellular growth causes dilution of metabolite concentrations

The General Case



- Intracellular mass balances

$$\frac{d[M_i]}{dt} = \sum_{j=1}^{N} a_{ij} v_j([M_1],\ldots,[M_n]) - \mu[M_i] \quad \Rightarrow \quad \frac{d[\mathbf{M}]}{dt} = \mathbf{Av}([\mathbf{M}]) - \mu[\mathbf{M}]$$

  - » [**M**]: *n*-dimensional vector of metabolite concentrations
  - » **v**: *N*-dimensional vector of fluxes; accounts for both reaction fluxes & membrane transport fluxes
  - » **A**: *n*x*N* matrix of stoichiometric coefficients

- Solve ordinary differential equation system for unknown metabolite concentrations [**M**]

Flux Balance Models

- Stoichiometric equations assuming intracellular steady state and neglecting dilution:

$$\mathbf{A}\mathbf{v} = \mathbf{0}$$

  - **v**: *n*-dimensional vector of intracellular fluxes
  - *m* balanced metabolites
  - **A**: *mxn* matrix of stoichiometric coefficients
- Flux balance model formulation
  - Given a set of measured/specified fluxes ($\mathbf{v}_m$)

$$\mathbf{A}\mathbf{v} = \mathbf{A}_c\mathbf{v}_c + \mathbf{A}_m\mathbf{v}_m = \mathbf{0} \quad \Rightarrow \quad \mathbf{A}_c\mathbf{v}_c = -\mathbf{A}_m\mathbf{v}_m \equiv \mathbf{b}$$

  - **b**: *m*-dimensional vector of measured transport rates & fluxes
  - Solve stoichiometric model for unknown fluxes ($\mathbf{v}_c$)
  - If the $\mathbf{A}_c$ is square and nonsingular: $\mathbf{v}_c = \mathbf{A}_c^{-1}\mathbf{b}$

Comparison of Metabolic Models

Flux balance models

- Assume an intracellular steady state
- Only require stoichiometric data
- Unknowns are the reaction fluxes
- The number of unknown fluxes should equal the number of balanced metabolites
- Easy to solve the linear algebra problem

