Nucleic Acids and Molecular Biology: *Part I*

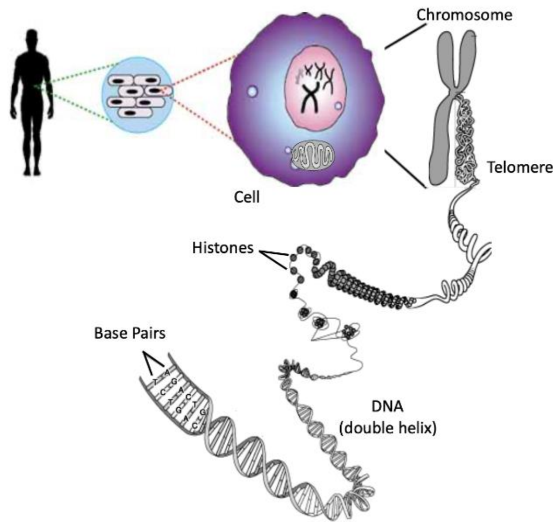
Biochemistry Boot Camp 2023 !

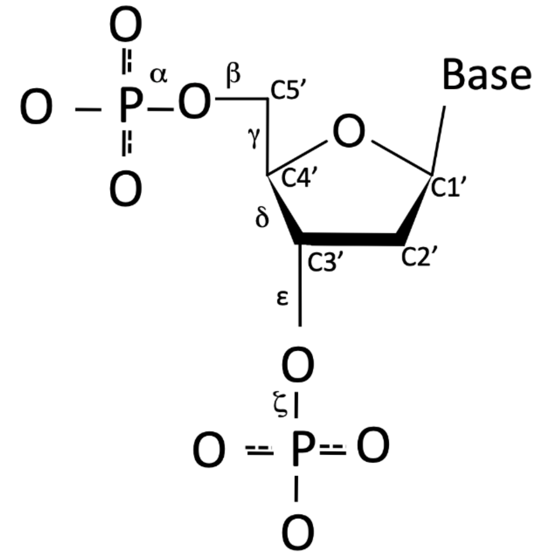
Lightning Bolt

SA node
Atrium
AV node
Purkinje fiber
Endocardium
Midmyocardium
Epicardium
Septum
RV
LV
Apex
400 msec
P
Q
R
S
T
QT

Chromosome
Telomere
Cell
Histones
Base Pairs
DNA
(double helix)

Deoxy-Ribose Nucleic Acids (DNA and RNA)

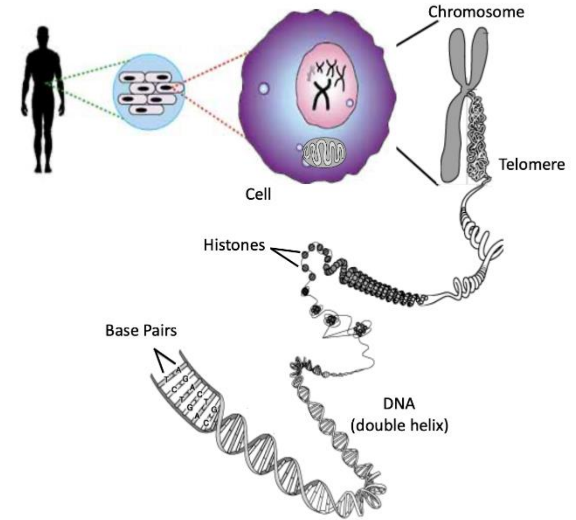




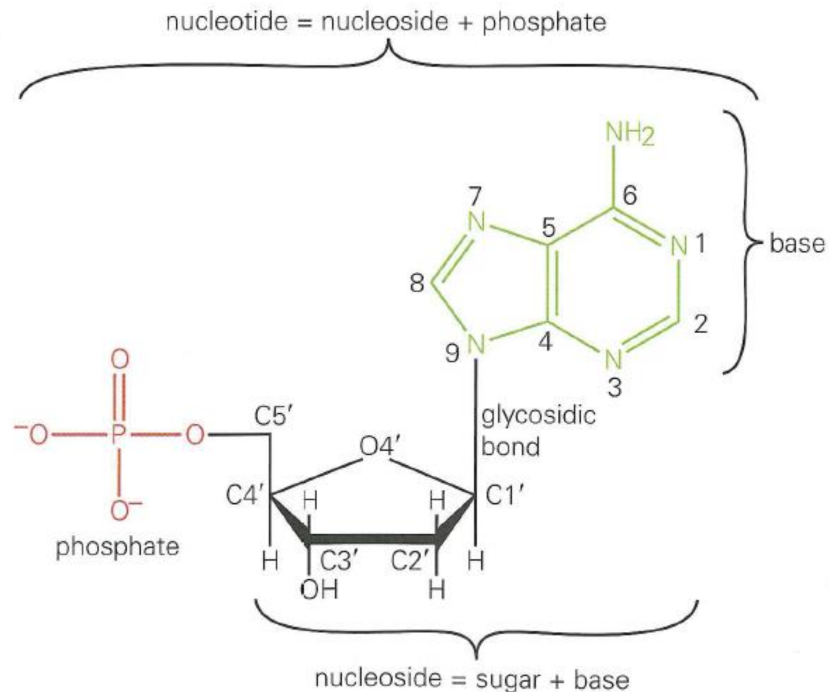
- DNA and RNA polymers of (deoxy) ribose nucleotides
- DNA - chromosomes, mitochondria and chloroplasts
- DNA - Carries the genetic information

- DNA ______________ -> RNA ______________-> Protein

Nucleotide Structure

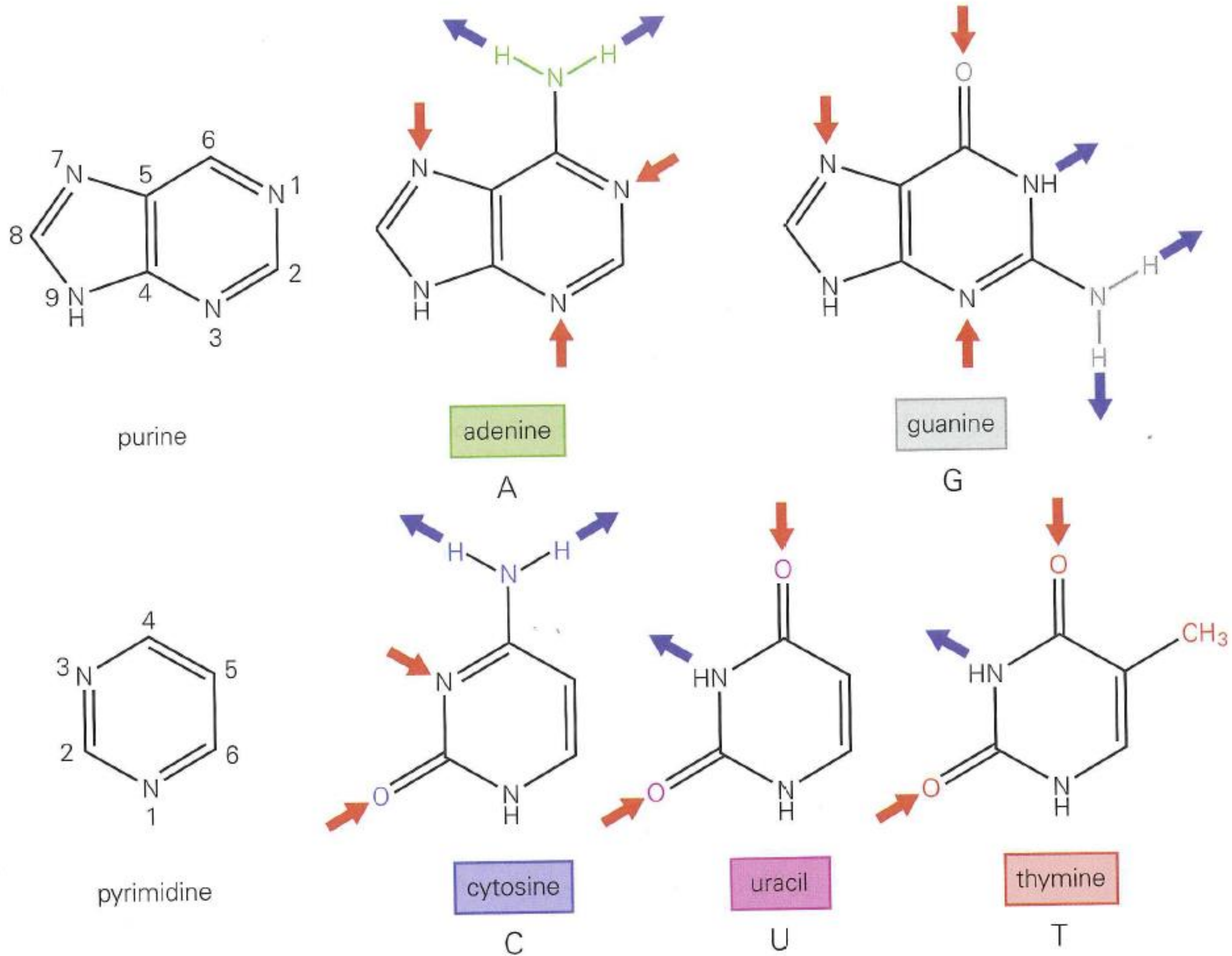


Question: Is this RNA or DNA?



