Standard Models and Approaches in Systems Biology

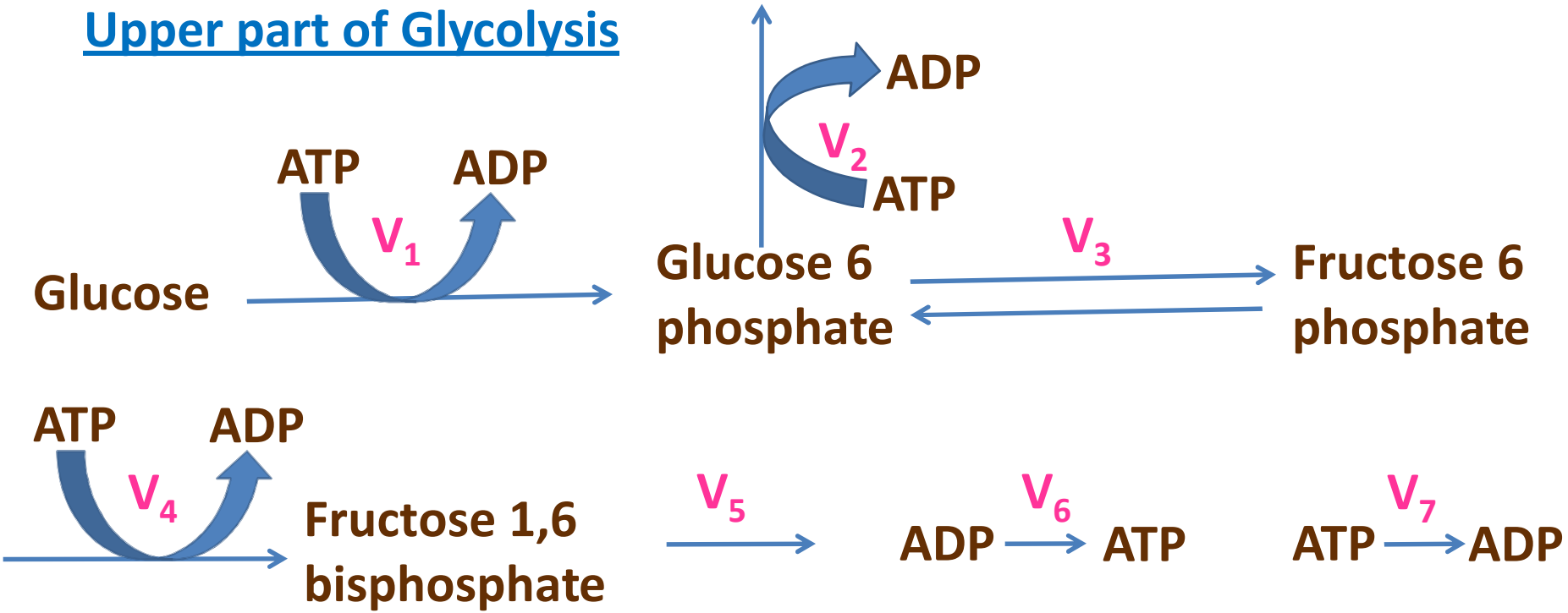
Metabolism

- Metabolism is the means by which cells survive and reproduce. –
- Metabolism – Two kinds of reactions:
  (1) catabolic reactions – breakdown of complex compounds to get energy and building blocks
  (2) anabolic reactions (construction of complex compounds used in cellular functioning).
- Metabolic networks consist of reactions transforming molecules of one type into molecules of another type.
- In modeling, the concentrations of the molecules (substrate + product) and their rates of change are studied.
- Metabolism is studied using three levels of abstraction;
  1. Enzyme kinetics – study the dynamic properties of the individual reactions in isolation.
  2. Network character - studied with stoichiometric analysis (studying the relative quantities of reactants and products) of compound production and degradation.

3. Metabolic control analysis - quantify the effect of disturbances in the network by measuring concentration changes and their effect in the network.

