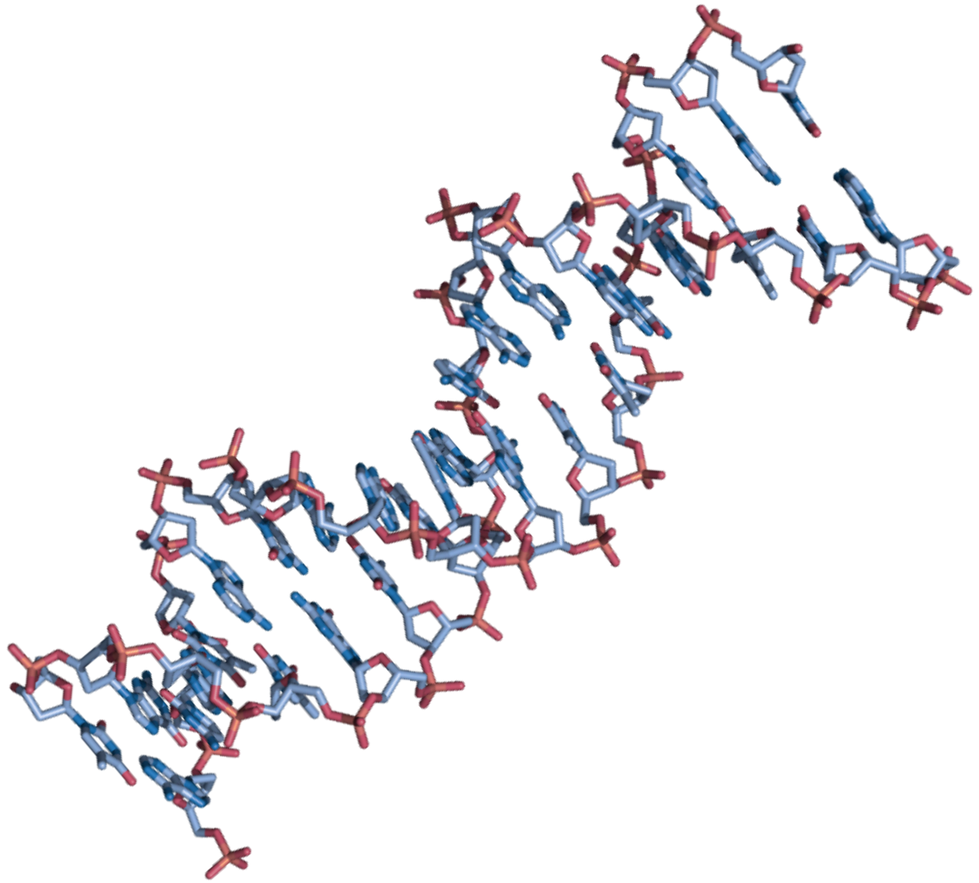
Universitat Pompeu Fabra Barcelona

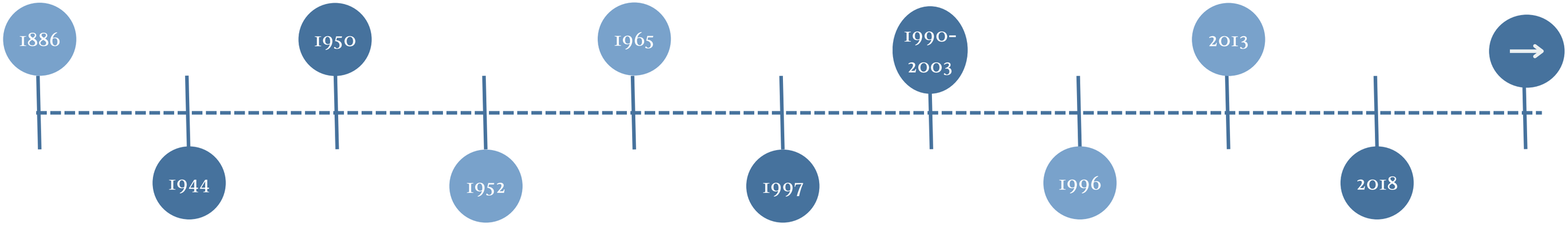
THE DNA STRUCTURE



Júlia Masip, Mar Oropesa, Sara Rica and John Torres

STRUCTURAL BIOLOGY

History & Discoveries



1886 — Gregor Mendel lays the foundations of genetic inheritance

1944 — Oswald Avery confirms that DNA makes up genes and chromosomes

1950 — Erwin Chargaff establishes the base-pairing rules (Chargaff's rules)

1952 — Rosalind Franklin obtains the X-ray diffraction image of B-DNA

1965 — Marshall Nirenberg deciphers the genetic code

1997 — Frederick Sanger develops the DNA sequencing method

1990-2003 — Human Genome Project: complete sequencing of the human genome

1996 — Cloning of Dolly the sheep

2013 — Discovery and application of CRISPR-Cas9

2018 — First genetically modified human babies using CRISPR-Cas9

→ Use of complex structures as therapeutic targets against cancer

DNA COMPOSITION

Nucleotides

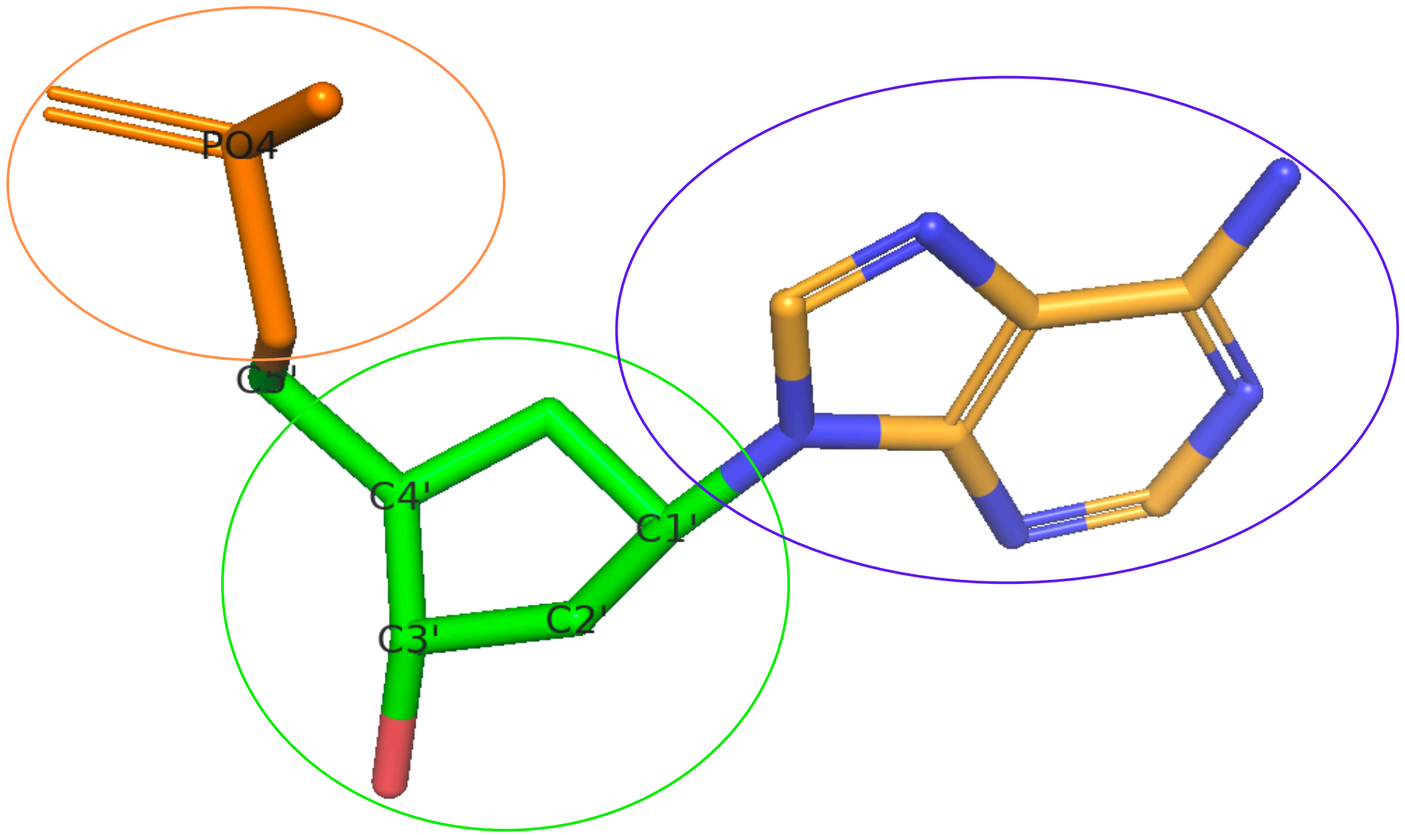
Phosphate Group

+

Deoxyribose Sugar

+

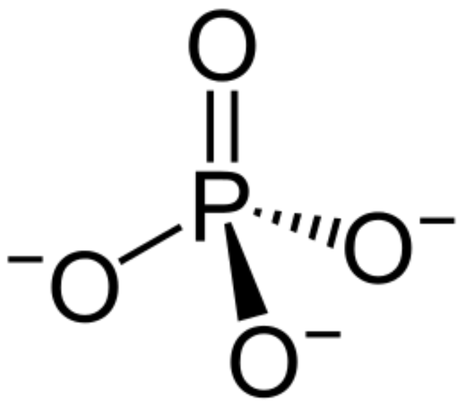
Nitrogenous Base

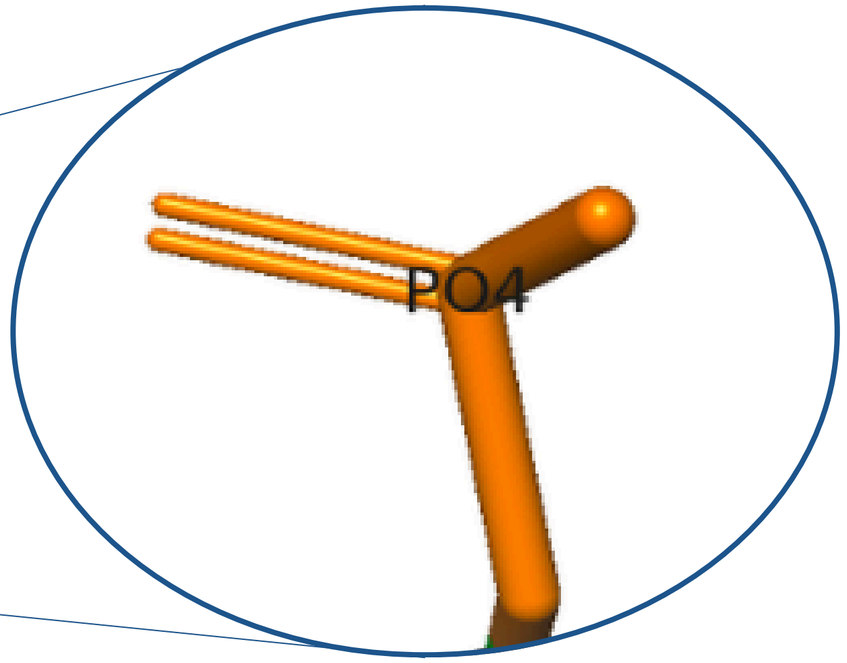


DNA COMPOSITION

Phosphate Group

- Key Property: High negative charge.
- Biological Role: Enables water solubility and binding to Histones.
- Responsible for the acidic nature of DNA.



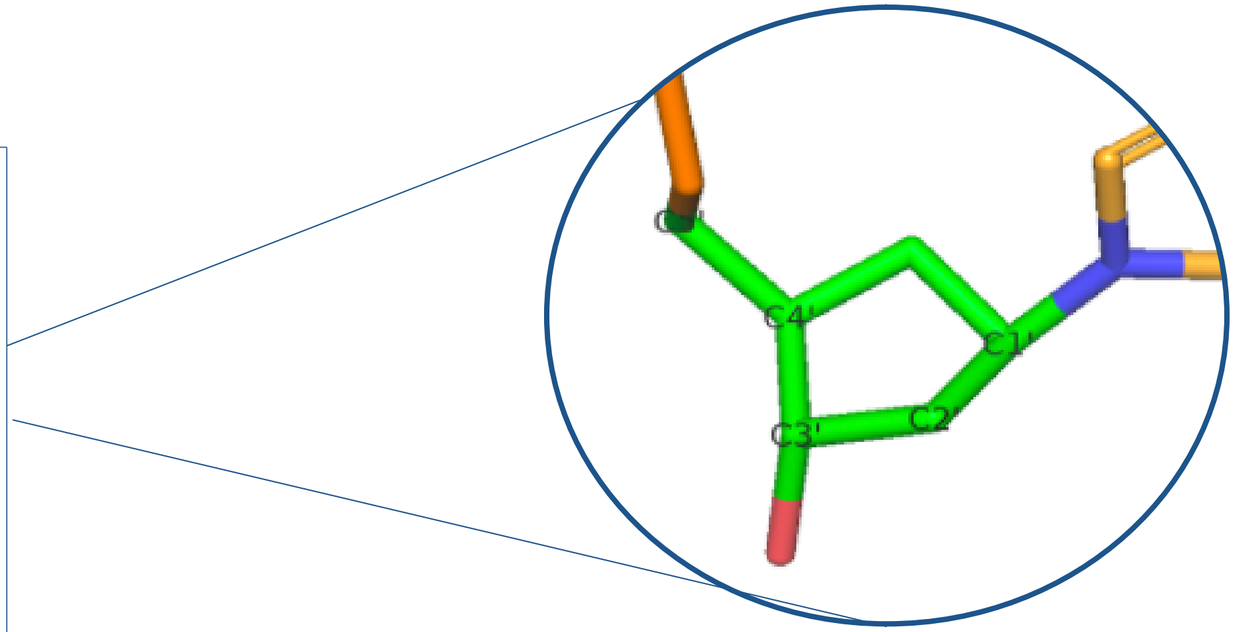


DNA COMPOSITION

Deoxyribose Sugar

- Missing an Oxygen at the 2' position.
- Stability: This single missing atom makes DNA chemically superior for long-term data storage compared to RNA.

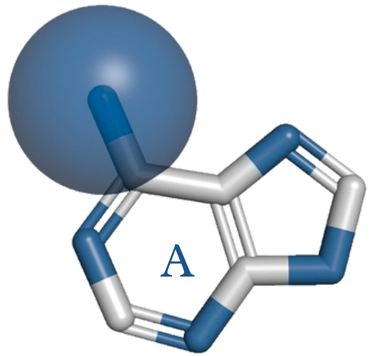
The Numbering System (1' to 5')
5' Carbon: Holds the Phosphate group.
3' Carbon: The attachment point for the next nucleotide (Directionality).
2' Carbon: Controls the Sugar Pucker (The "bend" that defines the helix shape).



DNA COMPOSITION

Types Of Nitrogenous Bases

Purines



Adenine

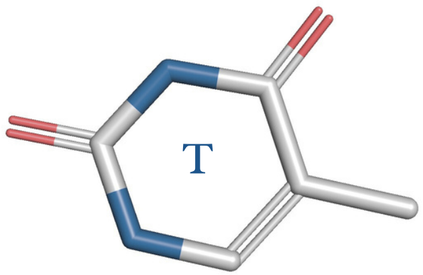
- Amino group but no oxygen
- ATP

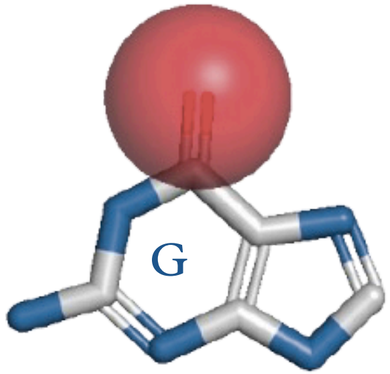
A=T

Thymine

- Methyl group

Pyrimidines





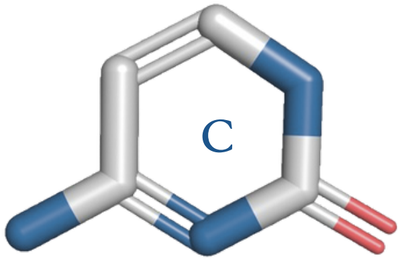
Guanine

- Carbonyl

G≡C

Citosine

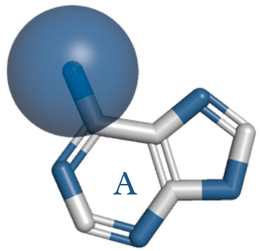
- Pairs with Guanine
- Unstable-Uracil

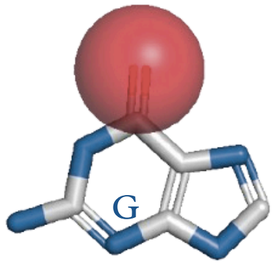


DNA COMPOSITION

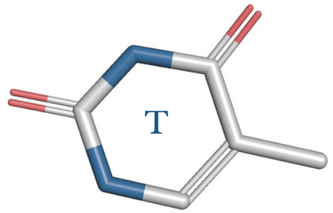
Types Of Nitrogenous Bases

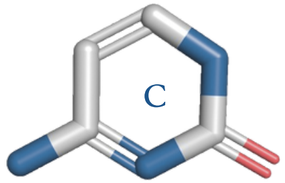
Purines

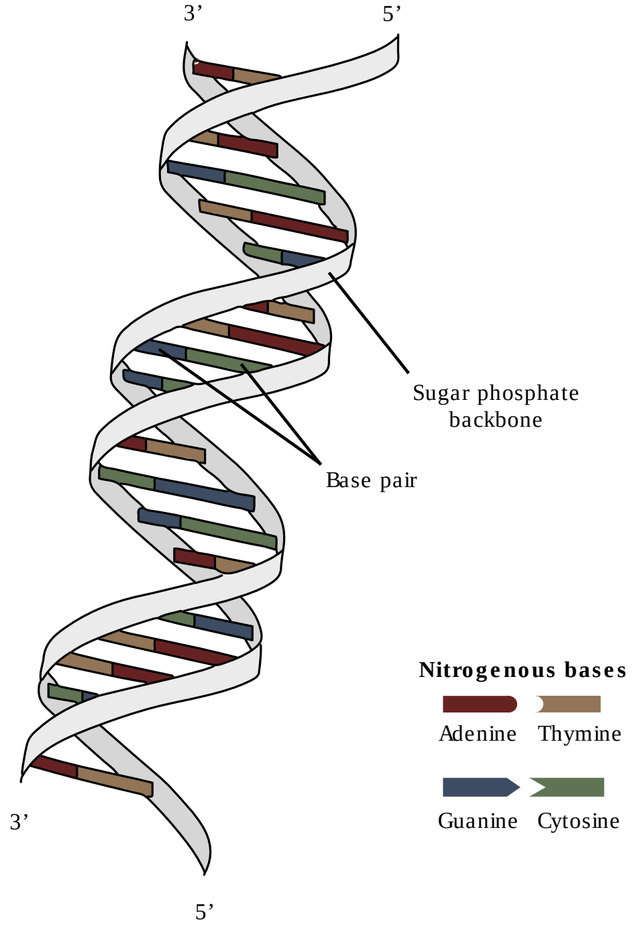


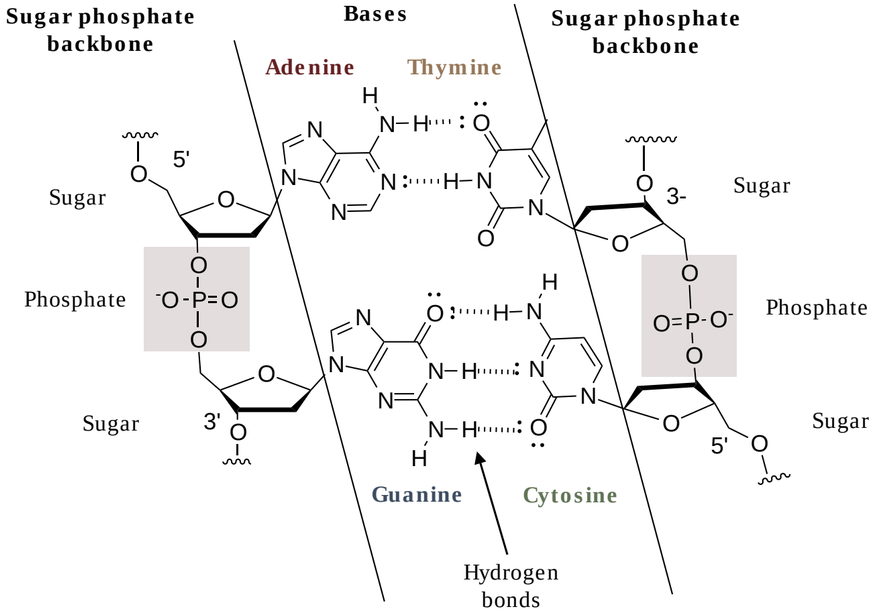


Pyrimidines







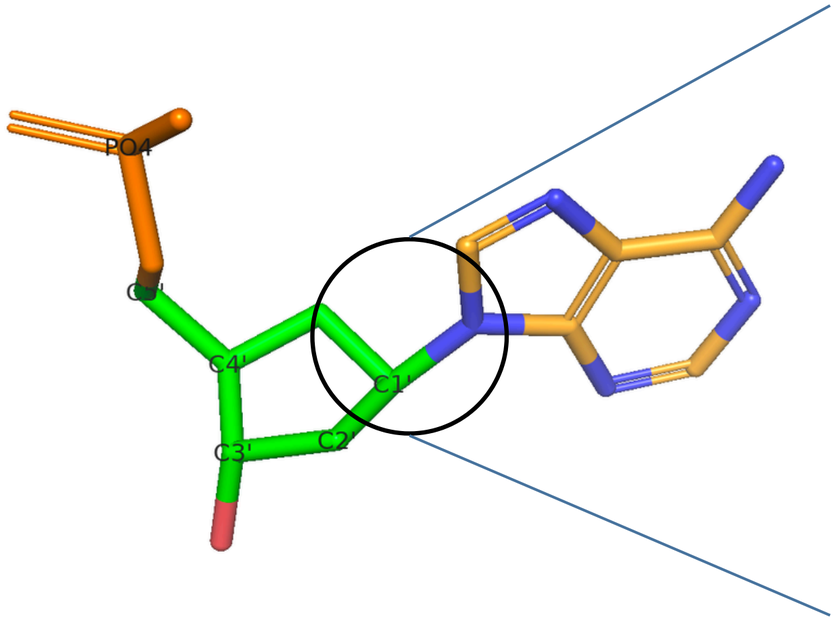


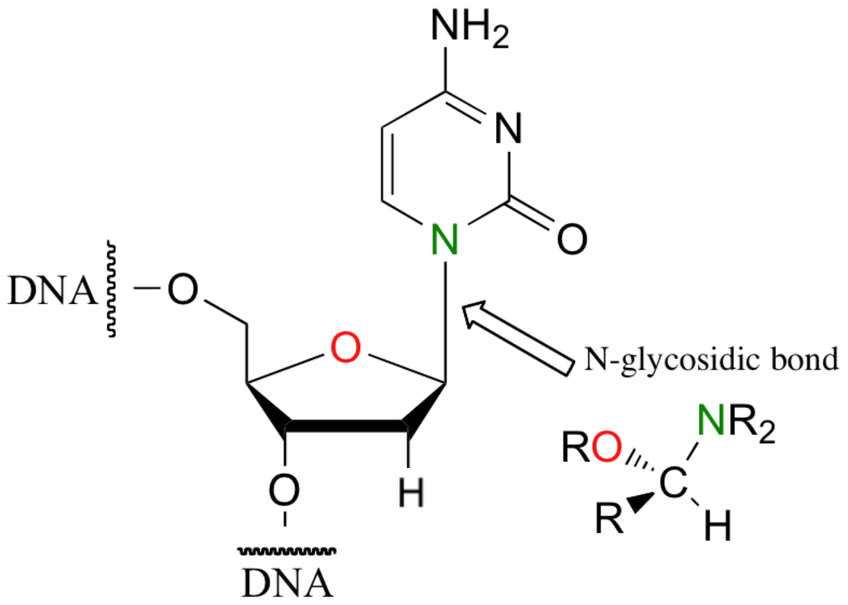
DNA Structure. Gris, Kindred & Jakubowski (2021).