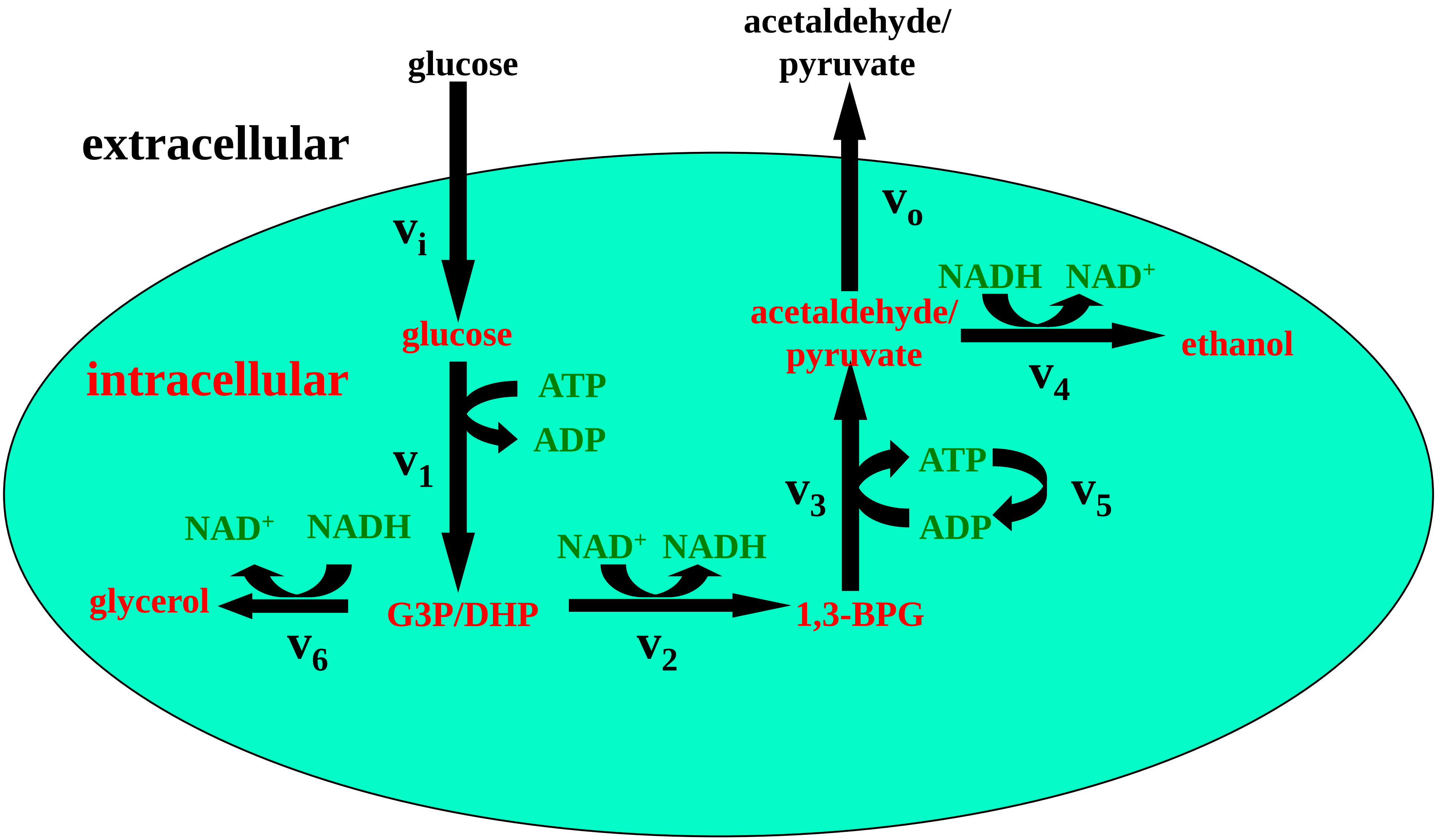
Kinetic models

- Capture dynamic intracellular behavior
- Require stoichiometric & enzyme kinetic data
- Unknowns are the metabolite concentrations
- No restrictions on the number of fluxes versus the number of balanced metabolites
- Must solve coupled set of nonlinear differential equations

Metabolic Models

Flux Balance Model of Yeast Glycolysis

Simplified Picture of Yeast Glycolysis



Wolf & Heinrich, Biochemical Journal, 345, 321-334, 2000.

Steady-State Mass Balances

Membrane transport (v_i)	Glucose (extracellular) → Glucose
Reaction 1 (v_1)	Glucose + 2ATP → 2G3P/DHP + ADP
Reaction 2 (v_2)	G3P/DHP+NAD$^+$ → 1,3-BPG + NADH
Reaction 3 (v_3)	1,3-BPG+ADP → Acetaldehyde/Pyruvate + 2ATP
Reaction 4 (v_4)	Acetaldehyde/Pyruvate+NADH → Ethanol + NAD$^+$
Reaction 5 (v_5)	ATP → ADP
Reaction 6 (v_6)	G3P/DHP+NADH → glycerol + NAD$^+$
Membrane transport (v_o)	Acetaldehyde/Pyruvate → Acetaldehyde/Pyruvate (extracellular)

$$\text{Glucose}:\ 0 = v_i - v_1$$

$$\text{G3P/DHP}:\ 0 = 2v_1 - v_2 - v_6$$

$$1-3-\text{BPG}:\ 0 = v_2 - v_3$$

$$\text{Acetaldeyde/Pyruvate}:\ 0 = v_3 - v_4 - v_0$$

$$\text{NADH}:\ 0 = v_2 - v_4 - v_6$$

$$\text{ATP}:\ 0 = -2v_1 + 2v_3 - v_5$$

Linear System Representation

- Assume measurements of glucose influx v_i and acetaldehyde/pyruvate efflux v_0 are available

$$\begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 2 & -1 & 0 & 0 & 0 & -1 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 1 & 0 & -1 & 0 & -1 \\ -2 & 0 & 2 & 0 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \end{bmatrix} = \begin{bmatrix} v_i \\ 0 \\ 0 \\ v_o \\ 0 \\ 0 \end{bmatrix}$$

