Creating and maintaining order requires work and energy

- The randomness or disorder of the components of a chemical system is expressed as **entropy**, ***S***.
- Any change in randomness of the system is expressed as **entropy change**, ΔS, which by convention has a positive value when randomness increases.
- The amount of energy available to do work is the **free-energy change**, ΔG; this is always somewhat less than the theoretical amount of energy released, because some energy is dissipated as the heat of friction.
- In closed systems, chemical reactions proceed spontaneously until **equilibrium** is reached.

- J. Willard Gibbs, who developed the theory of energy changes during chemical reactions, showed that the free-energy content, G, of any closed system can be defined in terms of three quantities: (a) enthalpy, H, reflecting the number and kinds of bonds; (b) entropy, S; and (c) the absolute temperature, T (in Kelvin scale).
- The definition of free energy is $G = H - TS$.
- When a chemical reaction occurs at constant temperature, the free-energy change, ΔG, is determined by the enthalpy change, ΔH, reflecting the kinds and numbers of chemical bonds and noncovalent interactions broken and formed, and the entropy change, ΔS, describing the change in the system's randomness:

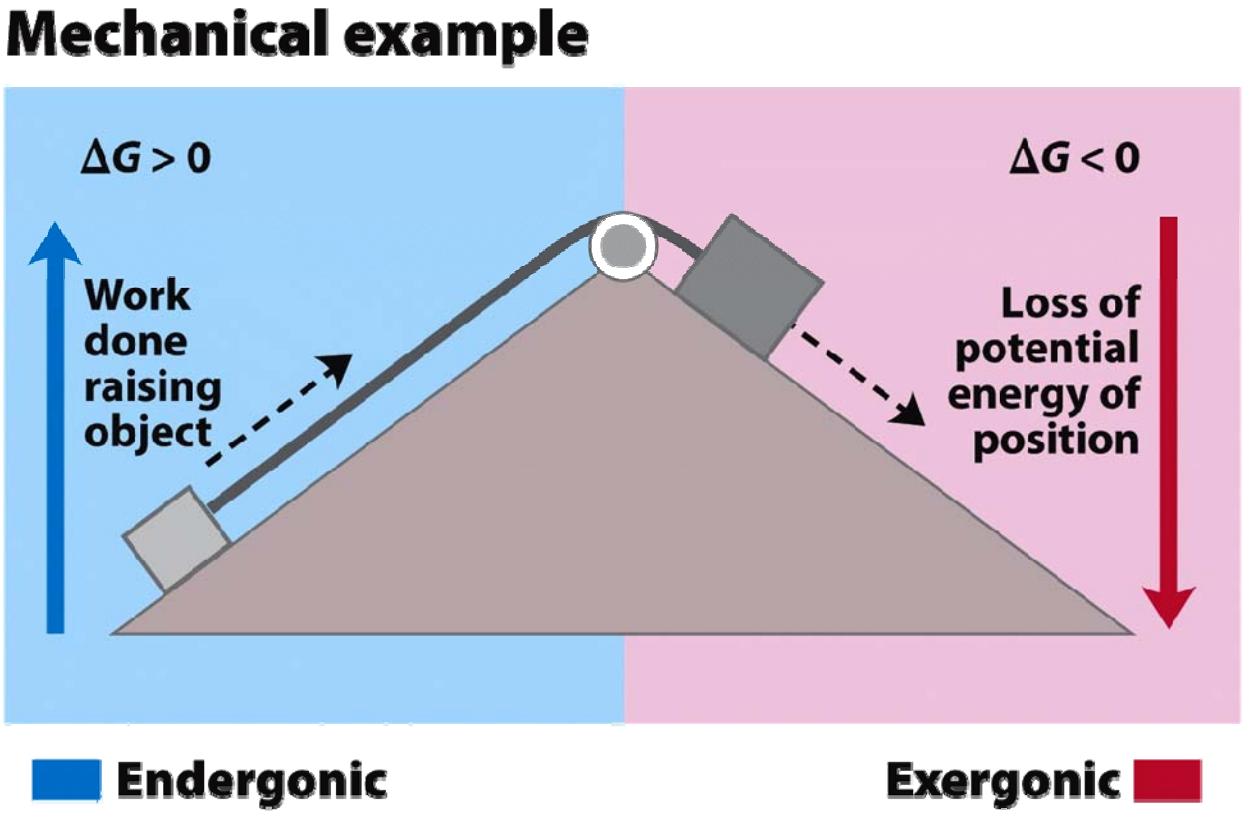
$$\Delta G = \Delta H - T\Delta S$$

- By definition, ΔH is negative for a reaction that releases heat, and ΔS is positive for a reaction that increase the system's randomness.
- A process tends to occur spontaneously only if ΔG is negative.