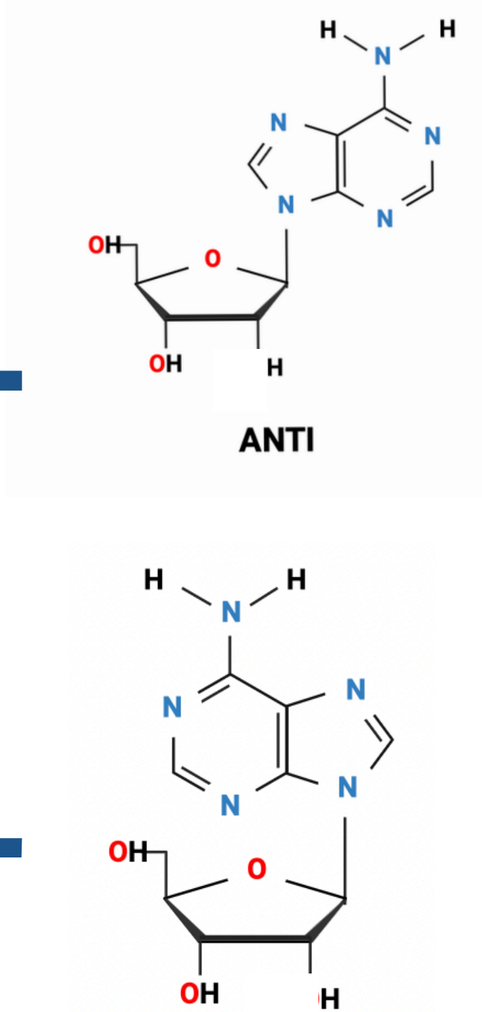
CHEMICAL BONDS IN THE CHAIN

N-Glycosidic Bond

Deoxyribose Sugar + Nitrogenous Base*





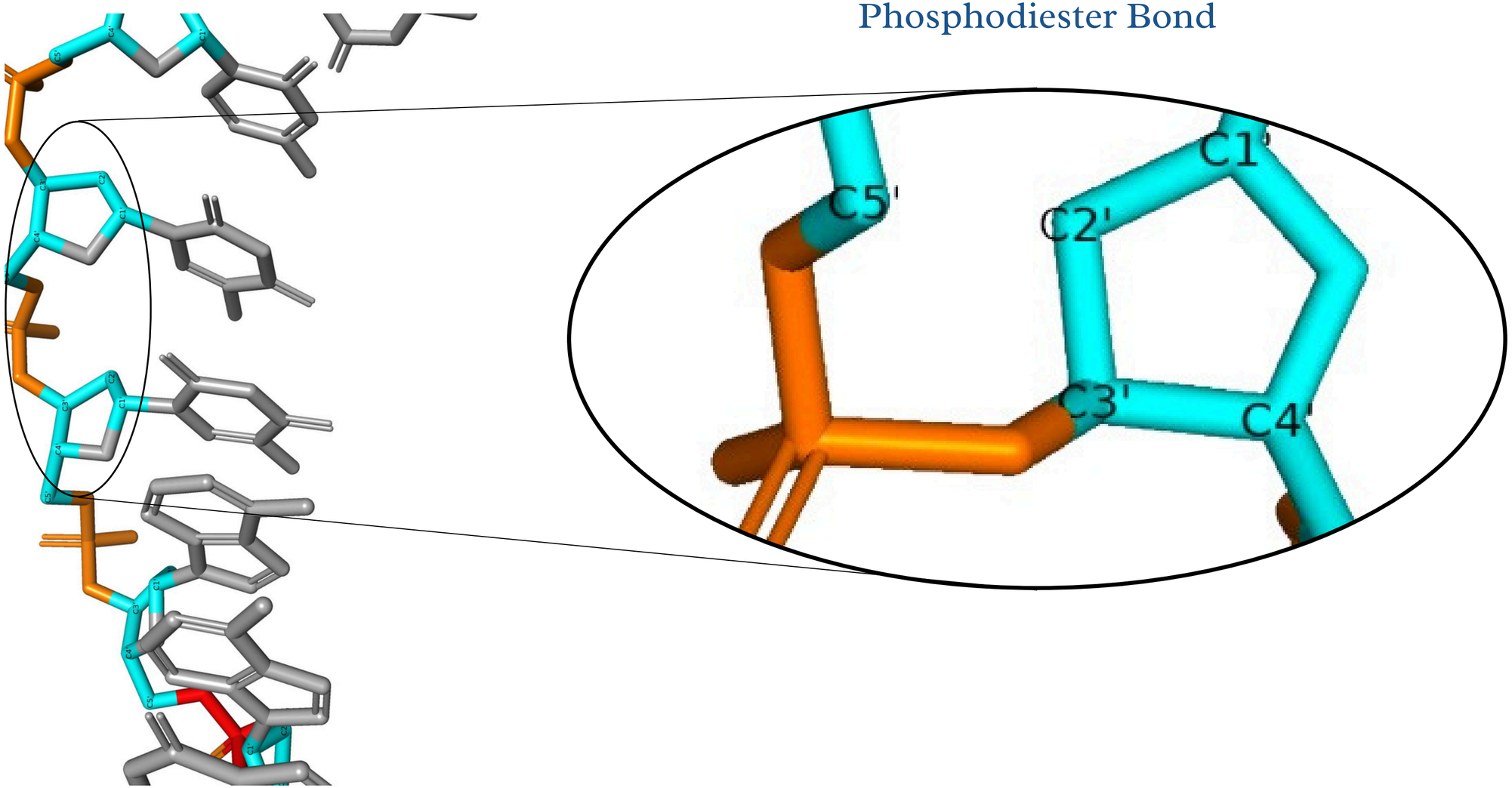


*Pyrimidines (C, T): Binds at the N1 nitrogen.
Purines (A, G): Binds at the N9 nitrogen.

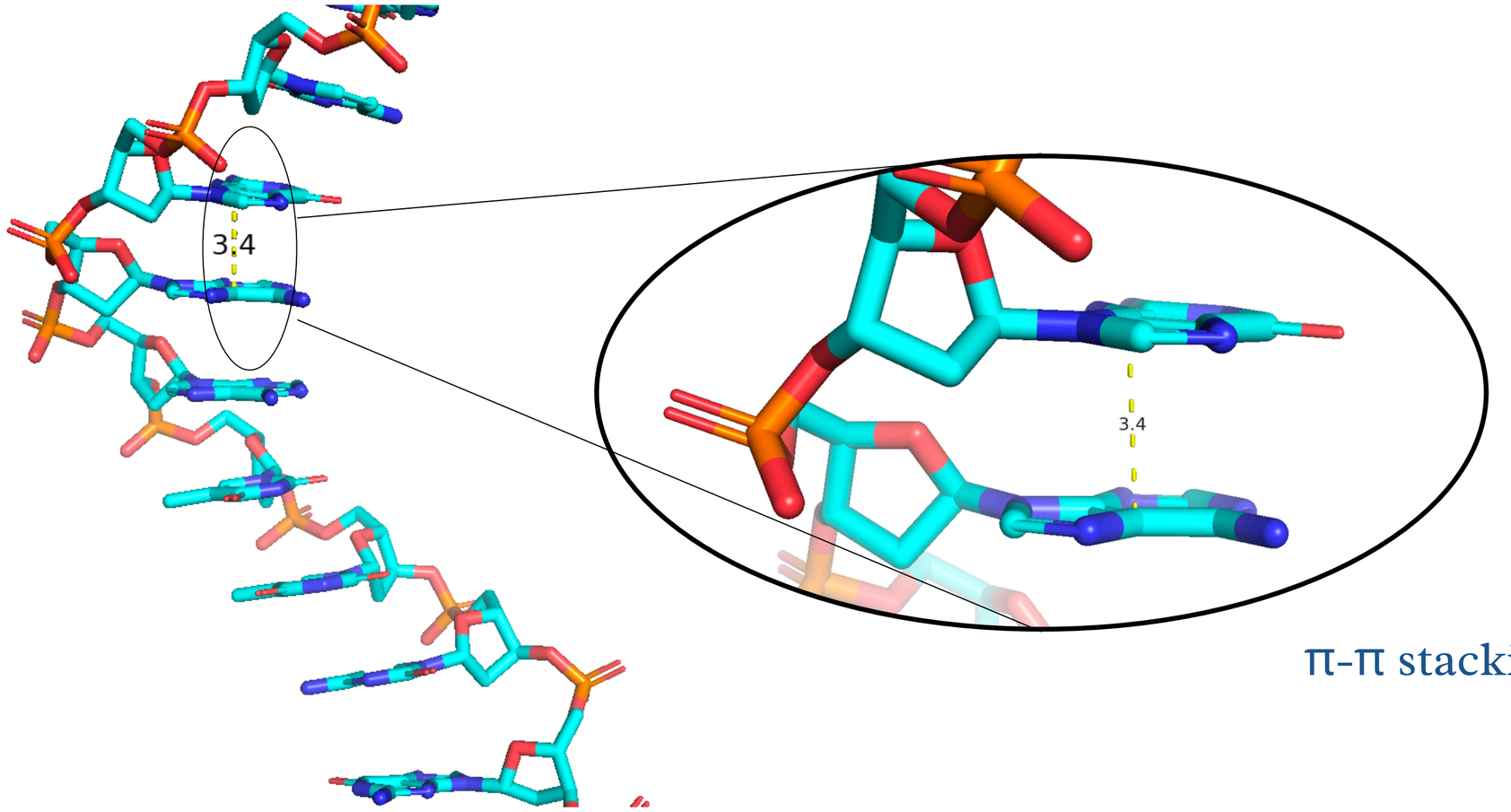
Image via Chemistry LibreTexts / Middle Georgia State University.

