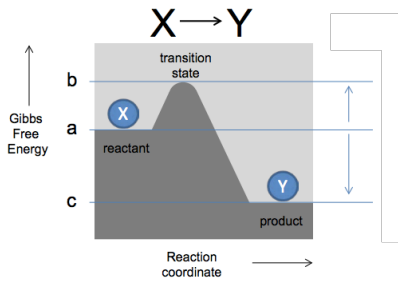
L1 – REACTIONS IN THE CELL

GIBBS FREE ENERGY (G)

- Energy of the reaction that is available to do work – also called 'available energy'



$$\Delta G = G_{products} - G_{reactants}$$

$$\Delta G = c\text{-}a$$

- Activation Energy Barrier (ΔG ‡) needs to be overcome before the reaction can progress

$$\Delta G^{\ddagger} = b - a$$

- Gibbs Free Energy takes into account enthalpy (heat) and entropy (disorder)
- ΔG is the change in Gibbs Free Energy: (where T is temperature in K)

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

Change in Gibbs free Energy	Change in enthalpy (heat)	Change in entropy (disorder)

SPONTANEOUS REACTIONS

- For spontaneous reactions, ΔG is negative; the Gibbs free energy of the products is lower than that of the reactants
- Negative ΔG can be given by either:
  - Large negative ΔH (exothermic)
  - Large positive ΔS (increased entropy)
- NB: T is always positive

Terms to describe reactions:

- Exergonic = negative ΔG, spontaneous, free energy is released, favourable
- Endergonic = positive ΔG, non-spontaneous, free energy is absorbed, unfavourable
- Exothermic = negative ΔH, heat released
- Endothermic = positive ΔH, heat absorbed

FREE ENERGY AND EQUILIBRIUM

- Thermodynamic Equilibrium Constant (K_{eq}) describes the position of the reaction at equilibrium (i.e. the relative concentrations of reactants and products at equilibrium)

$$aA + bB \rightleftharpoons cC + dD$$

$$K_{eq} = \frac{[C]_{eq}^{c}[D]_{eq}^{d}}{[A]_{eq}^{a}[B]_{eq}^{b}}$$

- ΔG is dependent on:
    - Standard Free Energy Change (ΔG°) = a constant for the particular reaction which is measured under standard conditions
    - The initial concentrations of reactions and products ($[X]_i$)
- R is the universal gas constant, T is temperature in K

$$\Delta G = \underbrace{\Delta G^o}_{\text{Standard Free energy Change}} + RT \ln \frac{[C]_i^c [D]_i^d}{[A]_i^a [B]_i^b}$$

- At equilibrium, ΔG=0, meaning that the reaction can do no more work
- The further away from equilibrium the reaction starts, the more work is done before equilibrium is reached and hence the larger ΔG (be it + or -)
- A reaction will proceed towards equilibrium (i.e. it will proceed towards ΔG=0)
- At equilibrium, it is possible to determine ΔG°:

$$\Delta G = \Delta G^o + RT \ln \frac{[C]_i^c [D]_i^d}{[A]_i^a [B]_i^b}$$

$$0 = \Delta G^o + RT \ln \frac{[C]_{eq}^c [D]_{eq}^d}{[A]_{eq}^a [B]_{eq}^b}$$

$$\Delta G^o = -RT \ln K_{eq}$$

STANDARD CONDITIONS

- Using standards conditions allows comparison of ΔG
- ΔG° describes free energy change required to reach equilibrium when the initial concentrations of all reactants and products are 1M
- Chemical and physical standard conditions:
    - 298K (25°C)
    - Gases at a partial pressure of 101.3kPa (1atm)
    - Reactants and products at a concentration of 1M
- Biochemical standard conditions include all described above, except biochemical reactions occur in a well buffered aqueous solution at pH 7, so $[H^+] = 10^{-7}M$
- When biochemical standard conditions are used, change is Gibbs free energy is denoted by Δ'G°

$$\Delta G'^o = -RT \ln K'_{eq}$$

- When the standard free energy change is known it is possible to determine:
    - Spontaneity of the reaction (and under what conditions)
    - If the reaction is favourable or unfavourable and needing to be coupled with a favourable reaction
    - The position of the reaction at equilibrium
    - How much work the reaction can theoretically do

FAVOURABLE/UNFAVOURABLE REACTIONS

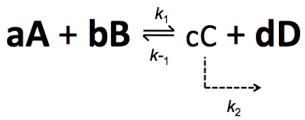
- Unfavourable reactions are non-spontaneous
- Measure of spontaneity of a reaction is ΔG not Δ'G° because spontaneity is determined by the initial reaction concentrations, not those at equilibrium
- If ΔG is negative, the reaction is spontaneous