Energy coupling links reactions in biology

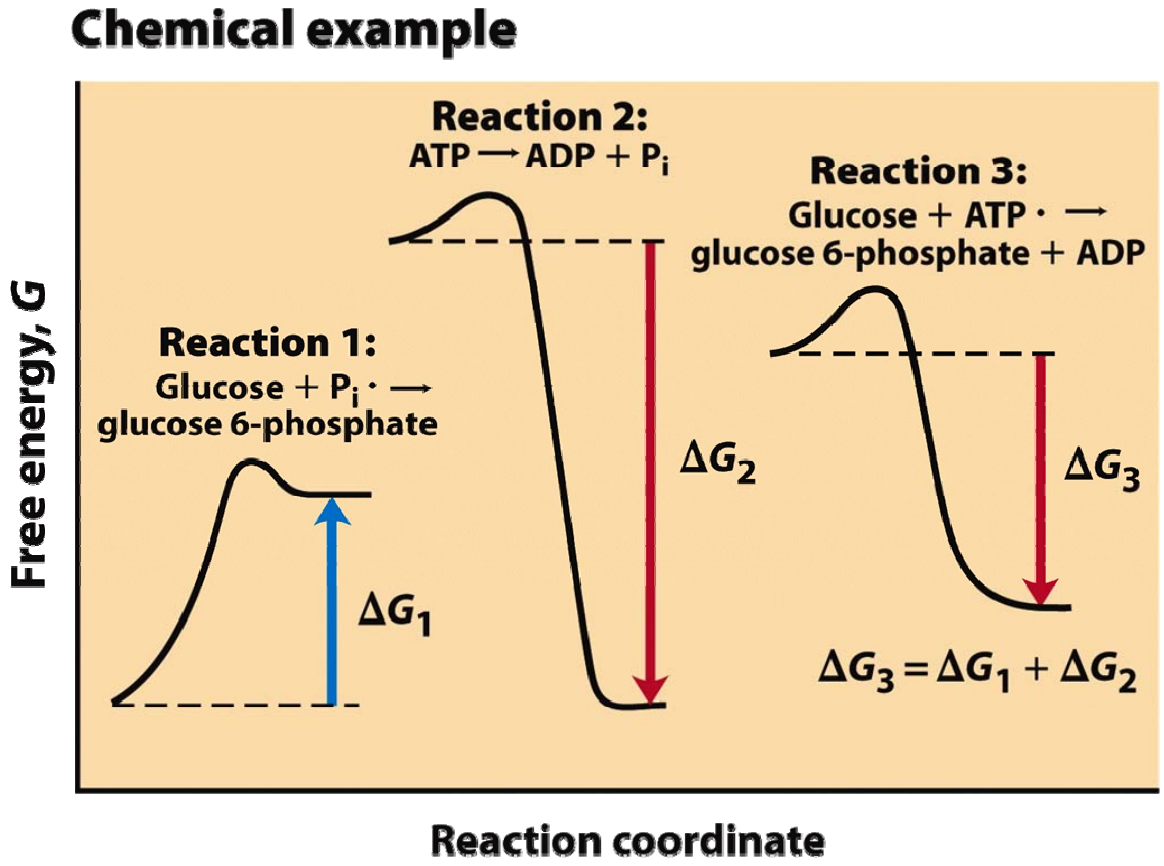
- Molecules are less stable and more highly ordered than a mixture of their monomeric components. To carry out these thermodynamically unfavorable, **energy-requiring (endergonic)** reactions, cells couple them to other reactions that **liberate free energy (exergonic)** reactions, so that the overall process is exergonic: the *sum* of the free-energy changes is negative.
- The reactions converting ATP to P_i and ADP or to AMP and PP_i are highly exergonic (large negative ΔG). Many endergonic cellular reactions are driven by coupling them, through a common intermediate, to these highly exergonic reactions.

Energy coupling in mechanical and chemical processes



- The downward motion of an object releases potential energy that can do mechanical work. The potential energy made available by spontaneous downward motion, an exergonic process (pink), can be coupled to the endergonic upward movement of another object (blue).

Energy coupling in mechanical and chemical processes

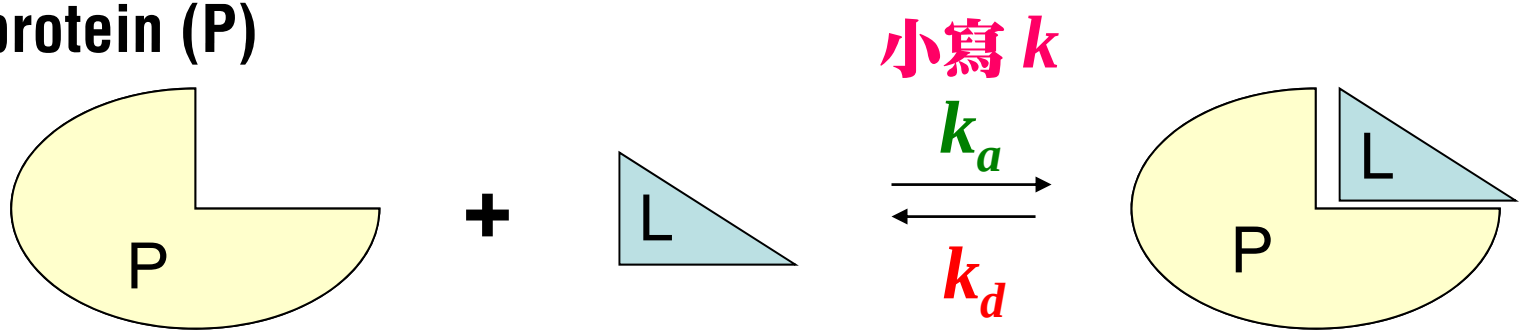


- In reaction 1, the formation of glucose 6-phosphate from glucose and inorganic phosphate (P_i) yields a product of higher energy than the two reactants. For this endergonic reaction, ΔG is positive.
- In reaction 2, the exergonic breakdown of adenosine triphosphate (ATP) has a large, negative free-energy change (ΔG_2).
- The third reaction is the sum of reactions 1 and 2, and the free-energy change, ΔG_3, is the arithmetic sum of ΔG_1 and ΔG_2. Because ΔG_3 is negative, the overall reaction is exergonic and proceeds spontaneously.

