# Lecture 26 (11/17/25)

## Carbohydrates
A. Definition
B. Roles
C. Monosaccharides-Chemistry
   1. Chirality
      a. One or more asymmetric carbons
      b. Linear and ring forms
   2. Derivatives: the chemistry of carbohydrates
      a. Oxidation
         i. C1
         ii. C6
      b. Reduction
         i. C1/C2
         ii. Other carbons
      c. Ester formation
      d. Amino sugars
   3. Polymerization
      a. The Glycosidic Bond
      b. Non-covalent bonds in macro-molecular structure
      c. Disaccharides
D. Oligosaccharides
   1. Glycoproteins & glycolipids
   2. O-linked
   3. N-linked
   4. Sequence determination-ABO
E. Polysaccharides
   1. Polymers of glucose
   2. Polymers of disaccharides

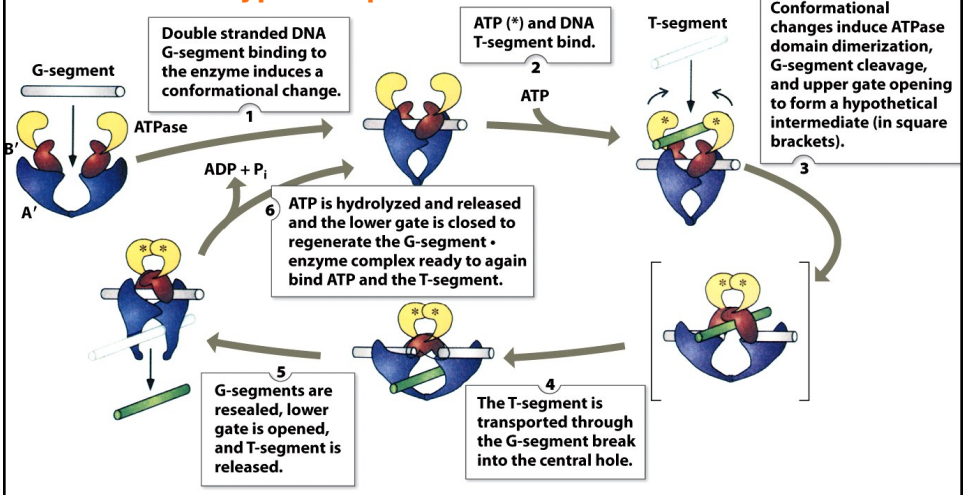
## Nucleic Acids
A. Nucleotides
   1. Parts-structure
   2. Nomenclature
   3. Numbering
   4. Properties
B. Nucleic Acids
   1. Polymer: phospho-diester bond
   2. H-bonds
   3. Roles
      a. Nucleotides
      b. Nucleic acids
C. The 4 S's
   1. Size
   2. Solubility
   3. Shape
      a. B-DNA
      b. A-DNA
      c. Z-DNA
      d. Topology
         i. Packaging
         ii. Supercoiling
         iii. Topoisomerases
   4. Stability
      a. Nucleotides: mutagenesis
         i. Tautomers
         ii. Acid/base
      b. Nucleic Acids
         i. Chemistry
         ii. Denaturation
         iii. Stability
         iv. Nucleases
D. Structure of the Information
   1. Exceptions to flow
   2. Structure
   3. Levels of Control
E. Recombinant DNA: Biochemical Basis of Biotechnology
   1. Restriction enzymes, DNA ligase
   2. Vectors and Inserts to make recombinant DNA (rDNA)
   3. Transformation of hosts
   4. Selection of transformants
   5. Expression
   6. Site-directed mutagenesis
F. Replication
   1. Polymerases
   2. Fidelity
   3. Sequence determination

