ENZYMES

THE AMAZING CATALYSTS

Chanati Jantrachotechatchawan (Book)

Outline for Today

- What is an enzyme? How important?
- Basic Chemistry Review
  - Thermodynamics
    - Gibb’s free energy
    - Reaction energy diagram, chemical equilibrium
  - Acidity
  - Bonding & Interaction
  - Protein & Amino acid

What is an ENZYME ?!

- Biological Catalyst
- Catalyst (positive): Speed up the reaction, but not consumed by the reaction.

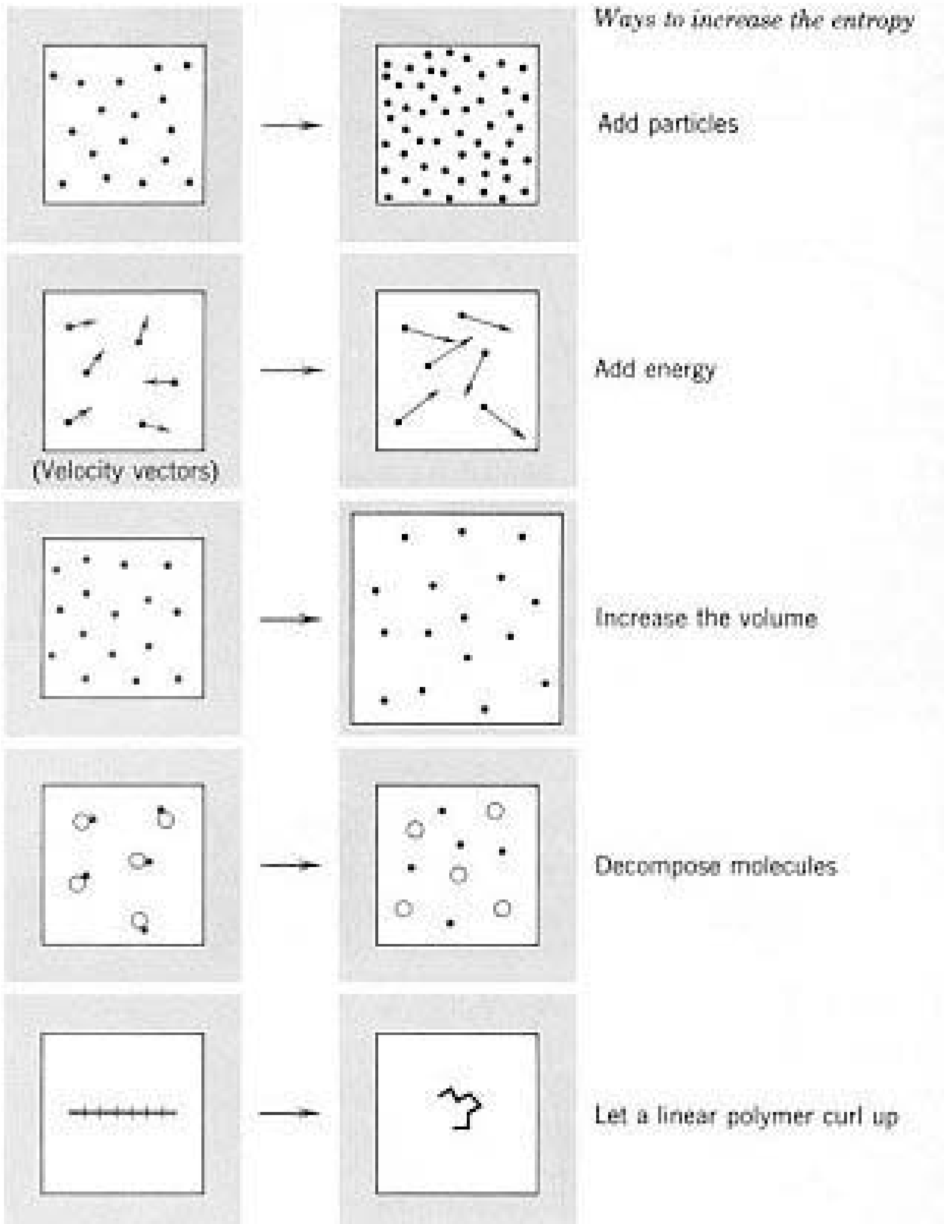
- It is “IN YEAST” ! ~ *ενζυμον*

Lactobacillus kimchii

- Most of the mechanisms inside the cells involve enzymes.

Thermodynamics

- 0^{th} : Thermal Equilibrium
- 1^{st} : Conservation of Energy
- 2^{nd} : Entropy cannot decrease
- 3^{rd} : Absolute Zero !

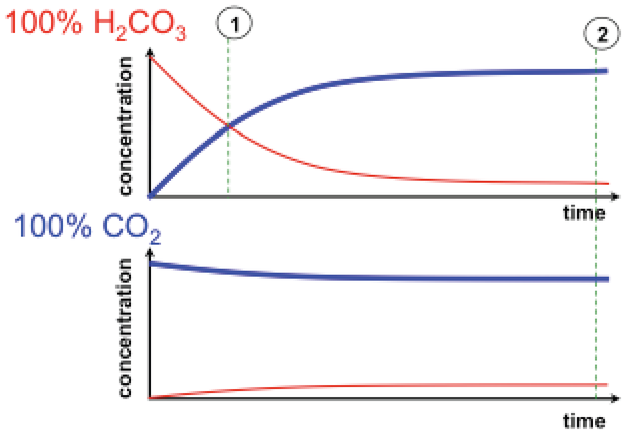


Factors that tend to increase the entropy of a system.