- The tendency for a chemical reaction to proceed toward equilibrium can be expressed as the free-energy change, ΔG, which has two components: enthalpy change, ΔH, and entropy change, ΔS. These variables are related by the equation $\Delta G = \Delta H - T\Delta S$.
- When ΔG of a reaction is negative, the reaction is exergonic and tends to go toward completion; when ΔG is positive, the reaction is endergonic and tends to go in the reverse direction. When two reactions can be summed to yield a third reaction, the ΔG for this overall reaction is the sum of the ΔGs of the two separate reactions.
- The standard free-energy change for a reaction, ΔG^0, is a physical constant that is related to the equilibrium constant by the equation $\Delta G^0 = -RT \ln K_{eq}$. ($R$: gas constant; T: absolute temperature)
- Most cellular reactions proceed at useful rates only because enzymes are present to catalyze them. Enzymes act in part by stabilizing the transition state, reducing the activation energy, ΔG^{++}, and increasing the reaction rate by many orders of magnitude.

Protein-ligand interactions can be described quantitatively

- Consider a process in which a ligand (L) binds reversibly to a site in the protein (P)



- The kinetics of such a process is described by:
  - ▲ the association rate constant k_a
  - ▲ the dissociation rate constant k_d

$$P + L \underset{k_d}{\overset{k_a}{\rightleftharpoons}} PL$$

- After some time, the process will reach the equilibrium where the association and dissociation rates are equal

$$k_a[P]\cdot[L] = k_d[PL]$$

- The equilibrium composition is characterized by the the association constant K_a

大寫 K

$$K_a = \frac{[PL]}{[P]\cdot[L]} = \frac{k_a}{k_d}$$

定義

- Interaction strength can be expressed as:
  - association constant K_a, units M^{-1}
  - dissociation constant K_d, units M, $K_d = 1/K_a$
  - interaction (binding) free energy ΔG^o, units: kJ/mol
- Definitions:

  $K_a = [PL]/[P][L]$ $\qquad$ $K_d = [P][L]/[PL]$

  $\Delta G^o = \Delta H^o - T\Delta S^o$: enthalpy and entropy
- Relationships:

  $\Delta G^o = -RT \ln K_a = RT \ln K_d$ $\qquad$ (RT at 25 ^oC is 2.48 kJ/mol)
- Magnitudes
  - Strong binding: $K_d < 10$ nM (10^{-9})
  - Weak binding: $K_d > 10$ μM (10^{-6})

Analysis in terms of the bound fraction

- In practice, we can often determine the fraction (θ) of occupied binding sites

$$K_a = \frac{[\mathrm{PL}]}{[\mathrm{P}]\cdot[\mathrm{L}]}$$

$$\theta = \frac{\text{binding sites occupied}}{\text{total binding sites}} = \frac{[\mathrm{PL}]}{[\mathrm{PL}]+[\mathrm{P}]}$$

- Substituting [PL] with K_a[L][P], we'll eliminate [PL]

$$\theta = \frac{K_a[\mathrm{L}][\mathrm{P}]}{K_a[\mathrm{L}][\mathrm{P}]+[\mathrm{P}]}$$

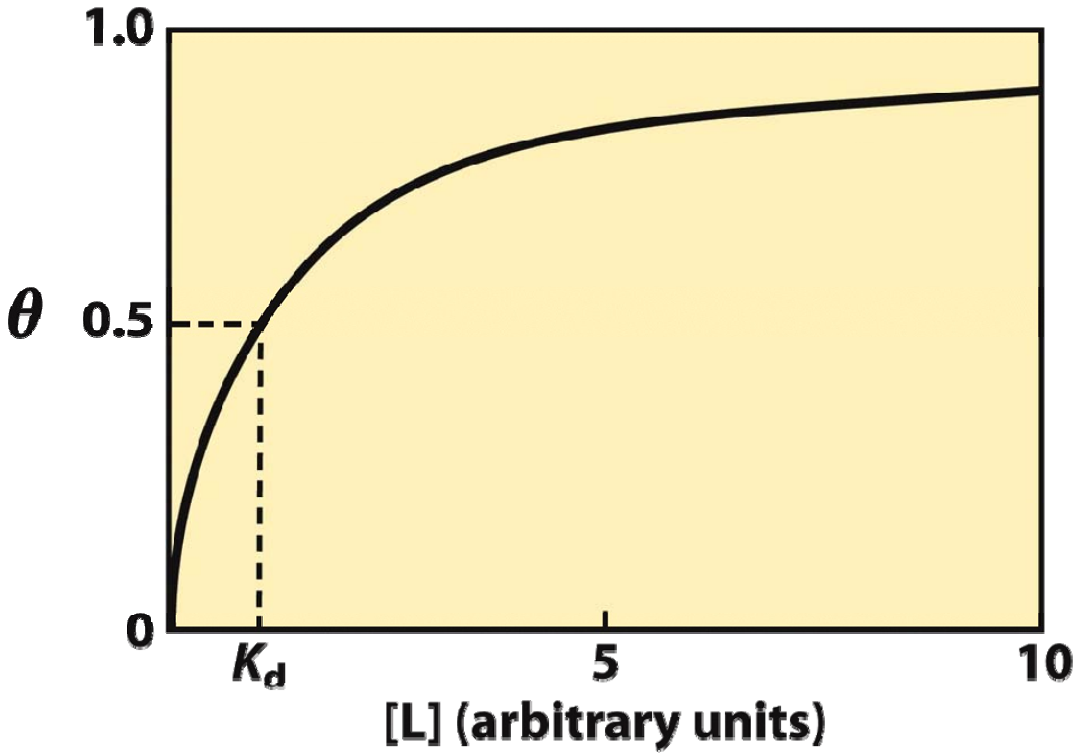
- Eliminating [P] and rearranging gives the result in terms of equilibrium association constant:

$$\theta = \frac{[\mathrm{L}]}{[\mathrm{L}]+\frac{1}{K_a}}$$

- In terms of the more commonly used equilibrium dissociation constant:

$$\theta = \frac{[\mathrm{L}]}{[\mathrm{L}]+K_d}$$

The fraction of occupied binding sites depends on the free ligand concentration and K_d



$$\theta = \frac{[L]}{[L] + \frac{1}{K_a}}$$

$$\theta = \frac{[L]}{[L] + K_d}$$

以 θ = 0.5 帶入公式

$$[L] = K_d$$

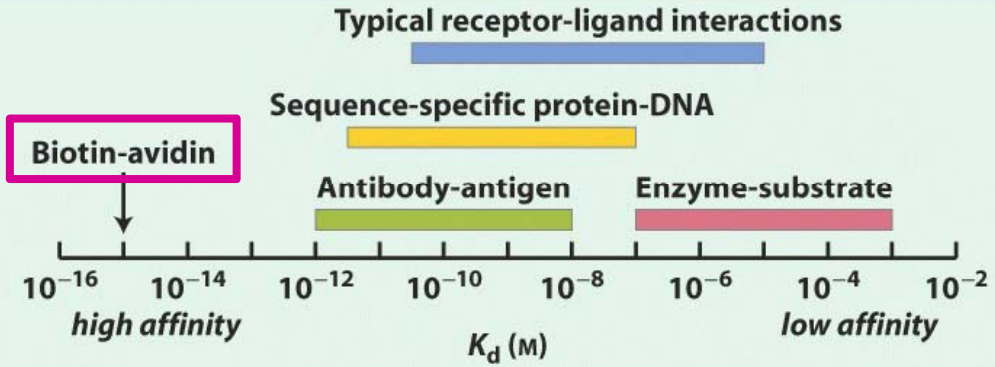
- K_d can be determined graphically or via least-squares regression
- The [L] at which half of the available ligand-binding sites are occupied (that is, θ = 0.5) corresponds to $1/K_a$, or K_d
- The curve has a horizontal asymptote at θ = 1 and a vertical asymptote at [L] = $-1/K_a$.

K_d

- K_d is the equilibrium constant for the release of ligand
- In practice, K_d is used much more often than K_a to express the affinity of a protein for a ligand.
- Kd is equivalent to the molar concentration of ligand at which half of available ligand-binding sites are occupied. At this point, the protein is said to have reached half-saturation with respect to ligand binding.
- The more tightly a protein binds a ligand, the lower the concentration of ligand required for half the binding sites to be occupied, and thus lower the value of K_d.
- Note that a lower value of K_d corresponds to a higher affinity of ligand for the protein.

TABLE 5–1 Some Protein Dissociation Constants

Protein	Ligand	K_d (M)*
* Avidin (egg white)†	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)‡	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)§	Ca^{2+}	3×10^{-6}
		2×10^{-5}



The range of dissociation constants for interactions in biological systems. Colors denote the range for each class of interaction. A few interactions, such as that between the protein avidin and the enzyme cofactor biotin, fall outside the normal ranges. The avidin-biotin interaction is so tight it may be considered irreversible. Sequence-specific protein-DNA interactions reflect proteins that bind to a particular sequence of nucleotides in DNA, as opposed to general binding to any DNA site.

*A reported dissociation constant is valid only for the particular solution conditions under which it was measured. K_d values for a protein-ligand interaction can be altered, sometimes by several orders of magnitude, by changes in the solution's salt concentration, pH, or other variables.

†This immunoglobulin was isolated as part of an effort to develop a vaccine against HIV. Immunoglobulins (described later in the chapter) are highly variable, and the K_d reported here should not be considered characteristic of all immunoglobulins.

‡Calmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

Cooperative ligand binding can be described quantitatively

- For a protein with *n* binding sites, the kinetics of protein-ligand interaction can be described as

$$\mathrm{P} + n\mathrm{L} \rightleftharpoons \mathrm{PL}_n \qquad K_a = \frac{[\mathrm{PL}_n]}{[\mathrm{P}][\mathrm{L}]^n} \qquad \theta = \frac{[\mathrm{L}]^n}{[\mathrm{L}]^n + K_d}$$

- Rearranging, then taking the log of both sides, yields

$$\frac{\theta}{1-\theta} = \frac{[\mathrm{L}]^n}{K_d}$$

$$\log\left(\frac{\theta}{1-\theta}\right) = n\log[\mathrm{L}] - \log K_d$$

- The **Hill equation**, and a plot of log [θ/(1 – θ)] versus log [L] is called a **Hill plot**.