- Even if Δ'G° is positive, ΔG can be negative by altering the initial conditions; how?
  - Concentration of products must be kept much lower than the concentration of reactants
  - Taking the log of a negative number (i.e. a fraction with a larger denominator) gives a negative number, making ΔG negative

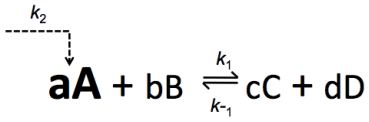
$$\frac{[C]_i^c[D]_i^d}{[A]_i^a[B]_i^b} < 1$$

$$\frac{[C]_i^c[D]_i^d}{[A]_i^a[B]_i^b} = \frac{smaller}{bigger}$$

- How to keep the concentration of products lower than that of the reactants?
  - Remove one or more of the products at a rate much faster than it is produced, making the reaction kinetically driven



  - Replenish one or more of the reactants at a rate much faster than it is removed, making the reaction kinetically driven



- Alternatively, couple the unfavourable reaction with a highly favourable reaction
- The 2 reactions need to be coupled in the same space (i.e. active site of the same enzyme)
- Highly favourable reactions will give off energy as they proceed, which the unfavourable reaction can use to drive to completion

A+*B*→**C**+*D*	$\Delta G_1°'$	unfavourable
E+*D*→F+*B*	$\Delta G_2°'$	highly favourable
A+~~*B*~~+~~*D*~~+E→~~*B*~~+**C**+~~*D*~~+F	$\Delta G_1°' + \Delta G_2°'$	favourable
A+E →**C**+ F		

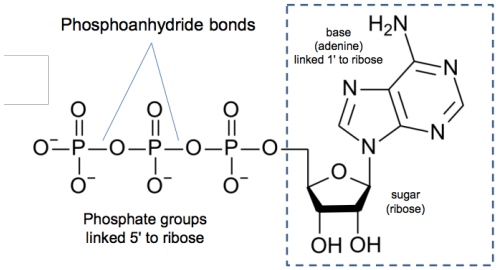
(1) **Glucose** + P_i → **Glucose 6-phosphate** + H_2O	$\Delta G_1'° = 13.8$ kJ/mol
(2) ATP + H_2O → ADP + P_i	$\Delta G_2'° = -30.5$ kJ/mol
Sum: Glucose + ~~P_i~~ + ATP + ~~H_2O~~ → Glucose 6-phosphate + ~~H_2O~~ + ADP + ~~P_i~~	$\Delta G_1'° + \Delta G_2'° = (13.8-30.5)$ kJ/mol
(3) **Glucose** + ATP → **Glucose 6-phosphate** + ADP	$\Delta G_1'° + \Delta G_2'° = -16.7$ kJ/mol

- e.g. production of GTP in the Krebs Cycle is couple with hydrolysis of a thioester bond in succinyl-CoA
  - GDP phosphorylation is highly unfavourable
  - Thioester hydrolysis is very highly favourable
  - Overall, the reaction is favourable (under standard conditions)

(1) **GDP** + P_i → **GTP** + H_2O	$\Delta G_1'° = 30.5$ kJ/mol
(2) Succinyl-CoA + H_2O → Succinate + CoA-SH	$\Delta G_2'° = -33.4$ kJ/mol
Sum: Succinyl-CoA + ~~H_2O~~ + GDP + P_i → Succinate + ~~H_2O~~ + GTP + CoA-SH	$\Delta G_1'° + \Delta G_2'° = (30.5-33.4)$ kJ/mol
Sum: Succinyl-CoA + GDP + P_i → Succinate + CoA-SH + GTP	$\Delta G_1'° + \Delta G_2'° = -2.9$ kJ/mol

ADENOSINE TRIPHOSPHATE

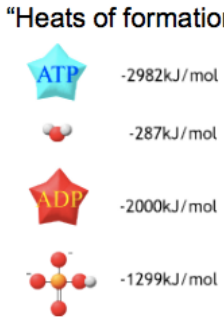
- ATP is composed of:
  - Adenosine (base and sugar)
  - 3 phosphate groups (bonded by phosphoanhydride bonds)



- Hydrolysis of the phosphoanhydride bonds releases a large amount of energy

Why does hydrolysis of ATP yield energy?