The position of a chemical equilibrium is independent of the initial state

$$CO_2\ (aq.) + H_2O\ (l) \rightleftharpoons H_2CO_3\ (aq.)$$

$K_{eq} = 3.09 \times 10^{-5}\ M^{-1}$



① State 1

$[H_2CO_3]/[CO_2] = 1 \neq K_{eq}[H_2O]$

② State 2 (equilibrium)

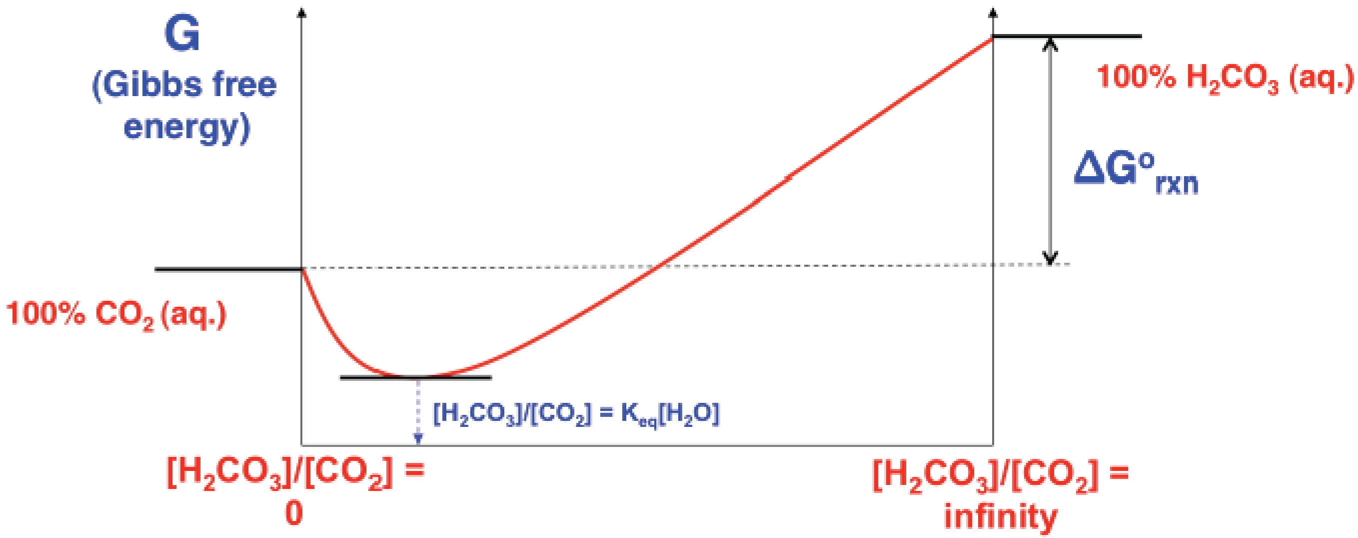
$[H_2CO_3]/[CO_2] = K_{eq}[H_2O]$

Equilibrium is reached when the ratio of concentrations of products to reactants stop changing.

What determines where the equilibrium lies?

All systems tend towards a state of lower Gibbs free energy

$$CO_2\,(aq.) \quad + \quad H_2O\,(l) \quad \rightleftharpoons \quad H_2CO_3\,(aq.)$$



There is an equilibrium state containing a mixture of CO_2 and H_2CO_3 that has the lowest Gibbs free energy.

There is a quantitative relationship between $\Delta G°_{rxn}$ and K_{eq}

It comes from the eqn that describes the slope of the red line (ΔG) associated with each state of products (C,D) and reactants (A,B)

$$A + B \rightleftharpoons C + D$$

$$\Delta G = \Delta G°_{rxn} + RT \ln \frac{[C][D]}{[A][B]}$$

How far we are from equilibrium at a particular state…

… equals the energy at a defined equilibrium state plus the energy of mixing at the particular state

At equilibrium, $0 = \Delta G°_{rxn} + RT \ln K_{eq}$

…and, therefore, $\Delta G°_{rxn} = -RT \ln K_{eq}$

$\Delta G°_{rxn}$ is just K_{eq} in units of energy

ΔG (Gibbs Free Energy)

Gibbs free energy (ΔG) is a measure of the favorability of a reaction.

ΔG is a composite of enthalpy ΔH (heat) and entropy ΔS (disorder).

Reactions proceed in the direction that causes the free energy to *decrease* ($\Delta G < 0$).

Reactions can only occur between parts of molecules that are capable of forming bonds

(you need electrons to make bonds and they are in restricted places – orbitals)

Even if the energy of the products is lower than the energy of the reactants there is a *barrier to reaction* that requires additional energy to get over

Rate of reaction = Collision frequency X Probability that molecules collide in the right orientation X Probability that molecules collide with enough energy to react

Collision frequency = [SM] depends on pressure X Molecular velocity depends on temperature X Reaction cross-section depends on size and shape

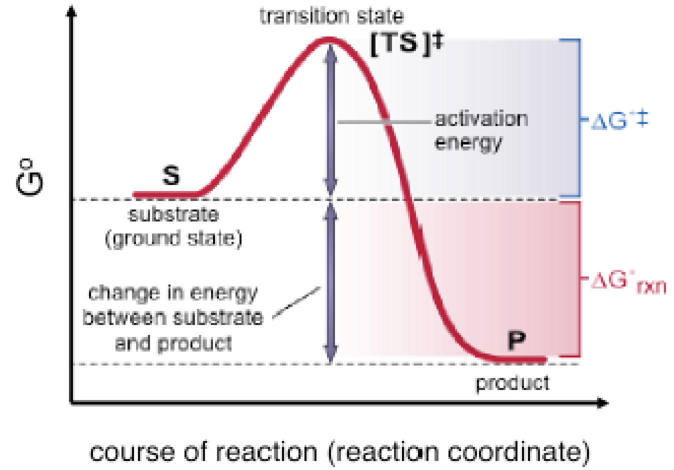
There are two ways to represent the reaction pathway: a molecular mechanism and a reaction-coordinate diagram

Reaction energy diagrams describe the energy landscape as a reaction proceeds

reactants (substrates) S $\longrightarrow$ $[TS]^{\ddagger}$ $\longrightarrow$ P products

transition state

- Reaction energy diagrams depict changes in G° as one substrate molecule becomes one product molecule
- X-axis: the *reaction coordinate*
- Y-axis: *free energy under defined conditions* (does not change with concentration of S or P)



At the transition state, bond breaking/making occurs with rearrangement of valence electrons

The difference between a fast reaction and a slow reaction depends on the magnitude of $\Delta G^{\ddagger}$

$$S_1 + S_2 \longrightarrow [TS]^{\ddagger} \longrightarrow P$$

For this reaction, the rate = $k\,[S_1][S_2]$

k (the ***rate constant***) tell you some thing about how quickly a given substrate becomes product. K_{eq} says which is more stable

k is related to $\Delta G^{\ddagger}$; $k = e^{-\Delta G^{\ddagger}/RT}$

It follows that the larger the $\Delta G^{\ddagger}$ the smaller the rate constant (k) and the slower the reaction