Created with biorender

CHEMICAL BONDS IN THE CHAIN

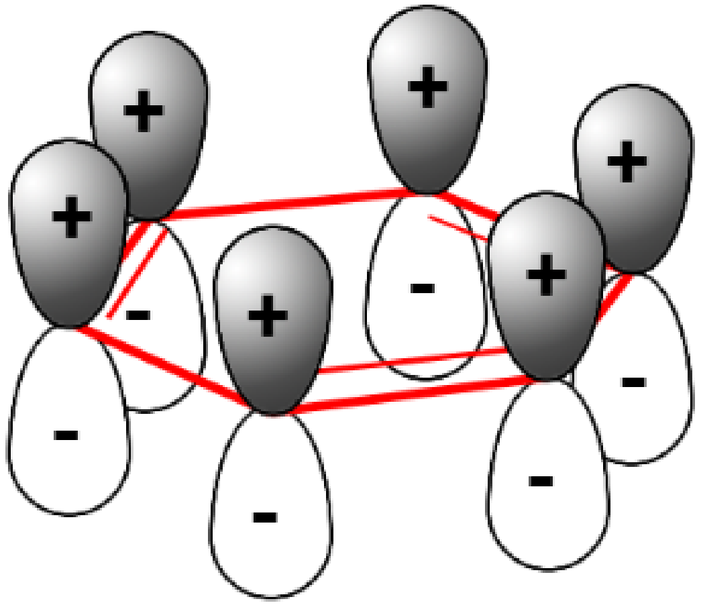


TERMODINAMICAL STABILITY: BASE STACKING

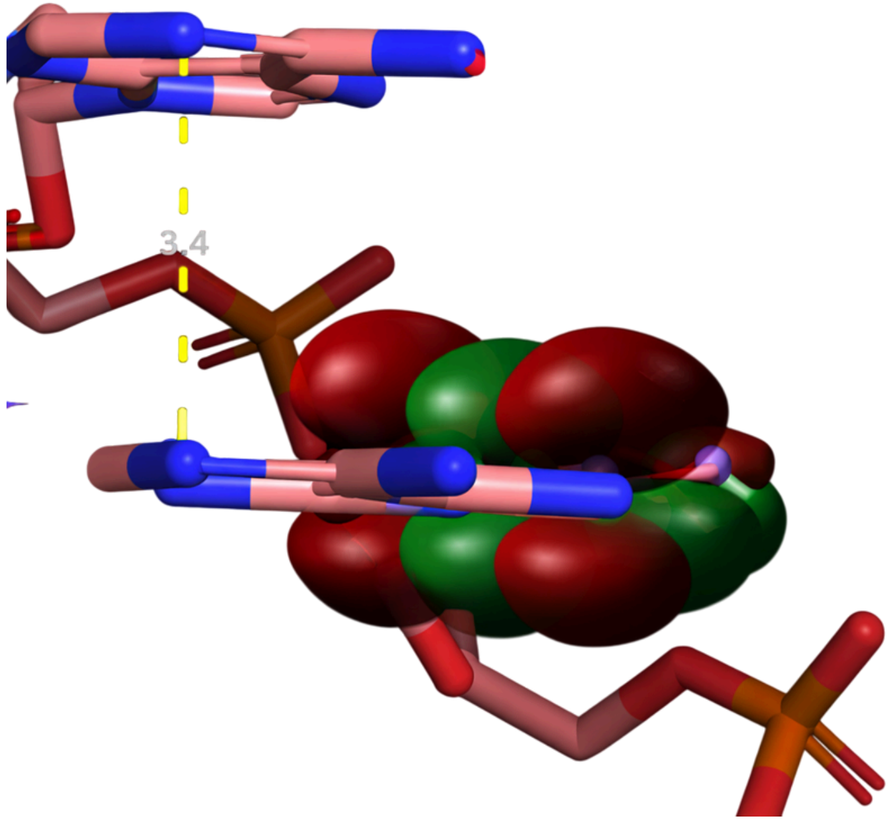


π-π stacking

TERMODINAMICAL STABILITY: PI-STACKING

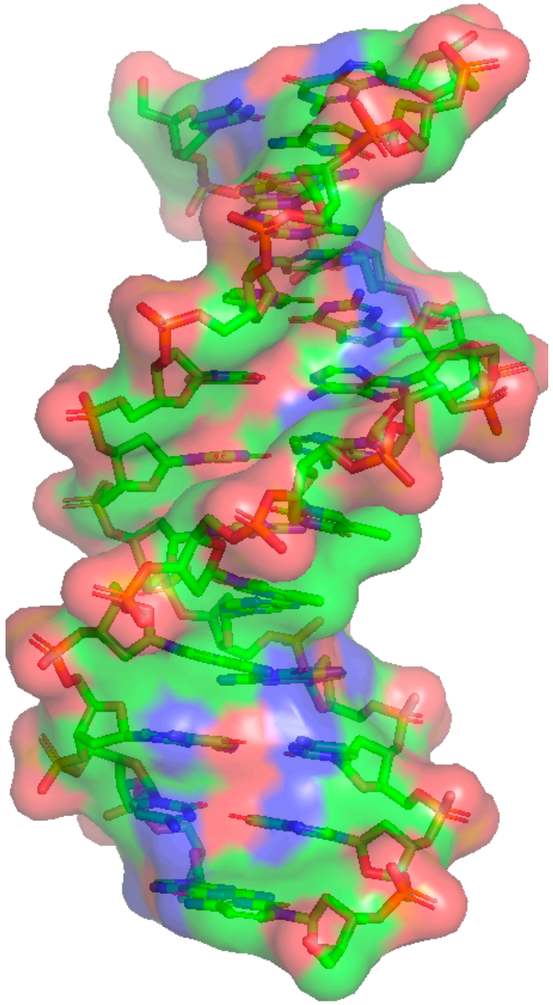


Created with biorender

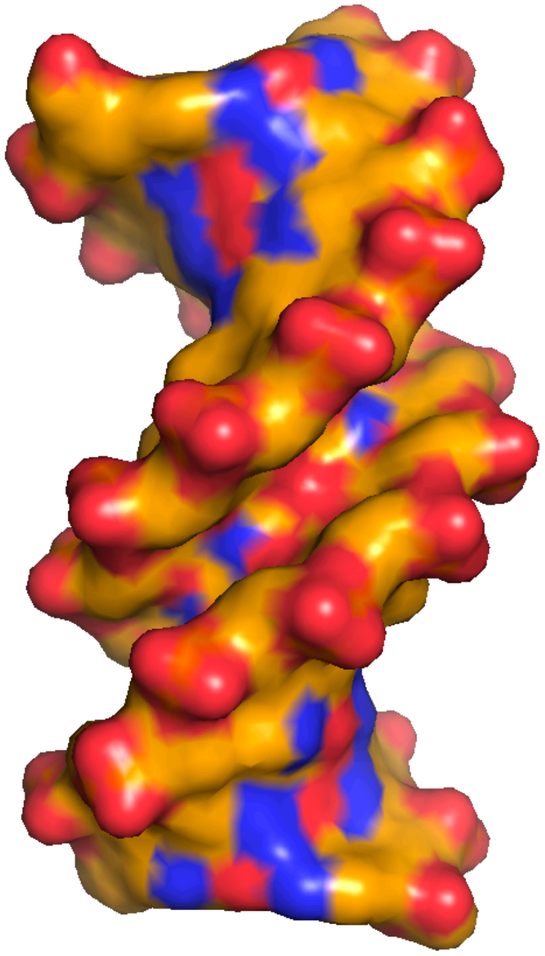


Hydrophobic effect

TERMODINAMICAL STABILITY: PI-STACKING



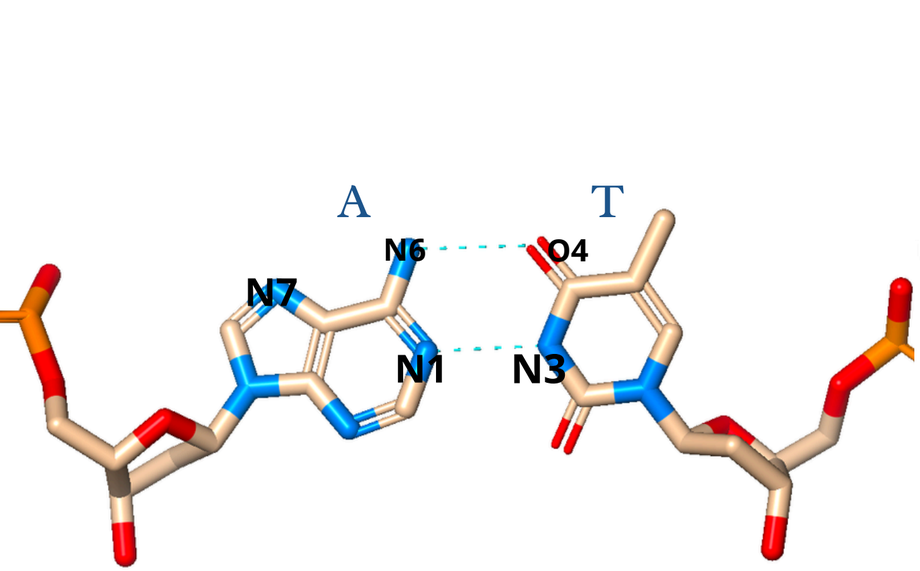
Electrostatic forces

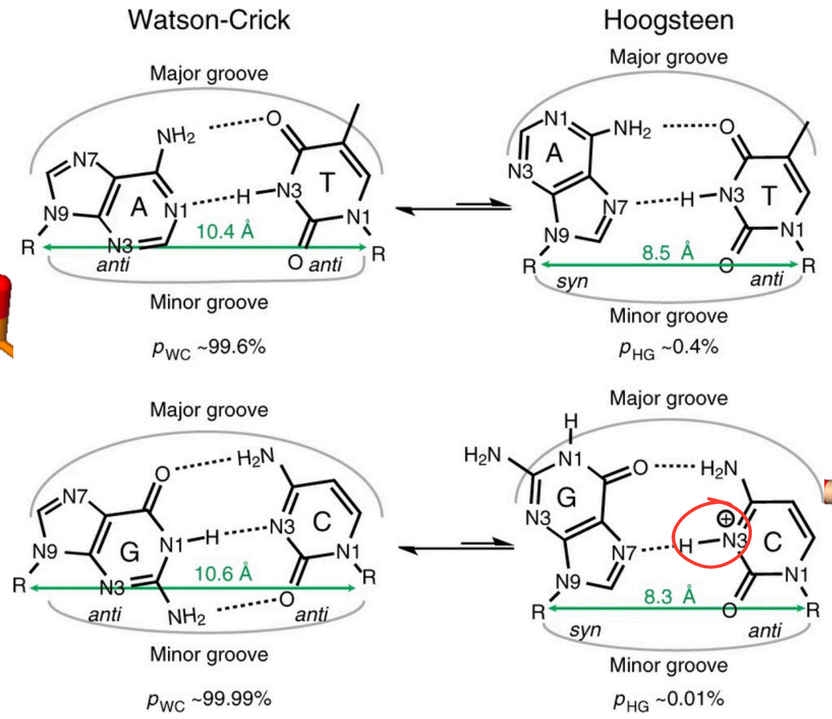


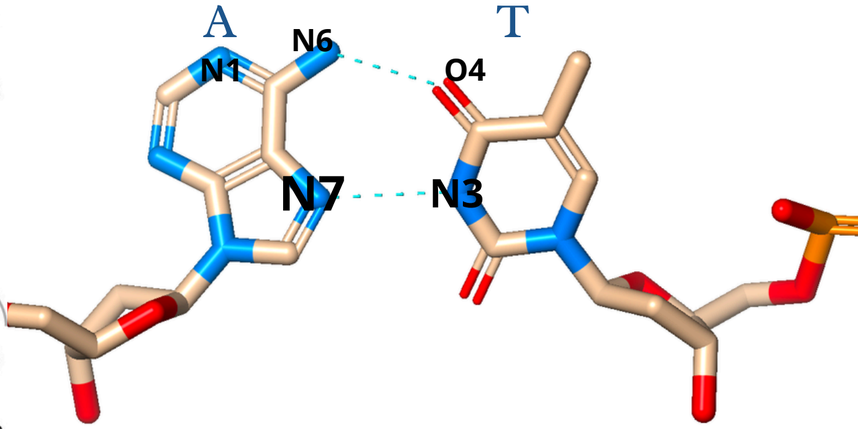
Hydrophobic effect

STRUCTURAL STABILITY: HYDROGEN BONDS

Watson & Crick .vs. HOOGSTEEN



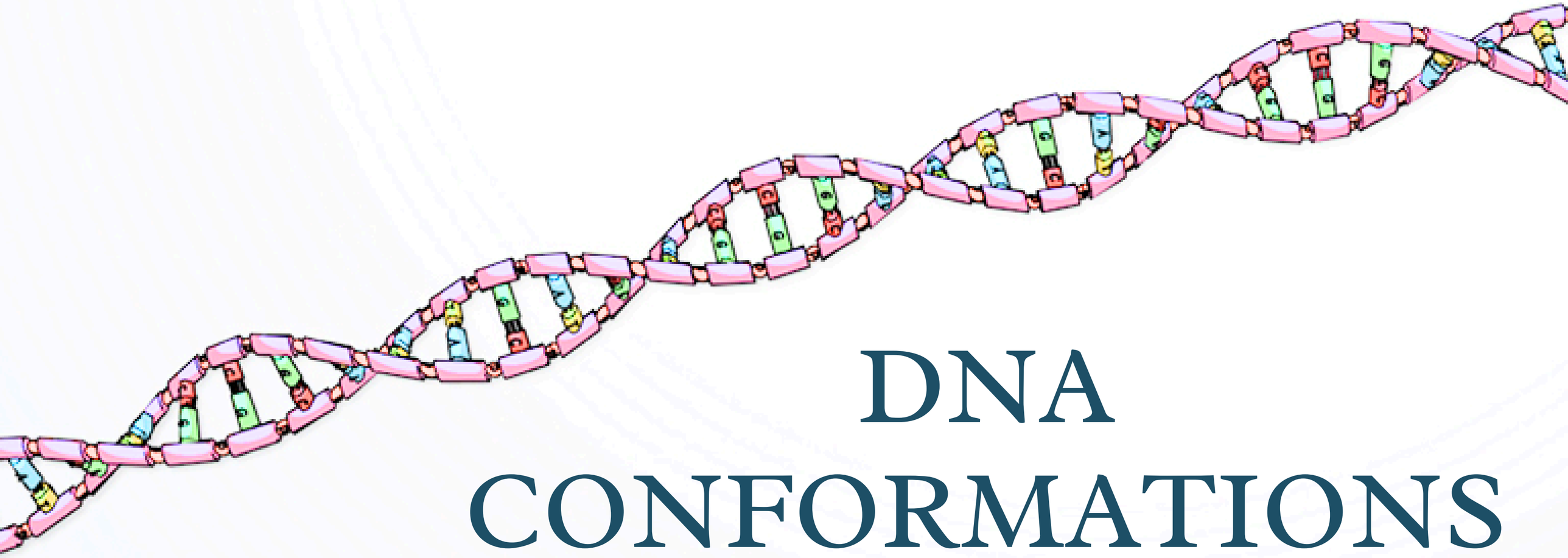




WC

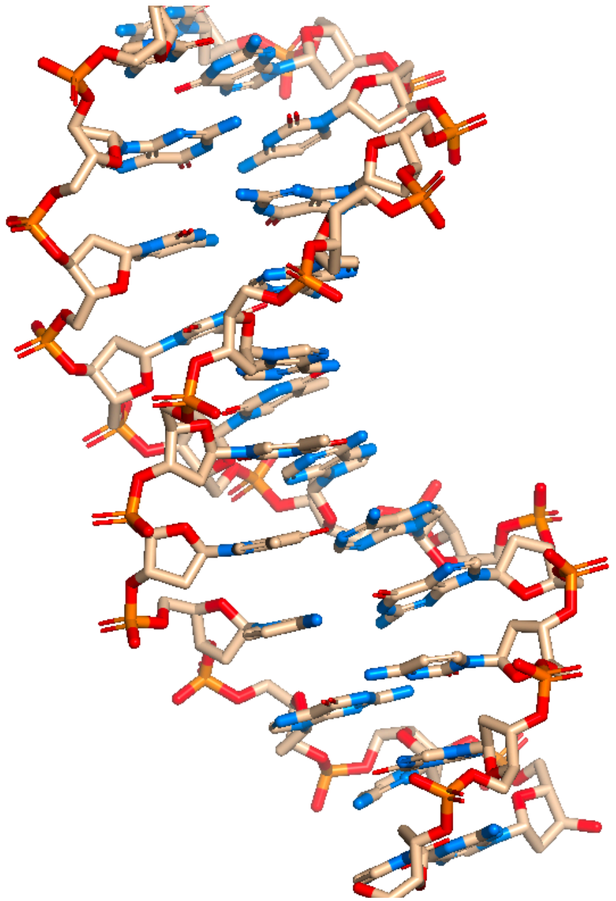
HG

Xu et al., 2018 (Nat. Commun.)



DNA CONFORMATIONS

B-DNA



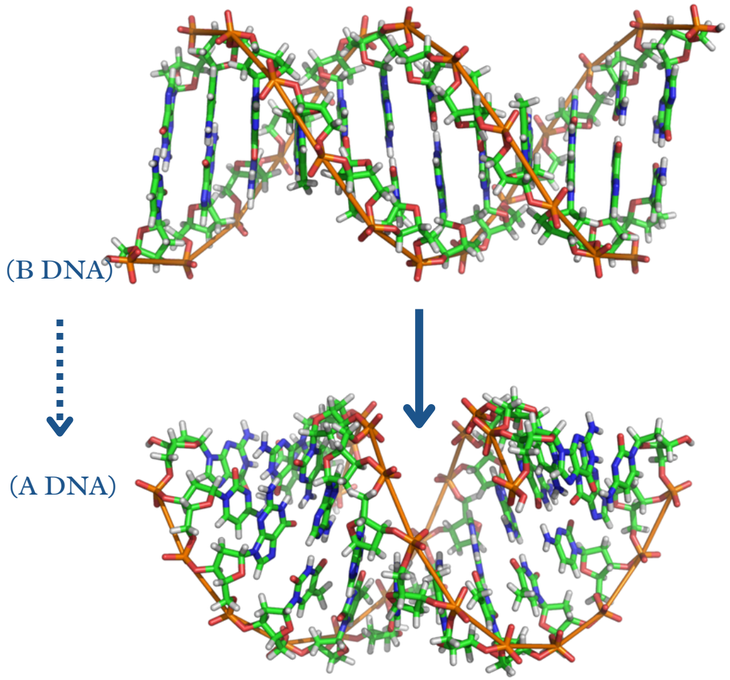
PDB 1BNA

- DNA's Physiological standard
- 10,5 bases/helix turn
- Anti conformation (glycosidic bond)
- Wide major groove: transcription factor access.
- Narrow minor groove

A-DNA

Dehydration

If water is removed, the helix collapses into a more compact form

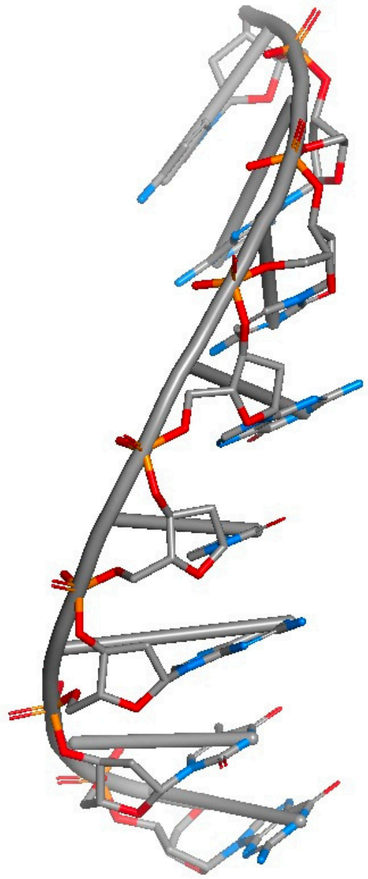


PDB 4IZQ

- <5% H_2O
- 11 bases/helix turn
- Anti conformation (glycosidic bond)
- Major groove: Narrow & deep
- Minor groove: Wide & shallow

Language of RNA

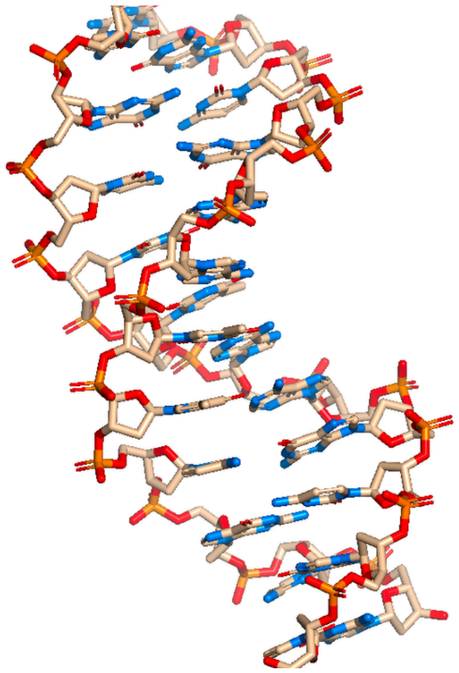
RNA's extra oxygen forces it into this A-shape



Z-DNA

Left-Handed Twist

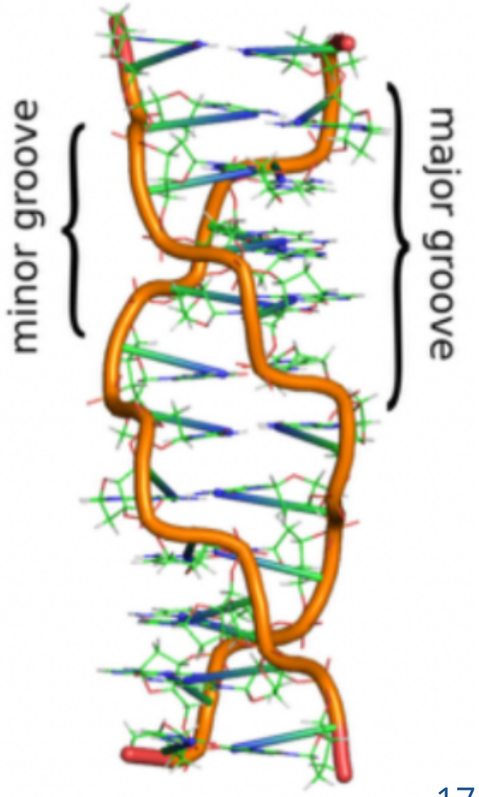
the most stretched and narrow form of DNA



PDB 6AQV

- Left-handed helix
- Specific sequence & conditions
- Zigzag pattern
- 12 bases/helix turn
- Syn/anti
- Gene silencing/viral defense

Zig-Zag Backbone

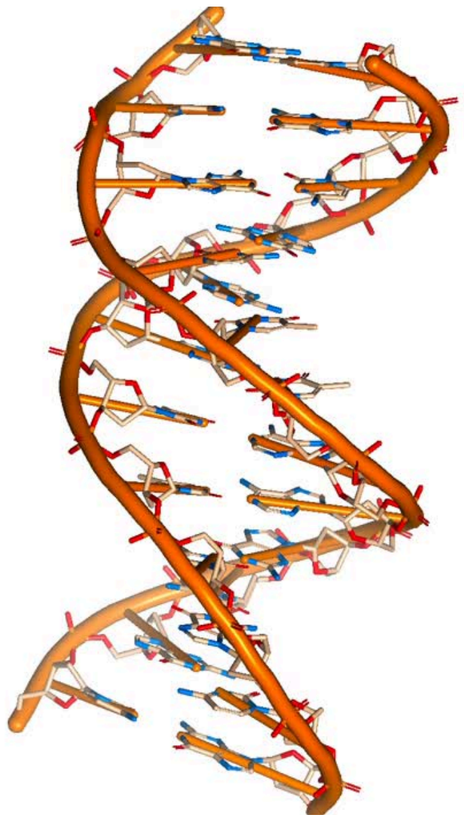


Obara et al., 2024 (Molecules)

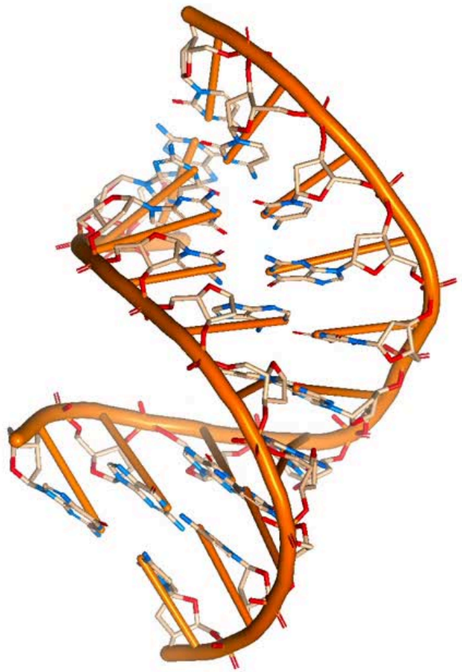
DIFFERENCES

Feature	B-DNA	A-DNA	Z-DNA
Helical Sense	Right-handed	Right-handed	Left-handed
Residues per Turn	10,50	11	12 (6 dimers)
Axial Rise (Å/bp)	3.4 Å	2.6 Å	3.7 Å
Helix Diameter (Å)	~20 Å	~23 Å	~18 Å
Sugar Pucker	C2'-endo	C3'-endo	C2'-endo / C3'-endo
Glycosidic Bond	Anti	Anti	Anti (Pyr) / **Syn (Pur)**
Major Groove	Wide & Deep	Narrow & Deep	Flat / Not distinct
Minor Groove	Narrow & Deep	Wide & Shallow	Narrow & Deep

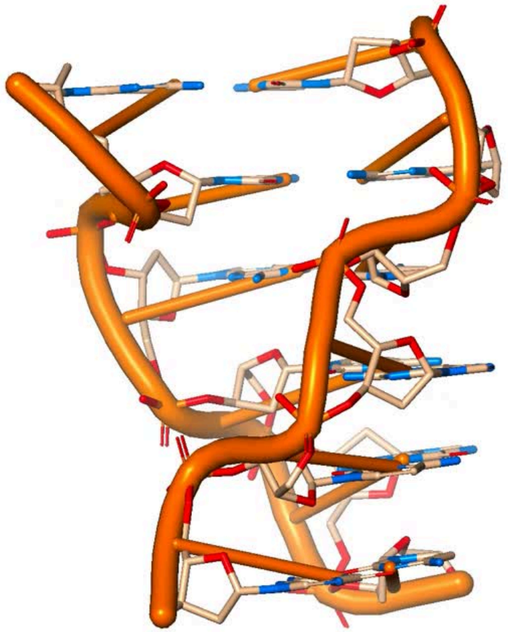
Feature	B-DNA	A-DNA	Z-DNA
Helical Sense	Right-handed	Right-handed	Left-handed



PDB 1BNA

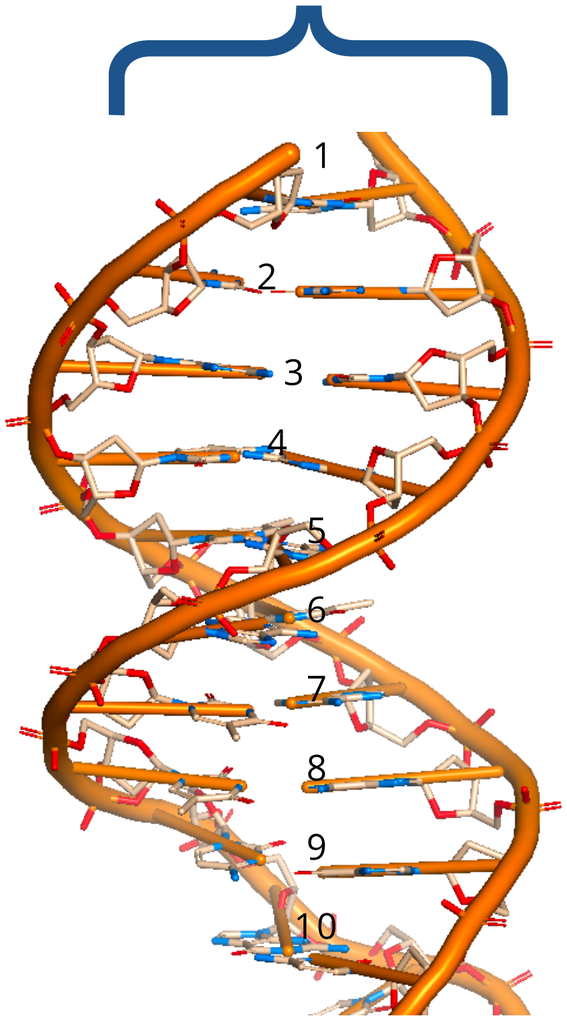


PDB 4IZQ

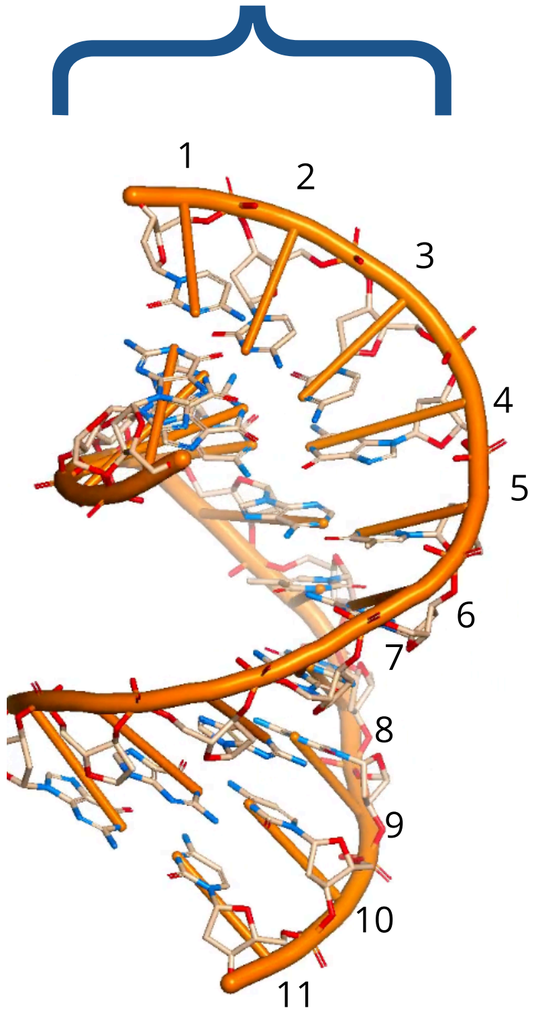


PDB 6AQV

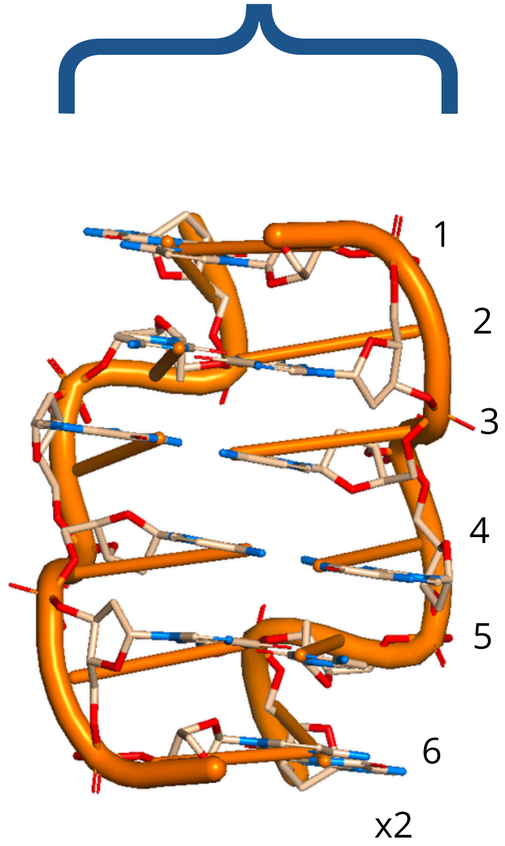
Feature	B-DNA	A-DNA	Z-DNA
Residues per Turn	10-11	11	12