Molecules of Life, pp. 15

Nucleic Bases



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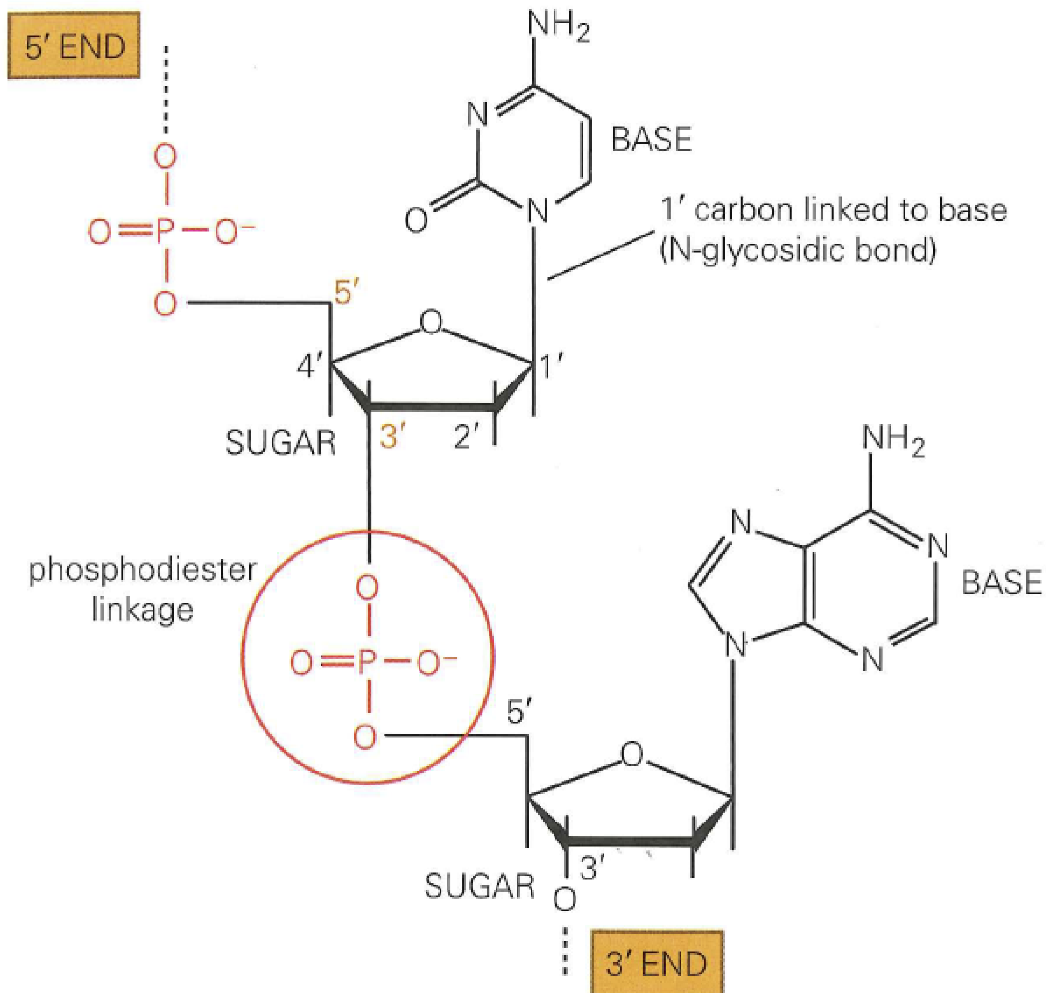
Nomenclature (Scientific Names)

	Base	Nucleoside	Nucleotide	Nucleic Acid
Purine	Adenine	Adenosine	Adenylate	RNA
		Deoxyadenosine	Deoxyadenylate	DNA
	Guanine	Guanosine	Guanylate	RNA
		Deoxyguanosine	Deoxyguanylate	DNA
Pyrimidines	Cytosine	Cytidine	Cytidylate	RNA
		Deoxycytidine	Deoxycytidylate	DNA
	Thymine	Thymidine	Thymidylate	
		Deoxythymidine	Deoxythymidylate	DNA
	Uracil	Uridine	Uridylate	RNA

Nucleic Acids are Polymers

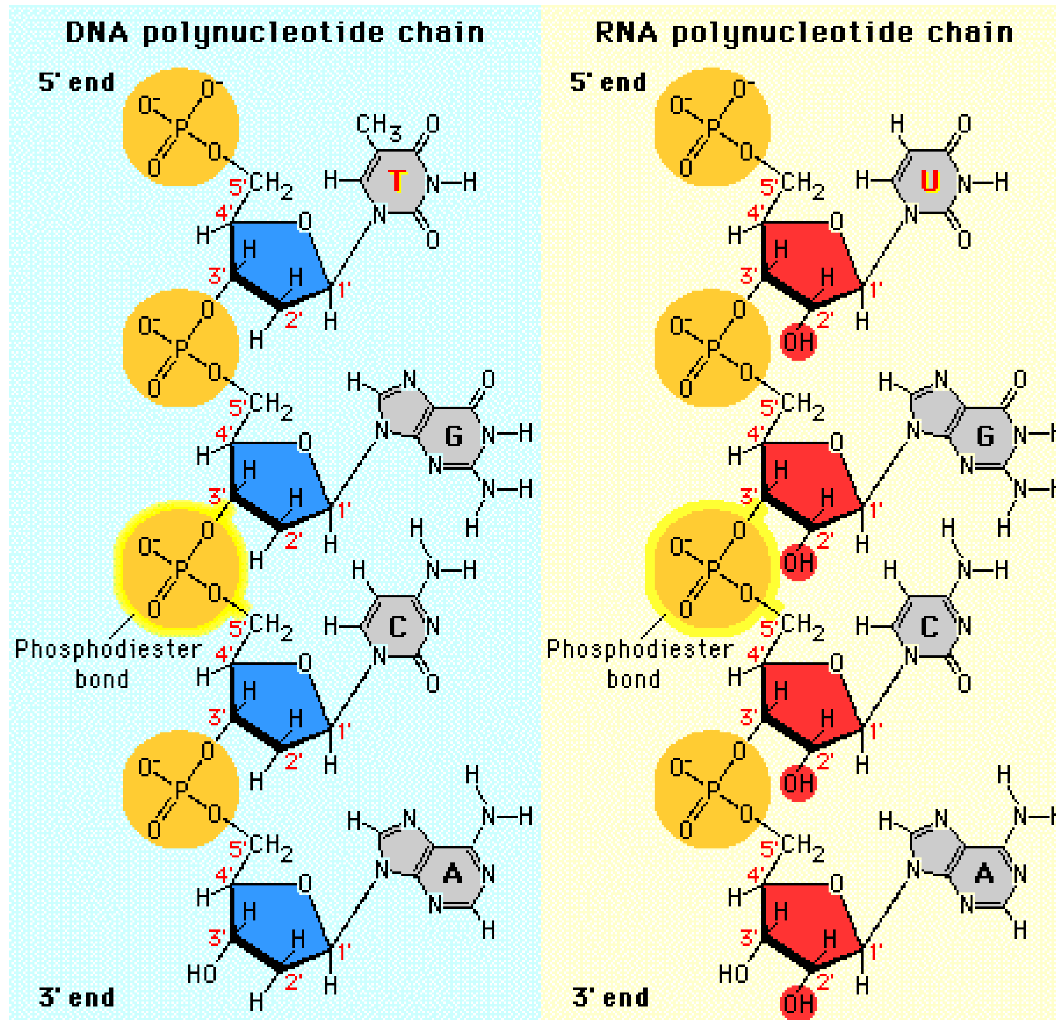


DNA & RNA Polymerase: Build up DNA and RNA from nucleoside triphosphates (5′ → 3′ synthesis)

Convention: RNA/DNA typically is read from 5′ to 3′ direction (e.g. 5′-ATTGCAAC-3′)