Adapt the Hill equation to the binding of oxygen to hemoglobin

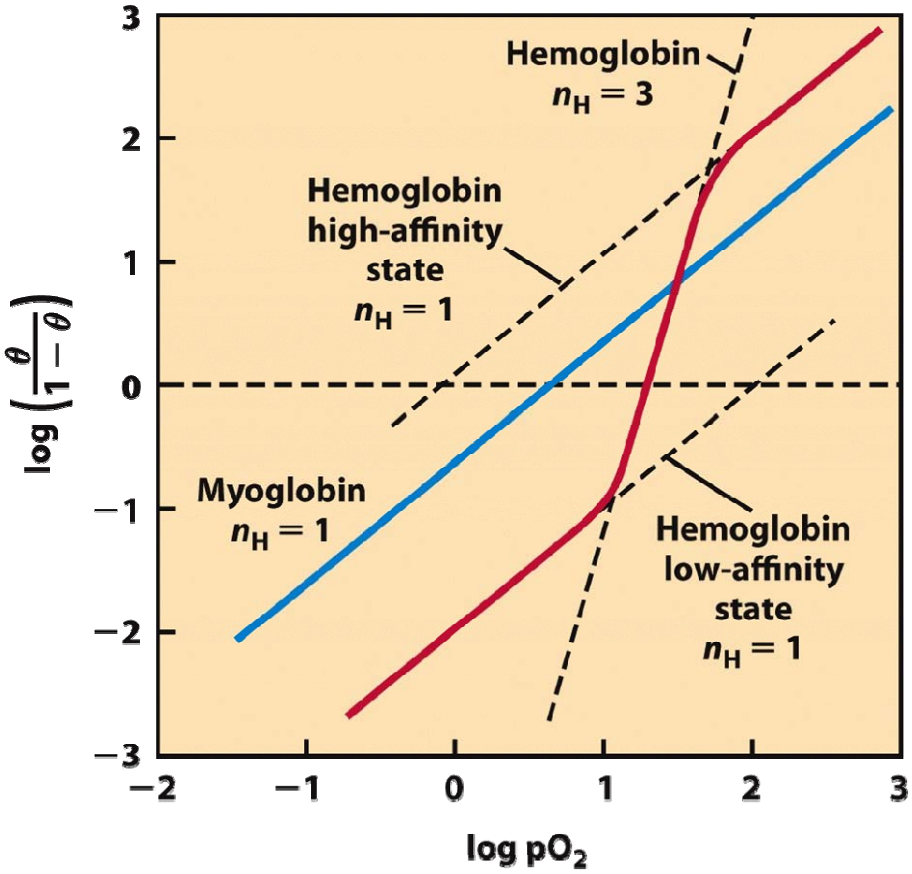
- **Based on the Hill equation, the Hill plot should have a slope of *n*. However, the experimentally determined slope actually reflects not the number of binding sites but the degree of interaction between them. The slope of a Hill plot is therefore denoted by n_H, the Hill coefficient, which is a measure of the degree of cooperativity.**

- **To adapt the Hill equation to the binding of oxygen to hemoglobin we must again substitute pO_2 for [L] and $P_{50}{}^n$ for K_d:**

$$\log\left(\frac{\theta}{1-\theta}\right) = n\log[\mathrm{L}] - \log K_d \qquad \textbf{Hill equation}$$

$$\log\left(\frac{\theta}{1-\theta}\right) = n\log \mathrm{pO}_2 - n\log P_{50}$$

Hill plots for oxygen binding to myoglobin and hemoglobin

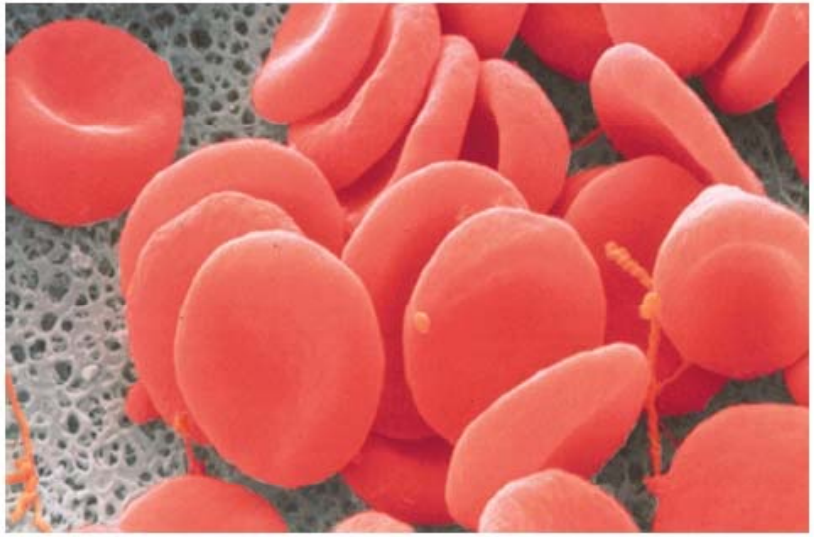


$$\log\left(\frac{\theta}{1-\theta}\right) = n\log \mathrm{pO}_2 - n\log P_{50}$$