**TODAY**
- Reading: Ch8; 278-283; Ch24; 895-897
  Ch9; 301-313, 326-327
  Ch8; 283-286
- Homework: #25 & #26

**NEXT**
- Reading: Ch25; 916-921
- Homework: #27

# Topoisomerase

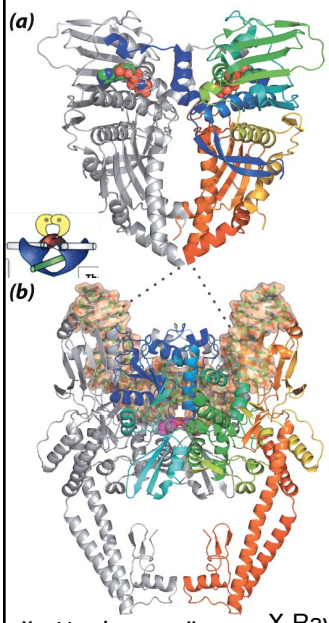
## Type II Topoisomerase Mechanism




**Type II Topoisomerase also called "gyrase"**

- Tetrameric ($\alpha_2\beta_2$; $\alpha$=105 kDa, $\beta$=95 kDa (ATPase))
- Creates double-stranded breaks
- Use of covalent Tyr intermediate
- Changes L by –2
- Uses ATP for energy to put in supercoils ($\Delta L = -2$)

# Topoisomerase

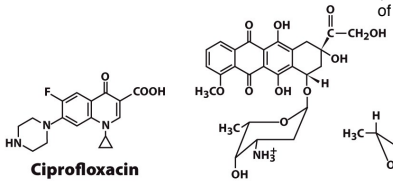


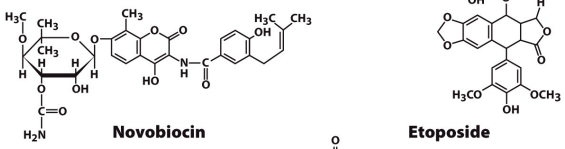

Yeast topoisomerase II
PDBids 1PVG and 2RGR

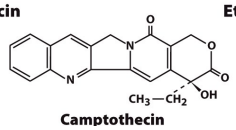
X-Ray structures of *yeast* topoisomerase II.

## Topoisomerase II

Related to Daunorubicin, which inhibits re-joining in (step #5 on previous slide) of Topo II (DNA gyrase)