- Yield of energy from ATP is highly exergonic, meaning free energy is released
- ATP is unstable and its hydrolysis products are more stable
- This is due to:
    - Inorganic phosphate molecules are more stable as they have electrons in pi orbitals and multiple resonance states
    - ATP has a high negative charge density and more repulsion along the phosphate chain compared to ADP
    - Phosphoanhydride bonds are relatively weak
- 2 non-bonded atoms have a free energy which is higher than the free energy when these 2 atoms are bonded and have overlapping orbitals
- The difference in free energy between the bonded and non-bonded states means that when these atoms bond, energy is released
- Energy is consumed in order to break a bond between atoms
- The energy consumed when bonds in ATP are broken is less than that released when the bonds in the products (ADP + P) are formed, giving a net energy yield



- In the human erythrocyte at steady state (because biochemical systems do not actually proceed to equilibrium, they're at steady state), the concentration of ADP is much lower than that of ATP, giving a higher ΔG

$ATP + H_2O \leftrightarrow ADP + P_i \qquad \Delta G'^{o} = -30.5\ kJ/mol$

$$\Delta G = \Delta G'^{o} + RT \ln \frac{[ADP]_i [P_i]_i}{[ATP]_i}$$

erythrocytes

$$\Delta G = -52\ \ kJ/mol$$

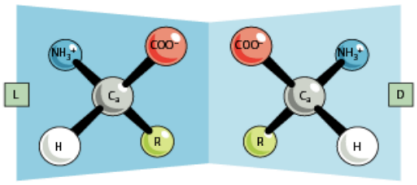
L2 – PRIMARY STRUCTURE AND THE PEPTIDE BOND

CHIRALITY OF α-CARBON

The α-carbon is asymmetric, which allows for non-superimposable mirror images (D and L)

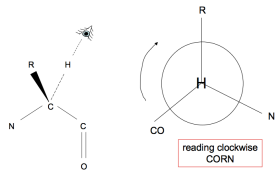
What do D and L mean?

- Naturally occurring proteins consist only of L-amino acids (so usually the isomeric designation is dropped)
- The allocation of D or L to an amino acid is based on the rotation of plane polarised light, viewed towards the light source
- Products which rotate light the same way (and hence have the same stereochemistry) as naturally occurring amino acids can be synthesised from L-glyceraldehyde → L-amino acids
- Products which rotate light the opposite way to naturally occurring amino acids can be synthesised from D-glyceraldehyde → D-amino acids
- Dextrotatory (+) = right/clockwise rotation
- Levorotatory (-) = left/anti-clockwise rotation
- Many, but not all, D amino acids are dextrotatory
- Many, but not all, L amino acids are levorotatory



How to determine the chirality of α-carbon?

- Look down the H-αC bond, with H at the front
- Read the substituents attach to the αC clockwise
- If they read CORN (carbonyl group → side chain → amine group) then it's an L-amino acid



ACIDITY AND AMINO ACIDS

K_a and pK_a

- Acid Dissociation Constant (K_a) is an equilibrium constant describing the reaction:

$$HA \leftrightarrow A^- + H^+$$

$$K_a = [H^+].[A^-]/[HA]$$

- pK_a is the negative log of K_a
- pK_a is useful because it's equal to pH when the concentration of the acid (protonated form) is equal to the concentration of its conjugate base (deprotonated form) → indicates 50% deprotonation

$$pK_a \text{ is pH when } [HA] = [A^-]$$

- The pK_a of bases tend to be high, as bases bind H+ which they pull off water, which generates OH-

$$BH^+ \leftrightarrow B + H^+$$

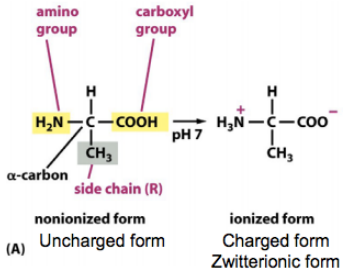
$$K_a = [H^+].[B]/[BH^+]$$

What does pK_a mean?