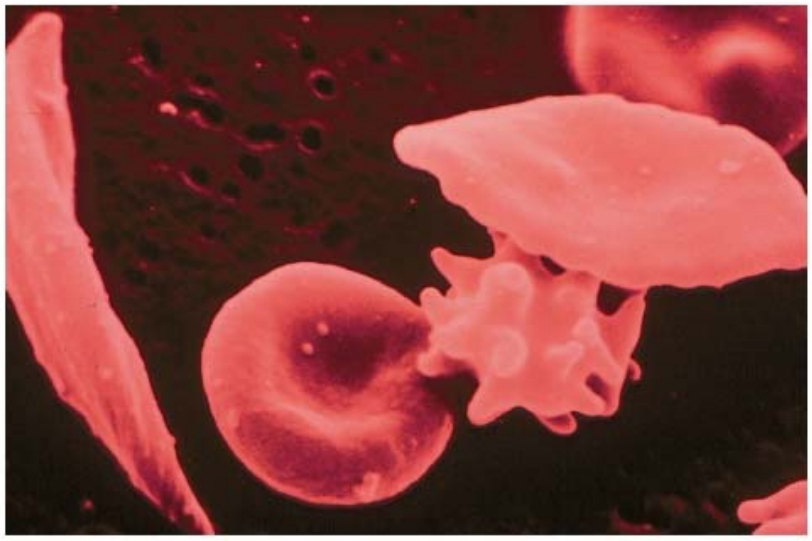
Cooperative binding of oxygen by hemoglobin was first analyzed by Archibald Hill in 1910

When $n_H = 1$, there is no evident cooperativity. The maximum degree of cooperativity observed for hemoglobin corresponds approximately to $n_H = 3$. Note that while this indicates a high level of cooperativity, n_H is less than n. This is normal for a protein that exhibits allosteric binding behavior.

Sickle-cell anemia ("lack of blood")



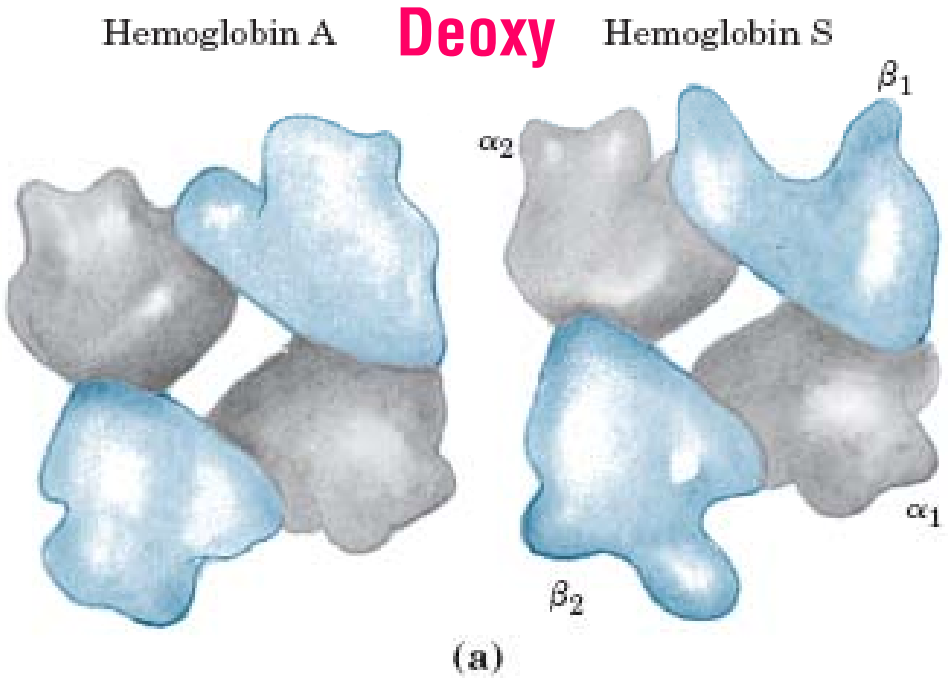
(a)



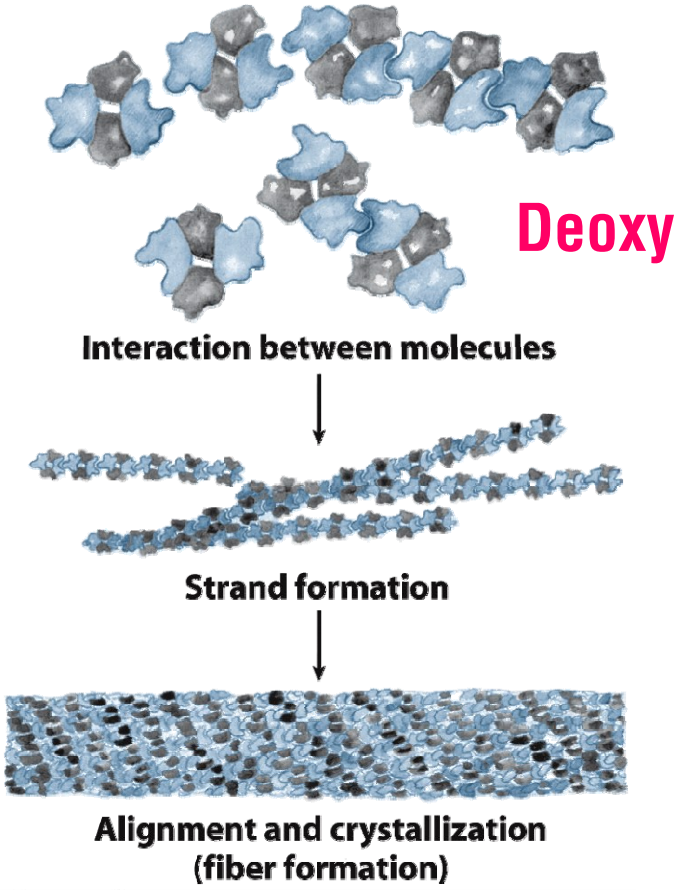
(b)