## Topoisomerase Inhibitors as Antibiotics/Anticancer Chemotherapeutics

# The 4 S's

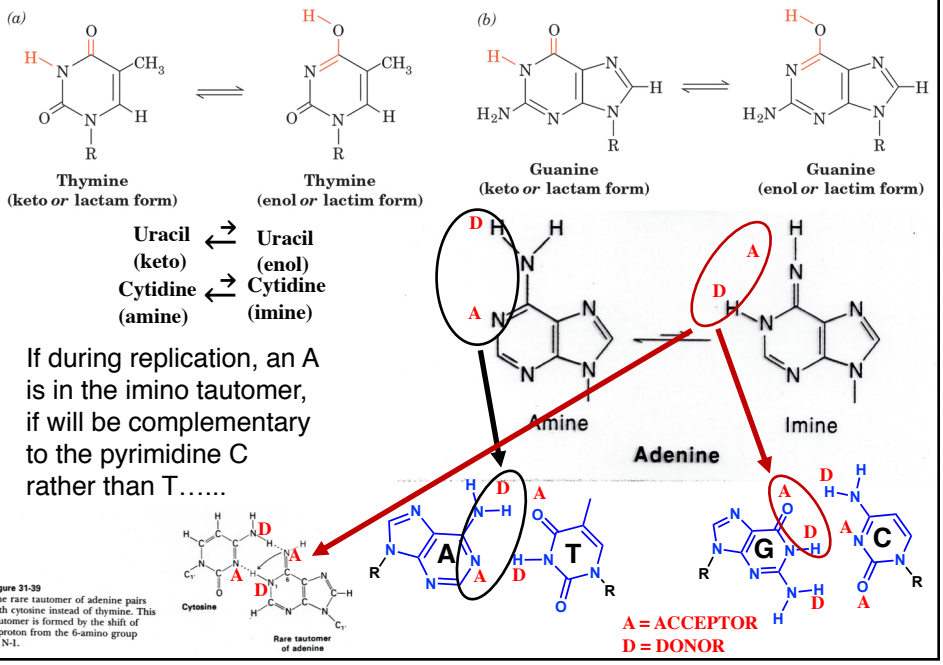
Size

Solubility

Shape

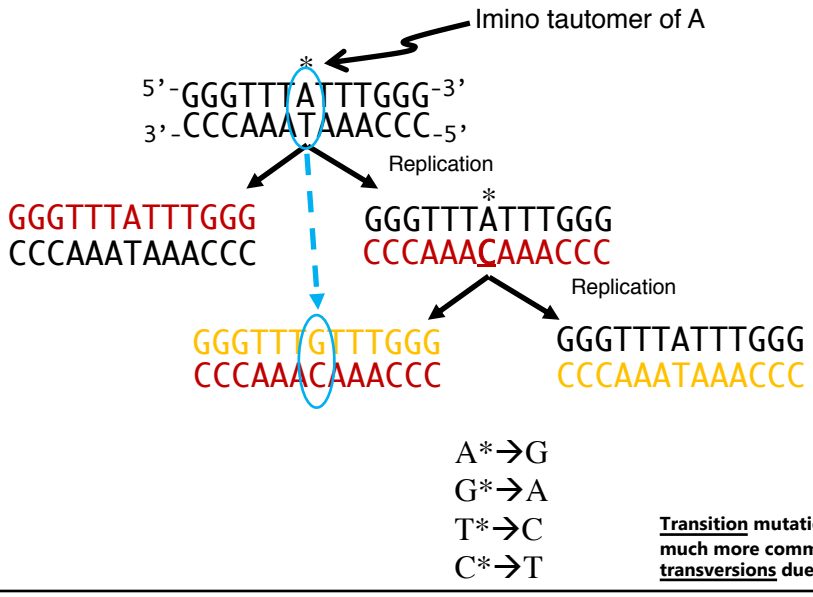
**Stability**

# Tautomeric Forms of Bases




# Tautomeric Forms of Bases

## Mutagenesis




Transition mutations are much more common than transversions due to this

# Tautomeric Forms of Bases

## Modified bases can stabilize the rare tautomer

enol tautomer of U

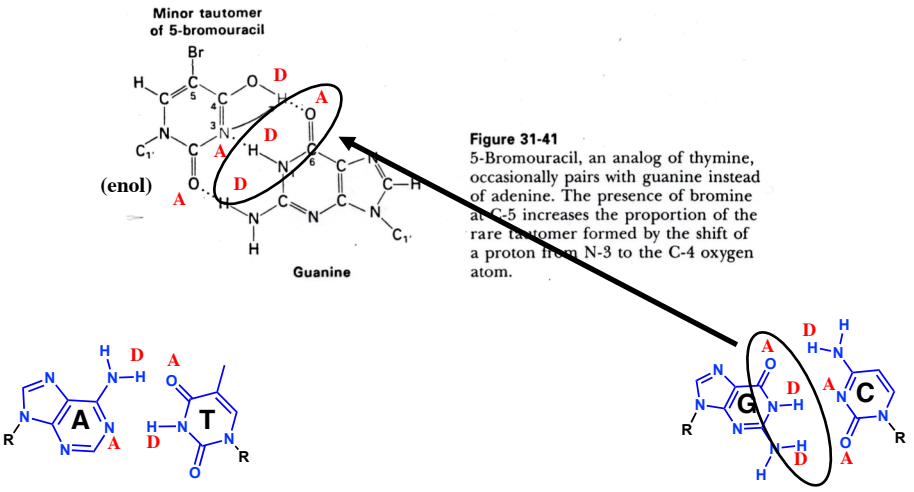



**Figure 31-41**
5-Bromouracil, an analog of thymine, occasionally pairs with guanine instead of adenine. The presence of bromine at C-5 increases the proportion of the rare tautomer formed by the shift of a proton from N-3 to the C-4 oxygen atom.

# Other Chemical Changes of Bases

DNA Damage:

- oxidation
- alkylation
- degradation

# Acid/base Properties of Bases

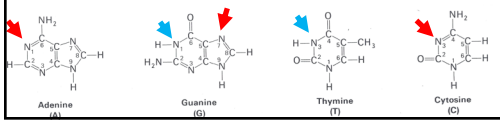
At pH 7, all bases are neutral. They can fit into middle of B-DNA without repulsions
But, acidic or basic treatment will protonate or deprotonate these bases.

At low pH, N7 of guanine, N1 of adenine, and N3 of cytosine become protonated.