Molecules of Life, pp. 21

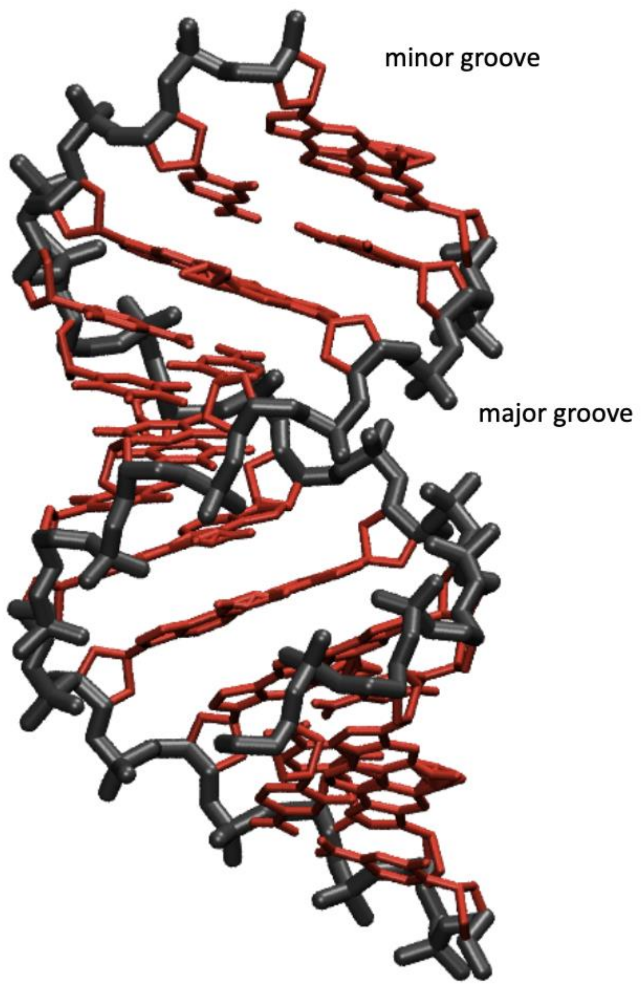
DNA vs RNA

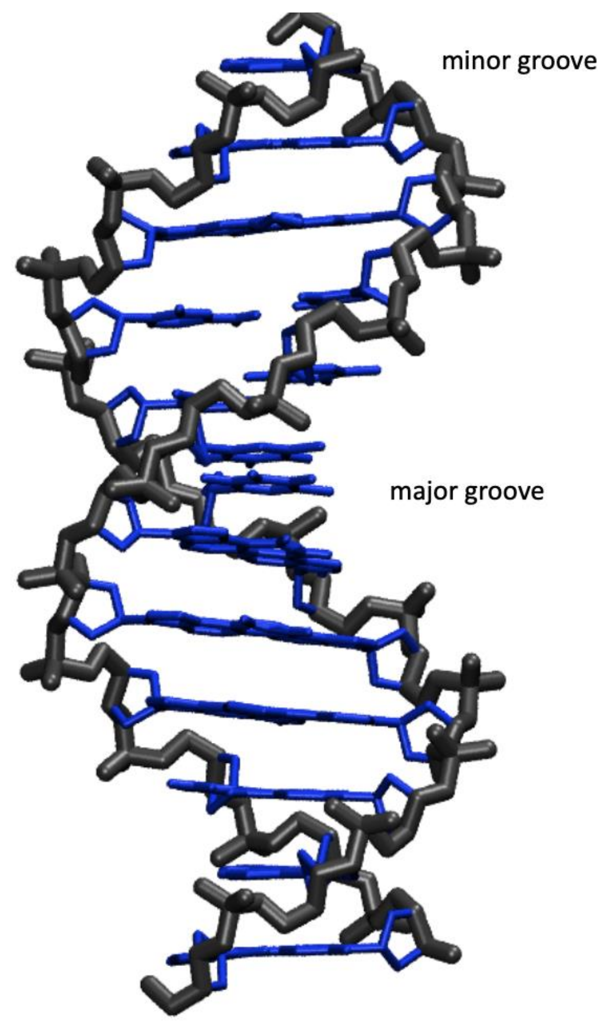


- DNA less reactive
- RNA is easily attacked by enzymes

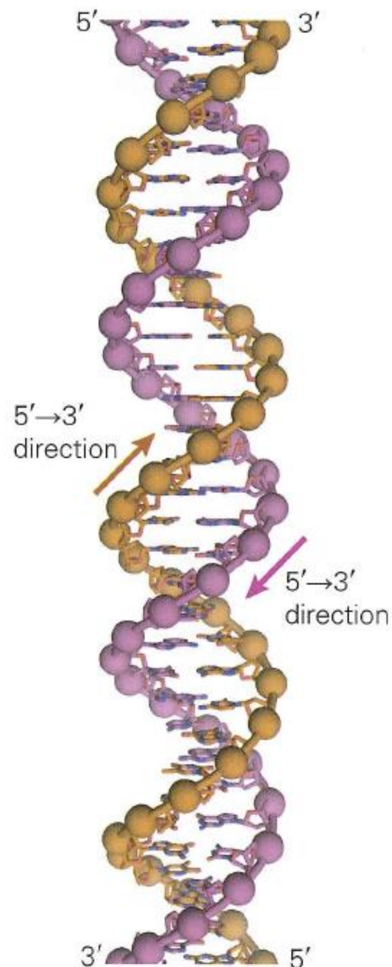
Science, www.phschool.com (Accessed on June 02, 2014)

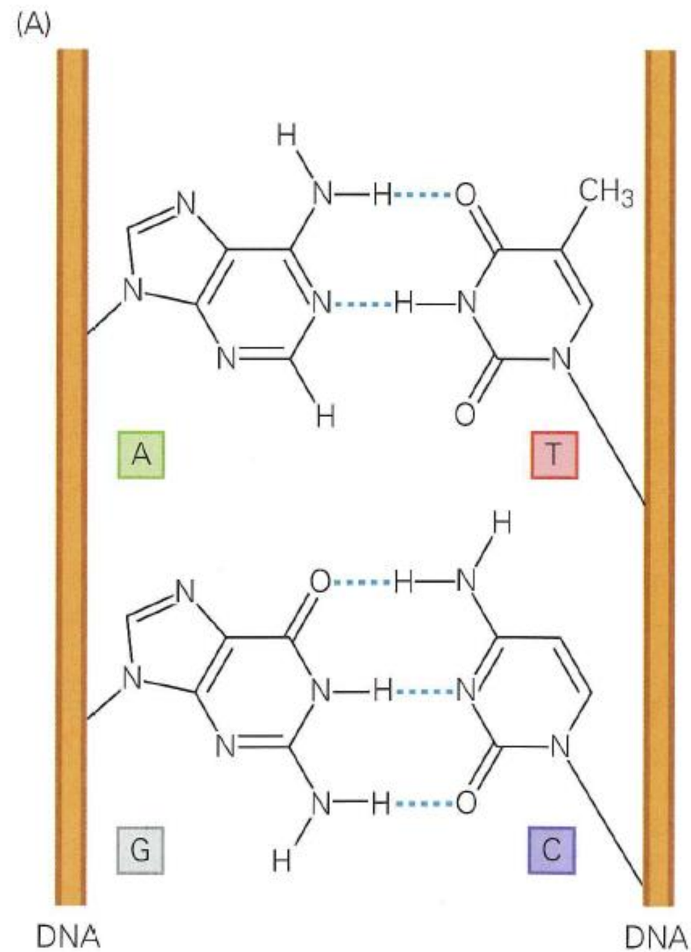
DNA and RNA are Similar but Different





Watson –Crick Base Pairing (Antiparallel) Double Helix





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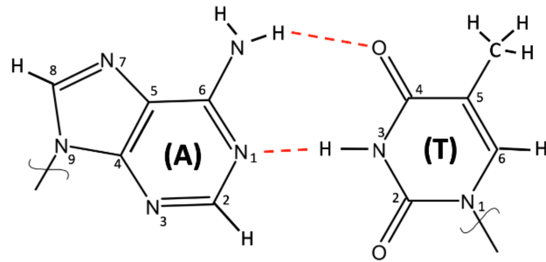
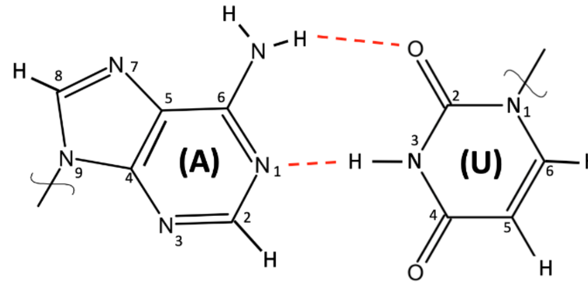
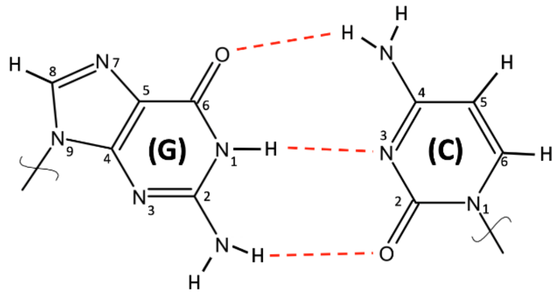
Base Pairing

Watson Crick

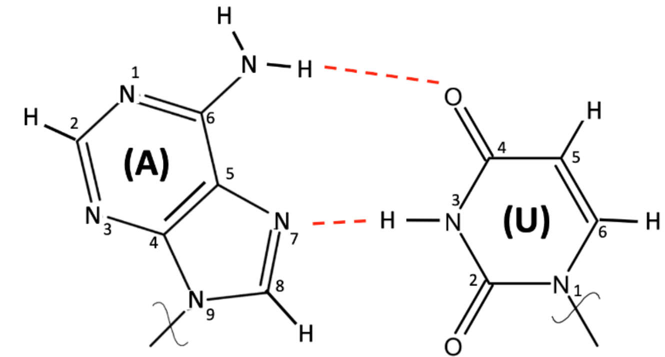


Reverse Watson Crick





Hoogsteen

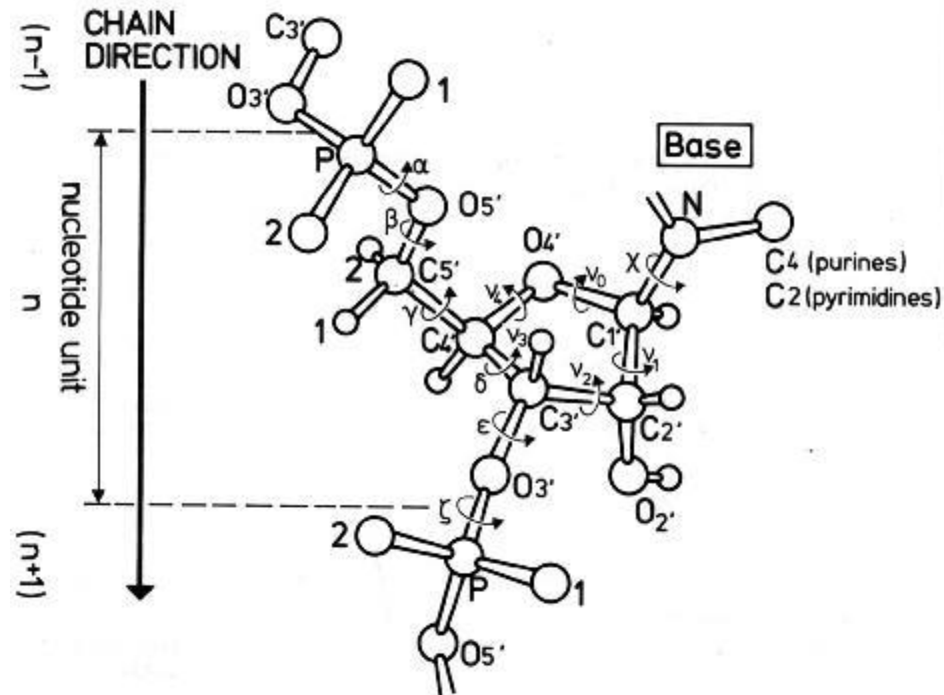


- Watson-Crick base pairing
- RNA can “hybridize” with DNA, forming mixed strands
- **Example:** What’s the reverse complement to AUCCGCCTT?

Nucleic Acid Structure

- Bases are planar
- Nucleic acids
  - 5 backbone torsion angles
- Proteins
  - 2 backbone torsion angles

- Nucleic acid structure can be much more complex compared to protein



Saenger, W. *Principles of Nucleic Acid Structure.*

Nucleic Acid Sugar Pucker

- ν angles are related, so sugar ring can be simplified

- Think “chair” and “boat” forms of cyclohexane

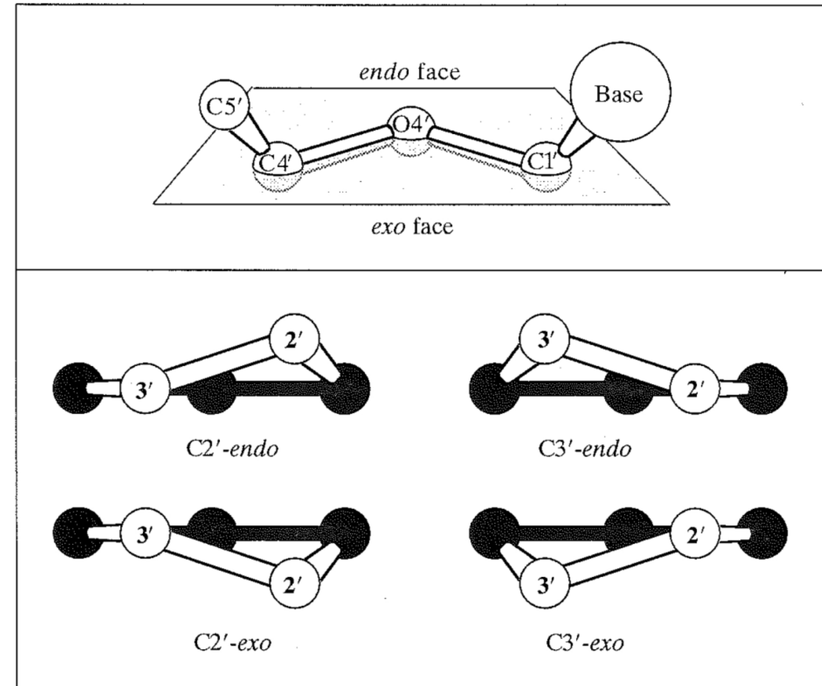


Figure 1.38 Sugar conformations of nucleic acids. The pucker of the sugar ring in RNA and DNA is defined relative to the plane formed by the C1′-carbon, C4′-carbon, and O4′-oxygen of the five-member ring. The *endo* face lies above the plane, toward the nucleobase, while the *exo* face lies below the plane.