- A genetic disease in which an individual has inherited the allele for sickle-cell hemoglobin from both parents. The erythrocytes of these individuals are fewer and also abnormal.
- Long, thin, crescent-shaped erythrocytes that look like the blade of a sickle.
- Sickle-cell trait (heterozygous): about 1% of erythrocytes become sickled on deoxy.
- Frequency of the sickle-cell allele in populations is unusually high in certain parts of Africa. Investigation into this matter led to the finding that in heterozygous individuals, the allele confers a small but significant resistance to lethal forms of malaria.

Normal and sickle-cell hemoglobin

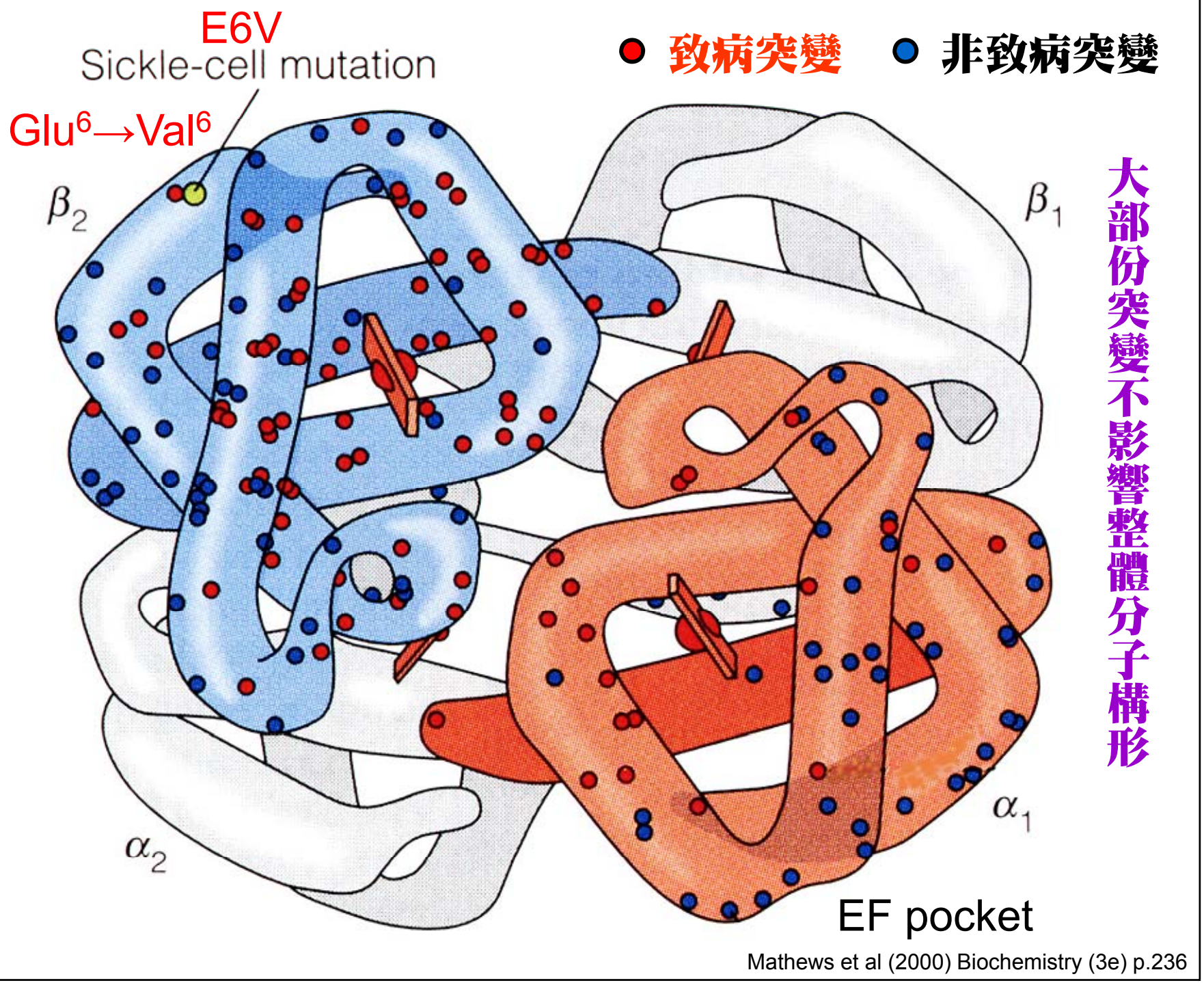


- The altered properties of hemoglobin S result from a single amino acid substitution, E6V in two β subunits.
- Val creates a "sticky" hydrophobic contact point at position 6 of the β chain, which is on the outer surface of the molecule.