To increase the rate of a reaction, you need to change (lower) the barrier to reaction ($\Delta G^{\ddagger}$)

The sum of all the acids and bases dissolved in a solution is defined as the pH

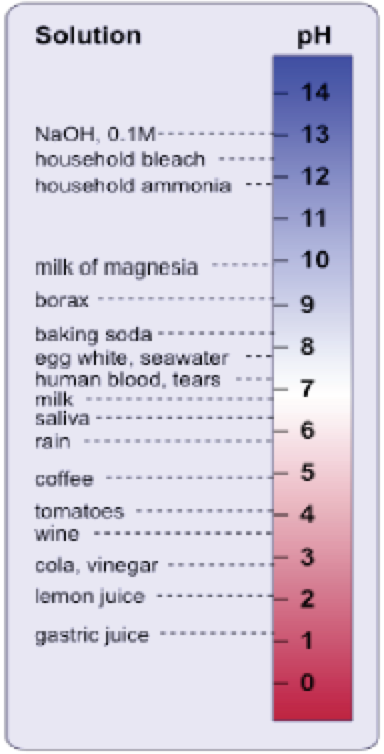
$$AH \rightleftharpoons A^- + H^+$$

(*e.g.* H_2CO_3) (*e.g.* HCO_3) (e.g. H_3O^+)

$pH = -\log [H^+]$

(In water, $pH = -\log [H_3O^+]$)

- The lower the pH, the higher the $[H^+]$, indicating a more acidic solution
- Each pH unit represents a 10-fold change in $[H^+]$



pH refers to a solution…like in the cell

The concentration of H_2O doesn't change significantly during ionization…

$$H_2CO_3\ (aq.) + H_2O\ (l) \rightleftharpoons H_3O^+\ (aq.) + HCO_3^-\ (aq.)$$

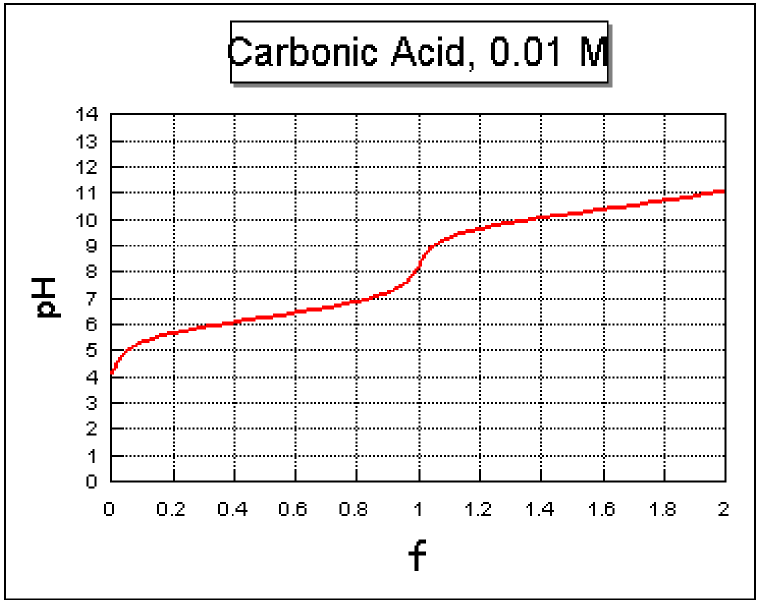
$$K_{eq} = \frac{[H_3O^+(aq.)][HCO_3^-(aq.)]}{[H_2CO_3(aq.)][H_2O(l)]} = 4.46 \times 10^{-6}$$

$$K_a = K_{eq}[H_2O(l)] = \frac{[H_3O^+(aq.)][HCO_3^-(aq.)]}{[H_2CO_3(aq.)]} = 2.5 \times 10^{-4}\ M$$

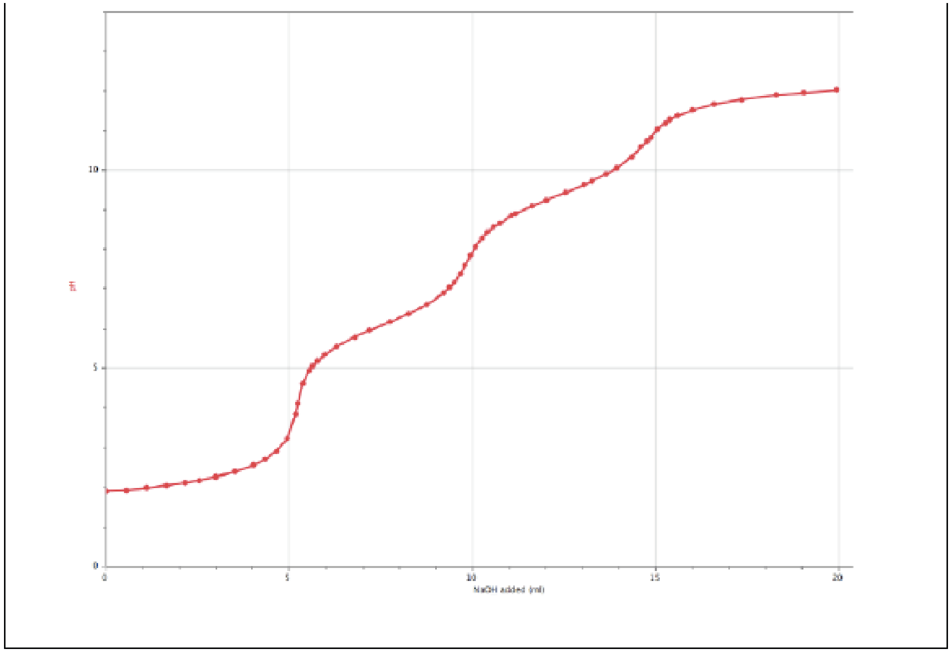
In general,

$$K_a = \frac{[H_3O^+(aq.)][A^-(aq.)]}{[HA(aq.)]}$$

The acid dissociation equilibrium constant (K_a), tells us how strong an acidic proton in a given molecule is… the larger the K_a the stronger the acid


Carbonic Acid, 0.01 M
pH
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
f

Histidine!