At high pH, N1 of Guanine and N3 of Uracil (Thymine) become deprotonated.

*Table 26.2*
Ionization Constants of the Ribonucleotides (Presented as p*K* Values)

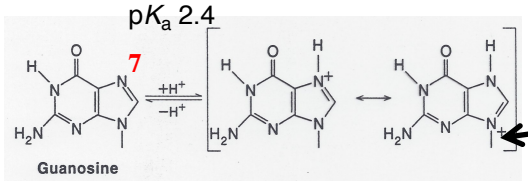
| | Base | Secondary Phosphate | Primary Phosphate |
|---|---|---|---|
| Adenosine-5′-phosphate (AMP) | 3.8 | 6.1 | 0.9 |
| Uridine-5′-phosphate (UMP) | 9.4 | 6.4 | 1.0 |
| Cytidine-5′-phosphate (CMP) | 4.5 | 6.3 | 0.8 |
| Guanosine-5′-phosphate (GMP) | 2.4, 9.4 | 6.1 | 0.7 |



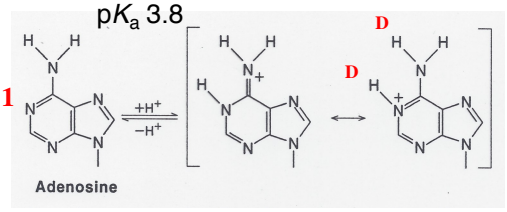

In Base: deprotonation at glycosidic bond, which lea

# Acid/base Properties of Bases

## Charged bases can de-stabilize the glycosidic bond and mutagenesis




Destabilizes glycosidic bond, leads to "AP-site" *




Leads to A → G mutations

* "AP-site" refers to a-purinic or a-pyridinic site

# Stability of the Polymers: Nucleic Acids

**BOTH acid or base lead to ssDNA or ssRNA**

## Acid/base treatment of DNA

In Base: deprotonation at G (N1) & U (N3) destabilizes the glycosidic bond, which leads to AP-sites.

In Acid: protonates at A (N1), G (N7), & C (N3). As we saw for G, this also destabilizes the glycosidic bond for C, which leads to AP-sites

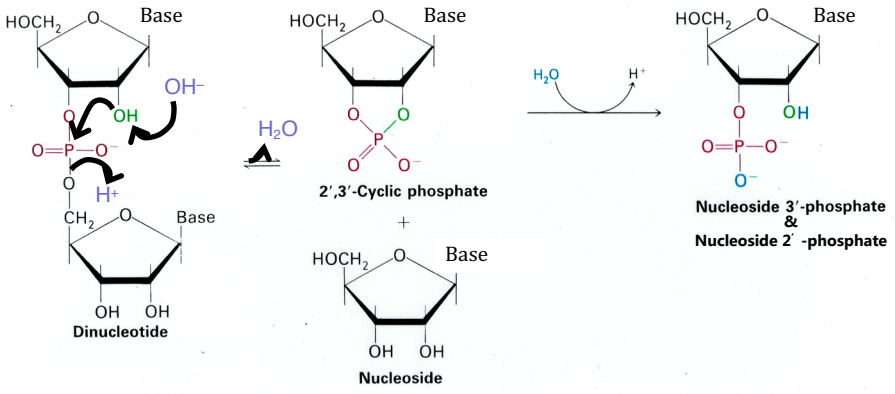
AP-sites can lead to cleavage of the phosphodiester bond

## Acid/base treatment of RNA

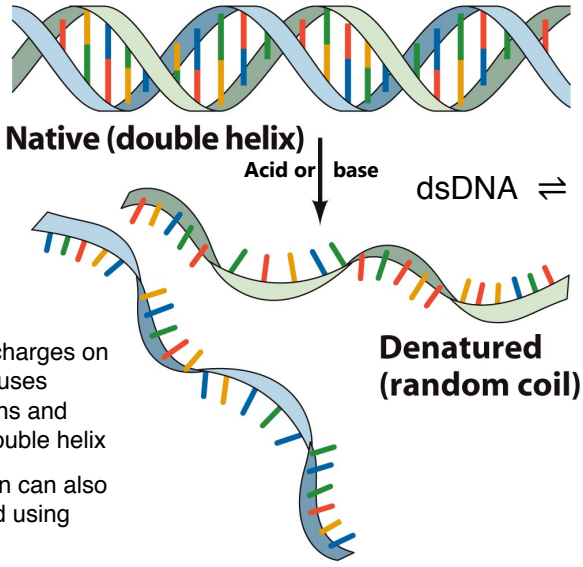
Similar generation of AP sites, except importantly in **Base**:
complete cleavage of ALL phosphodiester bonds!