MATLAB Exercise

- Consider the flux balance model **Av** = **b**:

$$\begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 2 & -1 & 0 & 0 & 0 & -1 \\ 0 & 1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 1 & 0 & -1 & 0 & -1 \\ -2 & 0 & 2 & 0 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \end{bmatrix} = \begin{bmatrix} v_i \\ 0 \\ 0 \\ v_o \\ 0 \\ 0 \end{bmatrix}$$

- Use MATLAB to solve for the unknown fluxes **v** given:
  - » $v_i = 6, v_o = 2$
  - » $v_i = 4, v_o = 4$
  - » $v_i = 2, v_o = 6$

Form Matrix and Check Rank

```
>> A = [1 0 0 0 0 0; 2 -1 0 0 0 -1; 0 1 -1 0 0 0; 0 0 1
-1 0 0; 0 1 0 -1 0 -1; -2 0 2 0 -1 0]

A =

     1     0     0     0     0     0
     2    -1     0     0     0    -1
     0     1    -1     0     0     0
     0     0     1    -1     0     0
     0     1     0    -1     0    -1
    -2     0     2     0    -1     0

>> rank(A)

ans =

     6
```

Compute Solutions

```
>> b1 = [6 0 0 2 0 0]';
>> v1 = linsolve(A,b1)
v1 =
    6
   10
   10
    8
    8
    2
```

```
>> b2 = [4 0 0 4 0 0]';
>> v2 = linsolve(A,b2)
v2 =
    4
    4
    4
    0
    0
    4
```

Compute Solutions cont.

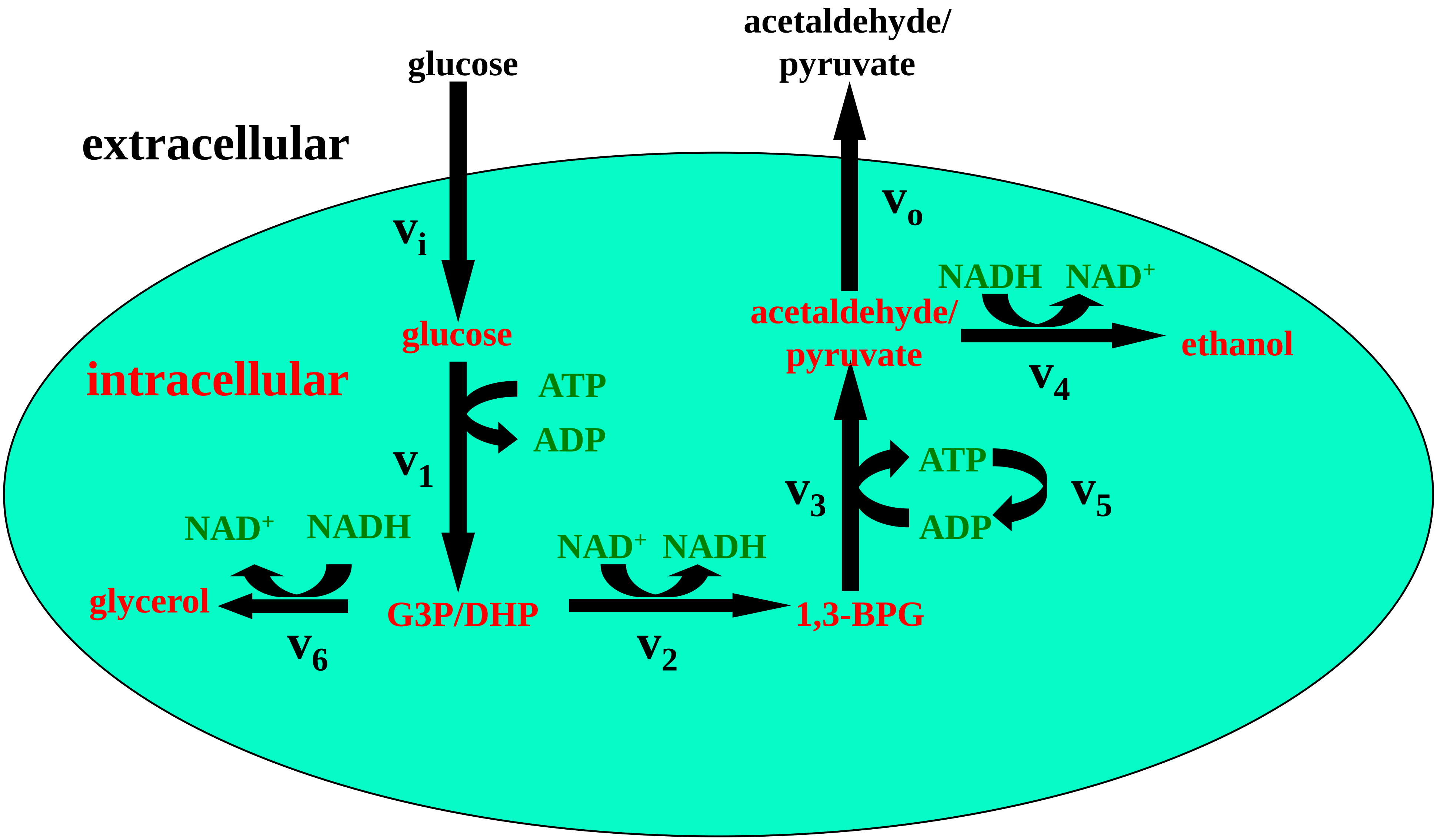
```
>> b3 = [2 0 0 6 0 0]';

>> v3 = linsolve(A,b3)

v3 =

     2

    -2

    -2

    -8

    -8

     6
```