10
5
0
0
5
10
15
20

Le Chatelier's Principle in action: the dissociation constant favors reactants but…

Dissolution

$$CO_2\ (g) \rightleftharpoons CO_2\ (aq.)$$

$K_{eq} = 0.8317$

Reaction

$$CO_2\ (aq.) + H_2O\ (l) \rightleftharpoons H_2CO_3\ (aq.)$$

$K_{eq} = 3.09 \times 10^{-5}\ M^{-1}$

Acid-Base

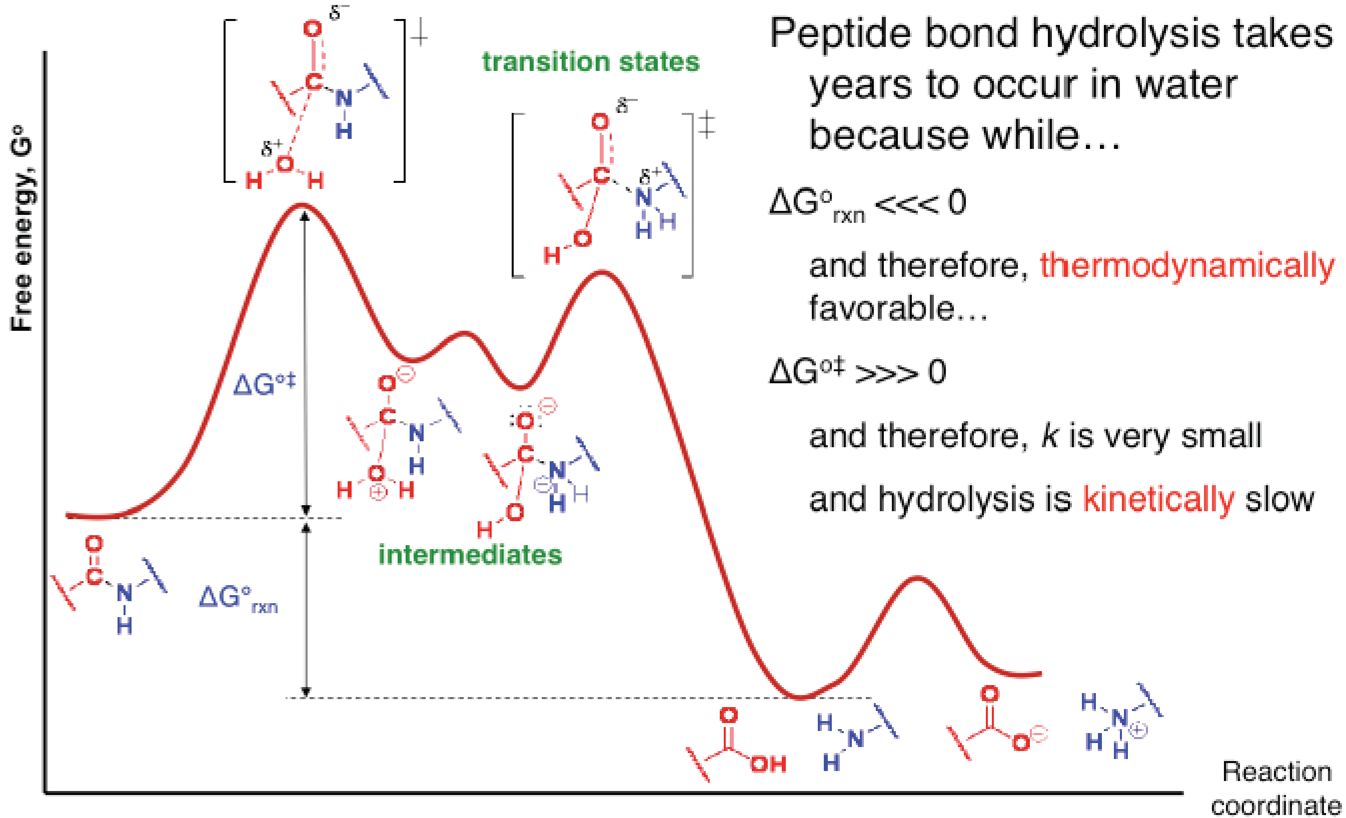
$$H_2CO_3\ (aq.) + H_2O\ (l) \rightleftharpoons H_3O^+\ (aq.) + HCO_3^-\ (aq.)$$

$K_{eq} = 4.46 \times 10^{-6}$ / $K_a = 2.5 \times 10^{-4}$

$[H_2CO_3]/[HCO_3^-] = 1{:}6300$ (pH 7.4)