PDB 1BNA

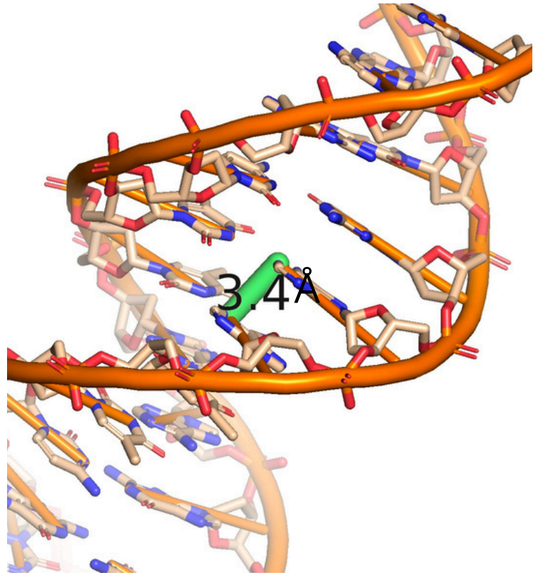


PDB 4IZQ

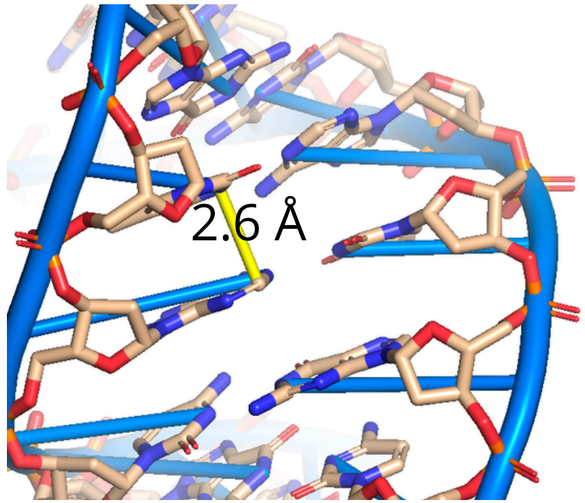


PDB 6AQV

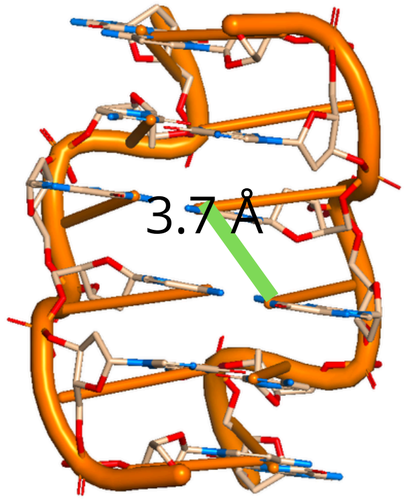
Feature	B-DNA	A-DNA	Z-DNA
Axial Rise (Å/bp)	3.4 Å	2.6 Å	3.7 Å



PDB 1BNA



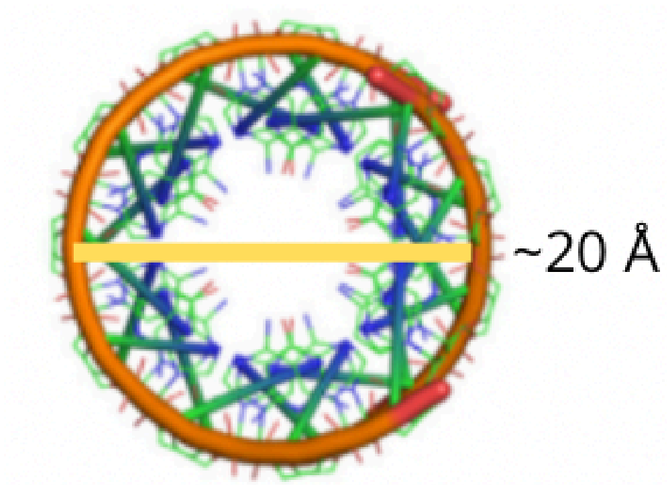
PDB 4IZQ



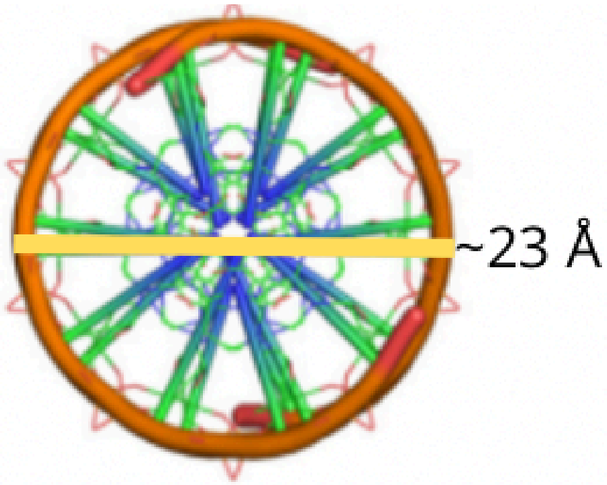
PDB 6AQV

Feature	B-DNA	A-DNA	Z-DNA
Helix Diameter (Å)	~20 Å	~23 Å	~18 Å

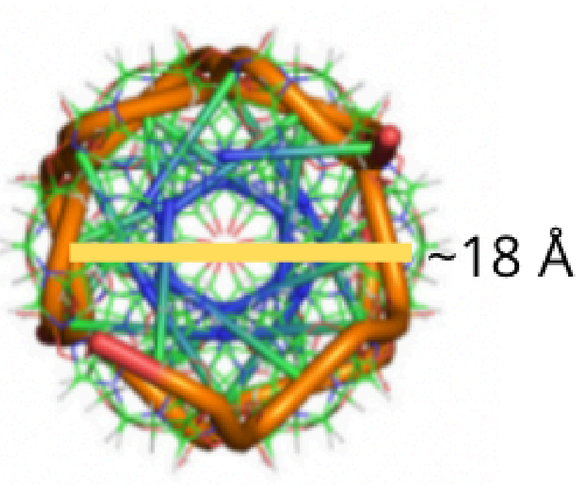
B-DNA



A-DNA



Z-DNA

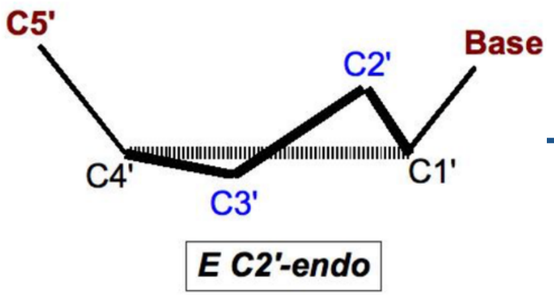


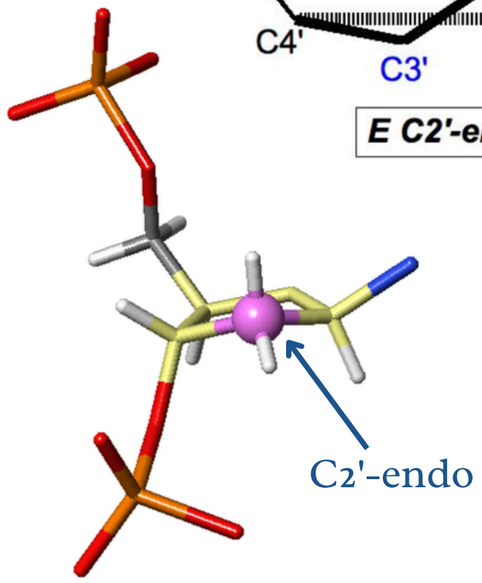
Adapted from Obara et al., 2024 (Molecules)

Feature	B-DNA	A-DNA	Z-DNA
Sugar Pucker	C2'-endo	C3'-endo	C2'-endo / C3'-endo
Base Pair Tilt	6°	20°	7°

C2'-endo Conformation

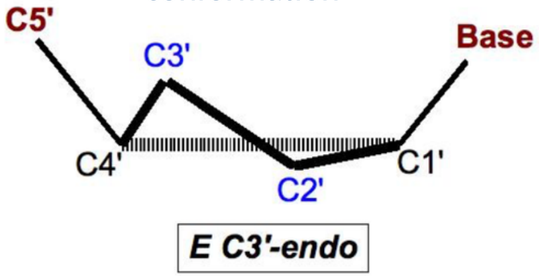
Specific "pucker" acts as a lever that stretches the entire

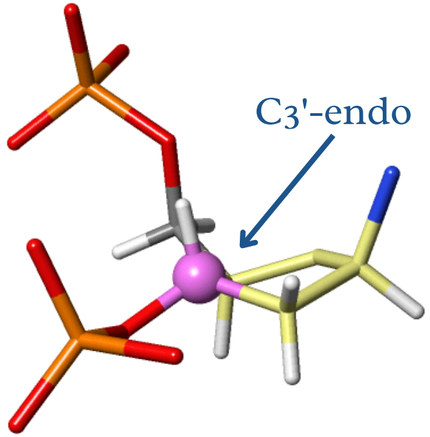




C3'-endo Flip

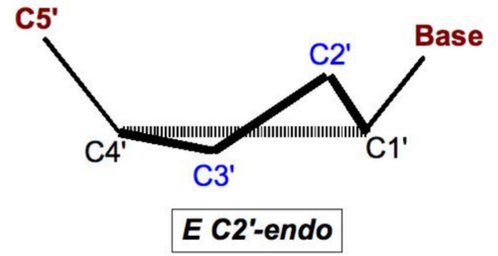
C3' atom now bends upward, replacing the C2'-endo conformation



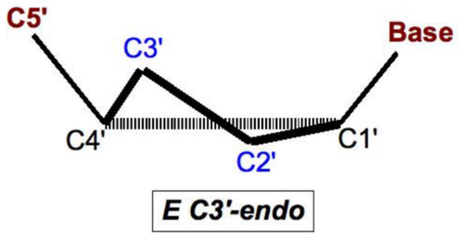


C3'-endo / C2'-endo

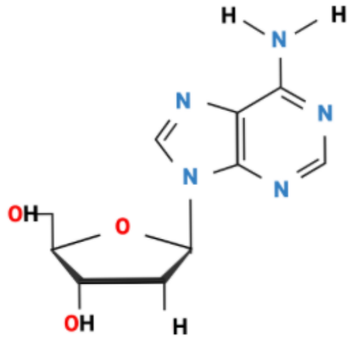
Purines: Adopt C3'-endo conformation
Pyrimidines: Adopt C2'-endo conformation



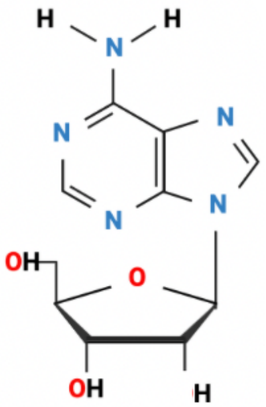
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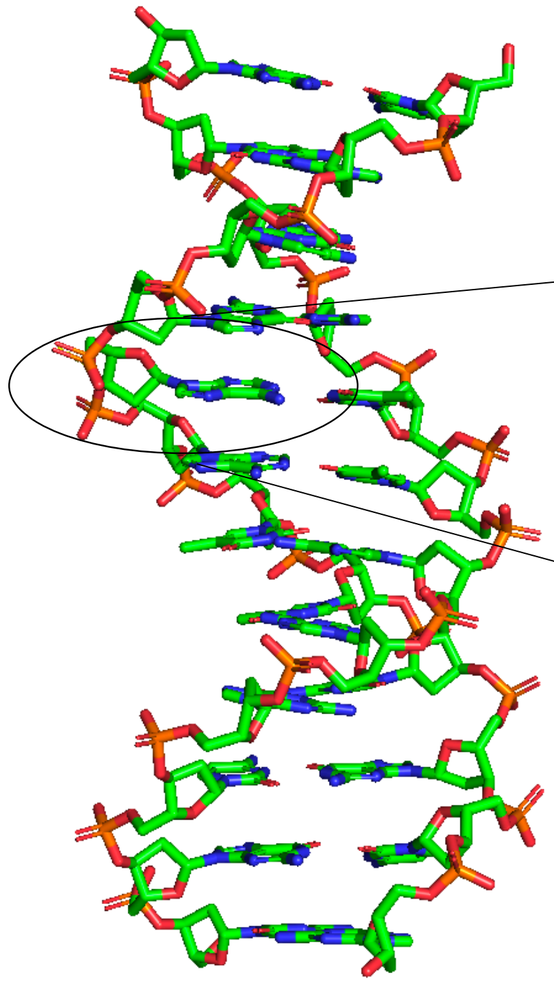
Feature	B-DNA	A-DNA	Z-DNA
Glycosidic Bond	Anti	Anti	Anti (Pyr) / Syn (Pur)



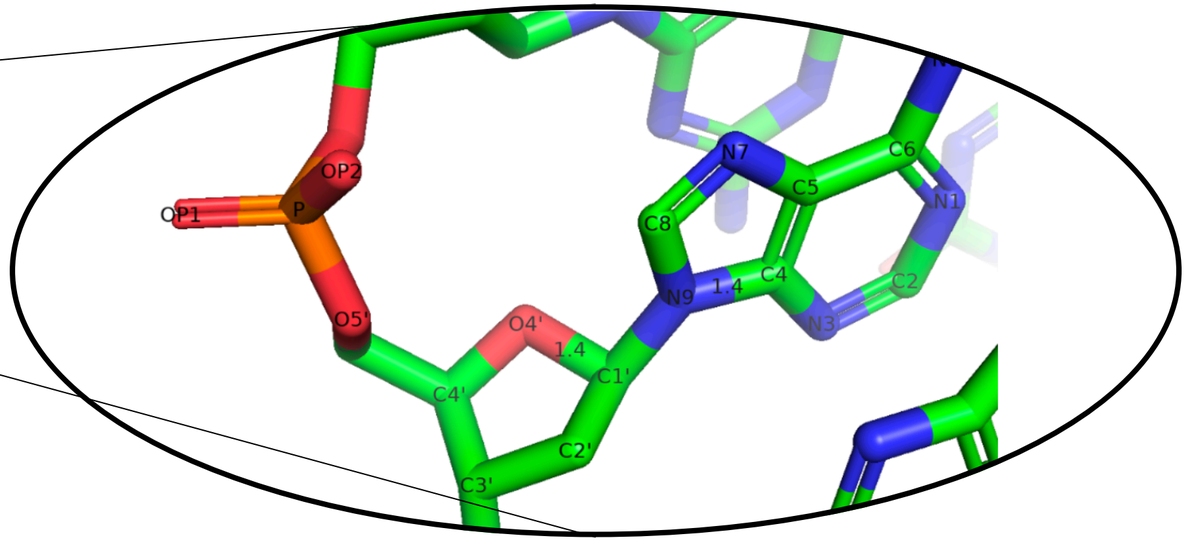
ANTI



SYN

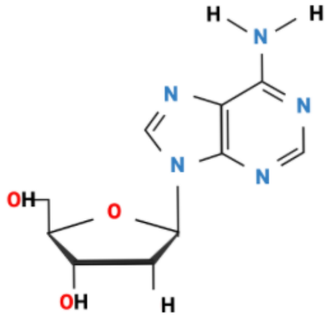


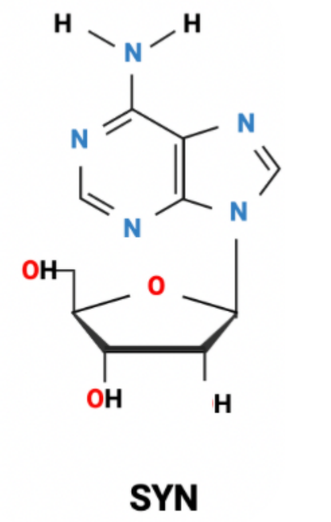
ANTI - ADENOSINE

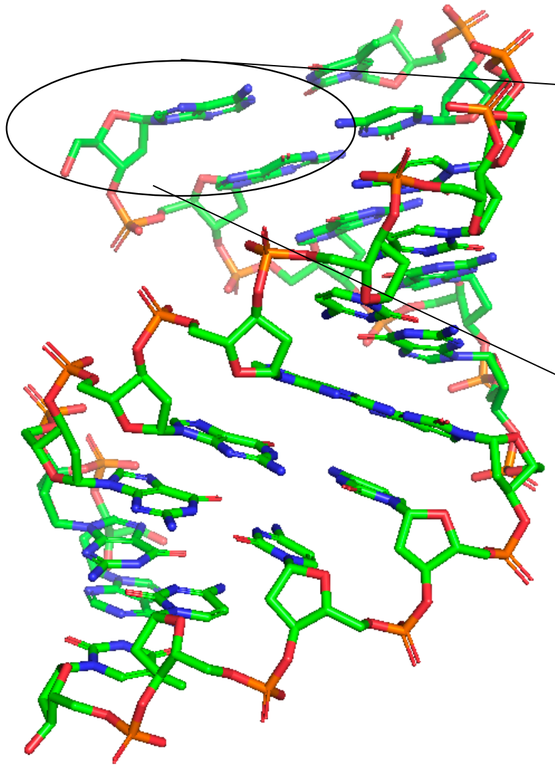


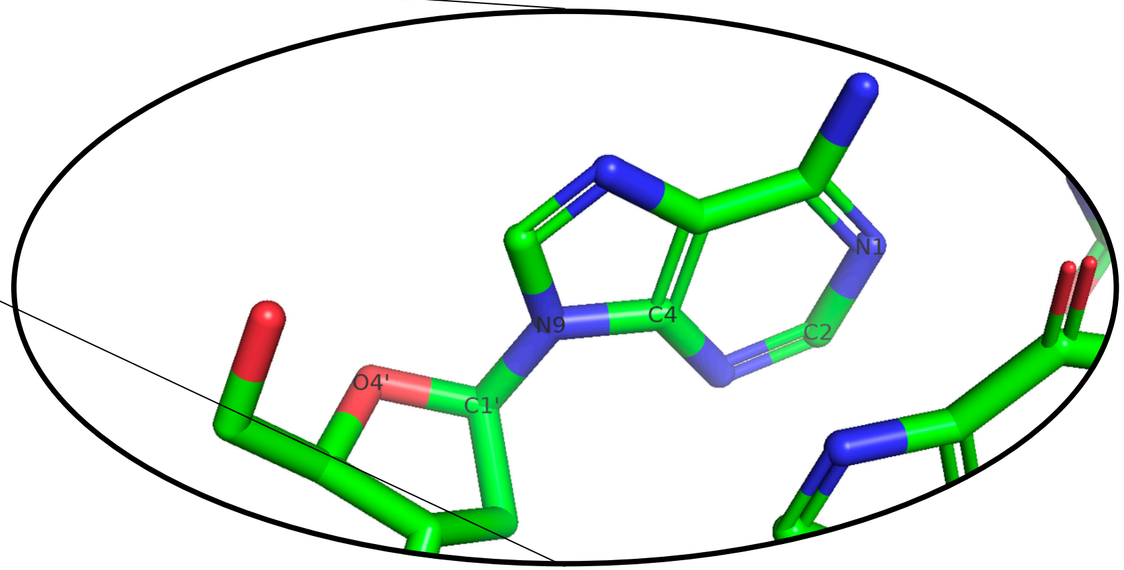
Created with Biorender

Feature	B-DNA	A-DNA	Z-DNA
Glycosidic Bond	Anti	Anti	Anti (Pyr) / Syn (Pur)



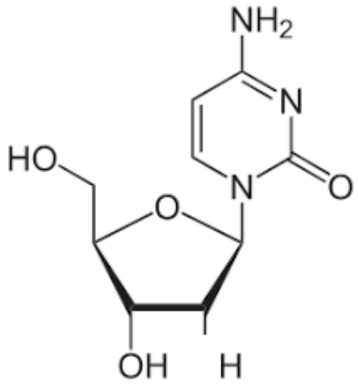




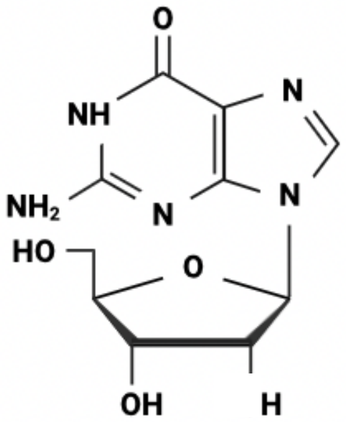


Created with Biorender

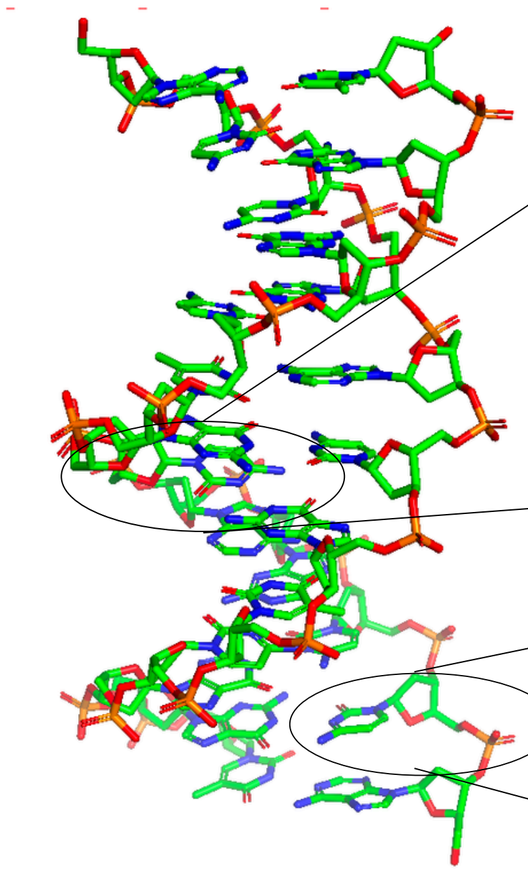
Feature	B-DNA	A-DNA	Z-DNA
Glycosidic Bond	Anti	Anti	Anti (Pyr) / Syn (Pur)

