The EGG!

Visualising Molecules

- In molecular biology and biochemistry it is better to view molecules as killer pythons rather than smarties.
- In an ideal solution the smarties do not interact with each other. The more dilute the solution the better.
If the molecules actually look more like killer pythons then…

- They interact with themselves and…
- They interact with each other.
- This complicates things…..
What determines the conformation a protein will assume?

- The amino acid sequence
- Anfinsen's experiment with ribonuclease
- The role of disulfide bridges
- Extrapolating Anfinsen's conclusions
  - Larger proteins
  - high [protein] inside the cell (~300 mg/ml)

Anfinsen's Experiment

Select a stable enzyme (ribonuclease) and measure its activity.

Denature reversibly (with a chemical like urea or guanidine) and measure activity.

Slowly remove the denaturant (by dialysis) and monitor the enzyme activity.

100% activity restored, particularly if a small amount of ~-SH added.

The role of disulfide bridges

- If the formation of the disulfide bridges was random.....
- There are 4 S-S so 8 cysteines (~-SH)
- 1 in 7 chance for the correct formation of the first... 1 in 5 for the next, followed by 1 in 3 and finally 1 in 1
- Should result in <1% activity restored
Why do proteins fold at all?

- Hydrophobic interactions drive proteins to fold.
- Consider the entropy of the whole system NOT just the protein.
- The role of chaperones
  - to protect the peptide during the folding
  - to prevent inappropriate association with other proteins

2 classes of proteins of particular interest to molecular biologists

- DNA binding proteins
- Enzymes

DNA binding Proteins

- Two classes of interaction
  - non-base sequence specific e.g. DNA packaging proteins, DNA polymerases
  - base sequence specific e.g restriction enzymes
- DNA is composed of nucleotides, protein is made of amino acids.
- The DNA sequence is buried in the core of the double helix.
Base sequence specific DNA binding Proteins

- Amino acid residues in the protein need to make contact with the sugar-phosphate backbone and the bases
- Most DNA binding proteins are one of 3 classes:
  - Helix-turn-helix (H-T-H)
  - Zinc finger
  - Leucine zipper

Alpha Helices and DNA

A perfect fit!

- Most DNA-binding proteins contact the DNA via an alpha helix.
- Diameter of helix is 1.2 nm
- Major groove of DNA is about 1.2 nm wide and 0.6 to 0.8 nm deep
- Proteins can recognize specific sites in DNA
- The side chains on the alpha helix face outwards
Enzymes:

- increase the rates of reactions
- are highly specific for their preferred substrate
- Can be regulated
- can be localized in certain organelles
- Can be organized into pathways.
**Rate Enhancement**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Non-enzymic $t_{1/2}$ (yr)</th>
<th>Rate Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMP decarboxylase</td>
<td>78 000 000</td>
<td>$1.4 \times 10^{17}$</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>120</td>
<td>$2.1 \times 10^{12}$</td>
</tr>
<tr>
<td>Cytidine deaminase</td>
<td>69</td>
<td>$1.2 \times 10^{12}$</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>5 sec</td>
<td>$7.7 \times 10^{6}$</td>
</tr>
</tbody>
</table>

**Context**

- The efficient functioning of your body requires certain pathways to be stimulated in certain circumstances and to be downregulated at other times.
- The way enzymes work and how they are regulated is crucial to all biochemical processes.
- Many diseases are the result of inherited or acquired deficiencies in a particular enzyme.

**Enzyme Characteristics**

- Enzymes are usually proteins (although recently RNA has been found to have catalytic activity – ribozymes).
- Enzymes usually have molecular weights between 10,000 and 1,000,000 D
- Some enzymes require co-factors and some are multi-enzyme complexes with regulatory functions (holoenzymes).
Enzyme Characteristics

- The molecule acted upon by the enzyme is termed the **substrate (S)**.
- The substrate is converted by the enzymatic reaction into **product(s)**.
- The enzyme is often named "trivially" by adding the suffix "ase" to the name of the substrate.

Living systems:

- Obey the laws of thermodynamics.
- Are an open system. They exchange both matter and energy with their surroundings.

Free Energy

- The change in free energy in a reaction is described by the term $\Delta G$.
- The change in free energy ($\Delta G$) is the amount of energy available to do useful work.
- The change in free energy ($\Delta G$) is a combination of enthalpy ($\Delta H$) and entropy ($\Delta S$): $\Delta G = \Delta H - T\Delta S$.
Free Energy

• The change in free energy ($\Delta G$) is a combination of enthalpy ($\Delta H$) and entropy ($\Delta S$):

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

Available energy to do useful work

Total energy

Energy utilised in wiggling and jiggling: the nervous energy

Equilibria

• “Living systems only achieve equilibrium upon death”
• Equilibrium occurs when there is no net flow of energy in either direction of a chemical reaction
• Equilibrium occurs when the $\Delta G = 0$ (no change in free energy)
• The concentration of reactants and products at equilibrium is described by the equilibrium constant $K_{eq}$

Equilibrium versus steady state

• As a reaction approaches equilibrium the amount of energy to do useful work decreases.
• The cell needs to operate far from equilibrium.
• You don’t want to lose the capacity to do useful work to an increase in entropy.
Equilibrium versus steady state

- Because organisms are an open system, matter and energy can continually flow in and out of the cell.
- The cell can exist in a steady state that is NOT at equilibrium.
- The product of one reaction becomes the reactant for the next. This can continue provided more starting material enters the pathway.