…the reaction favors formation of HCO_3^- at cellular pH

Energy diagram for peptide bond hydrolysis



Peptide bond hydrolysis takes years to occur in water because while…

$\Delta G^{o}_{rxn} \lll 0$

and therefore, thermodynamically favorable…

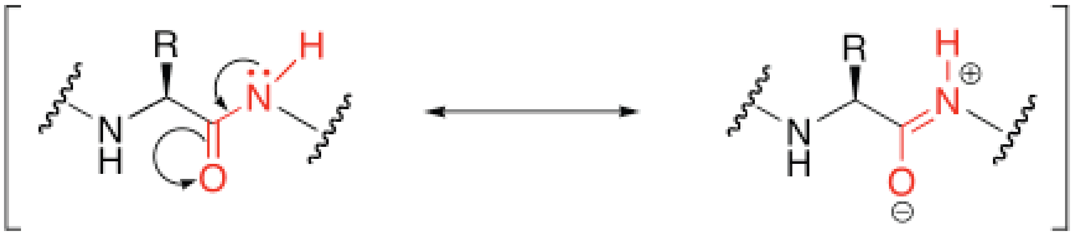
$\Delta G^{o\ddagger} \ggg 0$

and therefore, *k* is very small

and hydrolysis is kinetically slow

Peptide bonds have "partial" double bonds

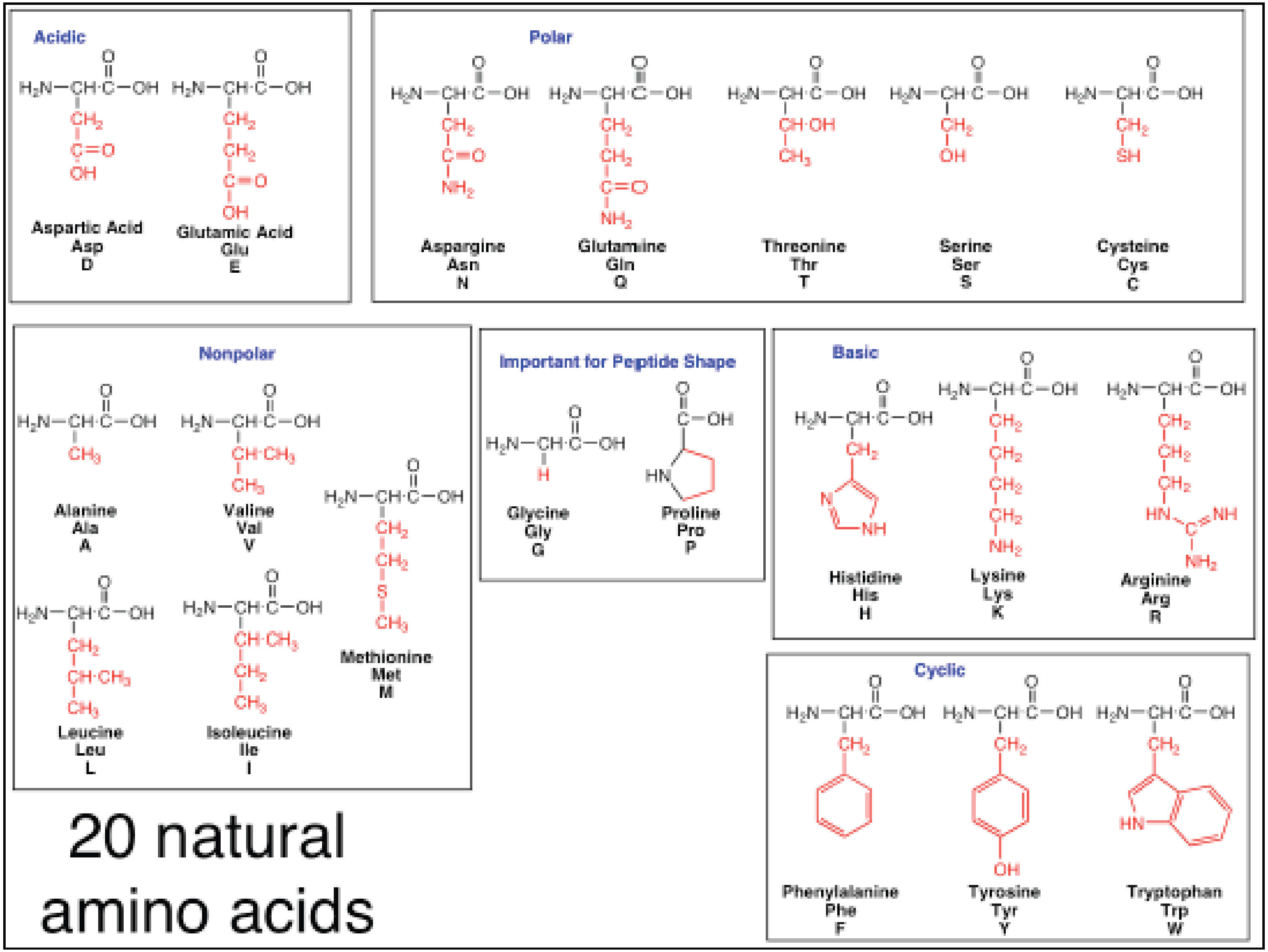
Due to differences in electronegativity, there is a tendency for the nitrogen to want to share its lone pair of electrons with the electro-positive carbon causing ***resonance stabilization***



60% 40%

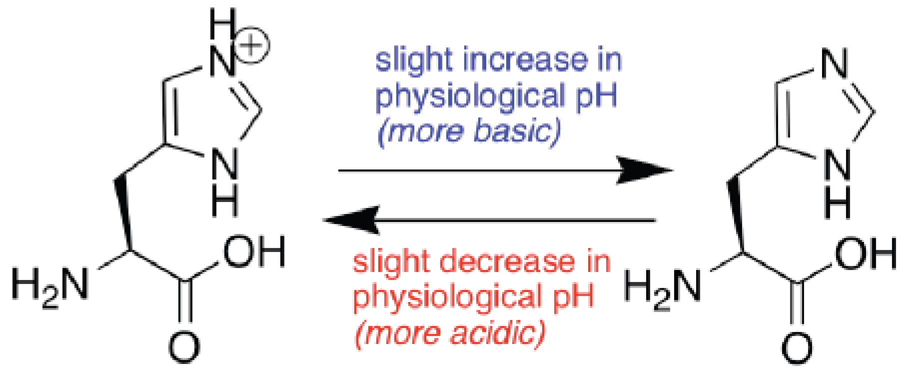
- We call these structures *resonance* structures
- Resonance structures have identical positions of all atoms, but the position of electrons differ.
- Resonance structures are drawn using DOUBLE-HEADED arrows.

These are not discrete structures (60/40 mixture), just a representation of the probable distribution of electrons



20 natural amino acids

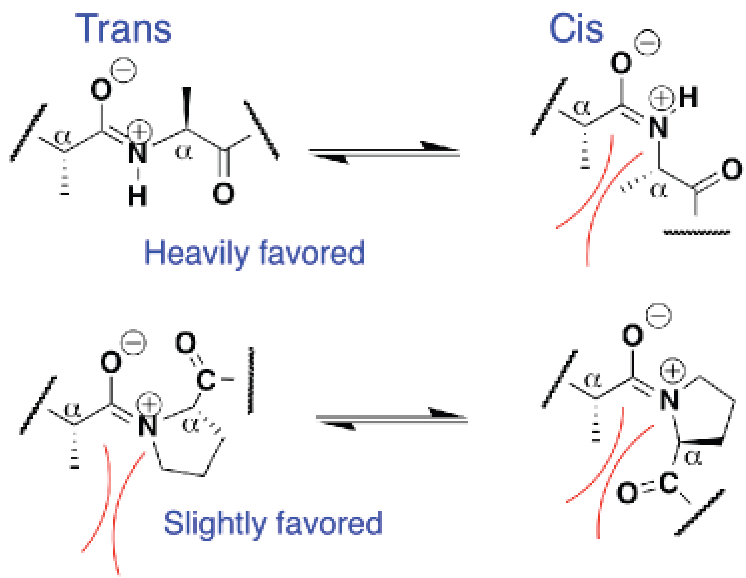
At physiological pH histidine is partially (10-50%) protonated



