Polynucleotide Structure

Function of a nucleic acid molecule is a direct consequence of its structure.

**Nomenclature**

By convention (unless otherwise indicated) a nucleic acid sequence is read from the 5' to 3' end.

 sequence designations

- AGCT (DNA)
- AGCU (RNA)

pKa = 

3' - 5' phosphodiester bond
Polynucleotide – Solvent Interaction

Which is the resulting polynucleotide conformation in H₂O??

bases (pseudoaromatic) ----> two conformational extremes ----> maximize

???? Which nucleotide base is the weakest stacker?

purines?? two rings (Pu) vs. one ring (Pyr)
pyrimidines??

?? Which base??
Doubled-Stranded Polynucleotide Structure

1. antiparallel strands
2. bases on the interior
3. ribose – PO₄⁻ backbone on the exterior
4. ribose – PO₄⁻ backbone is charged
5. hydrogen bonded chains

The “character” or structure of the hydrogen bond is critical in establishing double-stranded nucleic acid structure

hydrogen bond =
\[ \delta^- \delta^+ \delta^- \]
\[ \text{hydrogen bond} \rightarrow \]
\[ \text{covalent bond} \rightarrow \]

critical features for H-bond strength
1. \[ \delta^- \delta^+ \delta^- \]
2. \[ \text{(linearity maintains H-bond strength)} \]

double-stranded nucleic acids \[ \rightarrow \] two types of H-bonds

\[ \delta^- \delta^+ \delta^- \]
\[ \delta^- \delta^+ \delta^- \]
Pu – Pyr base pairing

A:T pair (DNA)
A:U pair (RNA)

G:C pair

Note: linear and defined length H-bonds gives double-stranded nucleic acid a base pairs frequently occur in RNA structure

base pair common in double stranded RNA (stable as an A:U pair)

base pair three nucleotides H-bonding ("base triples") (used in tRNA for 3-D folding)
1. **Solvent**

- Stacked bases at the helix interior
- Removed from solvent interaction
- H$_2$O molecules surrounding the helix
- Non-polar or decreasing polarity solvent
- Increased hydrophobicity

2. **Cations / Salt (Na$^+$/ K$^+$)**

- Salt
- Why??
- Negatively charged sugar-PO$_4^-$ backbone
- Effect of increased [Na$^+$] on single stranded polynucleotide??
3. Addition of Urea or Formamide to the Solvent

(urea and formamide are often added during gel electrophoresis or to nucleic acid hybridization reactions)

\[
\begin{align*}
\text{urea} & \quad \downarrow \\
\text{formamide} & \quad \downarrow
\end{align*}
\]

How do these molecules affect helix structure? ➔

WHY ?? ➔

4. pH Effects

\[
\begin{align*}
\text{DNA} & \quad \begin{cases} 
\text{acid pH} \quad \rightarrow \\
\text{alkaline pH} \quad \rightarrow 
\end{cases} \\
& \quad (\text{glycosidic bond cleavage})
\end{align*}
\]

\[
\begin{align*}
\text{RNA} & \quad \begin{cases} 
\text{acid pH} \quad \rightarrow \\
\text{alkaline pH} \quad \rightarrow 
\end{cases} \\
& \quad \begin{cases} 
\text{(no depurination)} \\
\text{(nucleotide monophosphate)} 
\end{cases}
\end{align*}
\]

\[
\begin{align*}
\text{DNA and RNA} & \quad \begin{cases} 
\text{strong acid (pH < 1)} \quad \rightarrow \\
12 \text{ M perchloric acid} \quad \rightarrow \\
100^\circ \text{C - 12 hrs} \quad \rightarrow 
\end{cases}
\end{align*}
\]
DNA

acidic pH \rightarrow depurination

(protonation at N3 causes N9 attack at glycosidic bond)

alkaline pH \rightarrow double-stranded helix denaturation

alkaline pH causes

(you should review nucleotide base pairing and ionizable protons (pKa values) to see which protons are lost)
RNA alkaline pH $\rightarrow$ hydrolysis of $\text{PO}_4^-$ backbone
Double-Stranded Nucleic Acid Denaturation and Renaturation

Denaturation →

(not degradation – covalent bonds are not broken)

Monitor denaturation by change in DNA / RNA absorbance

![Graph](image)

Why is there an increase in absorbance upon DNA denaturation???

Pyr and Pu base rings →

absorb incident radiation–UV light

π e⁻ jump to excited state

bases more accessible to light

Why is there a change in chromophore structure causes changes in e⁻ distribution resulting in change in absorbance

D.S. vs S.S.
What factors determine Tm??

\[ T_m \propto \] 

Can estimate \( T_m \)

\[ T_m(\,^\circ C) = \text{ + } \text{ + } \text{ - } \text{ - } \]

often used in DNA reannealing or hybridization experiments to lower the Tm incubation temperature

sometimes strands are not 100% complementary

can use to estimate % base mismatch

What is the energy contribution of base stacking and hydrogen bonding to the total energy of the helix??

\[ 100\% \quad \Delta G \quad ?\% \quad \text{denaturation} \quad ?\% \quad (x2) \]
rate-limiting rxn

kinetically reminds you of ??

rate of rxn dependent upon

increase rxn rate or

rate of DNA strand reassociation $\alpha$

can describe the kinetics of strand reassociation mathematically

$= \text{concentration of single strand in solution}$

decrease in [C] with respect to time is due to base pairing of complementary single strands
\[ \frac{-dC}{dt} = K_2 [C]^2 \]

decrease in complementary strand \( C \) over time

2\textsuperscript{nd} order rate constant

define original S.S concentration of \( C \) =

after integration \[ \frac{C}{C_0} = \frac{1}{1 + K_2 C_0 t} \]

when \( \frac{C}{C_0} \)

then time \( t \)

(The time at which half the single strands have become double stranded with their complementary strand)

Then the equation becomes:

This equation can be used to plot the reassociation of single stranded DNA to double stranded complementary DNA

This reassociation curve is defined as a
What can you say about the [conc] of complementary strands for the two reassociation curves??

Can use this approach to assess genomic organization of a given organism.