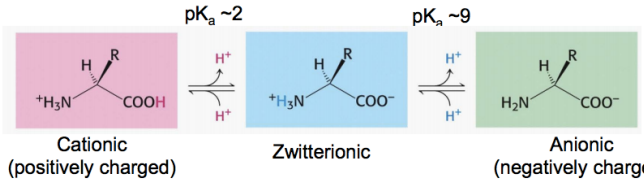
- Low pK_a means that the acid readily dissociated (i.e. H+ only stays on A- at a low pH, when [H+] is high)
- High pK_a means that the acid weakly dissociates (i.e. H+ is hard to remove from A-, needing high [OH-] to pull it off
- At a pH below pK_a, more of the protonated form of the acid is present
- At a pH above pK_a, more of the deprotonated form of the acid is present

Ionization Status of Amino Acids

- When crystalline, amino acids are uncharged
- When dissolved in water, at pH 7, the charged <u>zwitterionic form</u> is present
- Amino group accepts a proton and carboxyl group donates a proton
- Results in the amino acid having a positive charge at the N terminus, and a negative charge at the C terminus
- Overall the zwitterion is neutral, but it is still charges



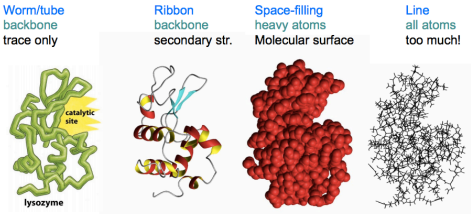
- pK_a of the carboxyl group is ~2, so at pH 2, there are equal concentrations of the cationic form and the zwitterionic form
- pK_a of the amino group is ~9, so at pH 9, there are equal concentrations of the anionic form and the zwitterionic form



- In solution, the non-ionized form doesn't exist → there is a very low probability of having the non-ionized form of both termini at the same pH

AMINO ACID SEQUENCE AND PROTEIN STRUCTURE

- Proteins are linear, unbranched polymers of amino acids
- 20 naturally occurring amino acids
- Proteins adopt a folded structure (they're folded polypeptides)
- The folded shape/conformation of a protein is specified by its amino acid sequence
- <u>Native Conformation</u> = fold with the lowest free energy
- Protein structure can be represented in different ways depending on the detail required :
    - Line model – shows all atoms
    - Space filling model – shows molecular surface (shows cleft for the active site in enzymes)
    - Ribbon model – shows the backbone and secondary structure elements
    - Worm/tube model – shows the backbone

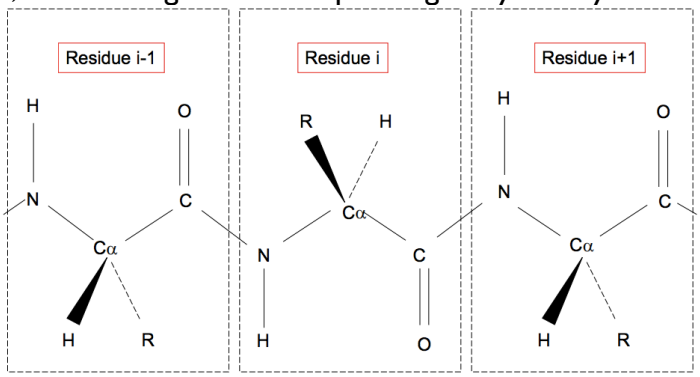


Side Chains

- Chemical properties of the backbone is largely uniform
- Chemical properties of the side chains vary
- Size, shape, charge, H bonding character, hydrophobicity and chemical reactivity all vary depending on the side chain

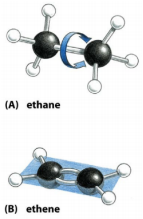
PEPTIDE BOND FORMATION

- Carboxyl group of 1 amino acid residue reacts with the amino group of another amino acid residue, and gives off water (condensation reaction)
- Forms a peptide bond
- N terminus is on the left and C terminus is on the right
- Order of atoms in the backbone: N-Cα-C
- Polypeptides are described as having 'n' residues, which describes the number of amino acids
- A single residue can be called an arbitrary letter (e.g. 'i') to describe the position of other amino acids relative to residue i
- Polypeptide with L-amino acids, in the trans orientation (central side chain pointing towards you, and flanking side chains pointing away from you:



ROTATION ABOUT BONDS IN PROTEINS

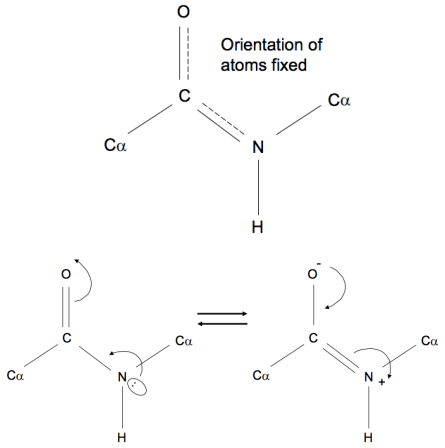
- Conformational freedom of a molecule depends on the nature of bonding between atoms
- Single bonds allow for free rotation
- Multiple bonds are planar and rotation is very limited