Standard Free Energy

- The standard free energy of a reaction, $\Delta G^\circ = \Sigma \Delta G^\circ_{\text{products}} - \Sigma \Delta G^\circ_{\text{reactants}}$ in their standard state (25°C, 1 M)
- This property reflects the difference in the chemical structure of the reactants and the products.

Standard Free Energy

- Exergonic reactions are those which release free energy i.e. $\Delta G$ is $-$ve. The product(s) have a lower $\Delta G^\circ$ than the reactant(s) and the reactions are thermodynamically favourable.
- Endergonic reactions are those that need an input of energy i.e $\Delta G$ is $+$ve.
The Relationship between Free Energy and Equilibrium

- The standard free energy of a reaction $\Delta G^o$ is related to the equilibrium constant as follows: $\Delta G^o = -RT\ln K_{eq}$
- Both $\Delta G^o$ and $K_{eq}$ relate to the chemical nature/structure of the reactant and the product.
The Thermodynamics of a Reaction

Reaction performed under standard conditions: 25°C and 1 atmosphere air pressure

Free Energy (G)

Substrate (1 M) \[ \Delta G^0 \]

Product (1 M)

Progress of the reaction

A negative \( \Delta G^0 \) makes the reaction thermodynamically favourable

The Thermodynamics of a Reaction

Reaction performed under standard conditions: 25°C and 1 atmosphere air pressure

Free Energy (G)

Substrate (1 M) \[ \Delta G^0 \]

Product (1 M)

Progress of the reaction

The \( \Delta G^0 \) is related to the \( K_{eq} \).

The Kinetics of a Reaction

Free Energy (G)

Substrate (1 M) \[ \Delta G^0 \]

Product (1 M)

Progress of the reaction

A negative \( \Delta G^0 \) makes the reaction thermodynamically favourable
The Kinetics of a Reaction

Free Energy ($G$)

Activation Energy

$\Delta G^*$

$\Delta G^0$

Substrate (1 M)

Product (1 M)

Progress of the reaction

A negative $\Delta G^0$ makes the reaction Thermodynamically favourable

The enzyme lowers the activation energy

$\Delta G^*$

$\Delta G^0$

A negative $\Delta G^0$ makes the reaction Thermodynamically favourable
Enzymes and Thermodynamics

- Enzymes do NOT change the thermodynamics of a reaction; $\Delta G^\circ$ or the $K_{eq}$ of a reaction.
- If a reaction is exergonic without an enzyme it will be with one.
- Likewise if the reaction is endergonic without an enzyme it will be with one.

![Reaction profile showing large $\Delta G^\circ$ for glucose oxidation, free energy change of -2,870 kJ/mol; catalyst lowers $\Delta G^\circ$, thereby accelerating rate.]

Enzymes and Thermodynamics

- To catalyse a thermodynamically unfavourable reaction it needs to be coupled to a source of energy, usually ATP hydrolysis.
- Enzymes increase the rate of the reaction BUT they do not change the equilibrium position. You just get there quicker.
- Enzymes lower the activation energy of the reaction (the hill you have to get over).
The relationship between $\Delta G^o$ and $K_{eq}$

- The standard free energy of a reaction $\Delta G^o$ is related to the equilibrium constant $K_{eq}$ as follows: $\Delta G^o = -RT\ln K_{eq}$
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$\Delta G^o$ and $K_{eq}$: an example where the $\Delta G^o$ is negative

A negative $\Delta G^o$ makes the reaction thermodynamically favourable

$\Delta G^o$ and $K_{eq}$: an example where the $\Delta G^o$ is negative

Under standard conditions

Free Energy (G) vs Progress of the reaction
$\Delta G^0$ and $K_{eq}$:

If the $\Delta G^0$ is negative then each substrate molecule contains much more free energy than each product molecule.

$\Delta G^0$ and $K_{eq}$:

So when there is an equal amount of both substrate and product the total free energy difference between the two, the $\Delta G^0$, is quite large.

$\Delta G^0$ and $K_{eq}$:

At equilibrium, when the total free energy difference, $\Delta G$, is zero, the ratio $K_{eq}$ is defined.

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At equilibrium, when the total free energy difference, $\Delta G$, is zero, the ratio $K_{eq}$ is defined.
At equilibrium, when the total free energy difference, $\Delta G$, is zero, there must be more product molecules and less substrate molecules to balance the equation. $\Delta G^0$ and $K_{eq}$

$K_{eq} = \frac{[\text{product}]}{[\text{substrate}]} = \frac{20}{3}$ or $>1$, hence the equilibrium favours product formation.