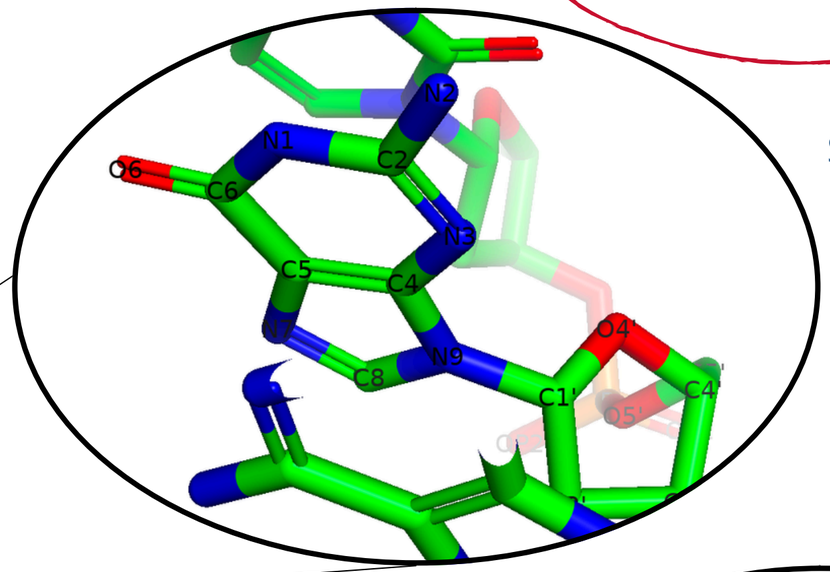


ANTI

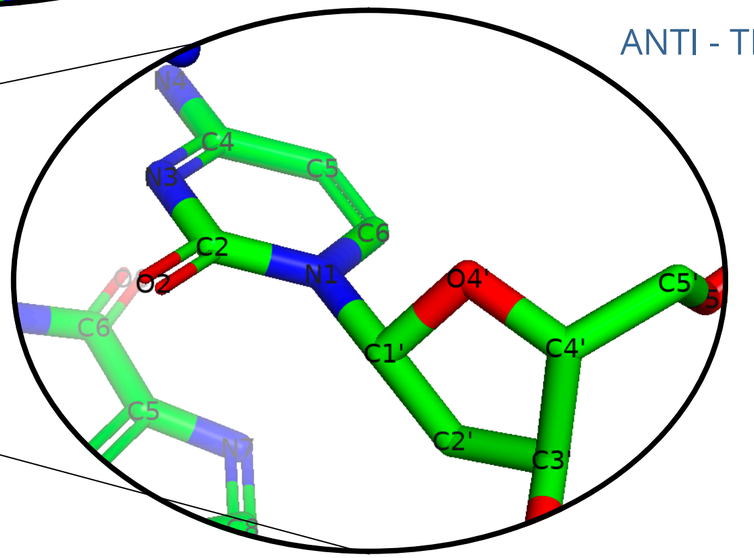


SYN





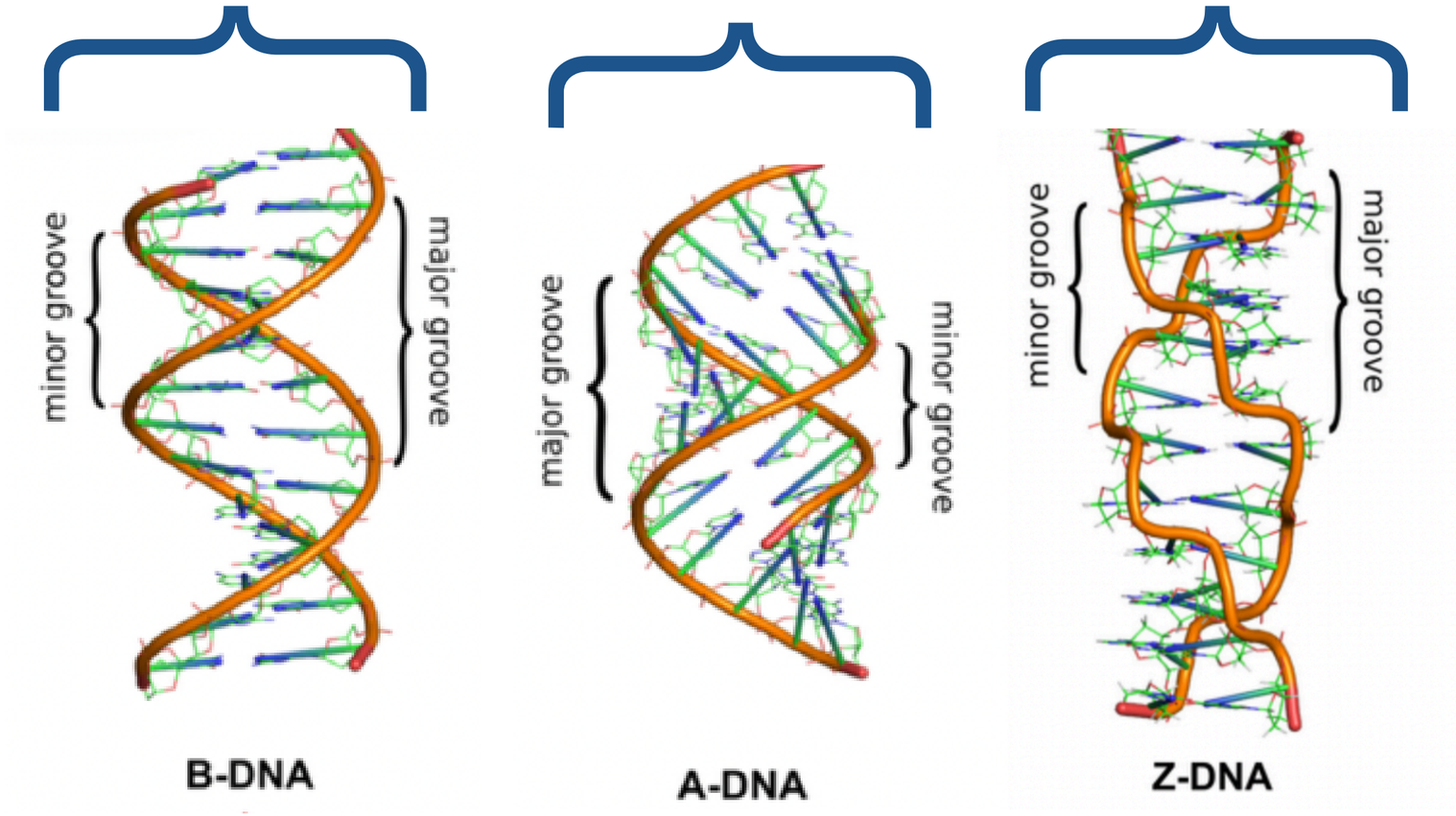
SYN - GUANOSINE



ANTI - THYMIDINE

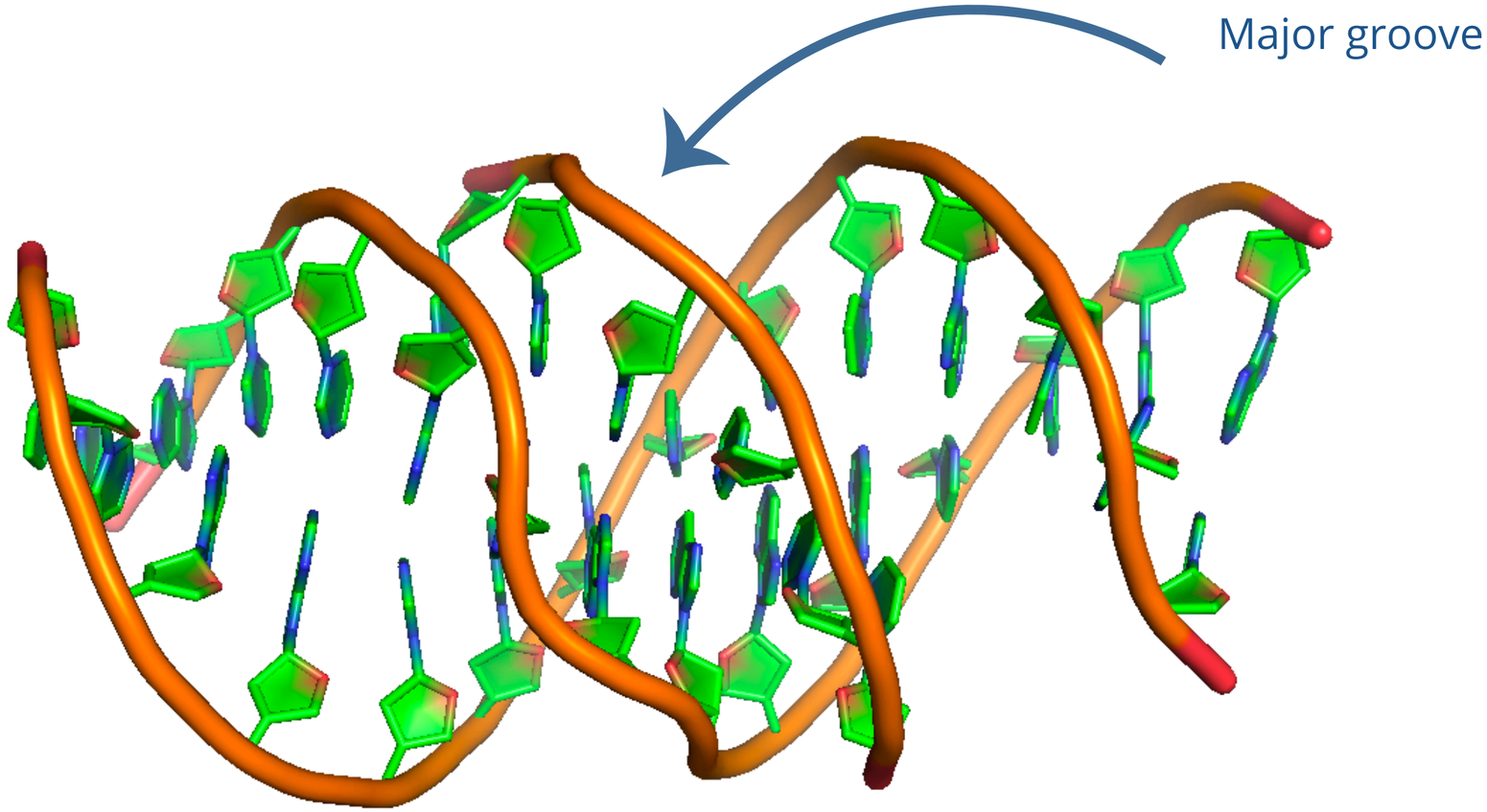
Created with Biorender

Feature	B-DNA	A-DNA	Z-DNA
Major Groove	Wide & Deep	Narrow & Deep	Flat / Not distinct
Minor Groove	Narrow & Deep	Wide & Shallow	Narrow & Deep

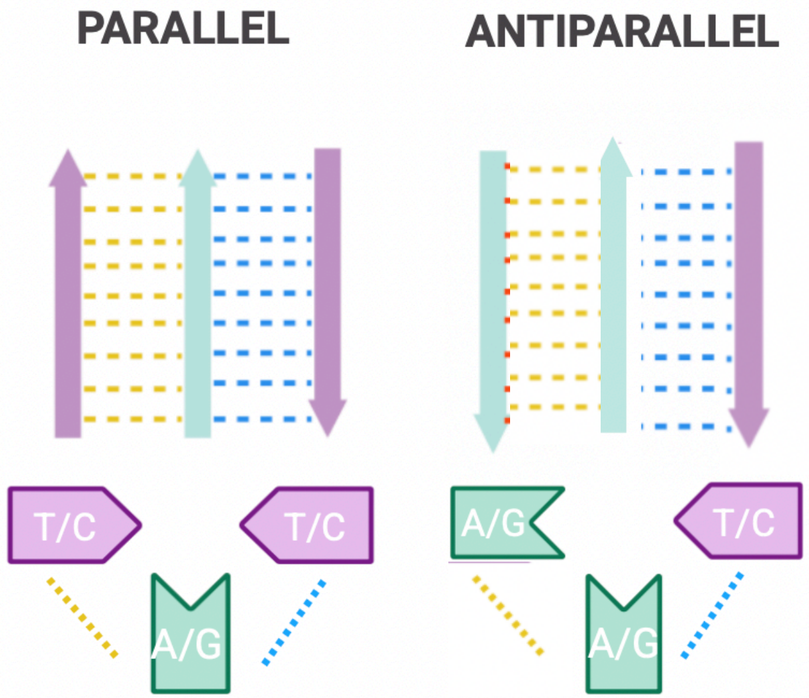


Obara et al., 2024 (Molecules)

COMPLEX DNA ARCHITECTURES: TRIPLE HELIX DNA



TRIPLE HELIX MOTIFS



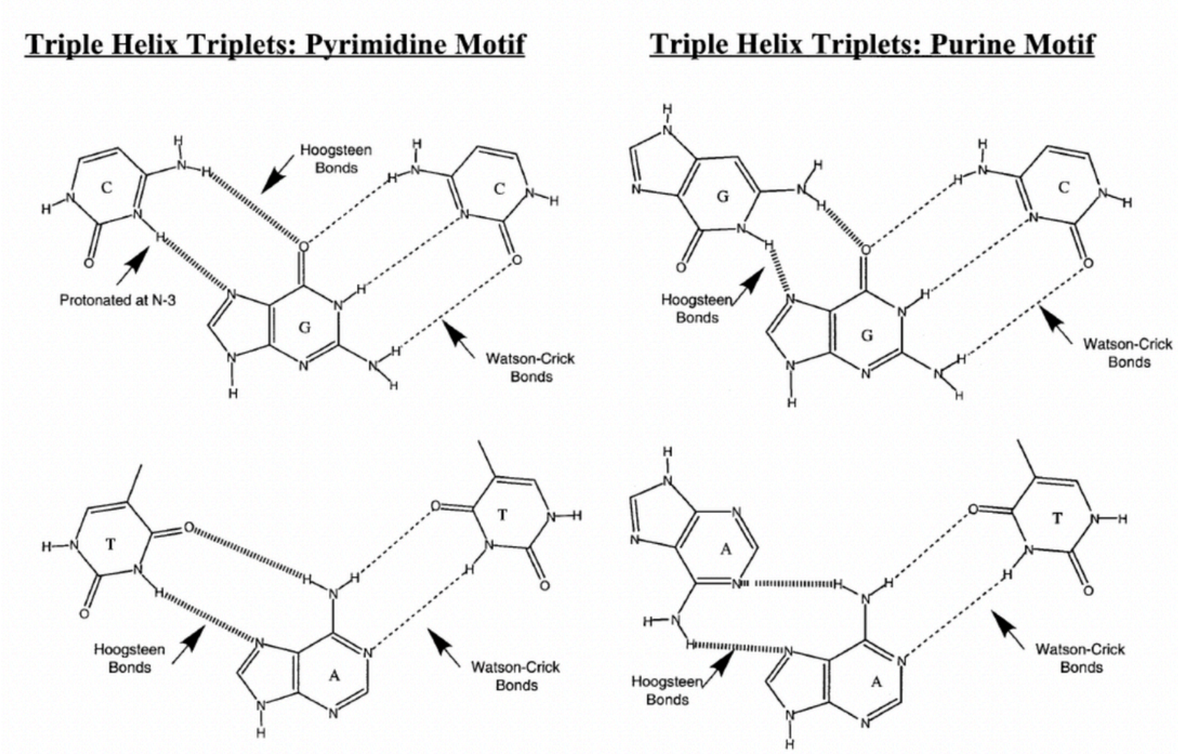
Interactions:

Hoogsteen

Watson & Crick

Pyrimidines

Purines



PYRIMIDINE MOTIF

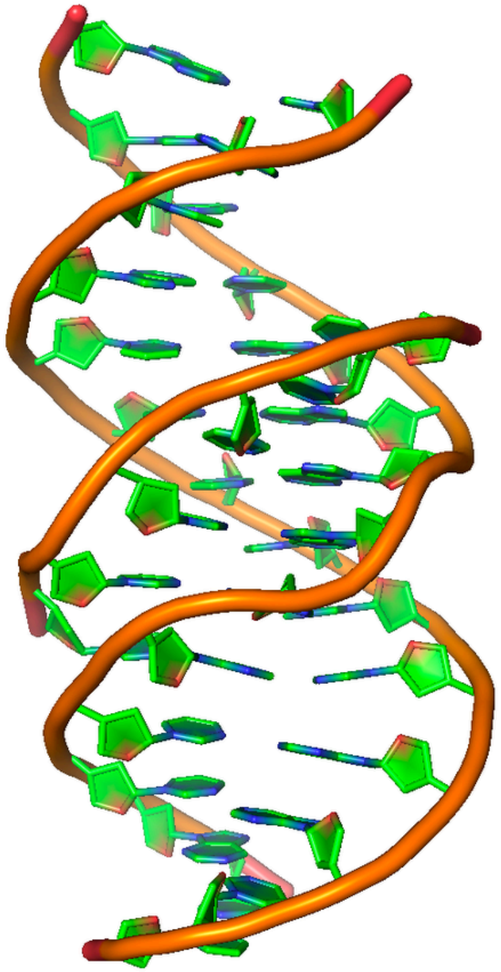
PURINE MOTIF

Created with Biorender

Melissa P. Knauert, Peter M. Glazer, Triplex forming oligonucleotides: sequence-specific tools for gene targeting, Human Molecular Genetics, Volume 10, Issue 20, 1 October 2001, Pages 2243–2251

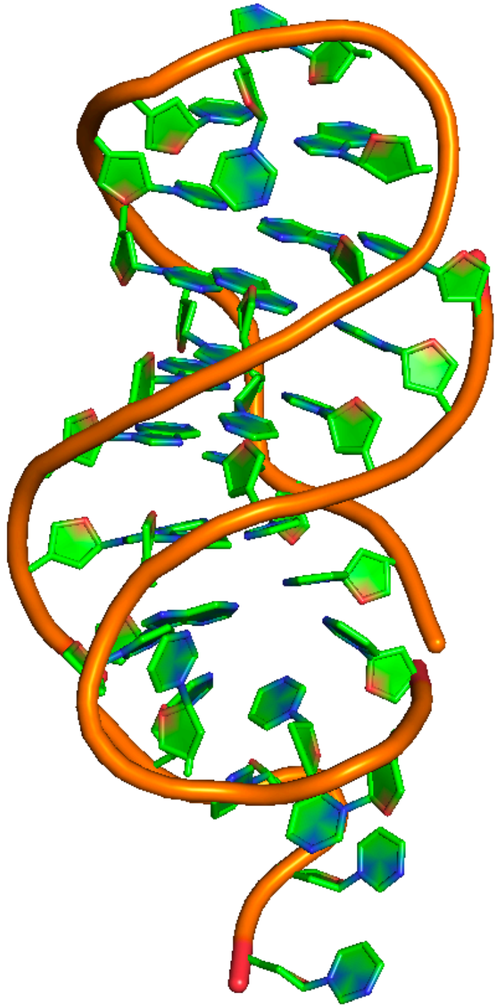
TYPES OF TRIPLEX DNA

INTERMOLECULAR



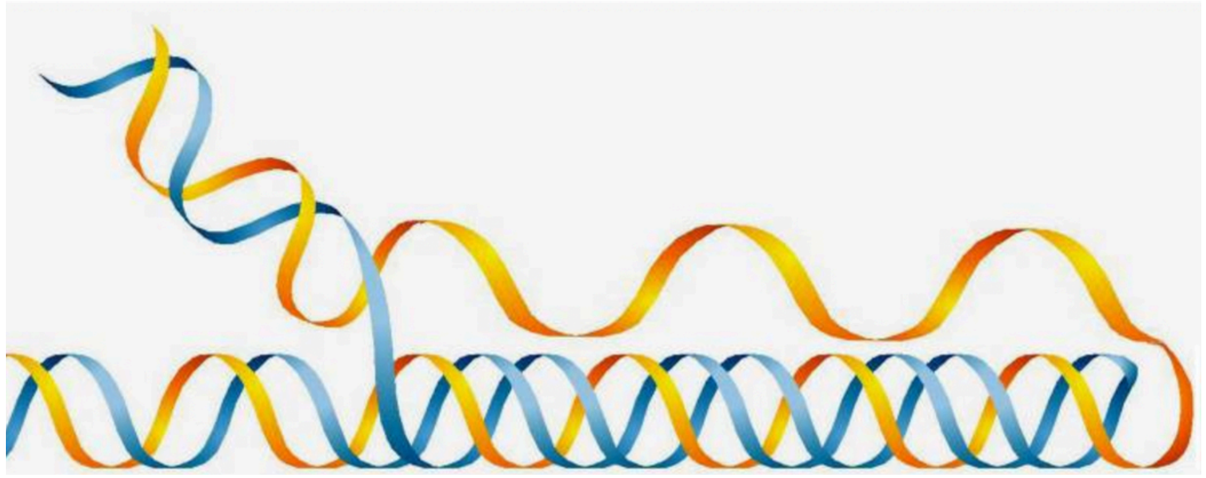
PDB:1BWG

INTRAMOLECULAR (H-DNA)