Histidine can act as both an acid and a base at physiological pH (it can encompass being more than one thing at the same time -- a true intellectual)

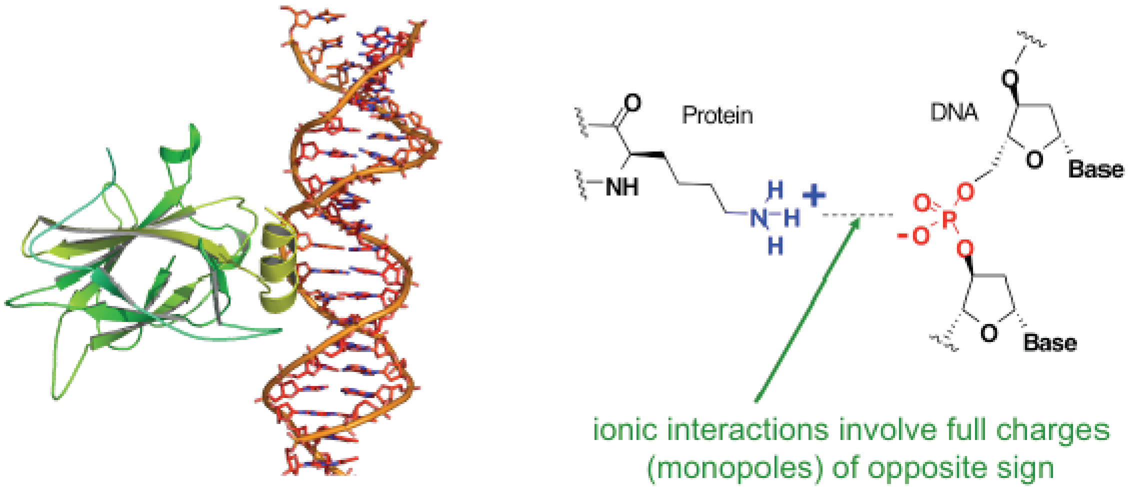
For 19 amino acids, the trans geometry around the peptide bond is favored

In proline, the *trans* isomer is only slightly favored over the *cis* isomer. Thus, proline can readily adopt the *cis* conformation.



Proline is the contrarian

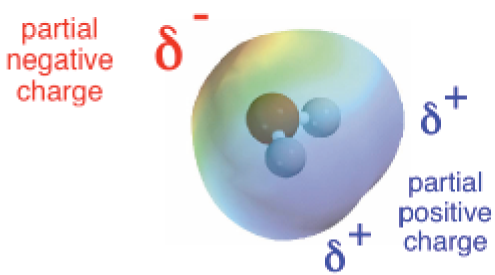
The strongest intermolecular forces are between ions of opposite charge.

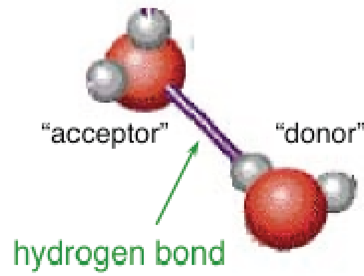


Ionic bonds (charge-charge interactions) between macromolecules in a cell mediate many important interactions in living systems

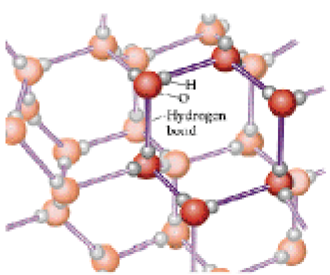
The strength of any ionic interaction depends heavily on the environment…

Polar bonds allow molecules to stick like mini magnets (e.g. a hydrogen bond)





(~0.2 to 10 kcal/mol)

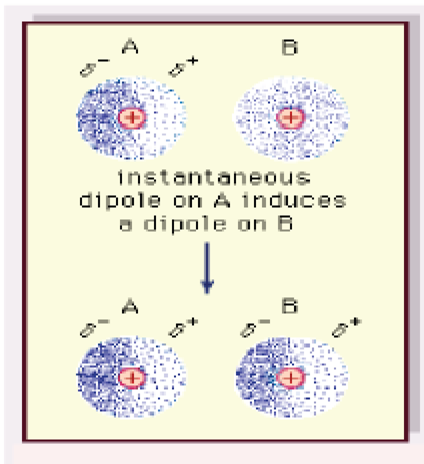


N 3.0	O 3.5	F 4.0
		Cl 3.0

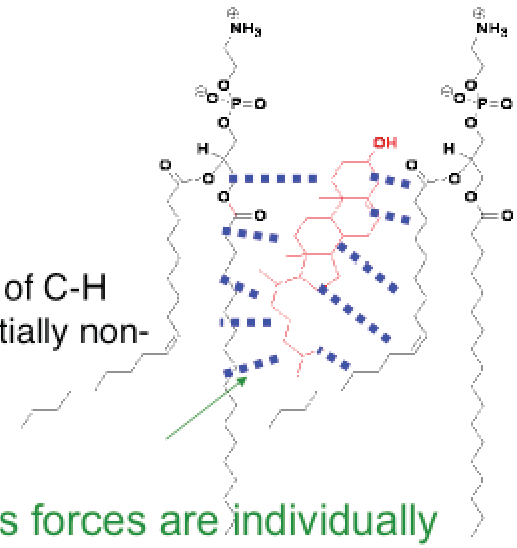
Intermolecular hydrogen bonding arises from the electrostatic attraction between partial positive and partial negative charges; H-bonds are directional

Even molecules with only non-polar bonds can stick to other molecules

An electron cloud can become unevenly distributed when it comes in contact with a polar molecule.



