Lecture 10: DNA Structure, Polymerases,Restriction Enzymes, Ligases.

Double Helical Structures: B-DNA (there is Z-DNA too)

a) The helix is right-handed; the chains are antiparallel
b) Rise 3.4Å/base pair; 10 bp/turn.
c) deoxyribose & phosphate on the outside. Negatively charged phosphates on opposite strands repel each other.
d) The helix interior is filled with stacked base.
e) Dissimilar grooves (phosphate spacing), one major, other minor.
f) T pairs with A via two "Watson-Crick H-bonds"
g) C pairs with G via three "Watson-Crick hydrogen bonds"
   - One strand is "the complementary strand" of the other.
   - Sequence of duplex DNA is written such that the top strand is 5' to 3'

Forces Stabilizing Nucleic Acid Structures. Double stranded DNA (& RNA) can be reversibly denatured ("melting"). Cooperative transition from double stranded helix → single stranded random coils; the change in light absorbance of the bases at λ=260 nm can be used to monitor this transition. The absorbance (A_{260}) increases when the DNA melts (hyperchromatic effect).
\[ T_m \propto \%GC \]
\[ T_m \propto [NaCl] \]

\[ \%GC \text{ or } [NaCl] \]

**Energetic Term** | dsDNA stability | Molecular Description of Energetic Terms in DNA stability.
--- | --- | ---
Hydrogen Bonds | ++ | 

\[
\begin{align*}
\text{A} & \text{=} \text{T} & \text{2 H-bonds} \\
\text{G} & \text{=} \text{C} & \text{3 H-bonds} \\
\end{align*}
\]

 Electrostatic interactions | -- | --

**DNA Annealing:** The two DNA molecules below are mixed under conditions that promote formation of double stranded DNA. Draw the double stranded (duplex) DNA that would form on the right.

\[
\begin{align*}
3' & -A-C-G-T-5' \\
5' & -T-G-C-A-3' \\
\end{align*}
\]

\[
\begin{align*}
1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 \\
\end{align*}
\]

**DNA Modification Tool Kit:**
- **Restriction endonucleases** to cut at specific locations.
- **DNA ligase** to join DNA fragments.
- **Polymerases** to convert and amplify RNA to DNA and to sequence DNA

**DNA-Protein Interactions**
- a) Non-seq. Specific: Electrostatic bonding to the backbone.
  - i) side chains of Lys and Arg to phosphates.
  - ii) Release of metal ions (e.g. K⁺) favors binding (large increase in entropy of the ions (ΔS>0)).

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**strong electrostatic forces**

\[
\begin{align*}
\text{Na}^+ & \text{ ordered low } S \\
\text{K}^+ & \text{ disordered high } S \\
\end{align*}
\]
b) Sequence Specific Non-Watson-Crick Hydrogen bonding to the polar edges of the bases and to sugars. (Major groove is large arc of a circle drawn through the basepair, minor groove is small arc).
   i) Side chains of Arg, Asn, Gln, Tyr...
   ii) Protein mainchain, C=O, NH.
   Note: WC acceptor C=O can participate in one non-WC H-bonds.

**Restriction Endonuclease:**
[endo - cut within, nuclease - cleave nucleic acid].
Used by bacteria to degrade invading viral DNA. Named after bacterial species the particular enzyme was isolated from.

1. Enzyme binds to specific **recognition sequences** with near absolute specificity and high affinity ($K_D = 10^{-16}$ M).

2. Homodimeric enzymes have 180-degree rotational symmetry. Because of the symmetry in the enzyme, the DNA sequence also symmetrical. The sequence is the same on the top and bottom strands (referred to as palindromic sequences).

3. Require Mg$^{2+}$ for cleavage, usually cleave both strands at the same position. Generating a 3’OH.

4. Cognate methylase adds a methyl group to the same sequence, preventing cleavage (otherwise bacteria would digest their own DNA).
DNA Ligase – Uses ATP to join 5'phosphate to 3'-OH, provided the two groups are in close proximity. Fragments created by the same restriction enzyme can always be joined to each other.

**Sticky-end ligation:**

![Diagram of DNA ligase reaction]

**DNA Polymerases:**

- Utilize a template to direct the order of added bases.
- Require a basepaired primer with a 3'OH. Primer can be DNA or RNA.
- Synthesize chains from 5'→3' direction. Adding new dNTP to the 3' hydroxyl of the existing polymer. Pyrophosphate (PP) is released.

- Fidelity of base incorporation is dependent on Watson-Crick base-pairing (A-T, G-C), plus purine-pyrimidine matching.
- Phosphodiester bond formation occurs quickly (1 msec) when the correct match is made. Bond formation is slow when the bases are incorrectly matched – allowing time for proofreading.

**Proofreading activity:** 3'→5' exonuclease activity
(exo – end, exonuclease – cut from the end)

![Diagram of proofreading activity]

**Note:** This activity is absent in many viral polymerases (e.g. HIV reverse transcriptase), leading to high mutation rates in the virus and a high number of viruses that are resistant to antiviral drugs.

**AZT is a potent inhibitor of HIV Reverse Transcriptase (1st HIV Drug)**

- Why does AZT inhibit the enzyme?
- Does AZT have to be modified before it can function as an inhibitor?