# Stability of the Polymers: Nucleic Acids

## Base treatment of RNA



# Stability of the Polymers: Nucleic Acids
## DNA Denaturation

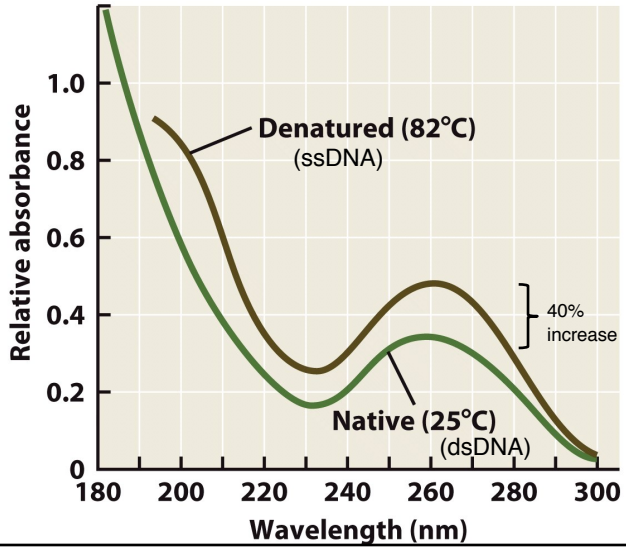



Acid/base puts charges on bases, which causes internal repulsions and breaks up the double helix

This denaturation can also be accomplished using heat.

# Stability of the Polymers: Nucleic Acids
## DNA Denaturation

UV Spectrum: Native vs. Denatured DNA



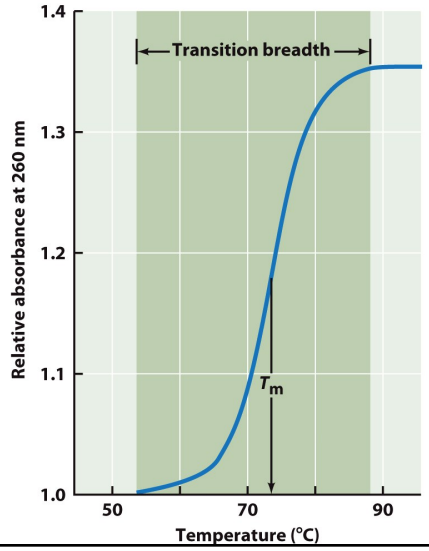

This denaturation causes a hyperchromic shift.

This makes a nice "observable" ($A_{260}$) and "perturbable" (heat).

What will such a plot of $A_{260}$ vs. Temp. look like?

# Stability of the Polymers: Nucleic Acids
# DNA Denaturation

DNA Melting Curve




This $T_m$ value is dependent on a number of parameters.

The shape is dependent on the sequence and size.

Like protein denaturation, this is a cooperative process.

# Stability of the Polymers: Nucleic Acids
# DNA Denaturation

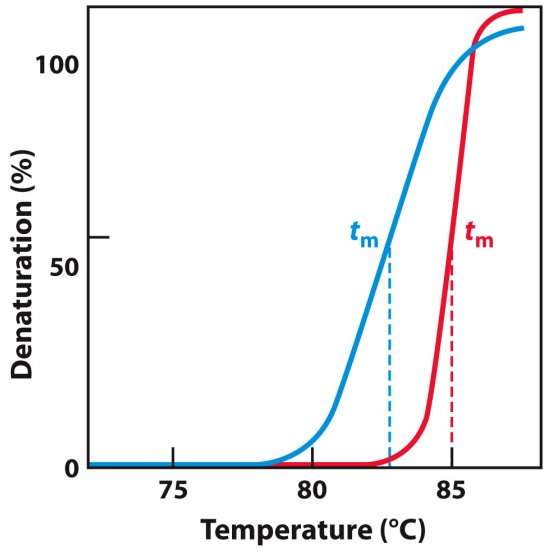



Figure 8-27a

# Stability of the Polymers: Nucleic Acids
## DNA Denaturation

This $T_m$ value is dependent on:

- [salt]
- solvents (urea, formamide, guanidine salts)
- G:C content of sequence

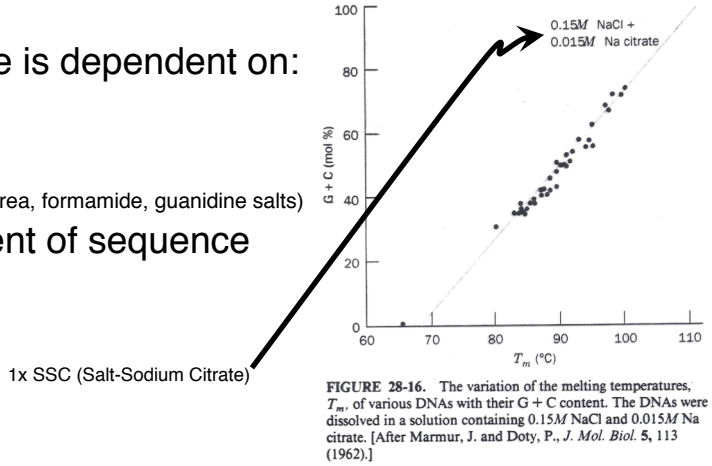
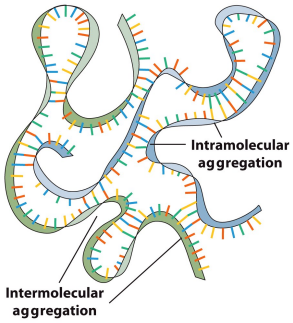



**FIGURE 28-16.** The variation of the melting temperatures, $T_m$, of various DNAs with their G + C content. The DNAs were dissolved in a solution containing 0.15*M* NaCl and 0.015*M* Na citrate. [After Marmur, J. and Doty, P., *J. Mol. Biol.* **5**, 113 (1962).]

# Stability of the Polymers: Nucleic Acids
## DNA *Renaturation*

ssDNA ⇌ dsDNA

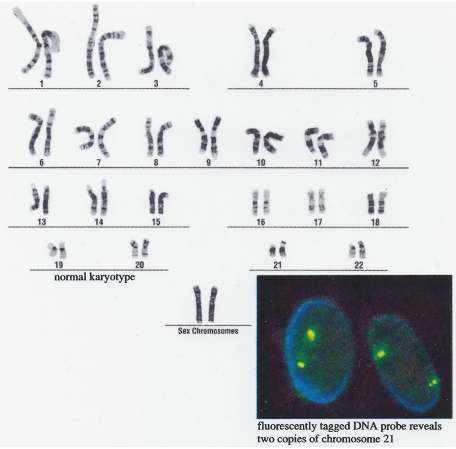
- Also called re-annealing or hybridization
- Depends on conditions (temp, [salt], solvent) that are maintained BELOW the $T_m$ value.
- In addition, the proper formation of the complete, pristine, double helix (completely double-stranded) requires the proper amount of TIME and CONCENTRATION of nucleic acid.
- Plots of this are called $C_o t$ curves, which are much like $T_m$ curves.
- $C_o t$ values are dependent on the complexity of the sequence.
- Not enough time, you get scrambled structures
- Given enough time, very specific annealing occurs….

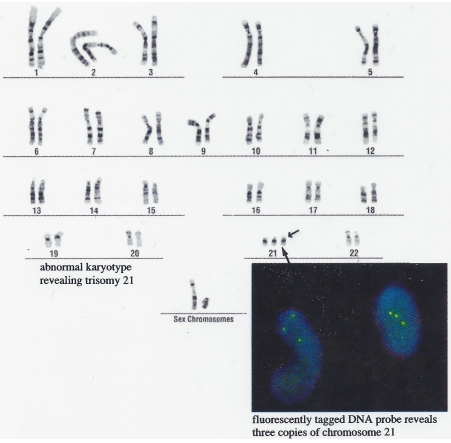



Partially Renatured DNA

# Stability of the Polymers: Nucleic Acids
## DNA *Renaturation*

EXAMPLE: Karyotyping




Fluorescence *in situ* hybridization (FISH)

FISH using fluorescence probe for gene on chromosome 21.