van Holde, *et al*. *Principles of Physical Biochemistry.*

Nucleic Acid Primary Structure

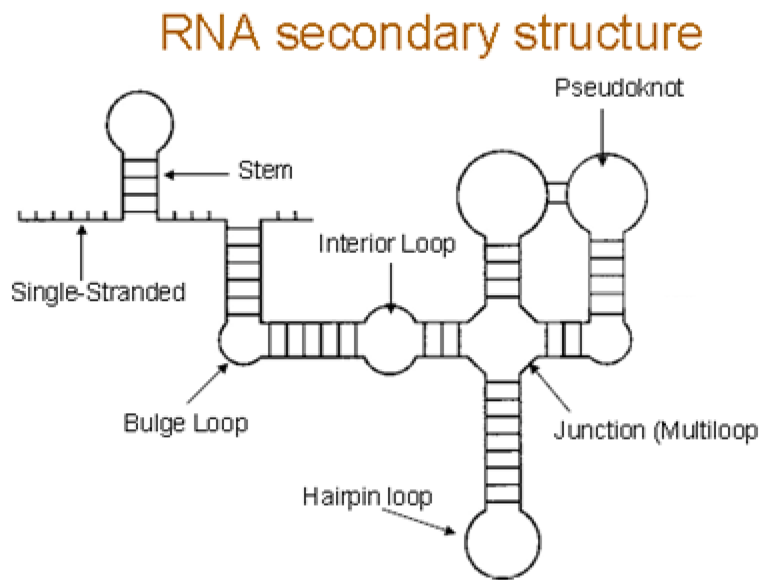
- **Just like proteins:** the sequence of bases

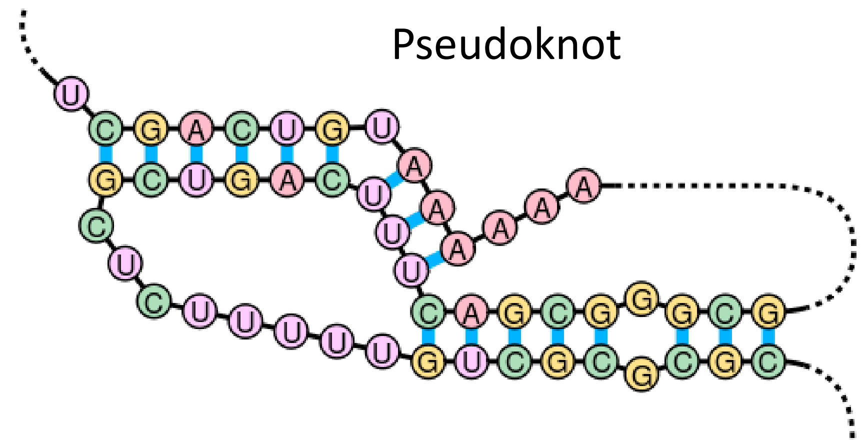
5'-dAdGdTdTdCdAdCdCdC-3' (DNA)

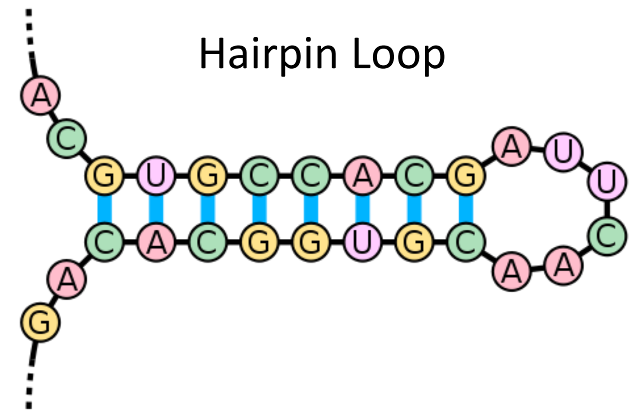
AGTTCACCC

5'-AGUUCACCC-3' (RNA)

Secondary Structure



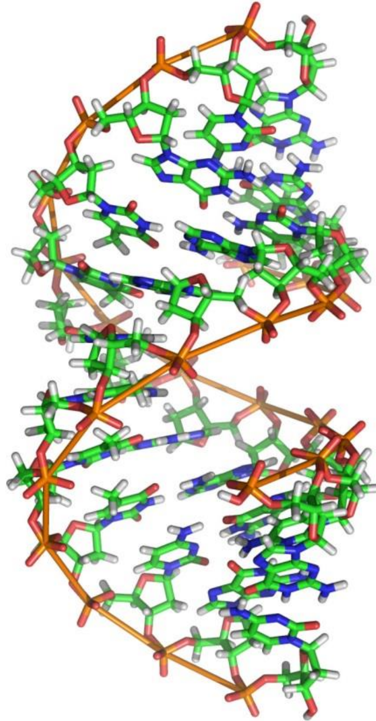




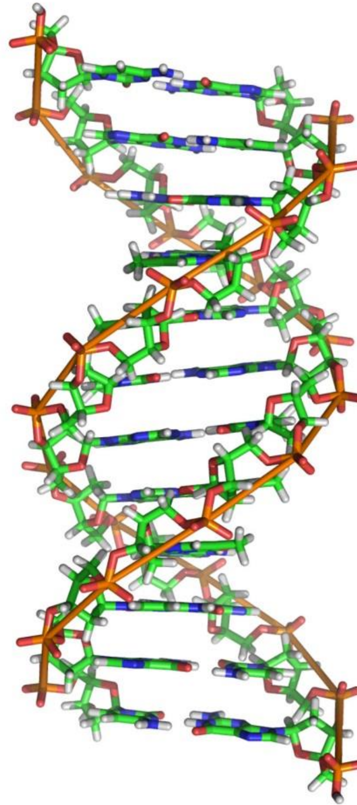
- Base pairing motifs

Source: Wikipedia, “RNA Secondary Structure,” “Nucleic Acid Secondary Structure”

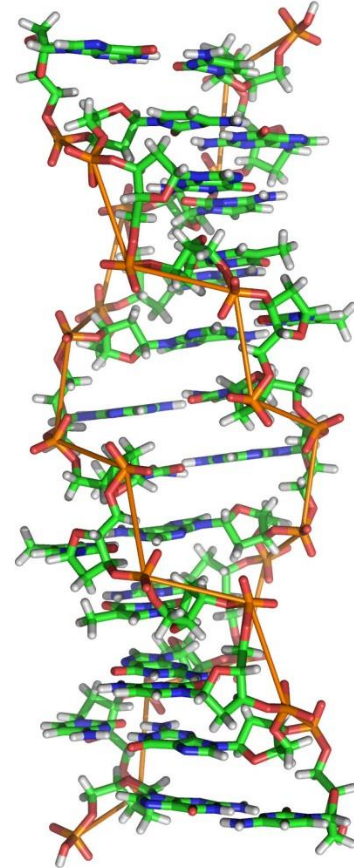
Tertiary Structure



A Form DNA



B Form DNA



Z Form DNA

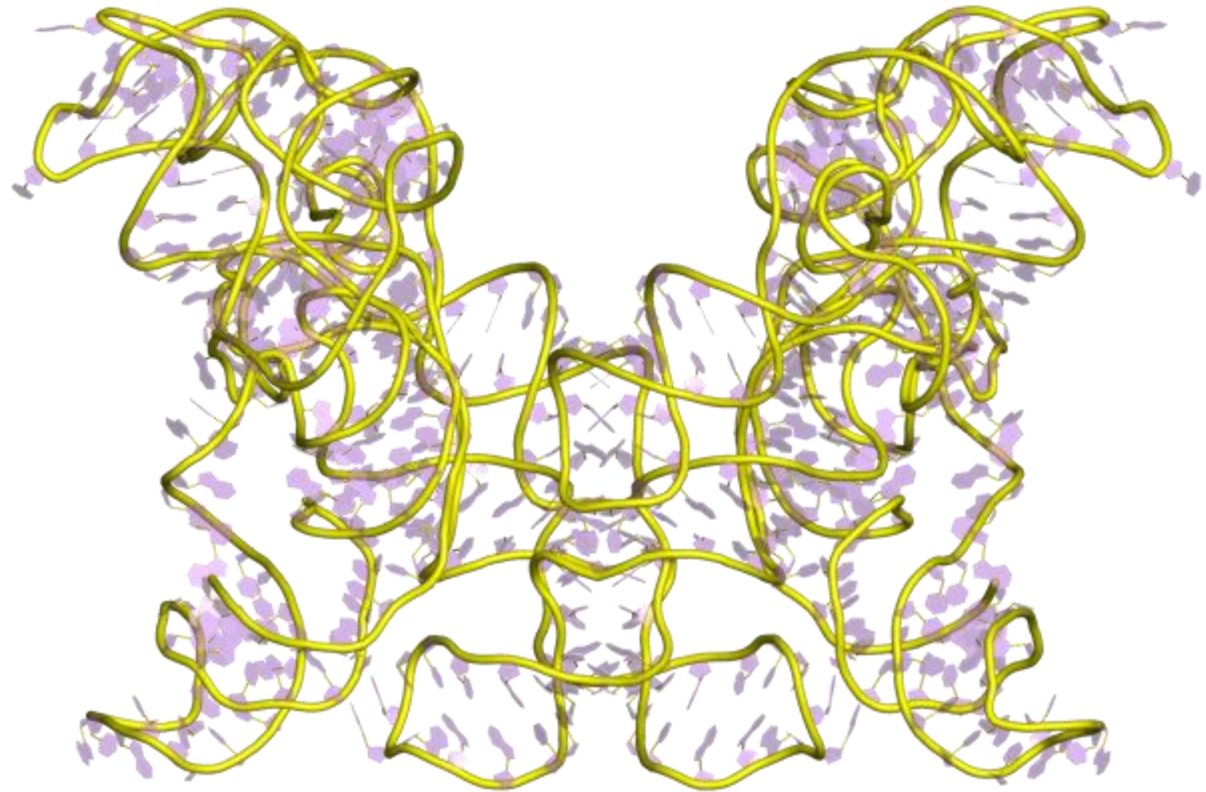
Source: Steven Carr, www.mun.ca

Tertiary Structure

	Average Torsion Angles for Nucleic Acid Helices (in °)						
Structure Type	**Alpha**	**Beta**	**Gamma**	**Delta**	**Epsilon**	**Zeta**	**Chi**
A-DNA (fibres)	-50	172	41	79	-146	-78	-154
GGCCGGCC	-75	185	56	91	-166	-75	-149
B-DNA (fibres)	-41	136	38	139	-133	-157	-102
CGCGAATTCGCG	-63	171	54	123	-169	-108	-117
Z-DNA (C residues)	-137	-139	56	138	-95	80	-159
Z-DNA (G residues)	47	179	-169	99	-104	-69	68
DNA-RNA decamer	-69	175	55	82	-151	-75	-162
A-RNA	-68	178	54	82	-153	-71	-158