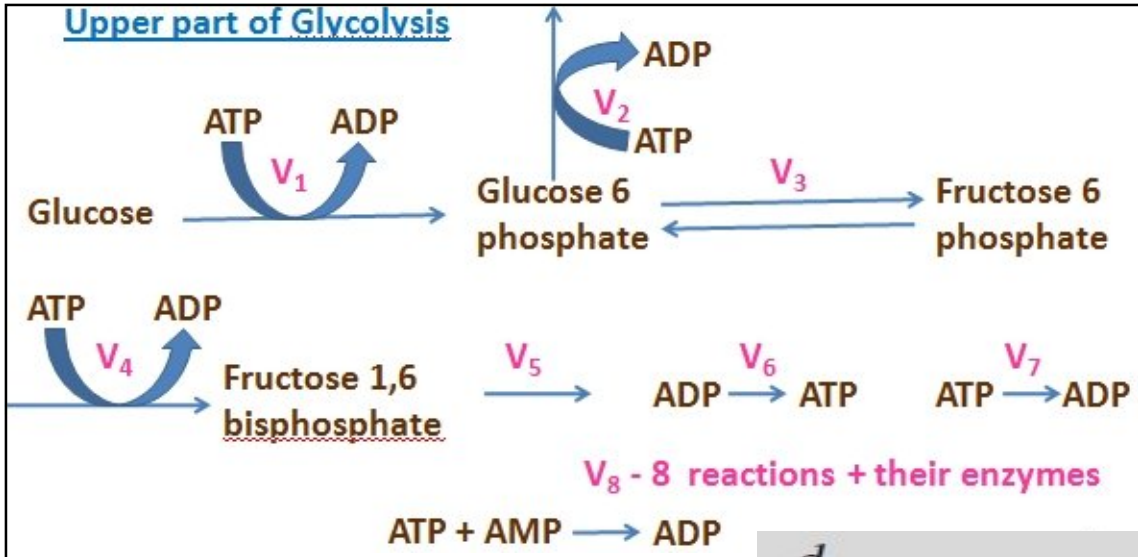
- Modeling of metabolic networks is the most elaborate in systems biology and hence we study it.
- An example for metabolic reaction;

Upper part of Glycolysis



V_8 - 8 reactions + their enzymes

$ATP + AMP \longrightarrow ADP$



Ordinary Differential Equation (ODE) → System

d/dt – rate of change over time

Actual concentrations could be included for calcln.

$$\frac{d}{dt}Gluc6P = v_1 - v_2 - v_3$$

$$\frac{d}{dt}Fruc6P = v_3 - v_4$$

$$\frac{d}{dt}Fruc1,6P_2 = v_4 - v_5$$

$$\frac{d}{dt}ATP = -v_1 - v_2 - v_4 + v_6 - v_7 - v_8$$

$$\frac{d}{dt}ADP = v_1 + v_2 + v_4 - v_6 + v_7 + 2v_8$$

$$\frac{d}{dt}AMP = -v_8 .$$

d – derivative – specifies change in quantity

Enzyme Kinetics and Thermodynamics

Deterministic kinetic modeling of biochemical reactions

- Basic quantities – 1) concentration S of a substance S (i. e., the number n of molecules of substance per volume V) 2) the rate v of a reaction (i. e., the change of concentration S per time t).
- This type of modeling is macroscopic or phenomenological – concentration is considered.
- In microscopic approach, single molecules and their interactions are considered.
- Classical enzyme kinetics assumes spatial homogeneity (the "well-stirred" test tube) and no direct dependency of the rate on time – this makes calculations simple.
- In more advanced modeling (like whole-cell modeling), spatial in homogeneities are taken into account.
- Because, many components are membrane-bound – further, cellular structures hinder the free movement of molecules.
- However, in most cases we can assume that diffusion is rapid enough to allow even distribution of all substances in space.

- One enzyme molecule catalyzes about a thousand reactions per second (the turnover number ranges from 10^2 s–1 to 10^7 s–1).
- This is an acceleration rate of about 10^6- to 10^{12}-fold when compared to the uncatalyzed, spontaneous reaction.
- This tremendous rate must be taken into account by the models we make.

The Law of Mass Action

- Guldberg and Waage – *The reaction rate is proportional to the probability of a collision of the reactants. This probability is in turn proportional to the concentration of reactants to the power of the molecularity, i. e., the number in which they enter the specific reaction.*
- Example - simple reaction $S_1 + S_2 \rightleftharpoons 2P$
- The reaction rate for the above is

$$v = v_+ - v_- = k_+ S_1 \cdot S_2 - k_- P^2$$

- Where, v is the net rate, v_+ the rate of the forward reaction, v_ the rate of the backward reaction, k_+ and k_ are the respective kinetic or rate constants.

