Recitation Section 2

Bi 110
DNA Structure
Major and Minor Grooves

A-DNA has a shallow minor groove and a deep major groove

https://www.youtube.com/watch?v=QD1TjeszTHQ
A, B, Z alpha-helices
A, B, Z alpha-helices

Figure 3: Properties of DNA bases. (a) The base pairs for guanine–cytosine (G•C) and adenine–thymine (A•T). (b) Twist angle for the A•T dinucleotide, and propeller twist for an A•T base pair. Modified from Sinden et al. (1998).
A, B, Z alpha-helices

Rise and twist determine the handedness and pitch of the helix. Slide and shift are typically small in B-DNA, but are substantial in A- and Z-DNA. Roll and tilt make successive base pairs less parallel, and are typically small.
A, B, Z alpha-helices

<table>
<thead>
<tr>
<th>Geometry attribute</th>
<th>A-DNA</th>
<th>B-DNA</th>
<th>Z-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helix sense</td>
<td>right-handed</td>
<td>right-handed</td>
<td>left-handed</td>
</tr>
<tr>
<td>Repeating unit</td>
<td>1 bp</td>
<td>1 bp</td>
<td>2 bp</td>
</tr>
<tr>
<td>Rotation/bp</td>
<td>32.7°</td>
<td>34.3°</td>
<td>60°/2</td>
</tr>
<tr>
<td>bp/turn</td>
<td>11</td>
<td>10.5</td>
<td>12</td>
</tr>
<tr>
<td>Inclination of bp to axis</td>
<td>+19°</td>
<td>−1.2°</td>
<td>−9°</td>
</tr>
<tr>
<td>Rise/bp along axis</td>
<td>2.3 Å (0.23 nm)</td>
<td>3.32 Å (0.332 nm)</td>
<td>3.8 Å (0.38 nm)</td>
</tr>
<tr>
<td>Pitch/turn of helix</td>
<td>28.2 Å (2.82 nm)</td>
<td>33.2 Å (3.32 nm)</td>
<td>45.6 Å (4.56 nm)</td>
</tr>
<tr>
<td>Mean propeller twist</td>
<td>+18°</td>
<td>+16°</td>
<td>0°</td>
</tr>
<tr>
<td>Glycosyl angle</td>
<td>anti</td>
<td>anti</td>
<td>C: anti, G: syn</td>
</tr>
<tr>
<td>Sugar pucker</td>
<td>C3'-endo</td>
<td>C2'-endo</td>
<td>C: C2'-endo, G: C2'-exo</td>
</tr>
<tr>
<td>Diameter</td>
<td>23 Å (2.3 nm)</td>
<td>20 Å (2.0 nm)</td>
<td>18 Å (1.8 nm)</td>
</tr>
</tbody>
</table>
Chromatography

• Ionic association of proteins with beads

• Elution phase required to release bound proteins
Polymerase chain reaction - PCR

1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72°C
Polymerases

thermostable DNA polymerase named after the thermophilic bacterium Thermus aquaticus from which it was originally isolated by Chien et al. in 1976.

T. aquaticus is a bacterium that lives in hot springs and hydrothermal vents, and Taq polymerase was identified as an enzyme able to withstand the protein-denaturing conditions (high temperature) required during PCR. Therefore, it replaced the DNA polymerase from E. coli originally used in PCR.
DNA Probes
Recombinant DNA

Host Plasmid
- Cleavage by Restriction Endonucleases
- Sticky ends
- Specified Genes

Site of cleavage
Annealing
Point of attachment and annealing
Recombinant Plasmid DNA

vector and donor DNA digested (cleaved) with restriction enzyme
mixing
DNA ligase added, seals overhangs

DNA introduced into bacterial cells
recombinant DNA molecules replicate and cells divide

Clones

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Analysis of ligation and digestion

1. Visual Analysis of Colony Fluorescence
   Which colonies were glowing?

2. Restriction Digest and Gel Electrophoresis
   Which bands did you see? How does that inform you of the number of insertions and the direction of insertion?

- pMAL vector
- purified PCR amplified GFP
- Eco R1
- Nco1

4 potential combinations/configurations

X  X  X  X  X
While lipid tails primarily modulate bilayer phase behavior, it is the headgroup that determines the bilayer surface chemistry.

**The Membrane Bilayer**

- **glycerol**
- **phosphoester headgroup**
- **fatty acid**

**Headgroups:**
- Phosphatidic acid
- Phosphatidylcholine
- Phosphatidyethanolamine
- Phosphatidylserine
- Phosphatidylglycerol
- Phosphatidylinositol

**Types of lipids:**
- Phospholipid
- Lysophospholipid
- Glycosyl diacylglycerol
- Plasmalogen
- Sphingomyelin

**Other membrane components:** cholesterol; small molecules?

From R.B. Gennis “Biomembranes”
phosphatidylcholine (PC)

Zwitterionic most common head group, accounting for half the phospholipids in mammalian cells
Lipids

Lipid phases

gel phase - molecules pack together more tightly together. Alkyl chains are more highly ordered - larger bilayer thickness

Liquid crystalline - represents bulk of lipids in biological membranes - considerable disorder in alkyl tails (Ioannou, pg. 48)

Critical packing shape

Cone

Spherical micelles

Truncated cone

Cylindrical micelles

Flexible bilayers, vesicles

Cylinder

Planar bilayers

Inverted truncated cone or wedge

Inverted micelles

Nagle & Tristem-Nagle COSB 10, 474 (2000)