- First two solutions seem reasonable since all the computed fluxes are positive
- Implies that glucose is use to make pyruvate
- That is glycolysis!
- Third solution seems unreasonable since some of the computed fluxes are negative
- Implies that ethanol is used to make pyruvate
- That is not glycolysis!

Metabolic Models

Kinetic Model of Yeast Glycolysis

Simplified Picture of Yeast Glycolysis



Wolf & Heinrich, Biochemical Journal, 345, 321-334, 2000.

Dynamic Mass Balances

Membrane transport (v_i)	Glucose (extracellular) → Glucose
Reaction 1 (v_1)	Glucose + 2ATP → 2G3P/DHP + ADP
Reaction 2 (v_2)	G3P/DHP+NAD^+ → 1,3-BPG + NADH
Reaction 3 (v_3)	1,3-BPG+ADP → Acetaldehyde/Pyruvate + 2ATP
Reaction 4 (v_4)	Acetaldehyde/Pyruvate+NADH → Ethanol + NAD^+
Reaction 5 (v_5)	ATP → ADP
Reaction 6 (v_6)	G3P/DHP+NADH → glycerol + NAD^+
Membrane transport (v_o)	Acetaldehyde/Pyruvate → Acetaldehyde/Pyruvate (extracellular)

$$\text{Glucose}: \frac{dS_1}{dt} = v_i - v_1 \qquad \text{G3P/DHP}: \frac{dS_2}{dt} = 2v_1 - v_2 - v_6$$

$$1-3-\text{BPG}: \frac{dS_3}{dt} = v_2 - v_3 \qquad \text{Acetaldeyde/Pyruvate}: \frac{dS_4}{dt} = v_3 - v_4 - v_0$$

$$\text{NADH}: \frac{\mathrm{dN}_2}{\mathrm{dt}} = v_2 - v_4 - v_6 \qquad \text{ATP}: \frac{dA_3}{dt} = -2v_1 + 2v_3 - v_5$$

Enzyme Kinetics

- Redundant to model ADP and NAD$^+$:

$$\mathrm{ATP} + \mathrm{ADP} = A_{tot} \qquad \mathrm{NADH} + \mathrm{NAD}^+ = N_{tot}$$