- The molecularity is 1 for each substrate of the forward reaction and 2 for the backward reaction. As $S_1 + S_2 \rightleftharpoons 2P$
- If we measure the concentra on in moles per liter (mol L^{-1} or M) and the time in seconds (s), then the rates have the unit M . s^{-1}.
- The general mass action rate law for a reaction with substrate concentrations S_i and product concentrations P_j is;

$$v = v_+ - v_- = k_+ \prod_i S_i^{m_i} - k_- \prod_j P_j^{m_j}$$

- where m_i and m_j denote the molecularities of S_i and P_j .
- The equilibrium constant K_{eq} (or simply q) characterizes the ratio of substrate and product concentrations in equilibrium (S_{eq} and P_{eq}), i. e., the state with equal forward and backward rates.
- The rate constants are related to Keq in the following way:

$$K_{eq} = \frac{k_+}{k_-} = \frac{\prod P_{eq}}{\prod S_{eq}}$$

- The change in concentration described in ODE format→

$$\frac{d}{dt} S_1 = \frac{d}{dt} S_2 = -v$$

$$\frac{d}{dt} P = 2v .$$

Reaction Kinetics and Thermodynamics.

- **An important purpose of metabolism is to extract energy from nutrients – necessary for growth.**
- **Some reactions are energy-supplying reactions (exergonic) , some are energy-demanding reactions (endergonic), and others are energetically neutral reactions.**
- **The laws of thermodynamics are applied to metabolism to understand the energy circulation in the cell.**
- **The first law of thermodynamics (law of energy conservation), - the total energy of a system remains constant.**
- **The second law of thermodynamics - a process occurs spontaneously only if it increases the total entropy of the system.**
- **Entropy (S)- The randomness or disorder of the components of a chemical system – not directly measureable.**
- **Suitable measure is the Gibbs free energy (G) – the energy capable of carrying out work under isotherm-isobar conditions (constant temperature, constant pressure).**

- Change in free energy is given as $\Delta G = \Delta H - T\Delta S$
  Where, ΔH is the change in enthalpy (heat content of a system);
  T is the absolute temperature in Kelvin; and
  ΔS is the change in entropy.
- If $\Delta G < 0$ then the reaction proceeds spontaneously under release of energy (exergonic process).
- If $\Delta G > 0$ then the reaction is energetically not favorable and will not occur spontaneously (endergonic process).
- $\Delta G = 0$ means that the system has reached its equilibrium.
- Endergonic reactions may proceed if they obtain energy from a strictly exergonic reaction by energetic coupling.
- The free energy difference for a reaction can be calculated from;
  $\Delta G = \Sigma G_P - \Sigma G_S$
- Enzymes cannot change the free energies of the substrates and products of a reaction - they change the reaction path and lower the activation energy.
- Many mixtures are thermodynamically unstable, $\Delta G \ll 0$; highly favorable for the reactions to occur, but reactions do not occur.

- **The reason: during the course of a reaction, the metabolites must pass one or more transition states of maximal free energy.**
- **In transition state the molecule configuration is called an activated complex.**