Blackburn and Galt. *Nucleic acids in chemistry and biology.*

Tertiary and Quaternary Structure



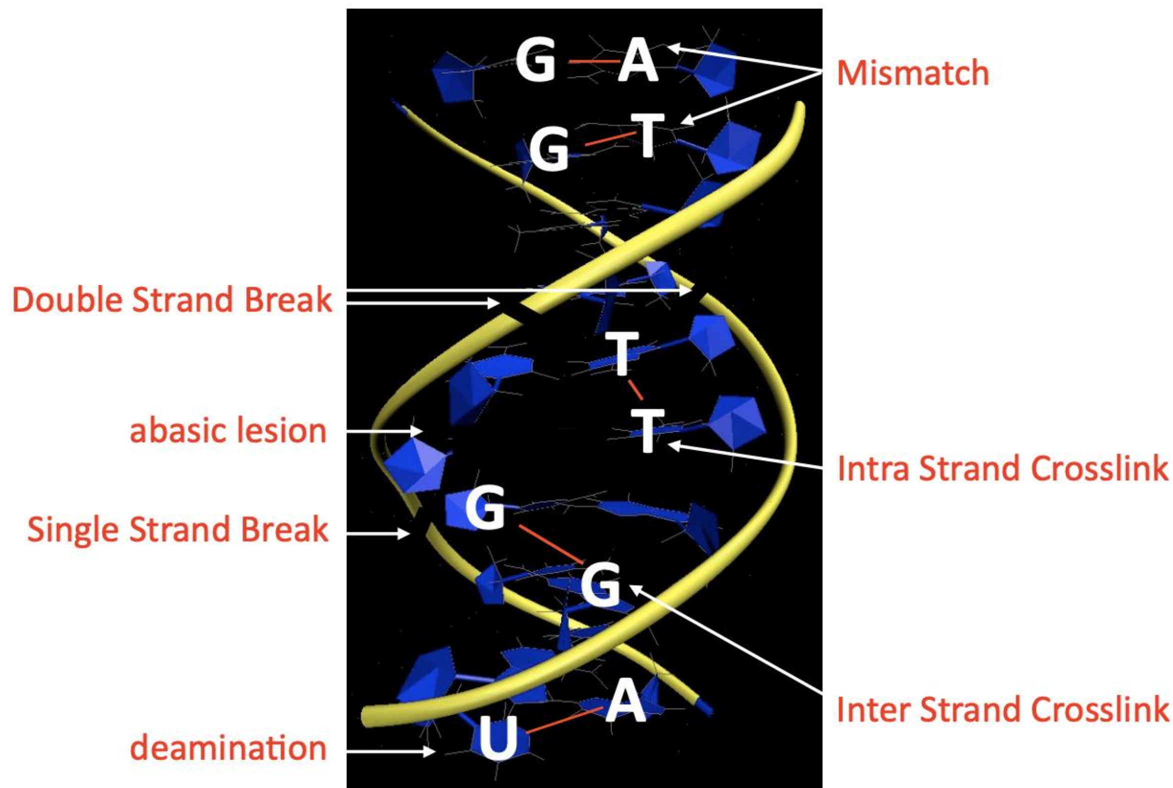
Ribozyme: An RNA capable of catalyzing a chemical reaction

The ribosome contains a significant amount of RNA as well as proteins

Macromolecules can perform incredibly diverse structures!
(And we haven't even mentioned lipids and sugars.)

Wikipedia, "Group I Catalytic Intron." Accessed 8/23/2012.

DNA Damage = Major Driving Force in Cancer



- **UV light can generate ~ 100,000 lesions per cell per hour.**
- **Healthy human cells generate ~ 10,000 lesions per cell / day.**
- **Repair pathways for fixing some but <u>NOT</u> all of this damage.**

Think and Discuss

Why is DNA damage bad?

Could DNA damage ever be good?

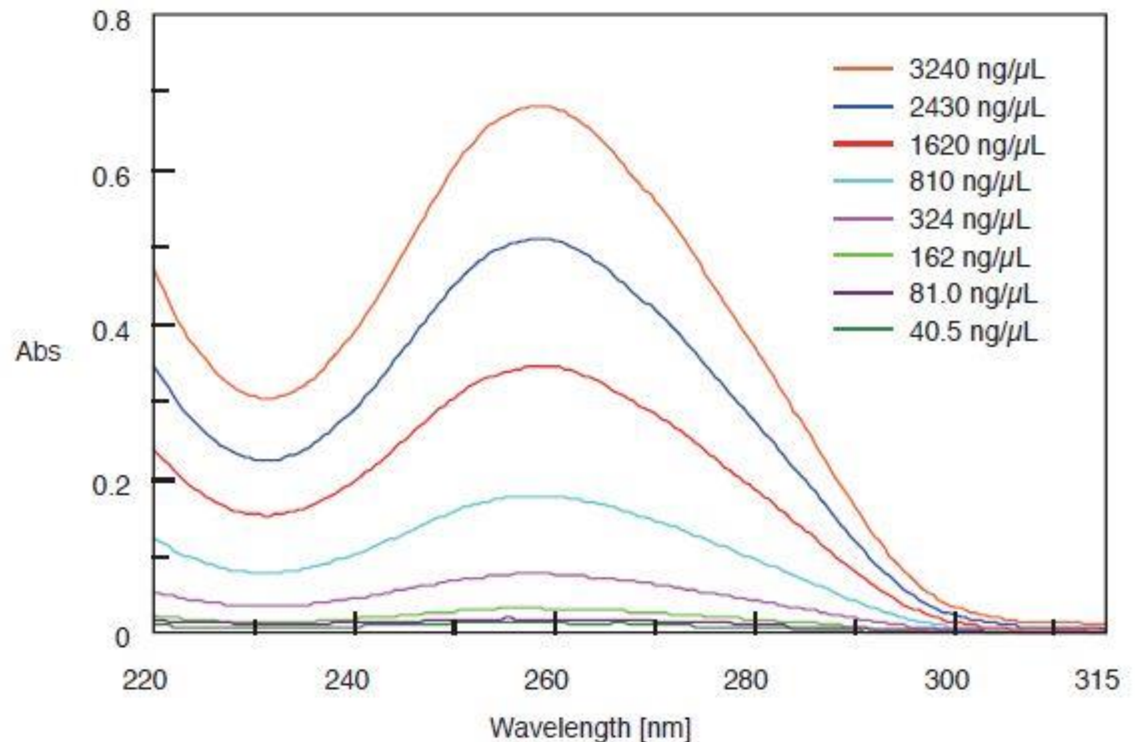
DNA and RNA Science Can Help!

Nucleic Acid Extinction Coefficient

DNA Concentrations: Often measured in µg/mL (or the equivalent ng/µL) instead of M, mM, etc. Also sequence isn't exactly known in many cases.

Rule of Thumb: For double-stranded, plasmid DNA, the extinction coefficient at 260 nm is

0.020 $(\mu g/mL)^{-1}\ cm^{-1}$



Source: www.jascoinc.com

DNA vs. Protein Absorbance

DNA Concentrations: At 260 nm, double-stranded DNA has an extinction coefficient of

$0.020\ (\mu g/mL)^{-1}\ cm^{-1}$

Protein Concentrations: At 280 nm, the GB3 protein has an extinction coefficient (in equivalent units) of

$0.0016\ (\mu g/mL)^{-1}\ cm^{-1}$

Which is more sensitive?

What are the implications?

Nucleic Acids – Smaller Molecules

OligoAnalyzer

Instructions | Definitions | Feedback

Sequence   5' MOD ▾ INTERNAL ▾ 3' MOD ▾ MIXED BASES ▾

CGA AGA ACA GGA AGC GGA ATT TAA AGA AG

Bases 29   CLEAR SEQUENCE

Try the new batch mode here

Parameter sets: SpecSheet (Default)

Target type: DNA

Oligo Conc: 0.25 μM

Na^{+} Conc: 50 mM

Mg^{++} Conc: 0 mM

dNTPs Conc: 0 mM

ANALYZE

HAIRPIN

SELF-DIMER

HETERO-DIMER

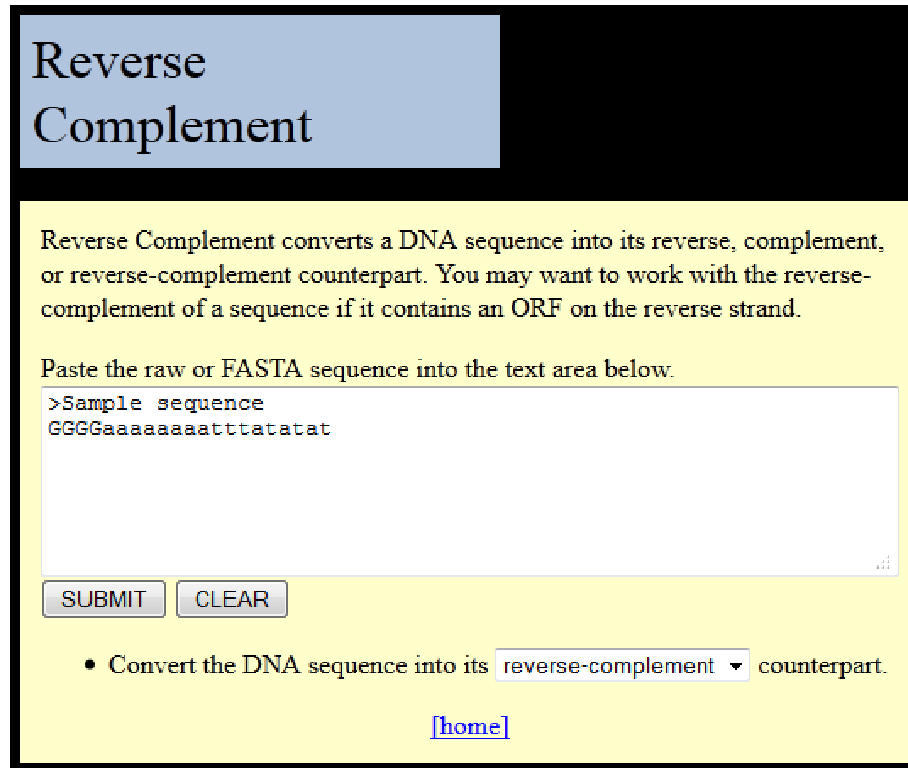
NCBI BLAST

TM MISMATCH

ADD TO ORDER

- IDT DNA Analyzer (extinction coefficient, Tm): https://www.idtdna.com/pages/tools/oligoanalyzer
  - Need to log in, create an account (free)