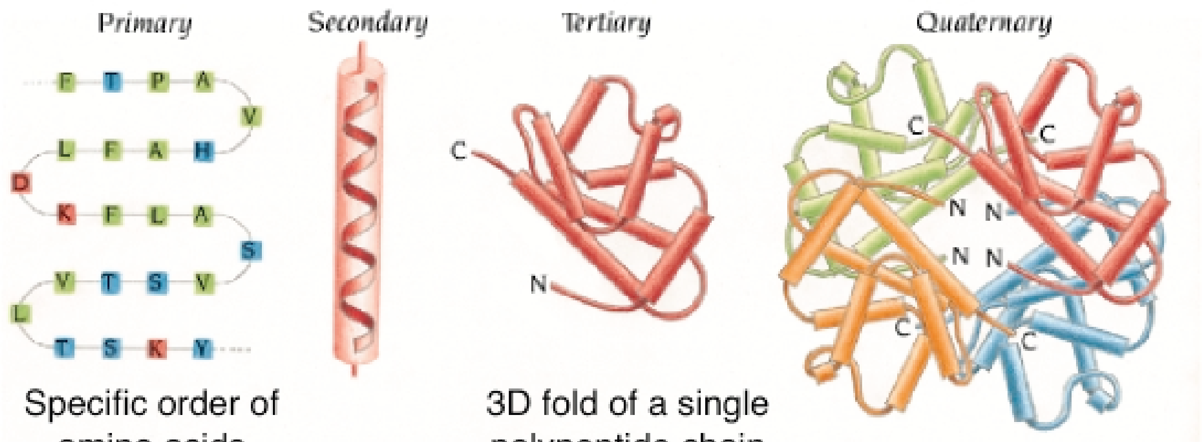
Molecules made of C-H bonds are essentially non-polar



Van der Waals forces are individually weak (~0.001 to 0.005 kcal/mol)…

…but over large surfaces, Van der Waals forces can sum to produce strong associations.

Four levels of protein structure



Specific order of amino acids

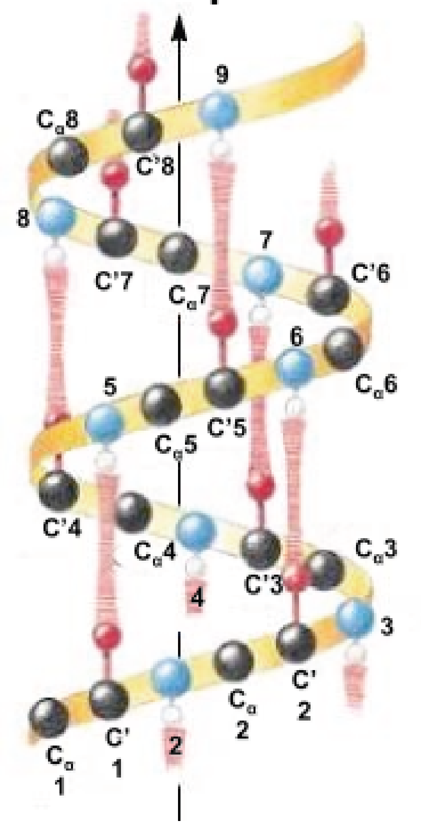
Local structure of stretch of AA's

3D fold of a single polypeptide chain

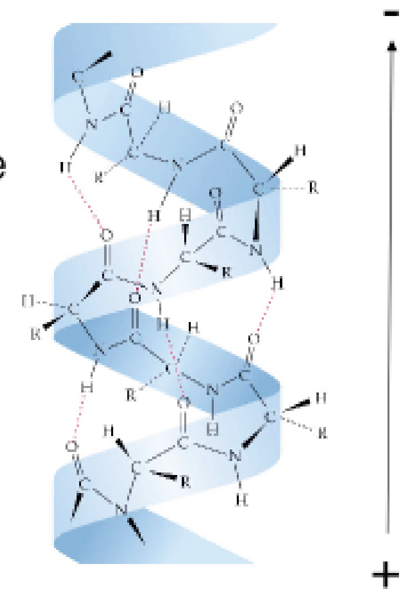
Higher order complex of polypeptide chains

The folded structure of a protein determines its function

In α-helices, adjacent N-H groups (blue) point in the same direction…

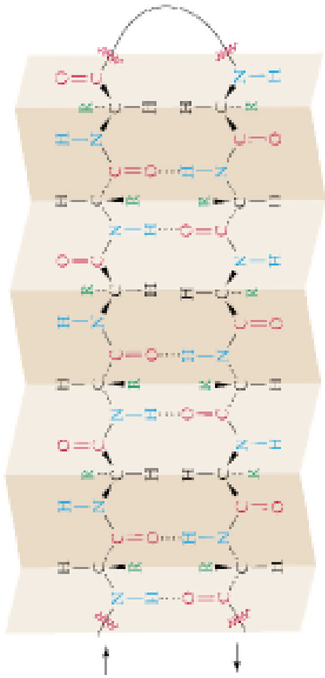


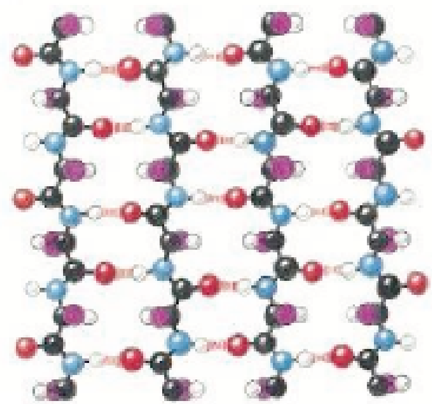
…and because of this arrangement of atoms, helices have a macrodipole

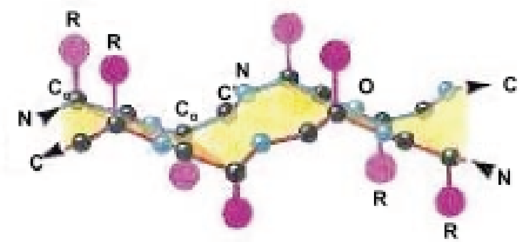


Side chains of AA's (R groups) point away from the helix

In β-sheets, adjacent N-H groups point in opposite directions



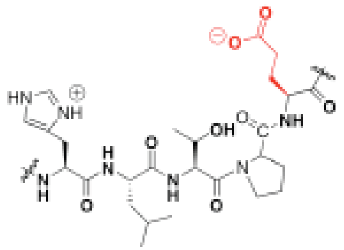




In β-sheets, there is no opportunity to form hydrogen bonds within one strand

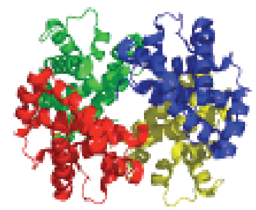
Why do some sequences form α-helices and others form β-sheets?