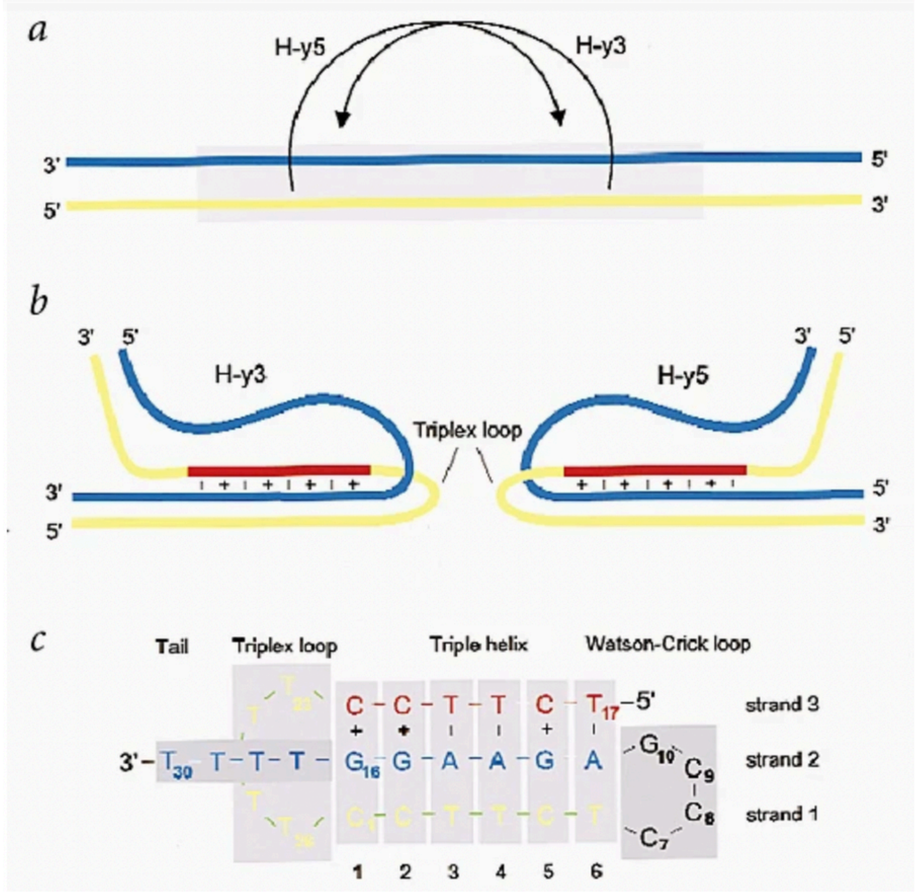


PDB:1B4Y

INTERAMOLECULAR TRIPLE HELIX

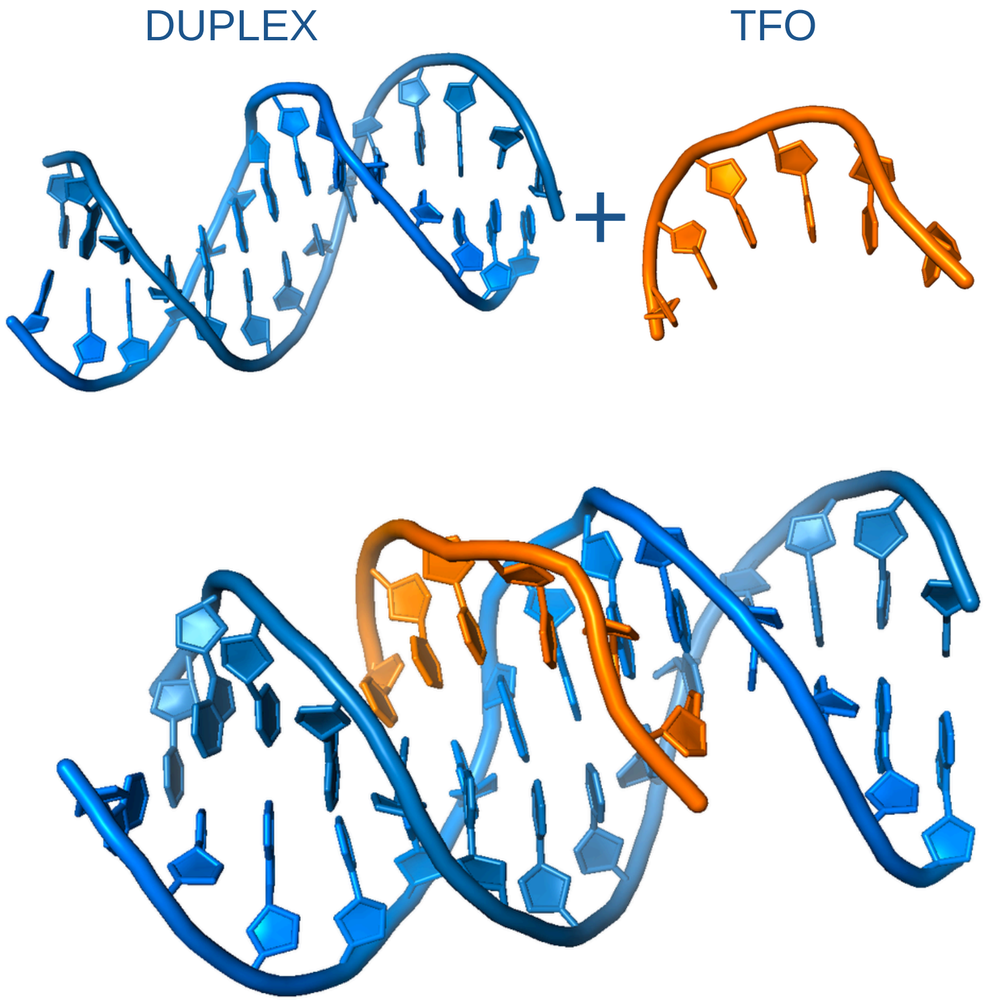


Jain A, Wang G, Vasquez KM. DNA triple helices: biological consequences and therapeutic potential. Biochimie. 2008 Aug;90(8):1117-30. doi: 10.1016/j.biochi.2008.02.011. Epub 2008 Feb 21. Erratum in: Biochimie. 2018 May;148:139.

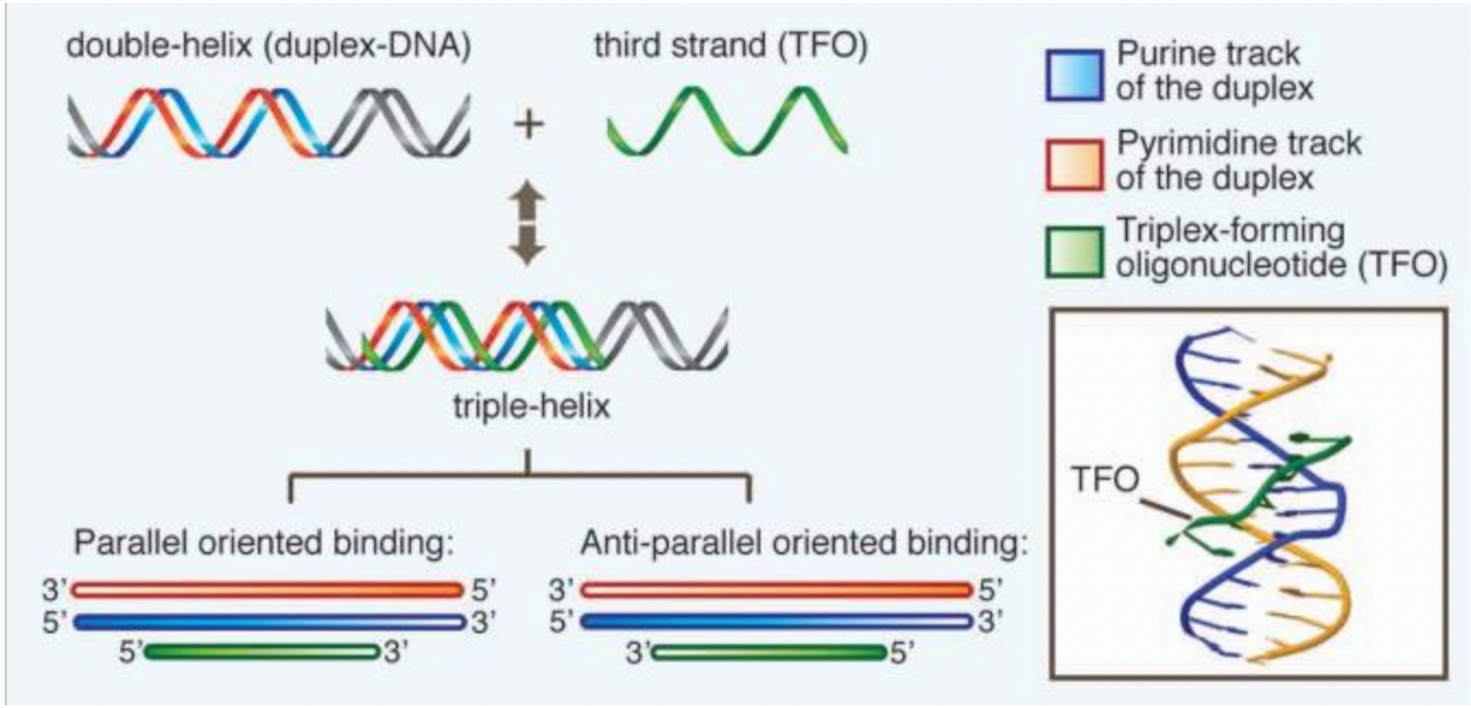


van Dongen, M., Doreleijers, J., van der Marel, G. et al. Structure and mechanism of formation of the H-y5 isomer of an intramolecular DNA triple helix. Nat Struct Mol Biol 6, 854–859 (1999).

INTERMOLECULAR TRIPLE HELIX

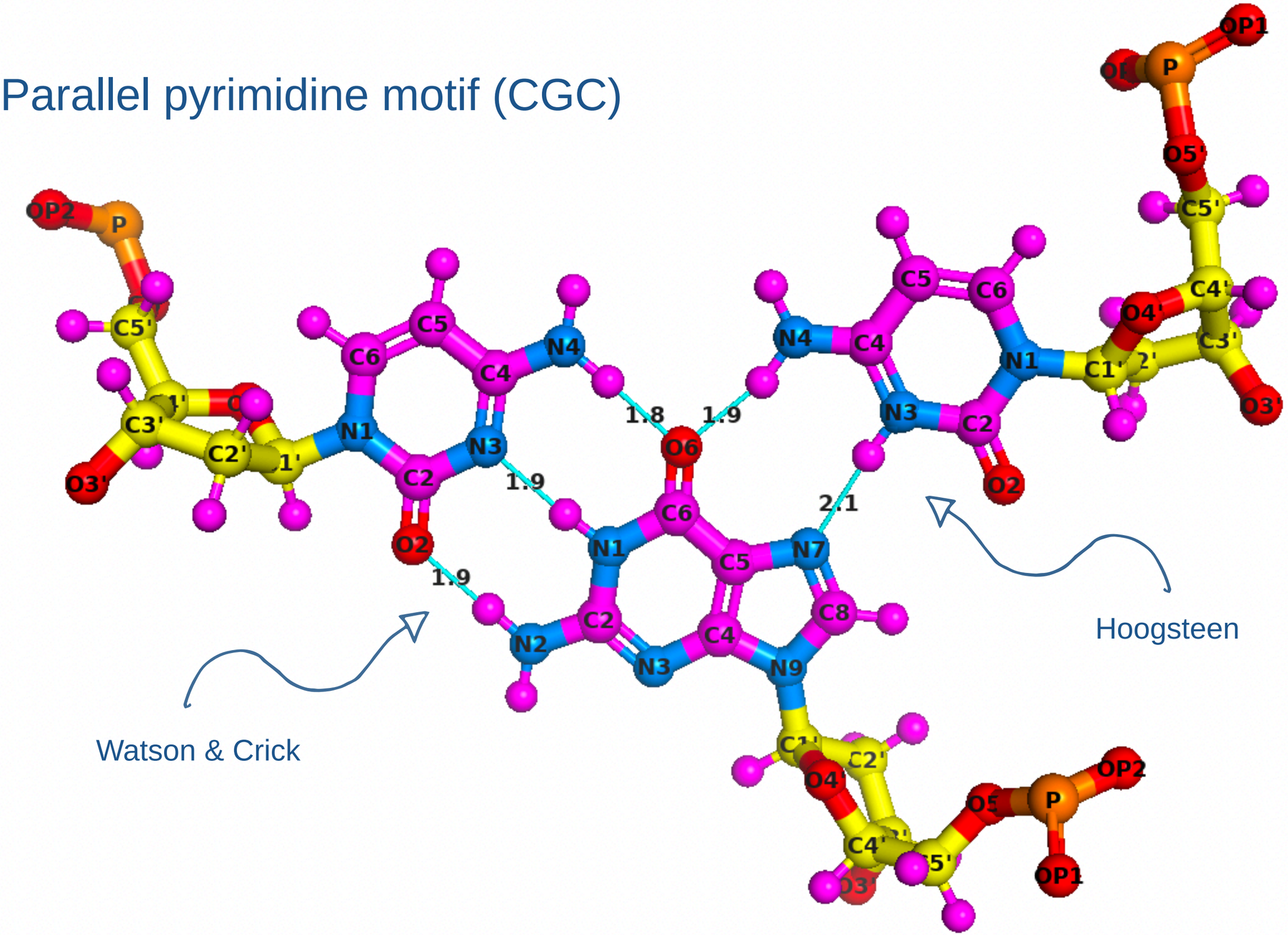


TFO = Triplex-Forming Oligonucleotide

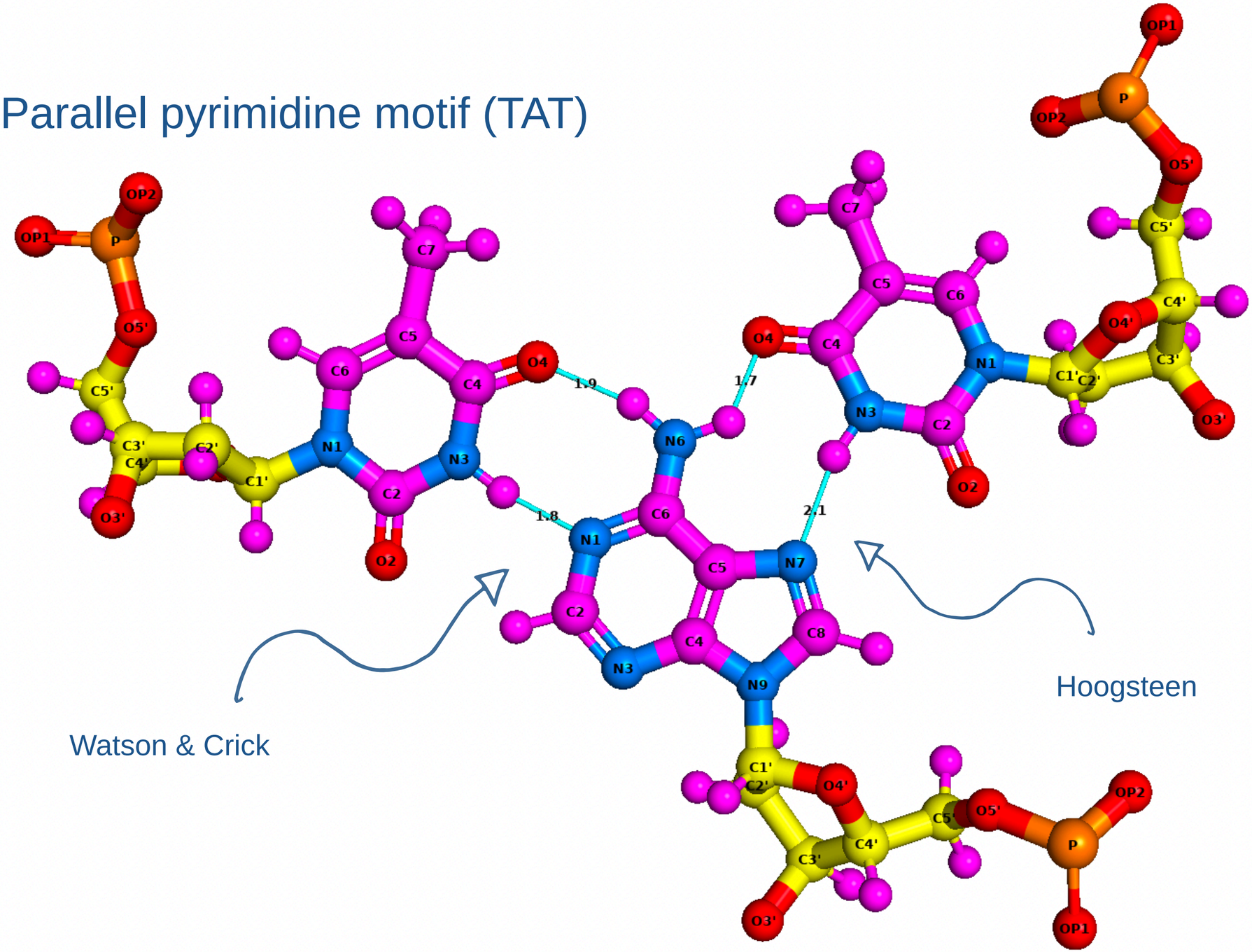


Buske FA, Mattick JS, Bailey TL. Potential in vivo roles of nucleic acid triple-helices. RNA Biol. 2011 May-Jun;8(3):427-39.

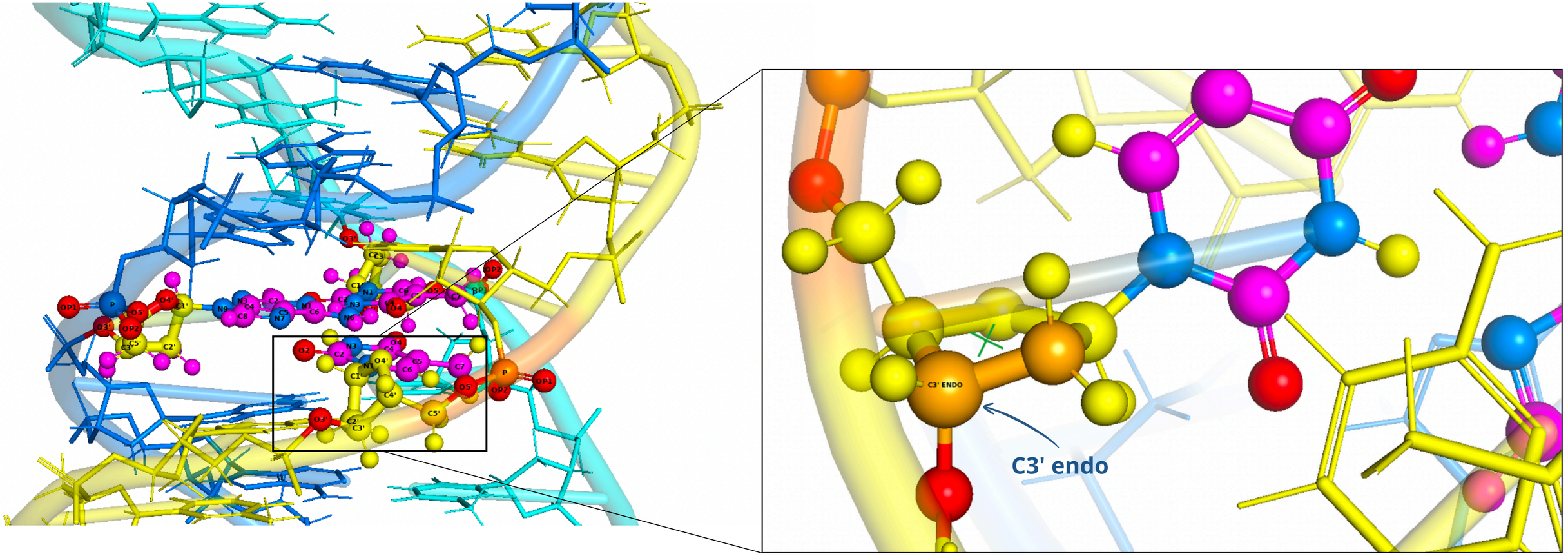
Parallel pyrimidine motif (CGC)



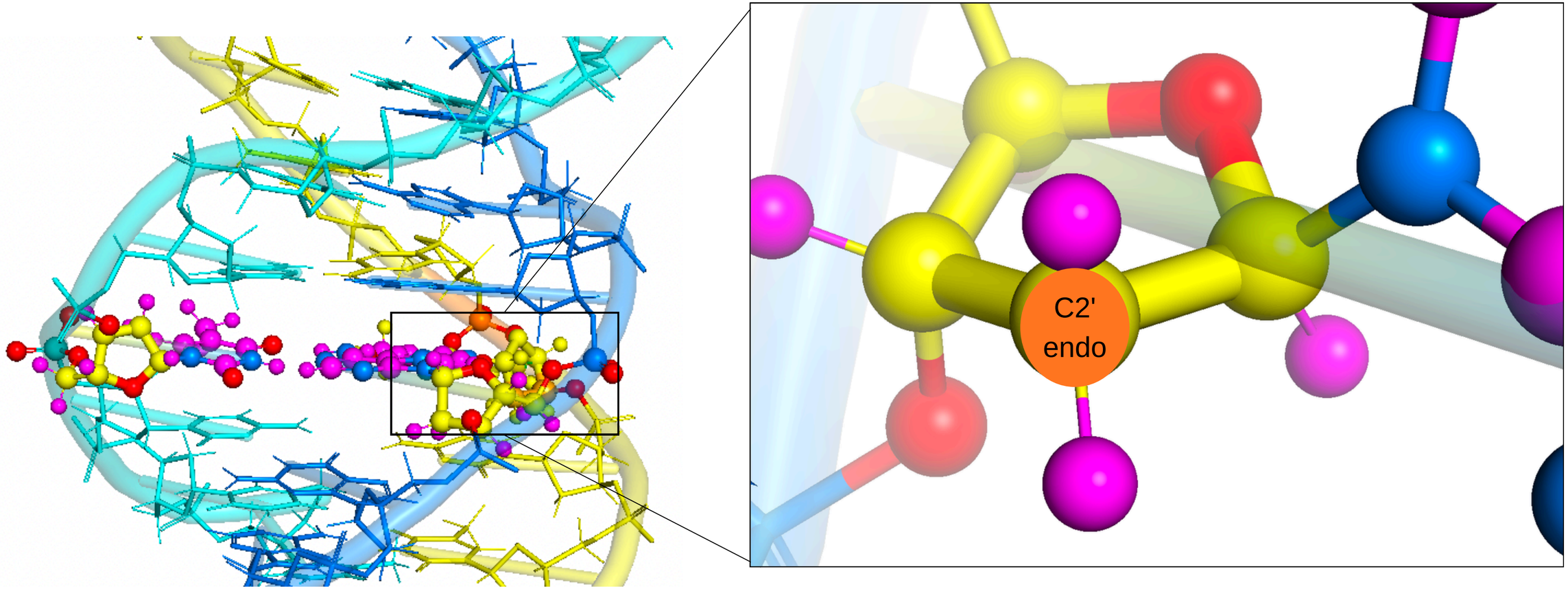
Parallel pyrimidine motif (TAT)