Calculating Reverse Complement



- Bioinformatics.org Calculator (no-frills): http://bioinformatics.org/sms/rev_comp.html

Source: www.jascoinc.com

DNA Translation Tool

- **Site:** http://web.expasy.org/translate/
- **Input:** DNA or RNA sequence (5’ → 3’ orientation)
- **Output:** All six possible translation frames

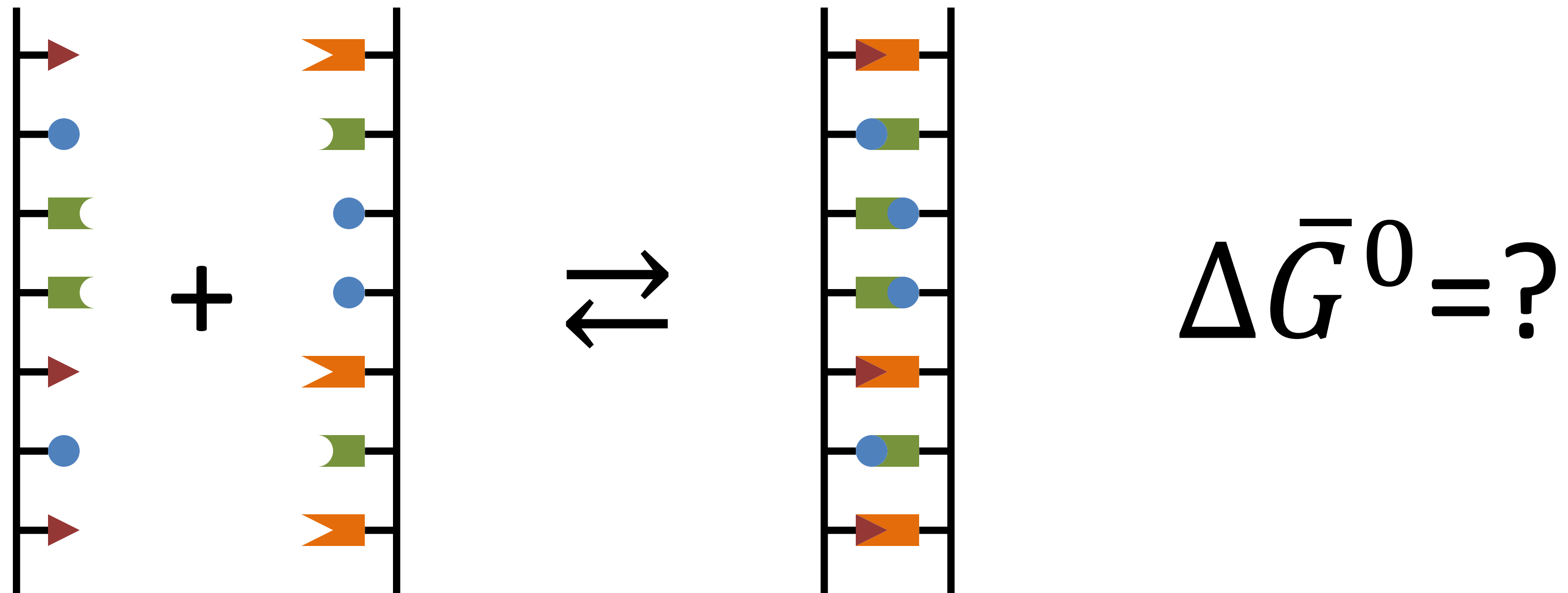
Other Databases

- NCBI Databases work for DNA sequences, too (reference sequences start with NM_)
- PDB also houses a number of RNA/DNA structures in addition to proteins

Think And Discuss

How can these databases be used to make your lab work easier? What are some practical examples?

DNA "Melting"



- Two strands come together:
  - How much work can be done?
  - Which side of the reaction does temperature favor?

Thermal Melts

- Adding heat favors highly random systems, DNA will separate at high temperature
  - Secondary and tertiary structure is lost, primary is maintained

- What will affect the melting temperature?

Predicting Melting Temperatures

- To calculate T_m, add 4 °C for each G-C pair, and 2 °C for each A-T
  - Not terribly accurate

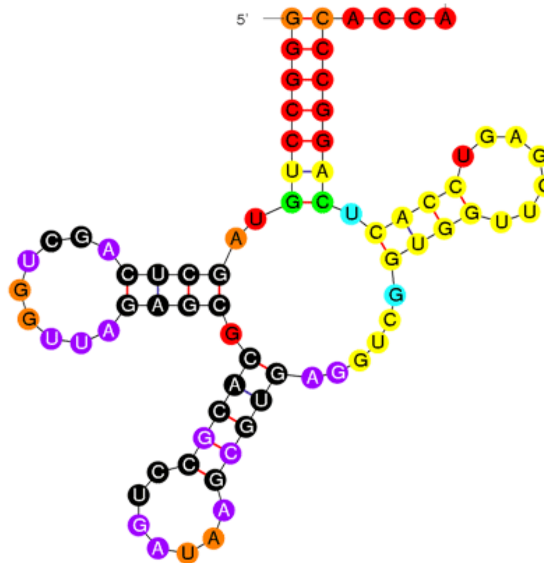
- **Example:** GCCCTGAAGGTCAAGTCCCCC
  - 14 G-C = 56 °C
  - 7 A-T = 14 °C
  - Prediction is 70

Predicting Melting Temperatures

- IDT OligoAnalyzer: https://www.idtdna.com/pages/tools/oligoanalyzer
- **Input:** Your DNA sequence of interest, salt concentration
- **Output:** T_m, extinction coefficient, %GC content

Predicting Secondary Structure

- `mfold` Web Server: http://mfold.rna.albany.edu/?q=mfold
- **Input:** RNA/DNA sequence
- **Output:**



Example: HIV TAR RNA

- Trans-Activation Response Element – Binds with a protein (Tat) to promote viral transcription

- Sequence:

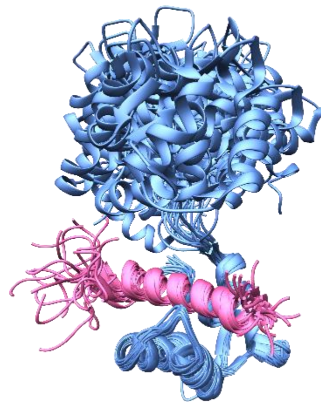
GGGUCUCUCUGGUUAGACCAGAUCUGAGCCUGGGAGCUCUCU
GGCUAACUAGGGAACCCAC

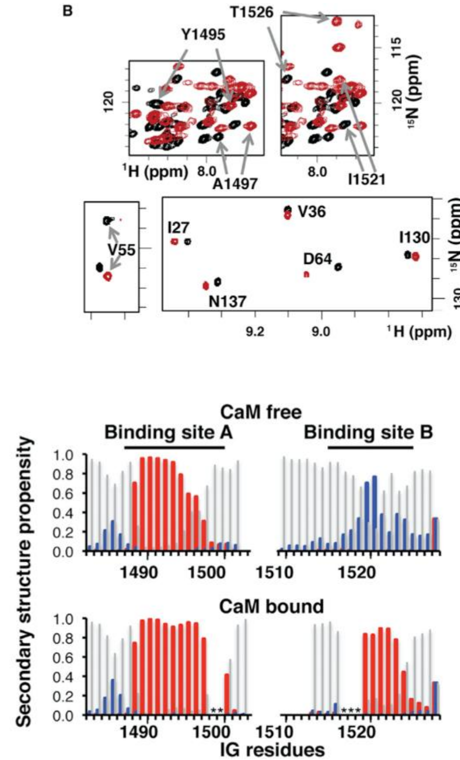
Nuclear Magnetic Resonance (NMR)

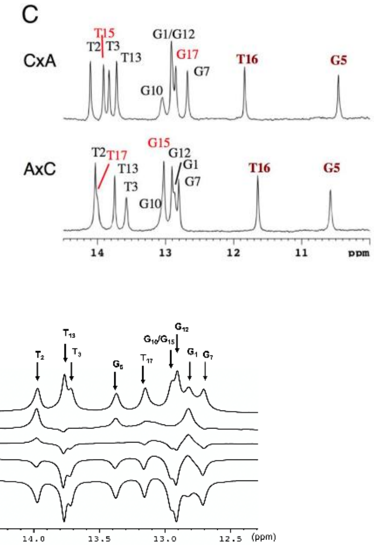
Discuss: What can you do with NMR

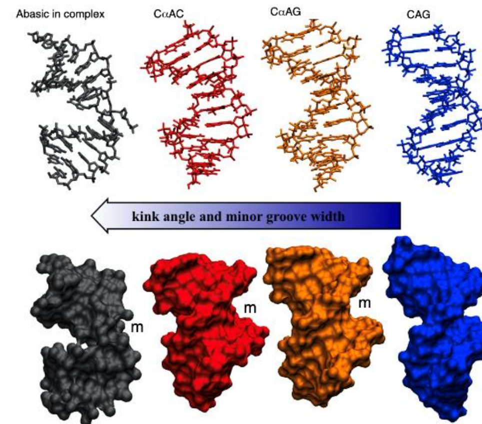
- Verify composition of small molecules
- Determine structure of protein and or protein complexes
- Directly investigate movement
  - (limited to specific time scales)
- Quantify conformational change

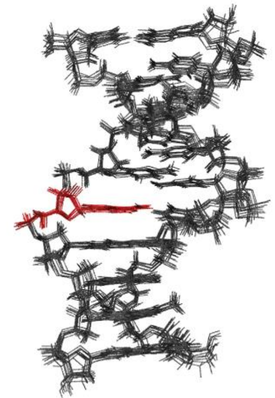
- and much more .....