- v_2-v_6: mass action kinetics:

$$v_2 = k_2 S_2 N_1 = k_2 S_2 (N_{tot} - N_2) \qquad v_3 = k_3 S_3 A_2 = k_3 S_3 (A_{tot} - A_3)$$

$$v_4 = k_4 S_4 N_2 \qquad v_5 = k_5 A_3$$

$$v_6 = k_6 S_2 N_2$$

- v_1: mass action kinetics plus ATP inhibition:

$$v_1 = k_1 S_1 A_3 \left[1 + \left(\frac{A_3}{K_I} \right)^q \right]^{-1}$$

MATLAB Exercise

- Solve the kinetic model for the following two values of the extracellular flux of acetaldehyde/pyruvate:
  - » $v_o = 1$
  - » $v_o = 2$
- For each v_o value, use the function fsolve to find the steady-state solution and the function ode45 to integrate the model with the steady-state solution as the initial condition

Parameter Values

Variable	Symbol	Value
v_1 rate constant	k_1	100 $\text{mM}^{-1}\text{min}^{-1}$
v_2 rate constant	k_2	6 $\text{mM}^{-1}\text{min}^{-1}$
v_3 rate constant	k_3	16 $\text{mM}^{-1}\text{min}^{-1}$
v_4 rate constant	k_4	100 $\text{mM}^{-1}\text{min}^{-1}$
v_5 rate constant	k_5	1.28 min^{-1}
v_6 rate constant	k_6	12 $\text{mM}^{-1}\text{min}^{-1}$
Glucose uptake flux	v_i	3 mM/min
Pyruvate/acetaldehyde secretion flux	v_o	2 mM/min
Total ATP+ADP	A_t	4 mM
Total NADH+NAD$^+$	N_t	1 mM
v_1 rate parameter	K_i	0.52 mM
v_1 rate parameter	q	4

MATLAB m-file

```
function f = yeast_glycolysis(x)

% Define model parameters
q = 4;
Ki = 0.52;
At = 4;
Nt = 1;
vi = 3;
vo = 2;
k1 = 100;
k2 = 6;
k3 = 16;
k4 = 100;
k5 = 1.28;
k6 = 12;

% Define state variables
S1 = x(1);
S2 = x(2);
S3 = x(3);
S4 = x(4);
N2 = x(5);
A3 = x(6);
```

```
% Calculate reaction rates
v1 = k1*S1*A3/(1+(A3/Ki)^q);
v2 = k2*S2*(Nt-N2);
v3 = k3*S3*(At-A3);
v4 = k4*S4*N2;
v5 = k5*A3;
v6 = k6*S2*N2;

% Calculate derivatives
f(1) = vi-v1;
f(2) = 2*v1-v2-v6;
f(3) = v2-v3;
f(4) = v3-v4-vo;
f(5) = v2-v4-v6;
f(6) = -2*v1+2*v3-v5;
f = f';
```

Solution for $v_o = 1$

- Find steady state for guess of 1 for each variable

```
>> xss = fsolve('yeast_glycolysis',[1 1 1 1 1 1])

Equation solved.

fsolve completed because the vector of function values is near zero as
measured by the default value of the function tolerance, and the
problem appears regular as measured by the gradient.

<stopping criteria details>

xss =

   12.5311    0.9167    0.3571    0.4400    0.0909    3.1250
```

- Define function handle to redefine number of function arguments to include time

```
>> df = @(t,x) yeast_glycolysis(x);
```

Solution for v_o = 1 cont.