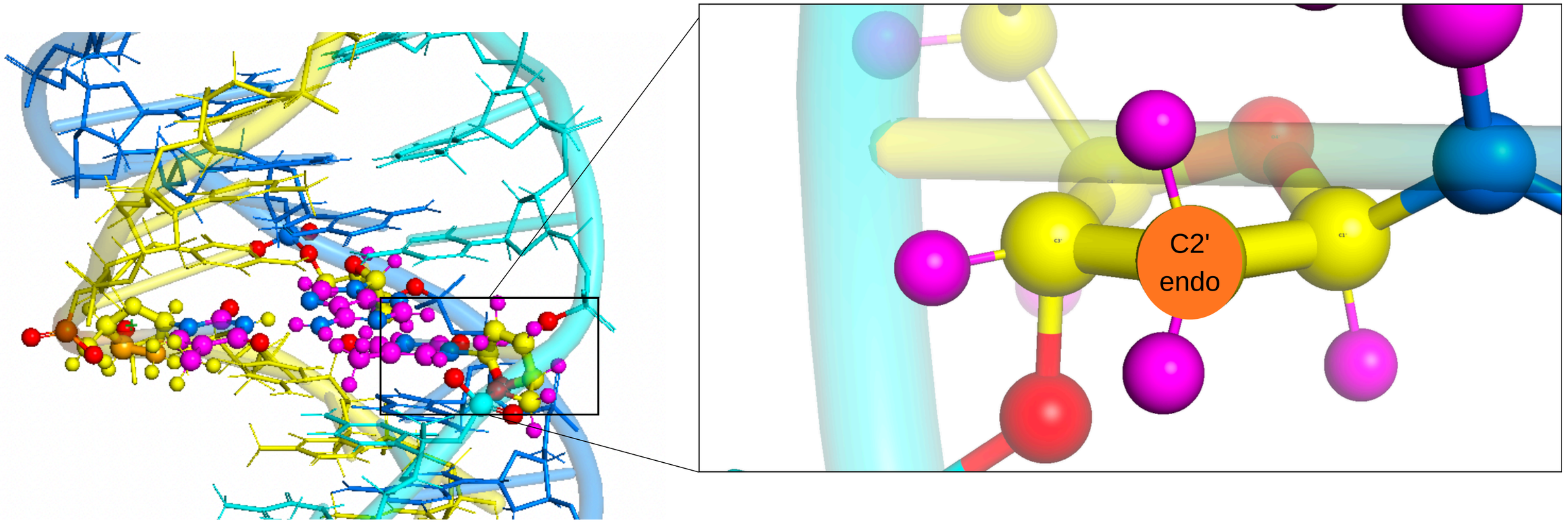
Sugar Pucker TFO chain C3' endo



Sugar Pucker chain A C2' endo

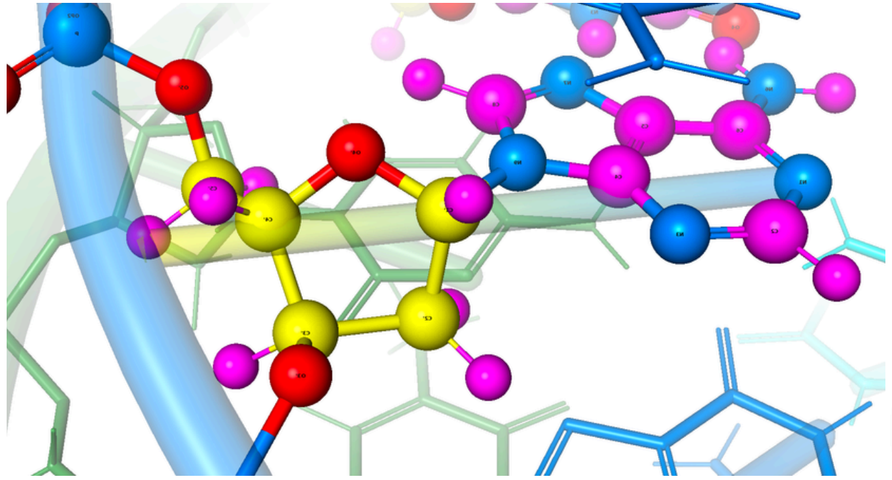


Sugar Pucker chain B C2' endo

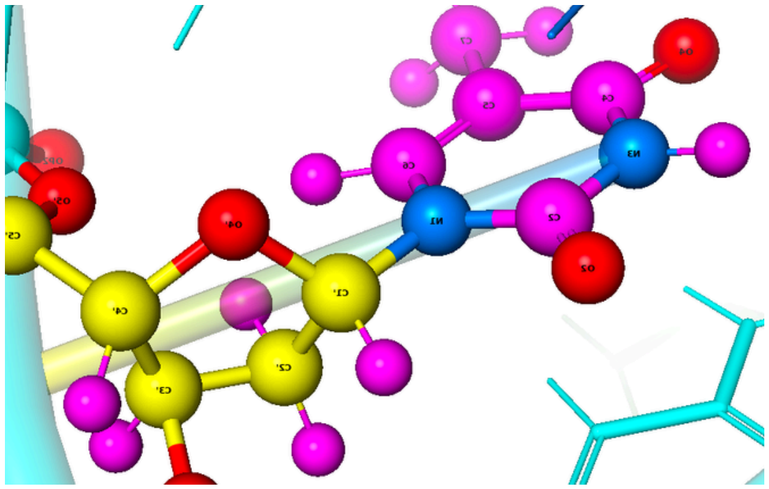


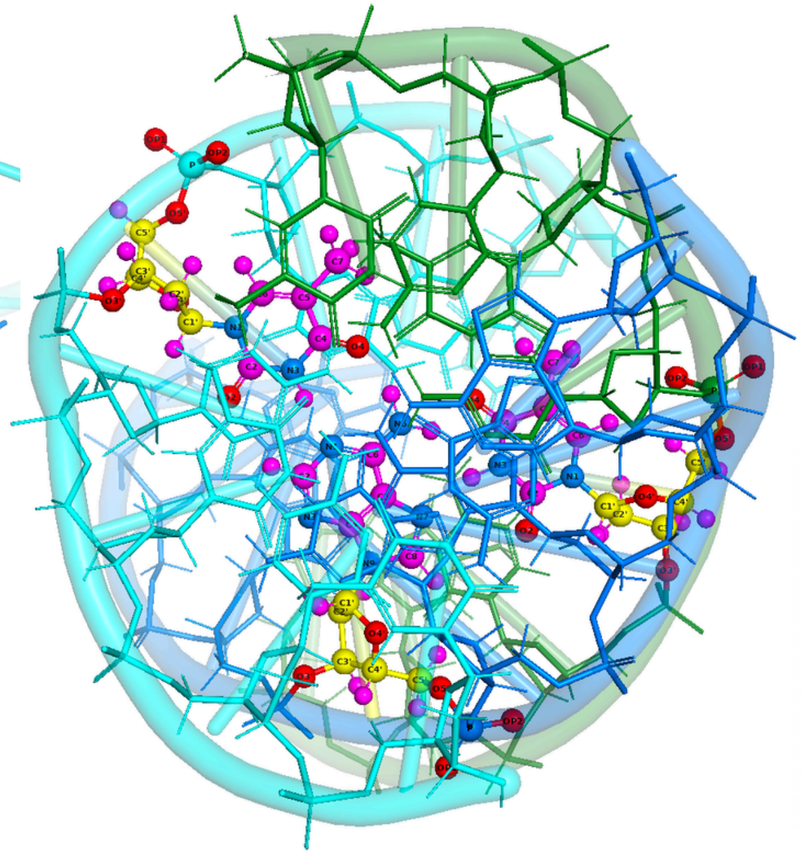
Glycosidic bond - Triplex DNA: Anti

Chain A - anti

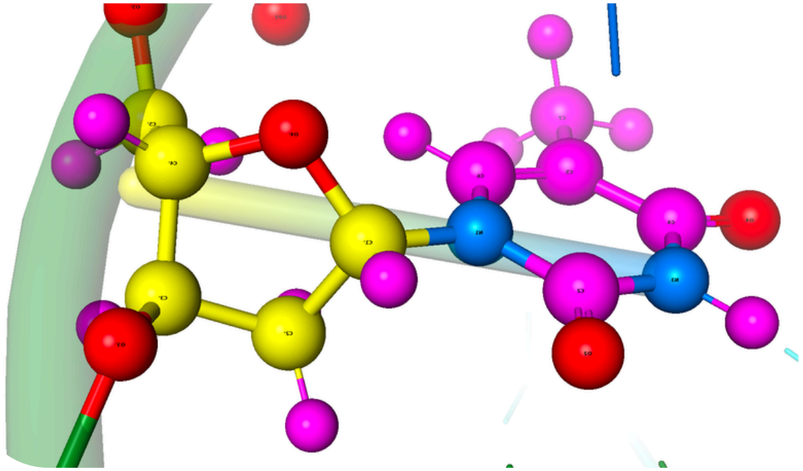


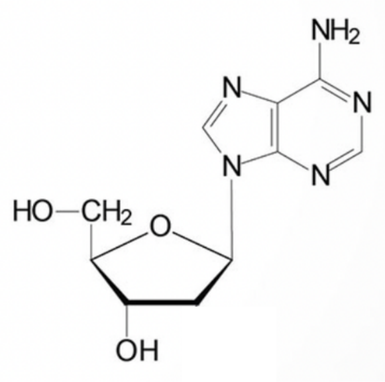
Chain B - anti



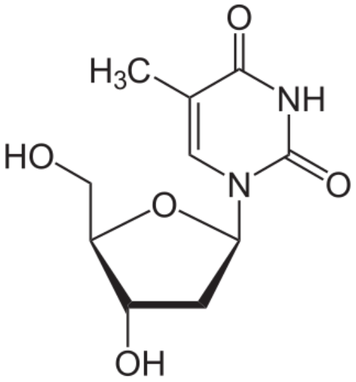


TFO - anti





Anti adenonosine



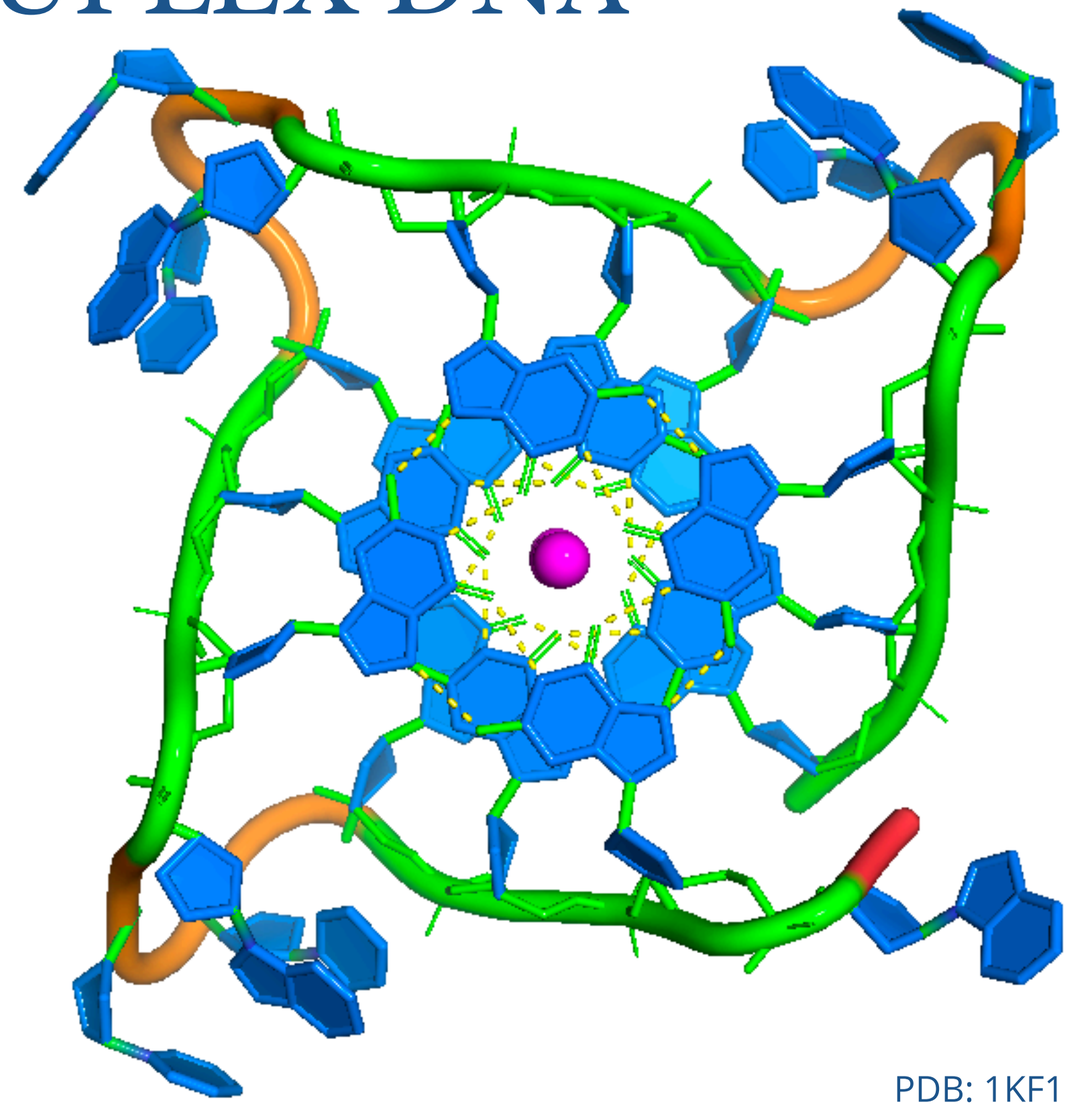
Anti timidine

Created with biorender

Biological aplications triplex-DNA

	TFO (External Tool)	H-DNA (Natural Structure)
Gene Regulation	Used as an **antigene strategy** to physically block transcription by binding to promoters.	Acts as a **natural regulator** that can either increase or decrease the expression of specific genes.
DNA Repair	Used to **intentionally trigger** repair pathways at a targeted genomic location.	Naturally recognized as a "lesion," activating **endogenous repair systems** like NER.
Gene Editing	Promotes **homologous recombination**, helping to swap or correct specific genetic sequences.	Can lead to **genomic instability**, causing natural deletions or translocations.
Mutagenesis	Directs chemical agents to create **precise, site-specific mutations** in the genome.	Acts as a "hotspot" for **spontaneous mutations** due to its structural fragility.
Cell Replication	Serves as a physical barrier to **stop replication forks**, which can induce cancer cell death.	Naturally **stalls replication**, which may compromise the genetic integrity of the cell.

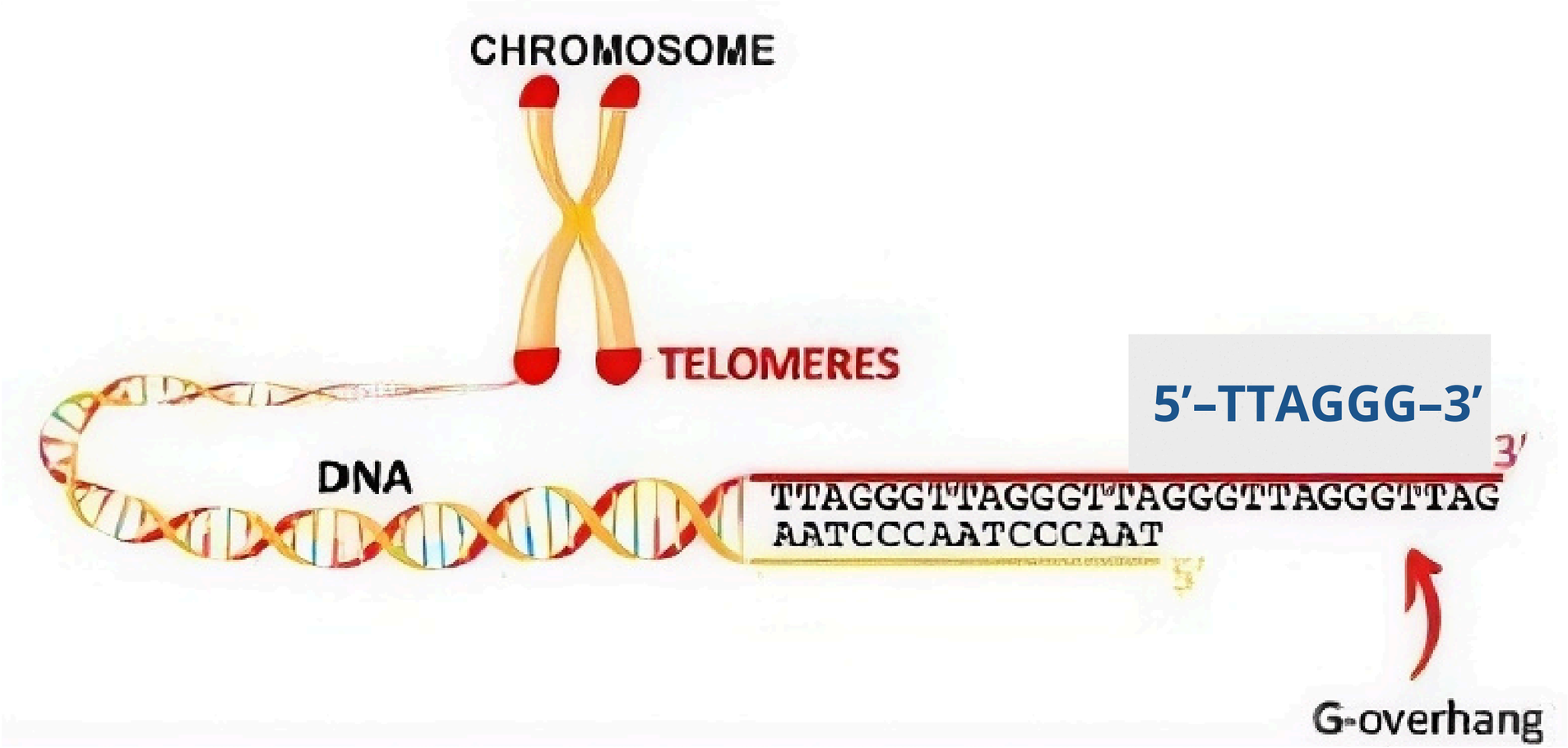
COMPLEX DNA ARCHITECTURES: G-QUADRUPLEX DNA



PDB: 1KF1

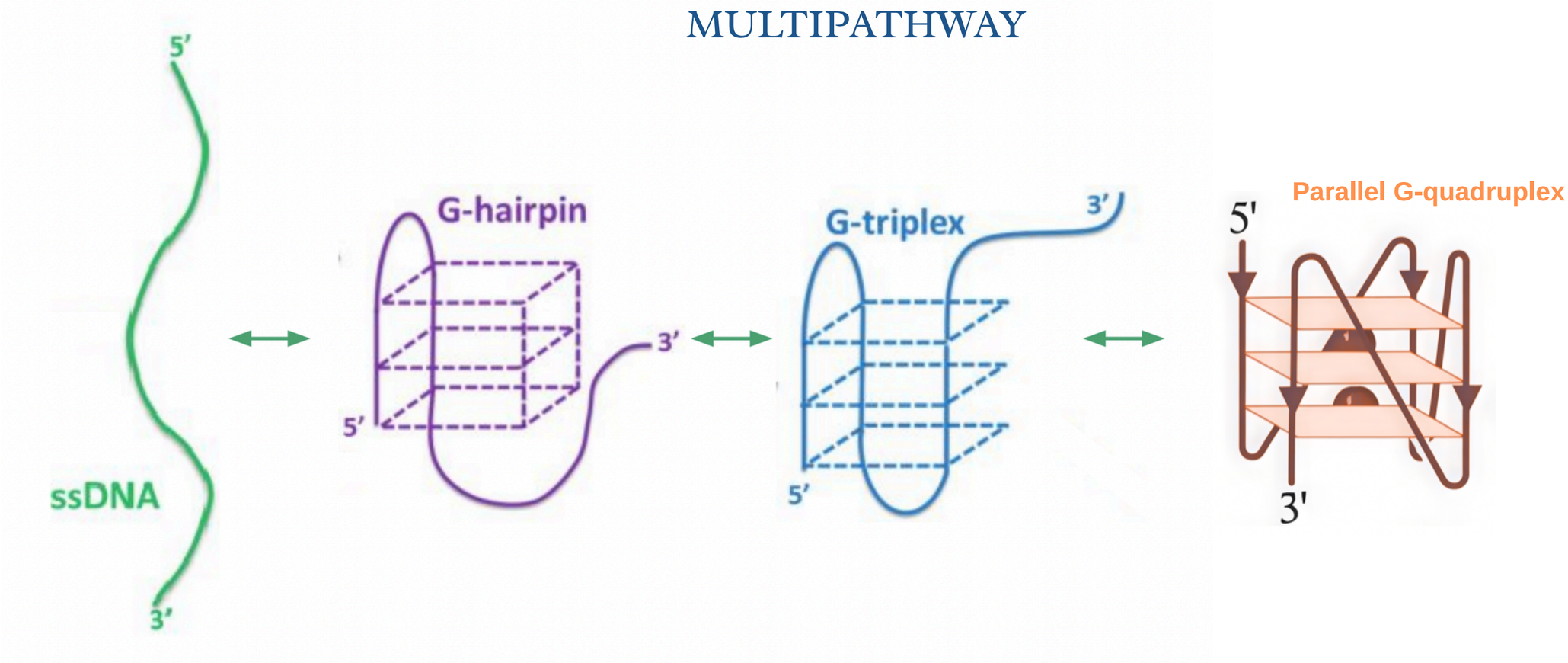
TELOMERES

Folding of the telomeric structures



Biochemical and structural analysis of Leishmania amazonensis Replication Protein A: a model to study the evolution of telomere-end binding proteins. (s/f). Sorbonne-universite.fr.

FOLDING



Hou XM, Fu YB, Wu WQ, Wang L, Teng FY, Xie P, Wang PY, Xi XG. Involvement of G-triplex and G-hairpin in the multi-pathway folding of human telomeric G-quadruplex. Nucleic Acids Res. 2017 Nov 2;45(19):11401-11412.