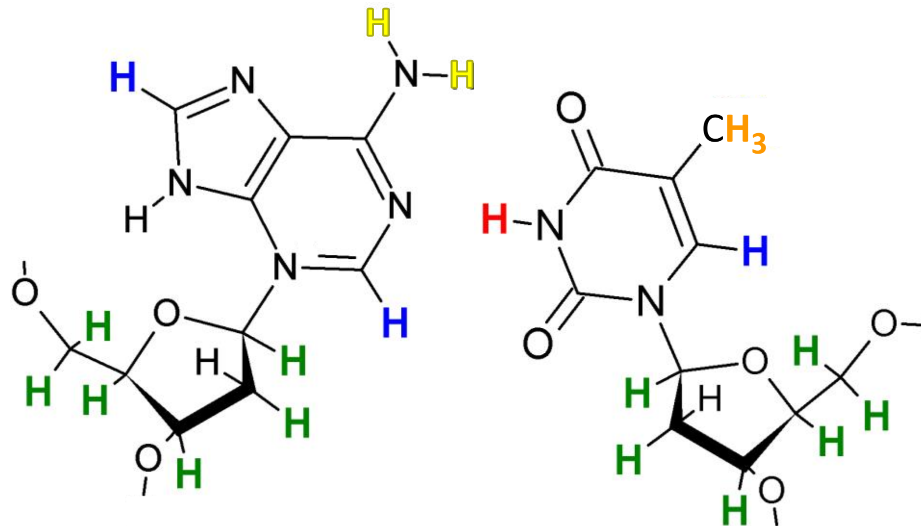


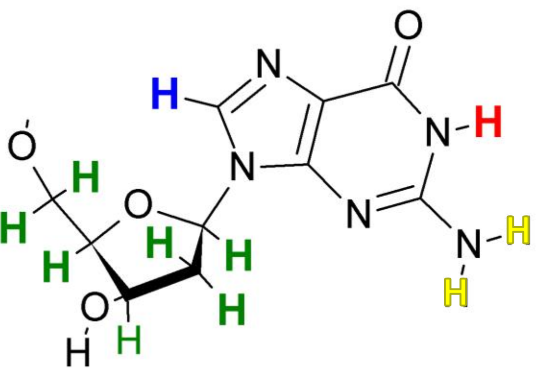


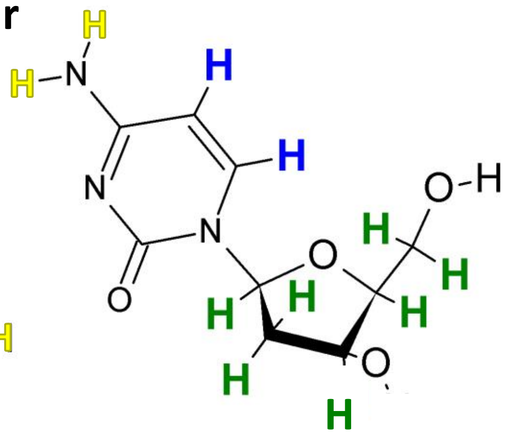


Protons provide information about structure and dynamics

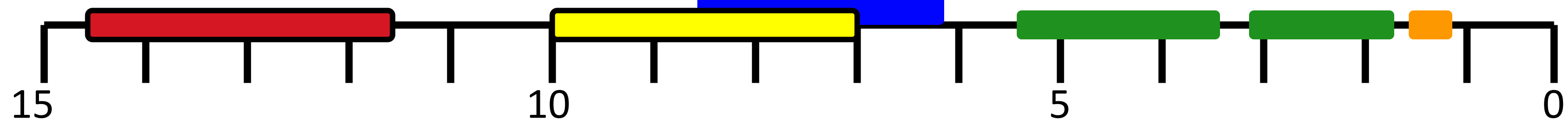
GC base pair





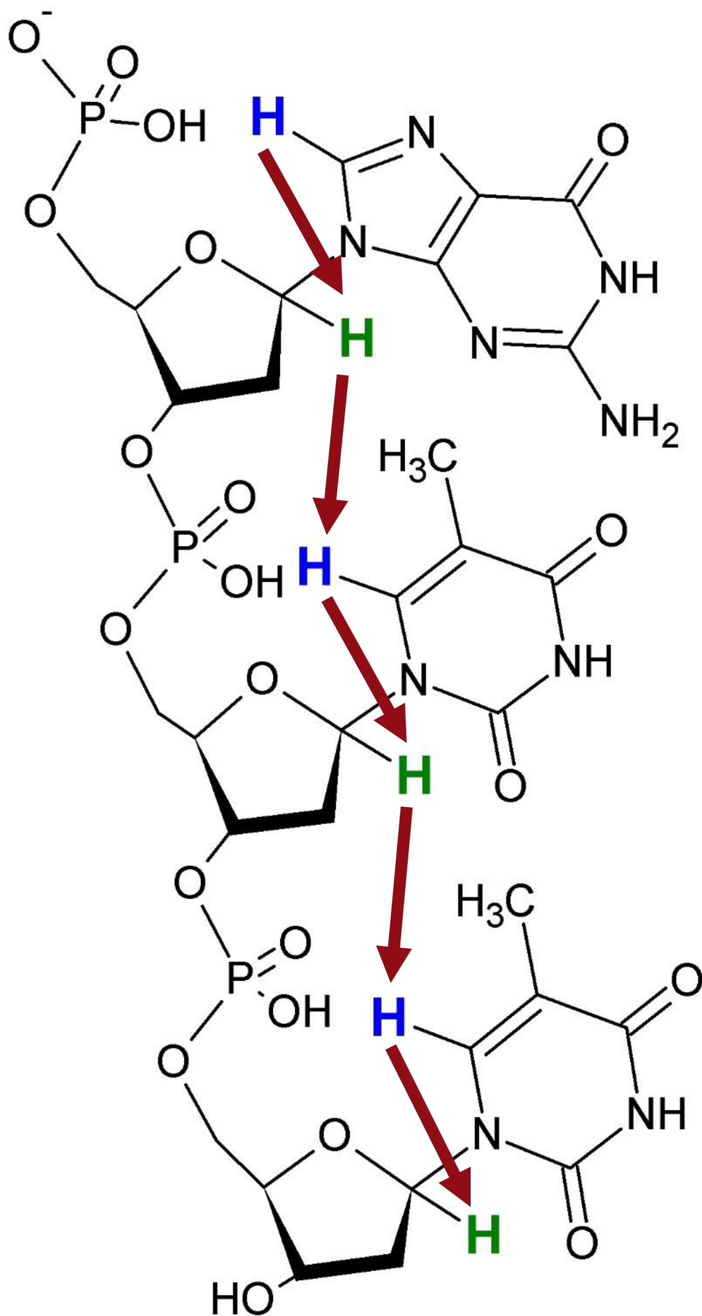


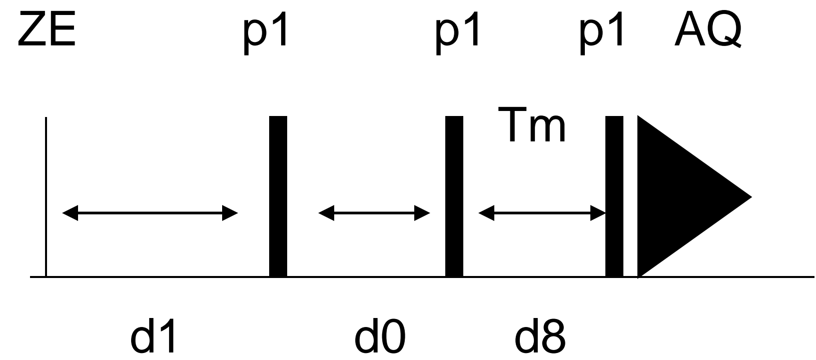
AT base pair

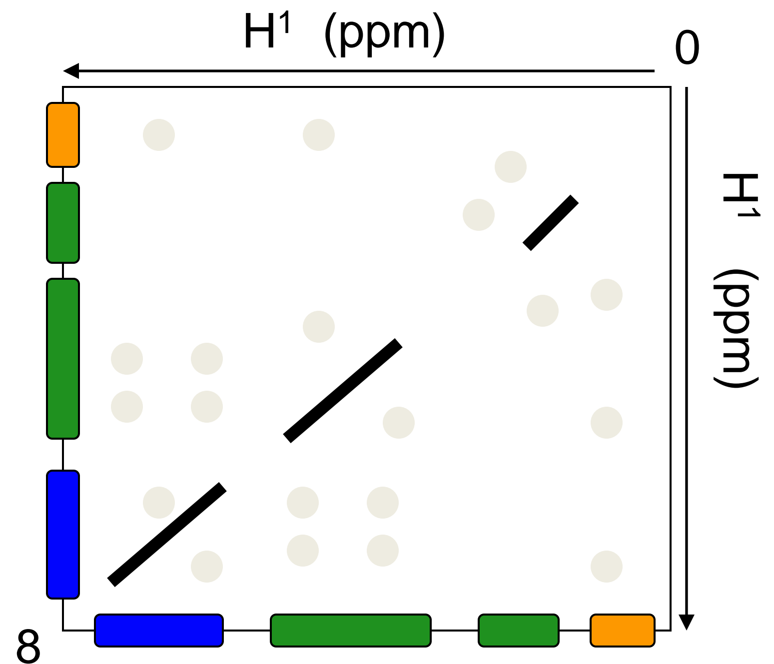


¹H chemical shifts (ppm)