Peptide Bond Geometry

- Peptide bonds (between A and N) appear to be single bonds, but don't have free rotation

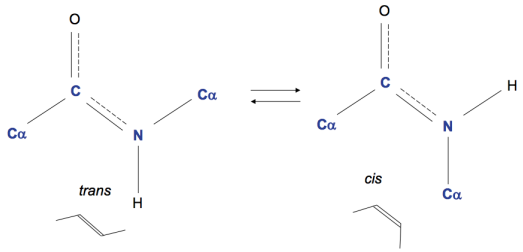
- The peptide bond has very limited rotation because it is a partial double bond (due to resonance)
- The groups involved in, and attached to the peptide bond are coplanar



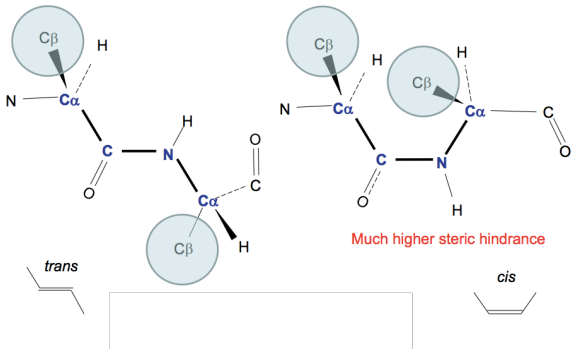
- The peptide bond is 1.33 angstroms which is shorter than a normal single bond, but longer than a normal double bond
- Therefore, the peptide bond is a partial double bond

Peptide Bond Isomerism

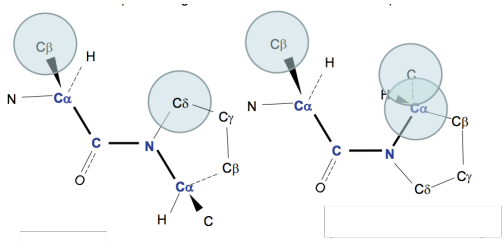
- Due to the peptide bond's planarity, it has cis and trans isomers
- Cis or trans refers to the orientation of α-Carbon atoms
    - Trans = α-Carbons on opposite sides of the double bond
    - Cis = α-Carbons on the same side of the double bond



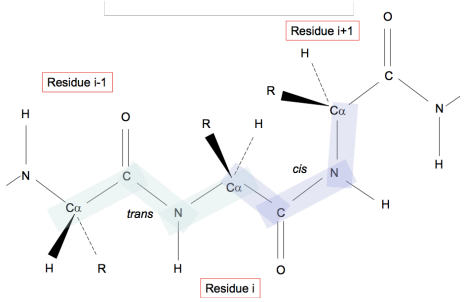
- The trans isomer is energetically favoured in proteins due to the steric hindrance of the side chains in the cis isomer
- Trans form is preferred over cis with a ratio of 1000:1



- There is an exception with the amino acid proline, which has less steric hindrance in the cis isomer, but still, the trans form is more favourable in a ratio of 4:1
- Trans is always more favourable

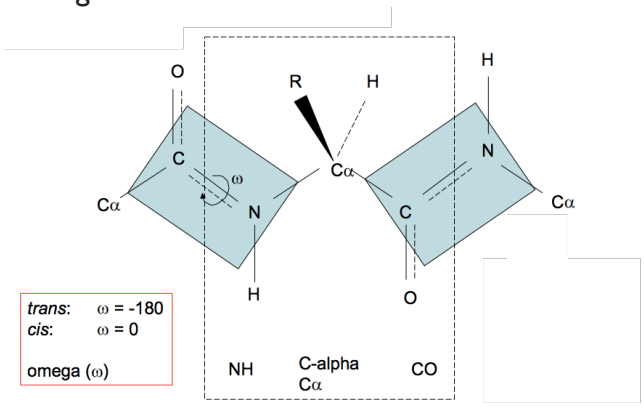


- The different isomers of the peptide bond can alter the structure of the polypeptide chain



Dihedral/Torsion Angles

- Rotation about the peptide bond can be described by the dihedral/torsion angle (ω)
- The dihedral angle spatially relates the αC-C bond to the N-αC bond
- ω is measures in degrees



How to determine the dihedral angle?

- Look along the peptide bond
- Measure the distance in degrees between the 2 αC's
- In the trans conformation, the αC's are on opposite sides of the partial double bond, and therefore, ω=-180°
- In the cis conformation, the αC's are on the same side of the double bond, and therefore, ω=0°