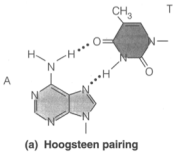
This FISH probe (a fluorescent DNA) found its complementary sequence among 3,000,000,000 bp in each cell!

# Stability of the Polymers: What is responsible for STABILITY of dsDNA?

| Base Pair | $K(M^{-1})^a$ |
|---|---|
| **Self-Association** | |
| A·A | 3.1 |
| U·U | 6.1 |
| C·C | 28 |
| G·G | $10^3 - 10^4$ |
| **Watson–Crick Base Pairs** | |
| A·U | 100 |
| G·C | $10^4 - 10^5$ |

[a] Data measured in deuterochloroform at 25°C.

Source: Kyogoku, Y., Lord, R. C., and Rich, A., *Biochim. Biophys. Acta* **179**, 10 (1969).




C:C nearly as stable as A:U

G:G more stable than A:U and as much as G:C

Hoogstein bp is very stable

# Stability of the Polymers: What is responsible for STABILITY of dsDNA

What forces operate? If it's not the H-bonds, then what is it?

- Ionic/electrostatics (salt-bridges)?

  It's a poly-anion; so charges actually de-stabilize

- Hydrophobic?

  Unlike proteins, where this is the driving force, experiments show that the ssDNA ⇌ dsDNA reaction is enthalpy driven process; ∴ bonds

- van der Waals?

  Yes! This is the most important. As uniform bp come together due to complementarity, the planer bases "stack" on each other and are close enough (<2 Å) to generate induced dipoles.
  Once started, it "zips" together as long as there are complementary bp being formed.

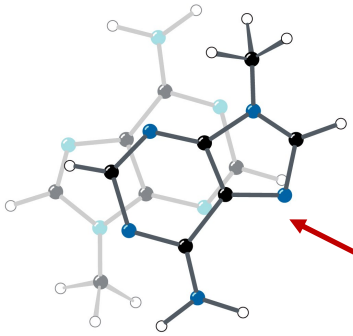
**Stacking energy = stability**

**H–bonds in complementary bp = specificity**

# Stability of the Polymers: What is responsible for STABILITY of dsDNA

**So, if its stacking energy, why are G:C rich sequences more stable than A:T rich sequences?**

## Stacking Energies in B-DNA



Adenine ring stacking

TABLE 24-2 Stacking Energies for the Ten Possible Dimers in B-DNA

| Stacked Dimer | Stacking Energy (kJ · mol⁻¹) |
|---|---|
| C · G<br>G · C | −61.0 |
| C · G<br>A · T | −44.0 |
| C · G<br>T · A | −41.0 |
| G · C<br>C · G | −40.5 |
| G · C<br>G · C | −34.6 |
| G · C<br>A · T | −28.4 |
| T · A<br>A · T | −27.5 |
| G · C<br>T · A | −27.5 |
| A · T<br>A · T | −22.5 |
| A · T<br>T · A | −16.0 |

*Source:* Ornstein, R.L., Rein, R., Breen, D.L., and MacElroy, R.D., *Biopolymers* 17, 2356 (1978).

## Stability of the Polymers: Biochemical

- Enzymes that catalyze the hydrolysis of the phosphodiester bonds:
- These enzymes are called “Nucleases”
  - Like proteases, if they cleave in the middle, they are called endonucleases (e.g., restriction endonucleases)
  - If they cleave at the ends, they are called exonucleases

    Exonucleases can be specific for either 5’-ends or 3’-ends, or either double-stranded or single-stranded nucleic acids (e.g., S1 nuclease)
- Also specificity for either DNA (DNases) or RNA (RNAses)
  - RNAases are very stable, DNAases require $Mg^{+2}$ cofactor
  - Can be inhibited by DEPC or EDTA, respectively