NMR: Base to sugar connectivity

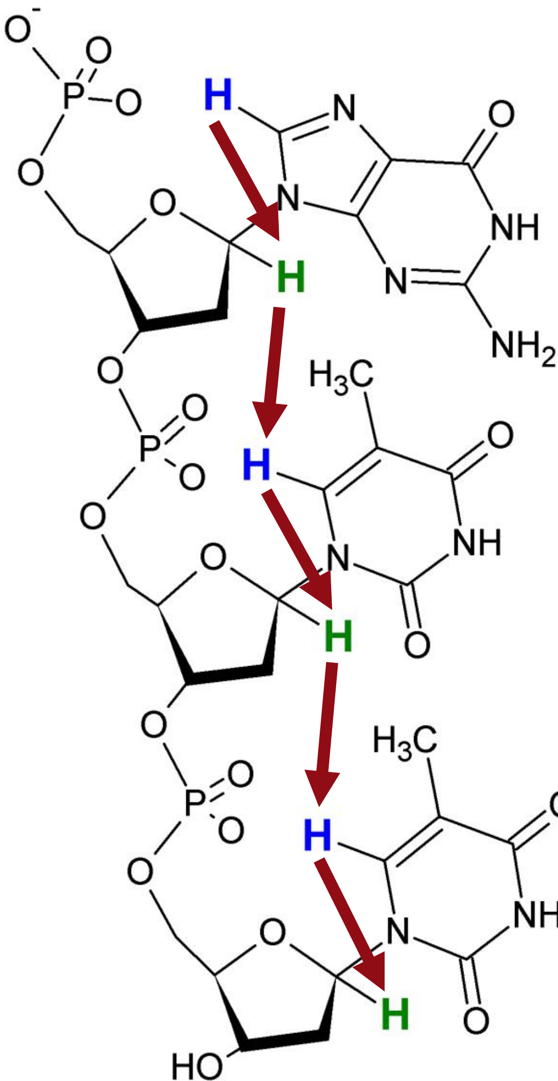


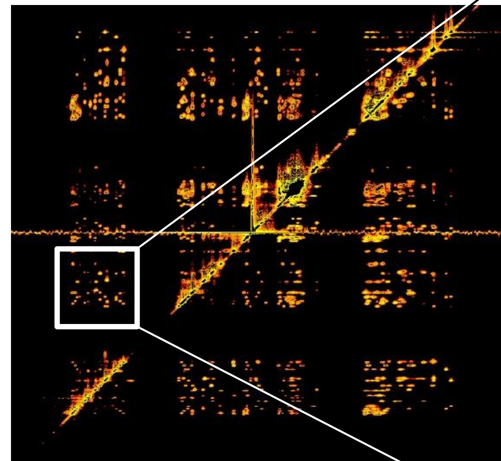


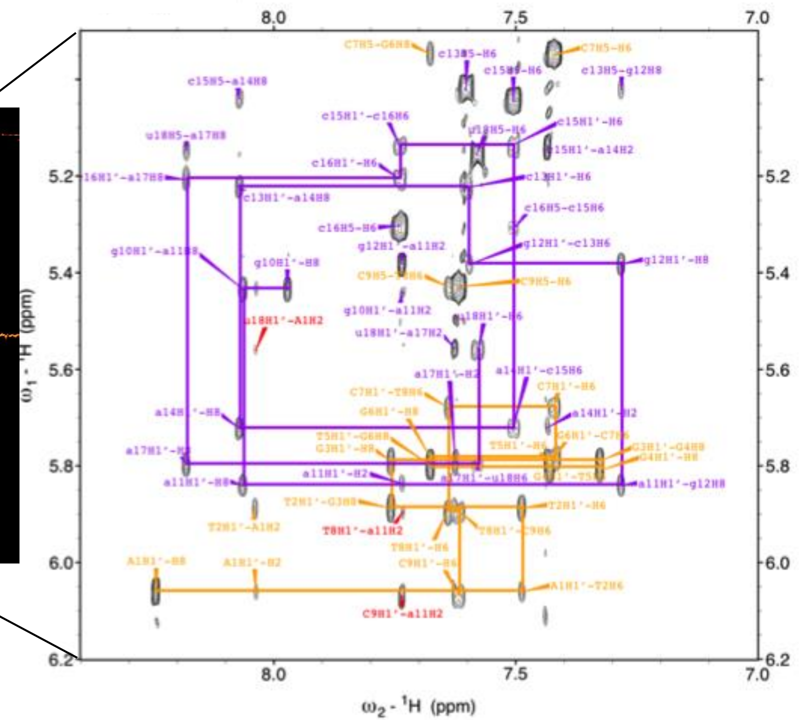


NMR: Base to sugar connectivity

A crazy game of connect the dots !!

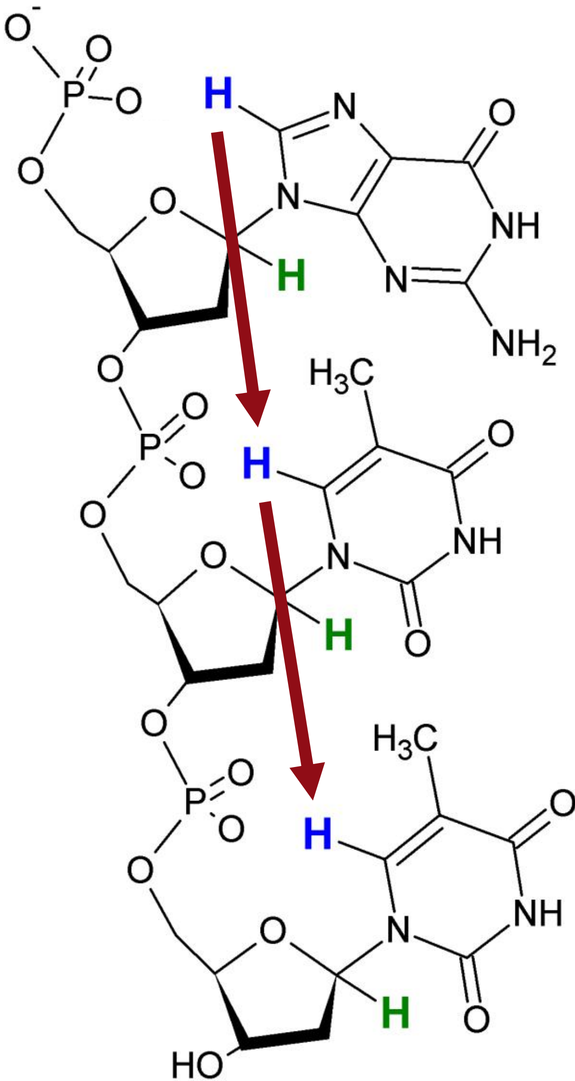






Question: How could we use this to understand DNA damage ?

How are damages sites recognized for repair?



5′

G1	T2	T3	C4	G5	A6	G7	A8	C9	G10
C20	A19	A18	G17	T16	T15	C14	T13	G12	C11

5′

5′

G1	T2	T3	A4	G5	C6	G7	A8	C9	G10
C20	A19	A18	T17	T16	G15	C14	T13	G12	C11

5′

5′

G1	T2	T3	C4	G5	A6	G7	A8	C9	G10
C20	A19	A18	G17	A16	T15	C14	T13	G12	C11

5′

5′

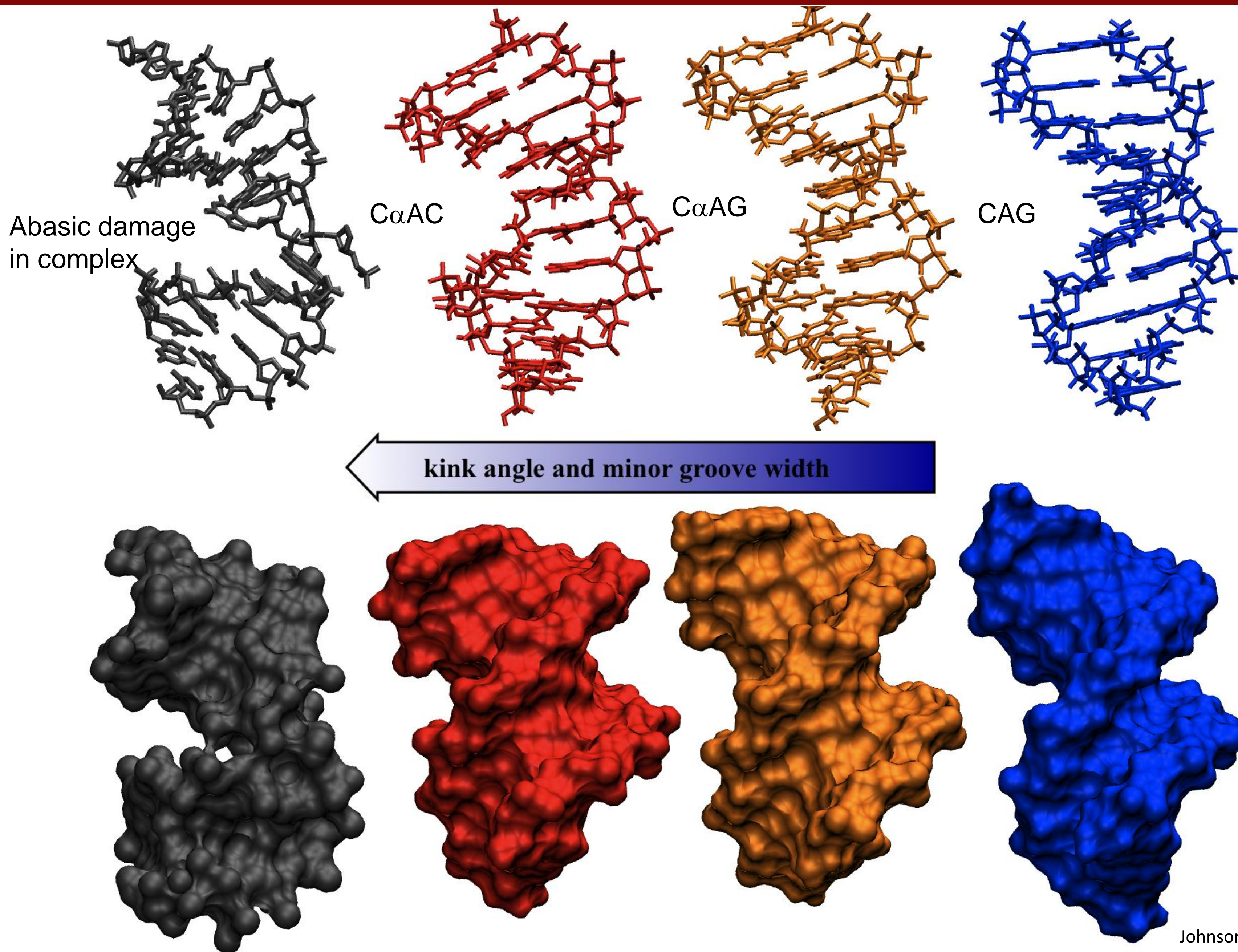
G1	T2	T3	A4	G5	C6	G7	A8	C9	G10
C20	A19	A18	T17	A16	G15	C14	T13	G12	C11

5′

Normal   Medium / Weak   Weak / No contact

Mazurek et al. PNAS 2009

DNA as a Molecular Wire?



Johnson et. al JMB 2012

Think and Discuss

What technologies have in part been developed based on DNA/RNA structural biology advancements?