- Integrate model from steady state over time interval of 20 minutes

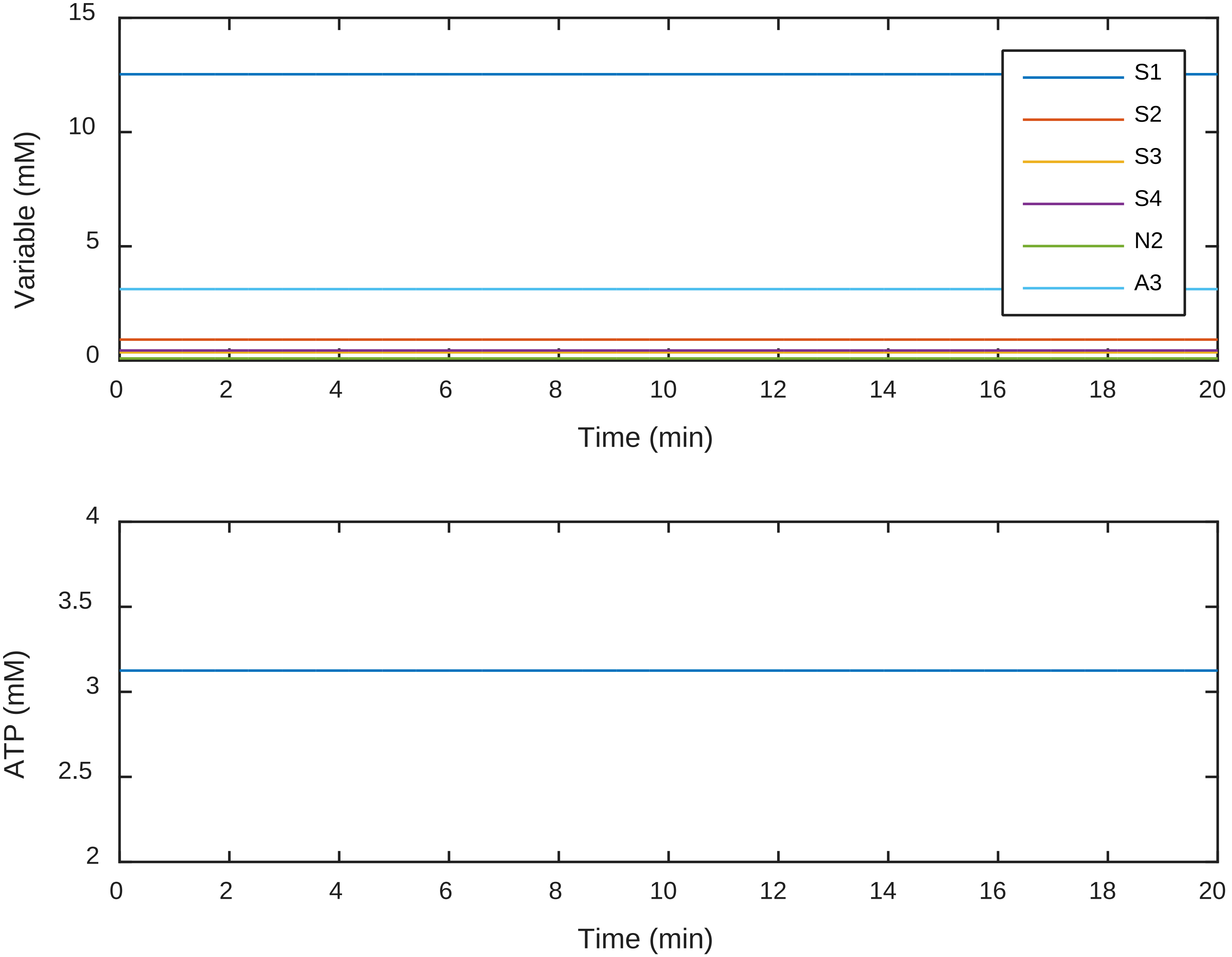
```
>> [t,x]=ode45(df,[0 20],xss);
```

- Plot time solutions for all variables and just ATP

```
>> subplot(2,1,1)
>> plot(t,x)
>> ylabel('Variable (mM)')
>> xlabel('Time (min)')
>> legend('S1','S2','S3','S4','N2','A3')
>> subplot(2,1,2)
>> plot(t,x(:,6))
>> ylabel('ATP (mM)')
>> xlabel('Time (min)')
>> axis([0,20,2,4])
```

- Steady state is stable!

Solution for v_o = 1 cont.



Solution for $v_o = 2$

```
>> xss = fsolve('yeast_glycolysis',[1 1 1 1 1 1])

xss =

    1.5844    0.8333    0.1026    0.1000    0.2000    1.5625

>> [t,x]=ode45(df,[0 20],xss);
>> subplot(2,1,1)
>> plot(t,x)
>> ylabel('Variable (mM)')
>> xlabel('Time (min)')
>> legend('S1','S2','S3','S4','N2','A3')
>> subplot(2,1,2)
>> plot(t,x(:,6))
>> ylabel('ATP (mM)')
>> xlabel('Time (min)')
>> axis([0,20,2,4])
```

- Solution is oscillatory → steady state is unstable!

Solution for v_o = 2 cont.