Israelachvili, *Intermolecular & Surface Forces*
Lipids

Saturated Fatty Acid

Unsaturated Fatty Acid
An acetylcholine receptor (green) forms a gated ion channel in the plasma membrane. This receptor is a membrane protein with an aqueous pore, meaning it allows soluble materials to travel across the plasma membrane when open. When no external signal is present, the pore is closed (center). When acetylcholine molecules (blue) bind to the receptor, this triggers a conformational change that opens the aqueous pore and allows ions (red) to flow into the cell.
The G protein–coupled receptor is activated by an external signal in the form of a ligand or other signal mediator. This creates a conformational change in the receptor, causing activation of a G protein.
Membranes, chemiosmotic theory

chemical potential energy, $\Delta \mu$, in transfer of contents across membrane (out to in)

\[
\begin{align*}
\text{< 0 (favorable)} & \quad \text{(energetically downhill)} \\
\text{when } (S)_{\text{out}} > (S)_{\text{in}} \\
\Delta \mu &= \mu_{\text{in}} - \mu_{\text{out}} \\
&= RT \ln \left( \frac{(S)_{\text{in}}}{(S)_{\text{out}}} \right) \\
\text{> 0 (unfavorable)} & \quad \text{(energetically uphill)} \\
\text{when } (S)_{\text{out}} < (S)_{\text{in}}
\end{align*}
\]
Diffusion & Transport

Simple vs. facilitated diffusion

- Rate of simple diffusion is limited by the surface area of the membrane and the size of the driving force
- Facilitated diffusion’s rate depends on number of integral membrane proteins
- Saturation kinetics: increase driving force for facilitated diffusion → increase rate of diffusion (only to a certain point) → No further increase in flux when all transport proteins are saturated
Diffusion & Transport

- Symports: carry two substances in the same direction
- Antiports: carry two substances in the opposite directions
- Selective permeability: only some things are allowed to pass through the proteins
- Channel proteins: narrow tunnels (ion channels) that pass specific ions to pass through
  - K+ channel: only potassium flows through down the gradient
  - Voltage-gated ion channels: channels open in response to electric potential changes across the membrane
  - Ligand-gated ion channels: opens in response to binding of a specific molecule like neurotransmitters
Diffusion & Transport

Active transport: movement of solutes against gradient
- Require energy input
- Always involves protein
- Primary active transport: ATP hydrolysis is coupled to transport molecules
- Secondary active transport: ATP is first used to create a gradient → potential energy is used to transport the molecules (indirectly use ATP)
Practice Problems
Design primers that will amplify the following region of DNA (assume this is one strand from a double stranded region of DNA). The primers should be 15 bases in length. Indicate the 5' and 3' ends of the primers.

5'
GGATCGATCAAGAACAATGACAGGATCGAGGAATTCAGCCTACGCAGCCCGTAGCTGG
AGGGA 3'

What other reagents are necessary to perform a PCR reaction?

PCR machines cycle between three temperatures. What is the purpose of each stage in the PCR cycle, and roughly what temperatures are used?

After 10 rounds of amplification approximately how many molecules of the amplified region should you have theoretically.
First determine the reverse complement sequence to this strand of DNA. Then design primers (shown in red) that will bind to the 3’ end of each strand of DNA.

3’ GGCATCGACCTCCCT  5’
GGATCGATCAAGAACATGACGGATCGAGGAATTCAACGCAGGCTAGCTGGAGGGAGA  3’

3’  CCTAGCTAGTTCTGTACTGTCTAGCTAGCTCTTAAAGTCGGATGCGTGGGCATCGACCTCCCT  5´
5´  GGATCGATCAAGAAC  3´

What other reagents are necessary to perform a PCR reaction?

Taq polymerase, nucleotides (dNTPs) and a buffer

PCR machines cycle between three temperatures. What is the purpose of each stage in the PCR cycle, and roughly what temperatures are used?

94°C - denatures template DNA
50-55°C - primer anneals to template DNA
72°C - Taq polymerase elongates DNA

After 10 rounds of amplification how many copies of the amplified region should you have theoretically.

Product is formed in the third round, 2 molecules of product (=2^1). After that it should double every round for the next seven rounds. Thus you would expect a total of 2^8=256 molecules. Note that you will continue to make more of the heterogeneous products each round, so this is really an underestimate.
The active transport of amino acids is also mediated by Na+ cotransport.

Problem:

How many sodium ions are needed to provide the free energy to transport a molecule of glutamic acid from a concentration of 0.1 mM outside the cell to 20 mM inside the cell?

Assume a temperature of 37°C (310°K).

Assume a concentration gradient (20/0.1 = 200) and an electrostatic gradient (moving a negative charge against a voltage of −70 mV).
At pH of ~7, glutamic acid molecules carry a net charge of minus 1.

Determining the movement of a molecule against an electrochemical gradient; that is, against both a concentration gradient (20/0.1 = 200) and an electrostatic gradient (moving a negative charge against a voltage of −70 mV).

\[ \Delta G = (R)(T) \times \ln(20/0.1) + (z)(F)(V_m) \]
\[ = [(2)(310) \times \ln(200)] + [(-1)(23,062)(-0.070)] \]
\[ = (620) \times (5.3) + 1614 \]
\[ = 3286 + 1614 \]
\[ = 4900 \text{ or } 4.9 \text{ kcal/mole} \]

Because sodium ions release only 3.3 kcal/mole (above), at least 2 Na+ are needed to cotrans...