- Deoxyhemoglobin S has a hydrophobic patch on its surface, which causes the molecules to aggregate into strands that align into insoluble fibers

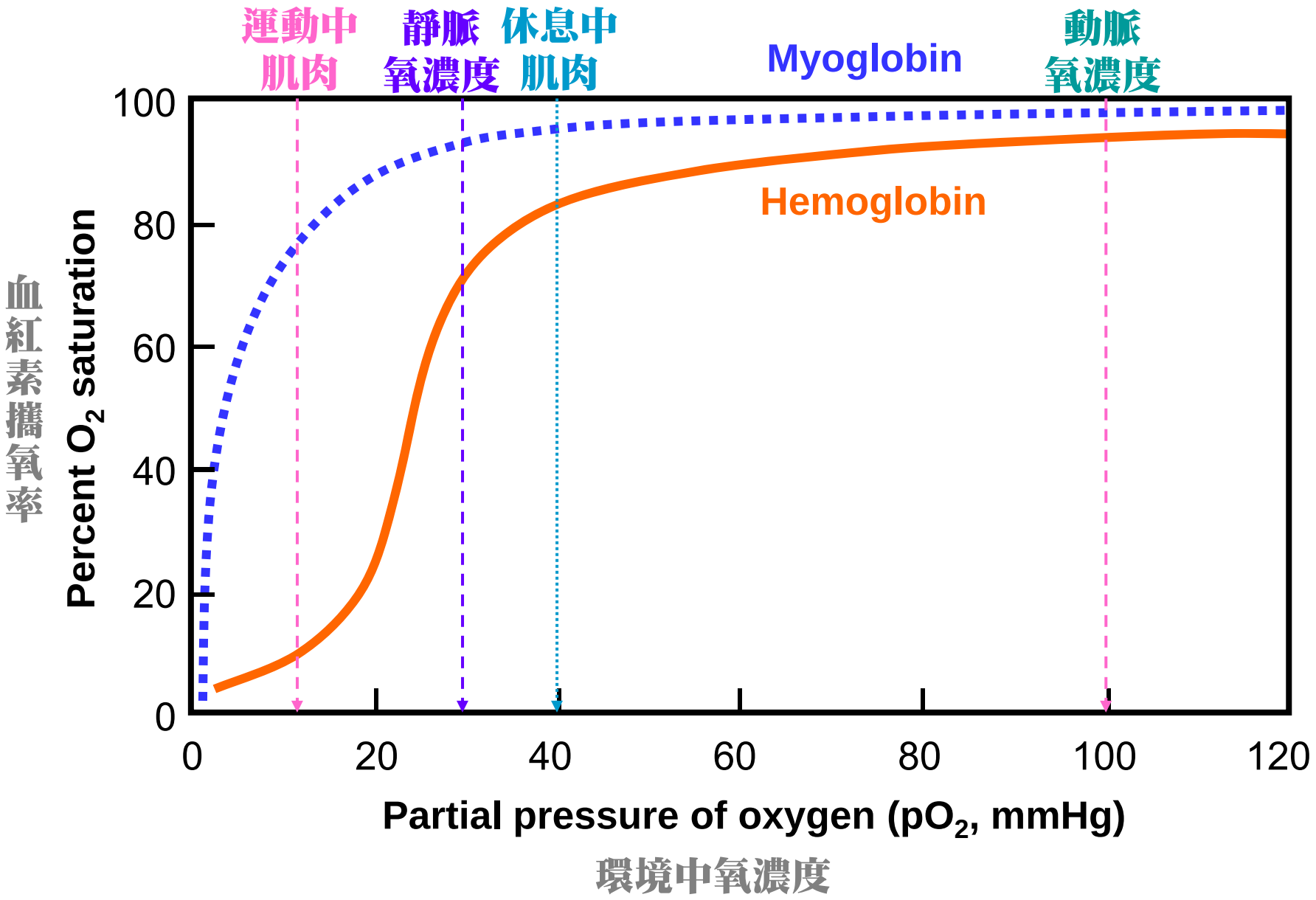
鐮形血球症只有一個胺基酸改變



大部份突變不影響整體分子構形

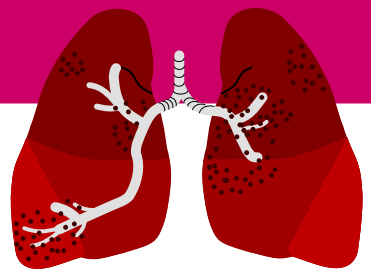
Mathews et al (2000) Biochemistry (3e) p.236

血紅素的攜氧率受環境氧濃度影響



Adapted from Garrett & Grisham (1999) Biochemistry (2e) p.480

血中氧分子的運送



任何一個次體接受氧分子後，會增進其它次體吸附氧分子的能力

氧分子

Lung

(R)
relaxed state

動 脈

環境氧濃度低時
Hb 迅速釋出氧分子

Muscle

Myoglobin

靜 脈

(T)
tense state

環境氧濃度高時
Hb 快速吸收氧分子

Hemoglobin