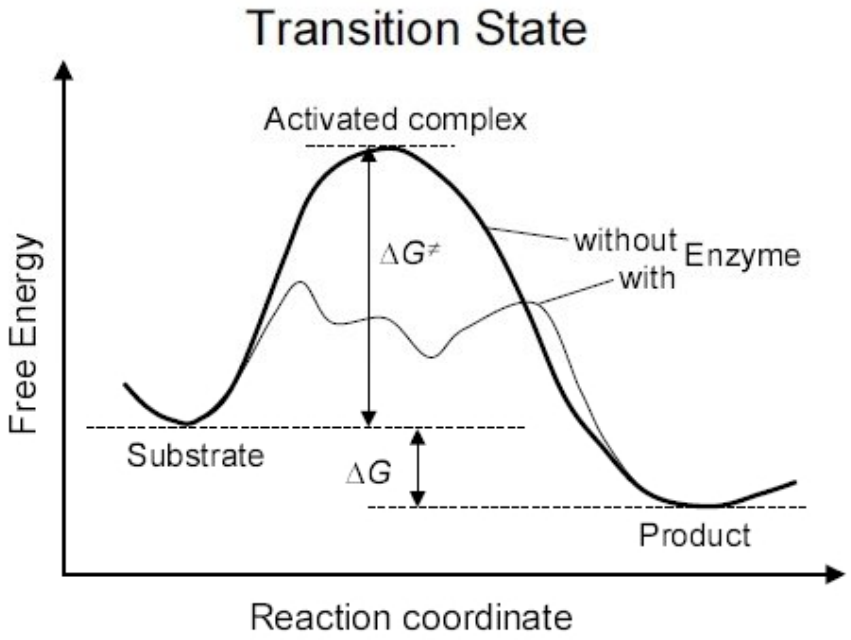


Fig. 5.3 Presentation of the change of free energy along the course of reaction. The substrate and the product are situated in local minima of the free energy; the active complex is assigned to the local maximum. The enzyme may change the reaction path and thereby lower the barrier of free energy.

- **The difference of free energy ($\Delta G^{\ddagger}$) between the reactants and the activated complex determines the dynamics of a reaction:**
- **The higher this difference, the lower the probability that the molecules pass this transition barrier and the lower the rate of the reaction.**

- The value of $\Delta G^{\ddagger}$ depends on the type of altered bonds, on steric, electronic, or hydrophobic demands, and on temperature.
- The interaction of the reactants with an enzyme may alter the reaction path and thereby lead to lower values of $\Delta G^{\ddagger}$.
- Furthermore, the free energy may assume more local minima and maxima along the path of reaction.

Michaelis-Menten Kinetics

- Reaction of invertase: A simple, one-substrate reaction without backward reaction and without effectors.

$$\mathrm{E+S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E+P} \quad \text{(Eq. 1)}$$

- A reversible formation of an enzyme-substrate complex ES from the free enzyme E and the substrate S
- Irreversible release of the product P from the enzyme E.

System of ODEs →

$$\frac{dS}{dt} = -k_1\, E \cdot S + k_{-1} ES \qquad \text{(Eq. 2)}$$

$$\frac{dES}{dt} = k_1\, E \cdot S - (k_{-1} + k_2)\, ES \qquad \text{(3)}$$

$$\frac{dE}{dt} = -k_1\, E \cdot S + (k_{-1} + k_2)\, ES \qquad \text{(4)}$$

$$\frac{dP}{dt} = k_2\, ES\,. \qquad \text{(Eq. 5)}$$

- **The rate of reaction :** $v = -\frac{dS}{dt} = \frac{dP}{dt}$ (Eq. 6)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- **All these ODEs cannot be solved analytically – hence, some assumptions were made to make things simple.**
- **Assumption by Michaelis and Menten – the conversion of E and S to ES and vice versa is much faster than the decomposition of ES into E and P *(quasi-equilibrium* between the free enzyme and the enzyme-substrate complex)** $k_1, k_{-1} \gg k_2$ (Eq. 7)
- **Assumption of Briggs and Haldane – during the course of reaction a state is reached where the concentration of the ES complex remains constant.**
- **This assumption is justified only if the initial concentration of the substrate is much larger than the concentration of the enzyme, S(t = 0) >> E; otherwise, this steady state will never be reached. More general assumption – *quasi-steady state of the ES complex*: The concentration of the ES complex does not change** $\frac{dES}{dt} = 0$ (Eq. 8)

$$\frac{dES}{dt} + \frac{dE}{dt} = 0 \quad \text{or} \quad E_{total} = E + ES \qquad \text{(Eq. 9)}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- **Introduce Eq. (9) into Eq. (3) under the steady-state assumption (Eq. (8)).**

$$ES = \frac{k_1 E_{total} S}{k_1 S + k_{-1} + k_2} = \frac{E_{total} S}{S + \frac{k_{-1} + k_2}{k_1}} \qquad \text{(Eq. 10)}$$

- **The reaction rate.**

$$v = \frac{k_2 E_{total} S}{S + \frac{k_{-1} + k_2}{k_1}} \qquad \text{(Eq. 11)}$$

or simply

$$v = \frac{V_{max} S}{S + K_m} \qquad \text{(Eq. 12)}$$

- **Eq 12 is the expression for Michaelis and Menten kinetics where;**

$$\textit{maximal velocity}, \quad V_{max} = k_2 E_{total},$$

$$\textit{Michaelis constant}, \quad K_m = \frac{k_{-1} + k_2}{k_1},$$

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