Small changes at the amino acid level can affect structure: sickle cell anemia

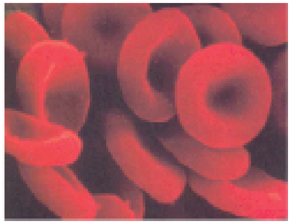


His Leu Thr Pro Glu

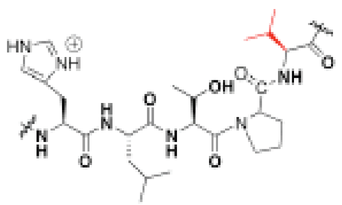
Hemoglobin:
(Glutamate at 6 position)



helical, globular structure
that forms a tetramer

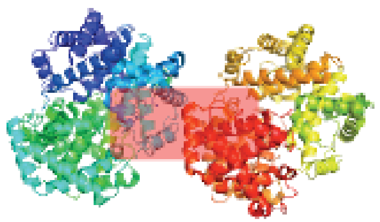


normal red blood cells

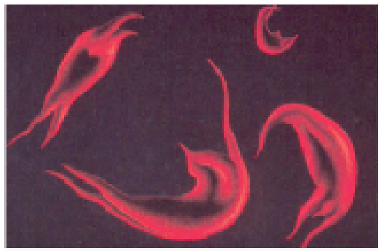


His Leu Thr Pro Val

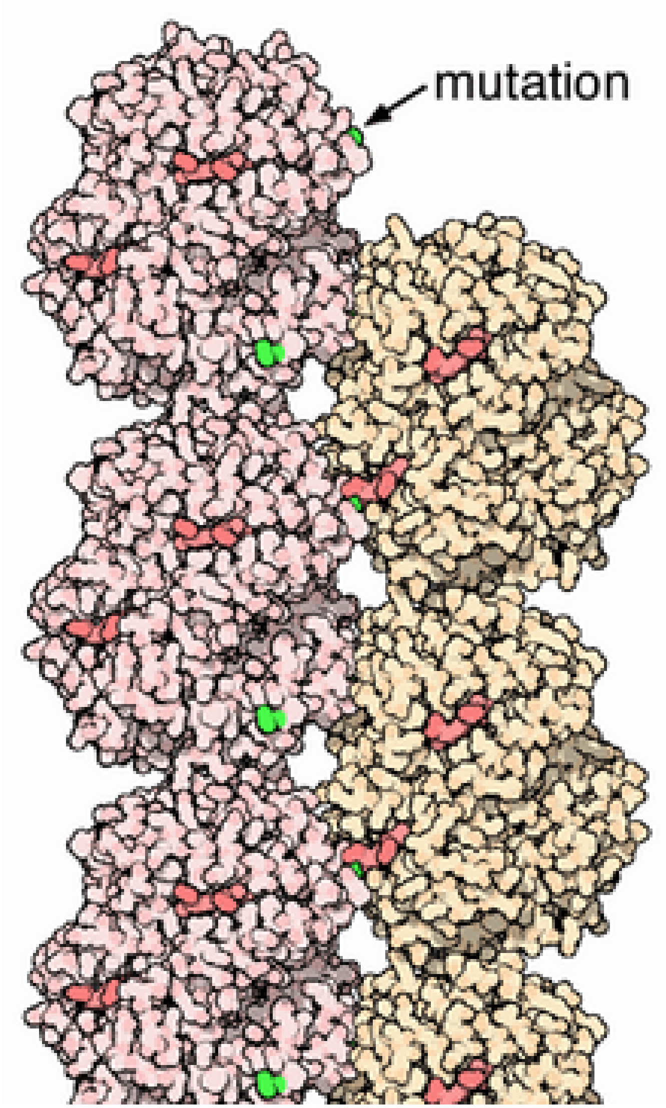
Sickle -Hemoglobin:
Valine at 6 position

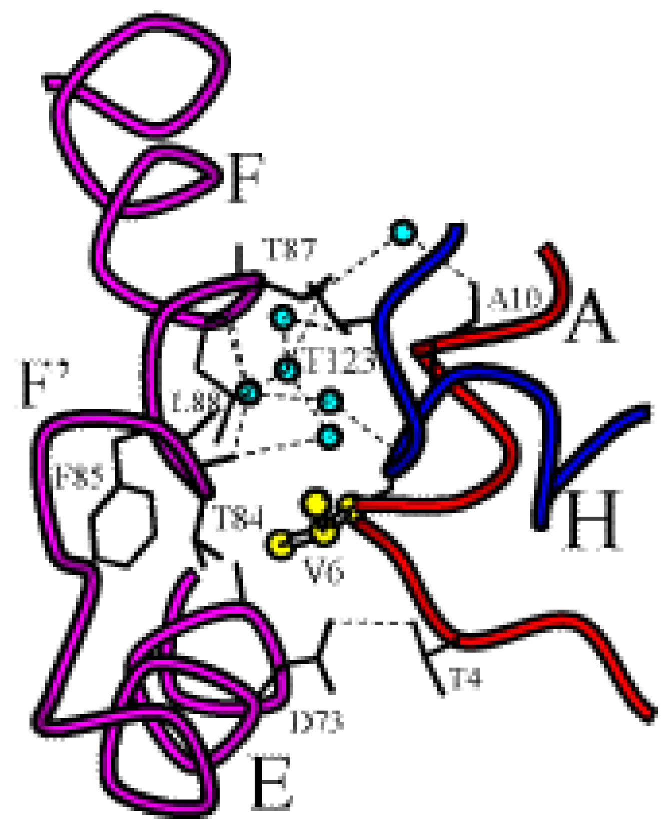


protein clumps together



sickle cell red blood cells


mutation


F
T87
A10
A
F'
T123
L88
F85
T84
V6
H
T4
D73
E

- Great Thanks to Life Science 1a course website
- See you guys next week

  (^_^)