HUMAN TELOMERIC SEQUENCE 22

- 22 nucleotides
- 4 GGG tracts → G QUADRUPLEX
- 3 natural TTA loops

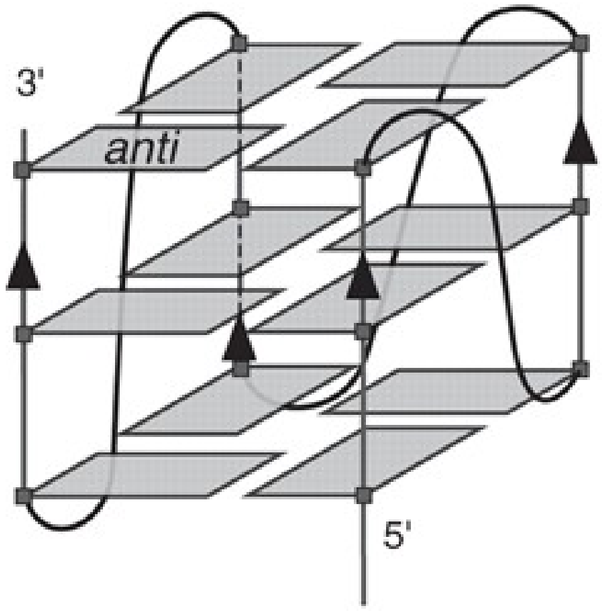
Tel22

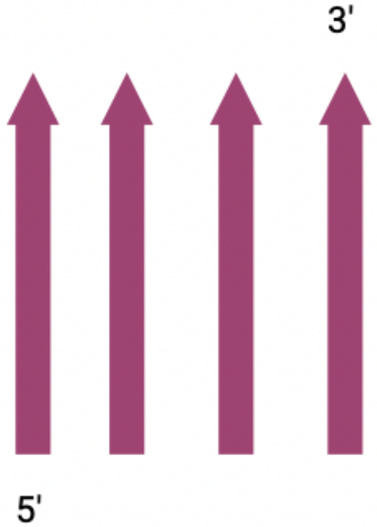
1 2 3 4 8 9 10 14 15 16 20 21 22

AGGGTTAGGGTTAGGGTTAGGG

T1 L1 T2 L2 T3 L3 T4

Folding topology of Tel22 → PARALLEL FOLDING





GGG tracts:

- G2–G4
- G8–G10
- G14–G16
- G20–G22

A. Ambrus, D. Chen, J. Dai, T. Bialis, R. A. Jones, D. Yang, Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution, Nucleic Acids Research,1 May 2006.

Tel22

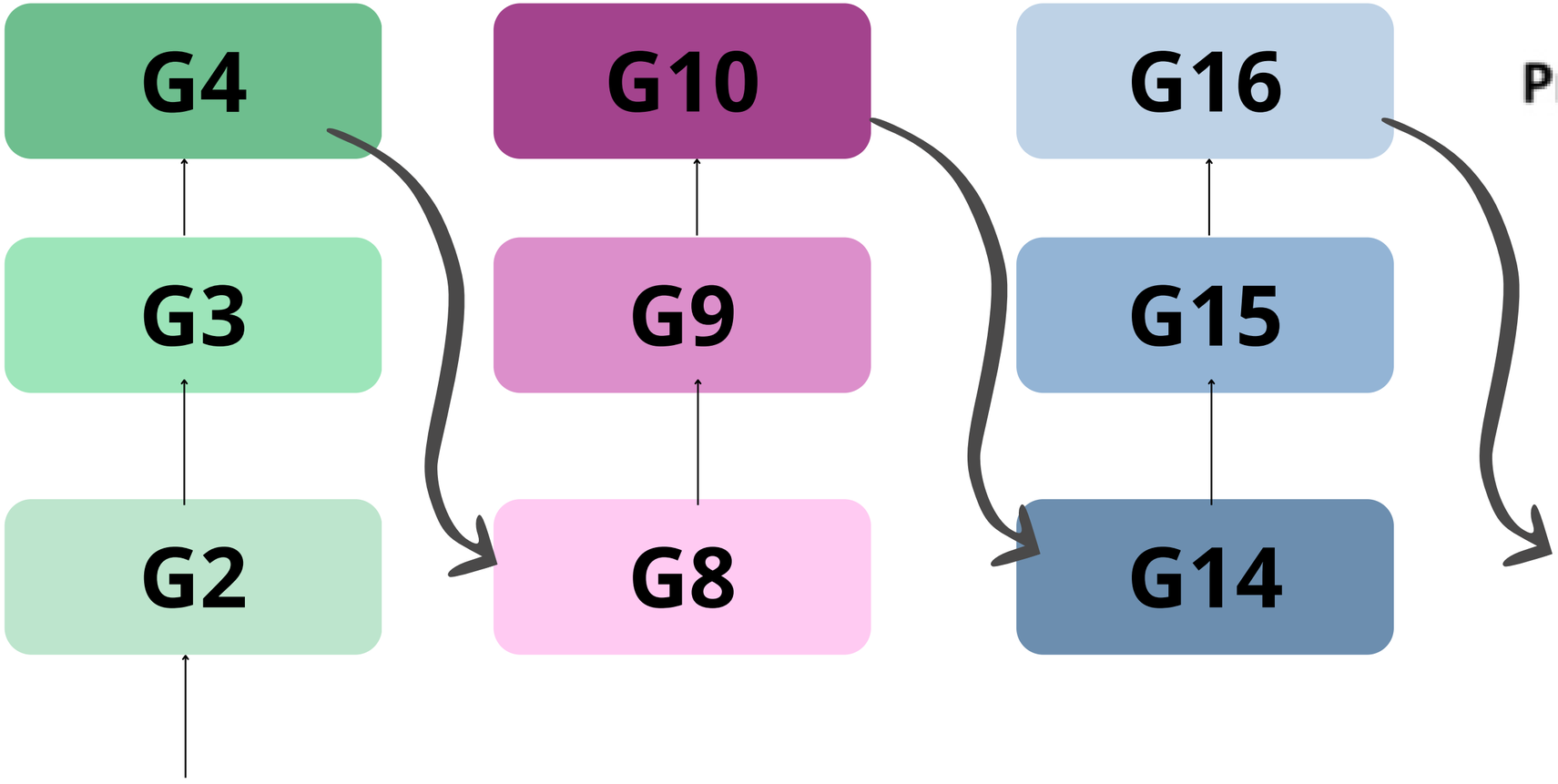
1 2 3 4 8 9 10 14 15 16 20 21 22

AGGGTTAGGGTTAGGGTTAGGG

T1 L1 T2 L2 T3 L3 T4



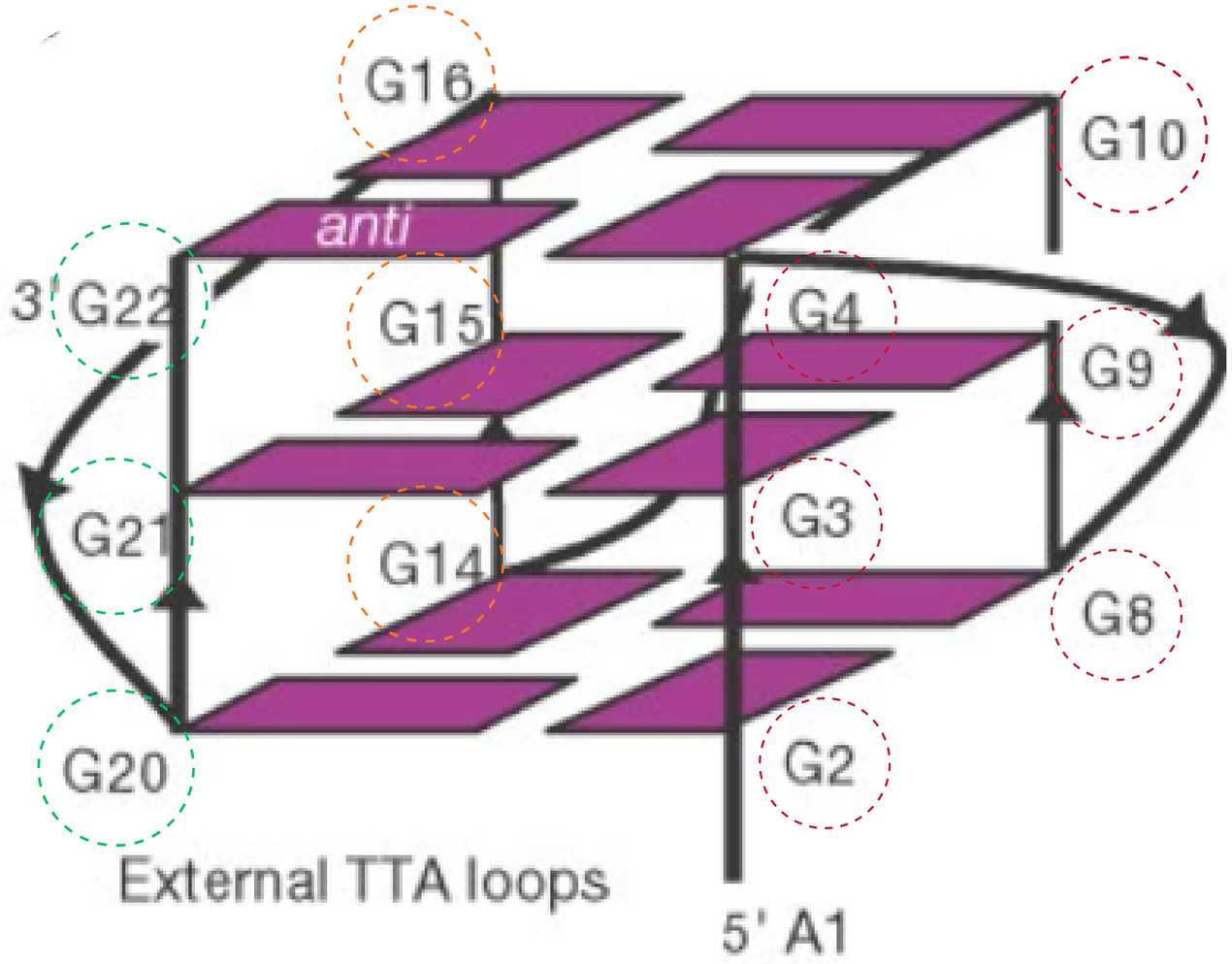
Propeller-type loop



Tel22 AGGGTTAGGGTTAGGGTTAGGG

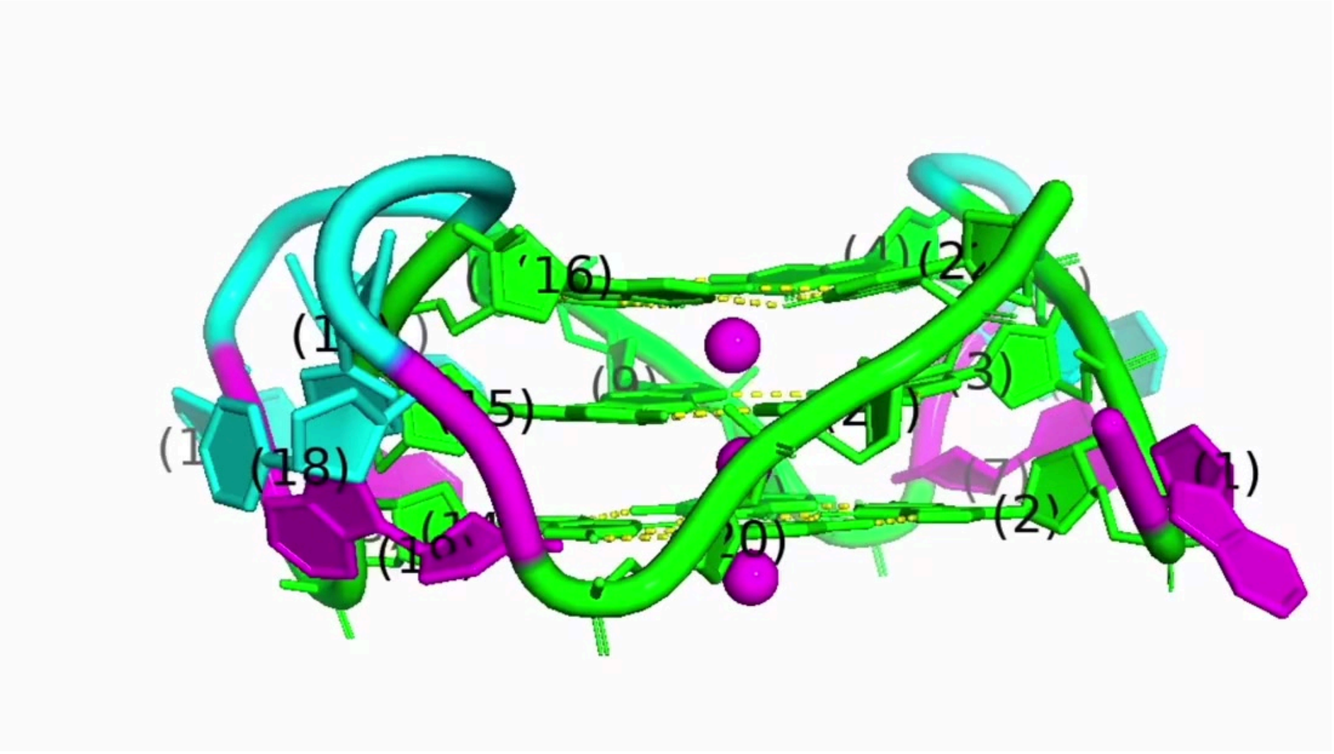
T1 L1 T2 L2 T3 L3 T4

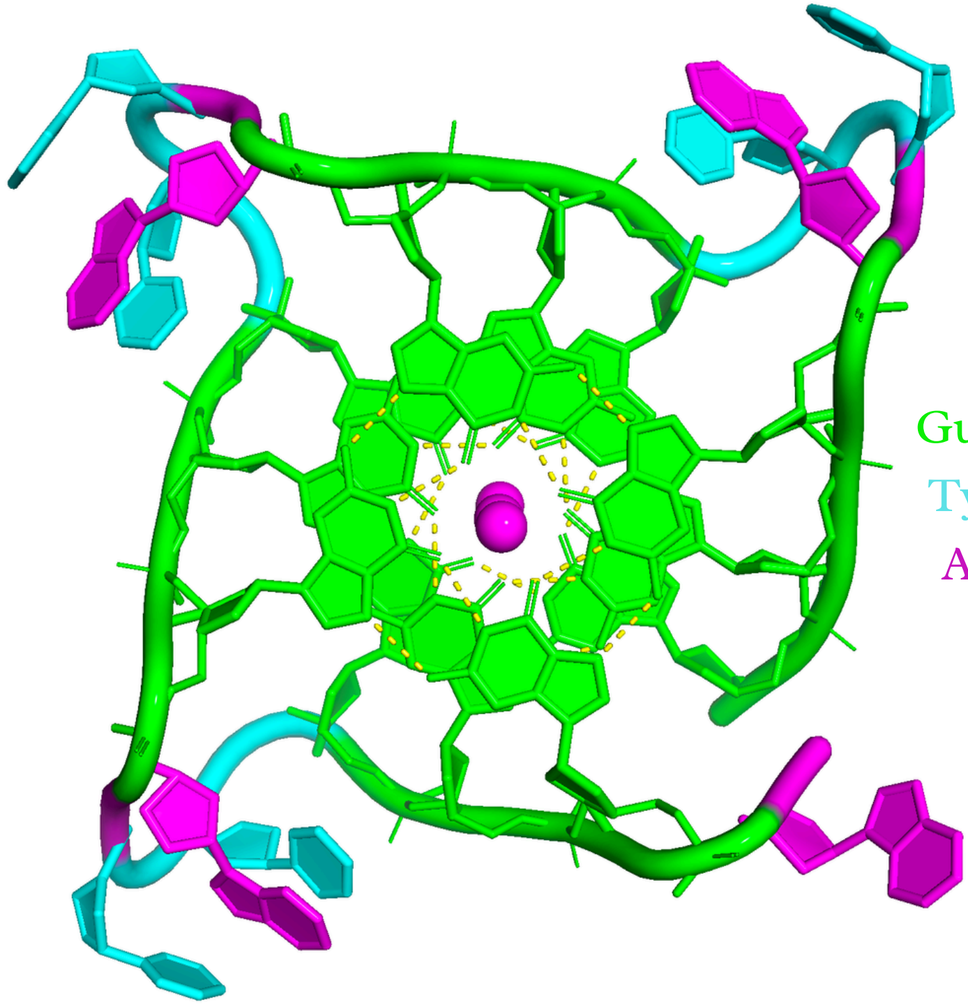
22 nucleotides



Parkinson, G., Lee, M. & Neidle, S. Crystal structure of parallel quadruplexes from human telomeric DNA. Nature 417, 876–880 (2002).

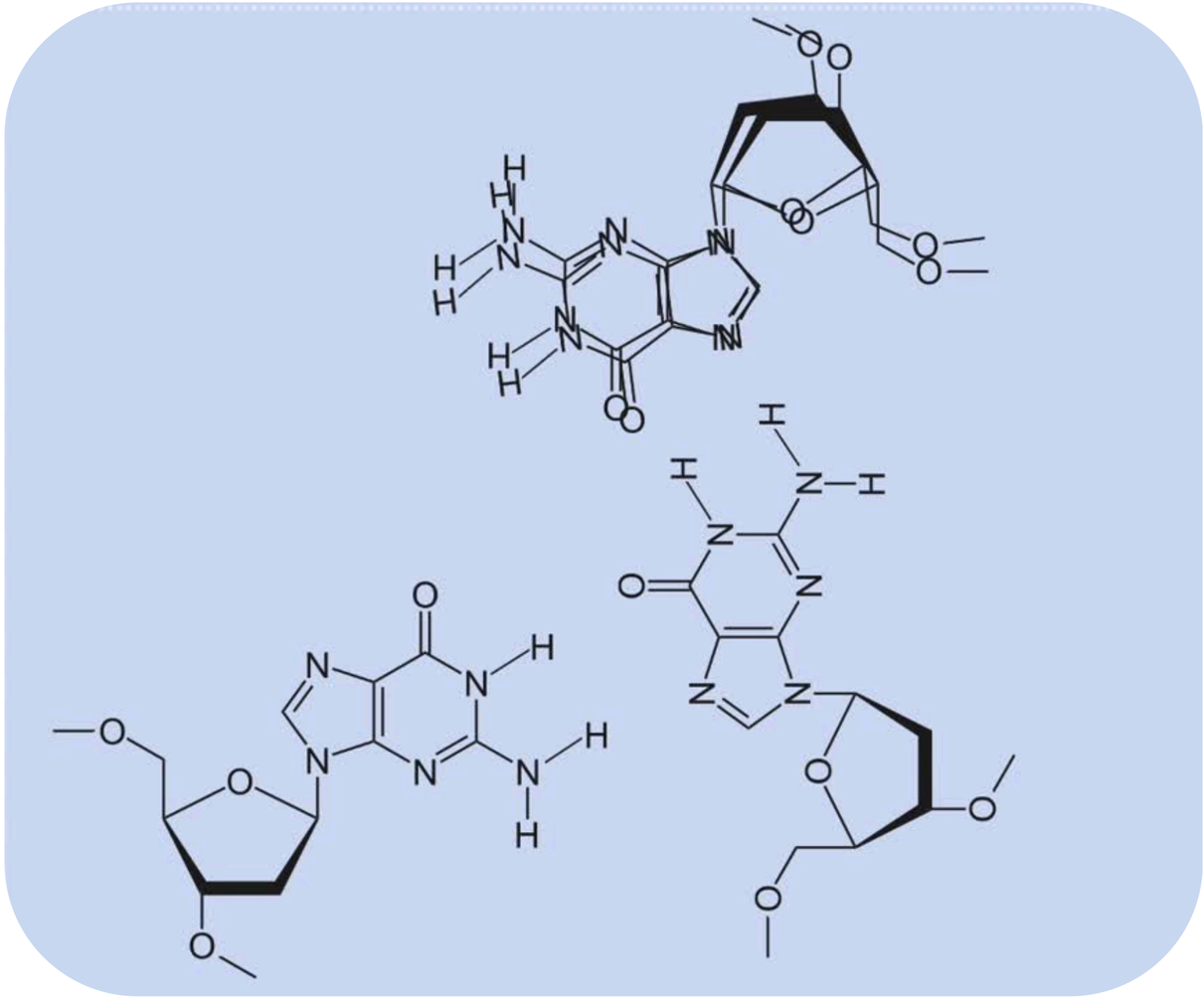
G QUADRUPLEX STRUCTURE





Guanosine
Tymidine
Adenine

G QUADRUPLEX



Created with Biomodel

K^+ ion

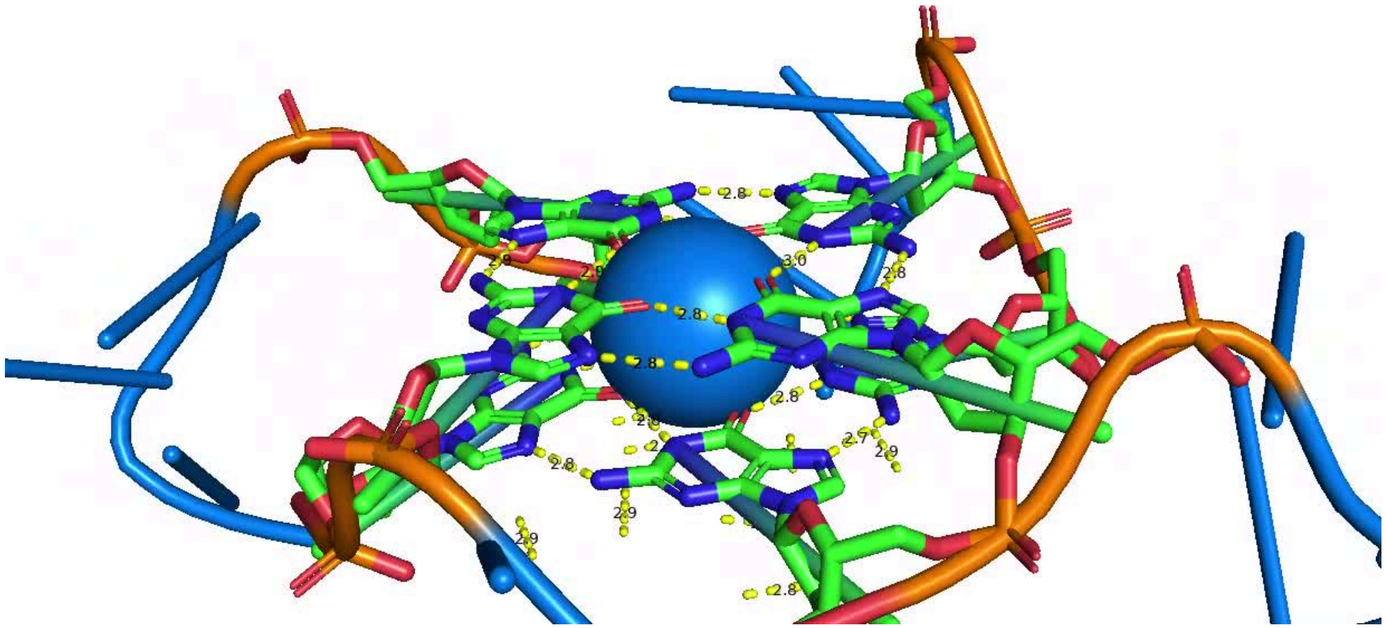
electrostatic interactions

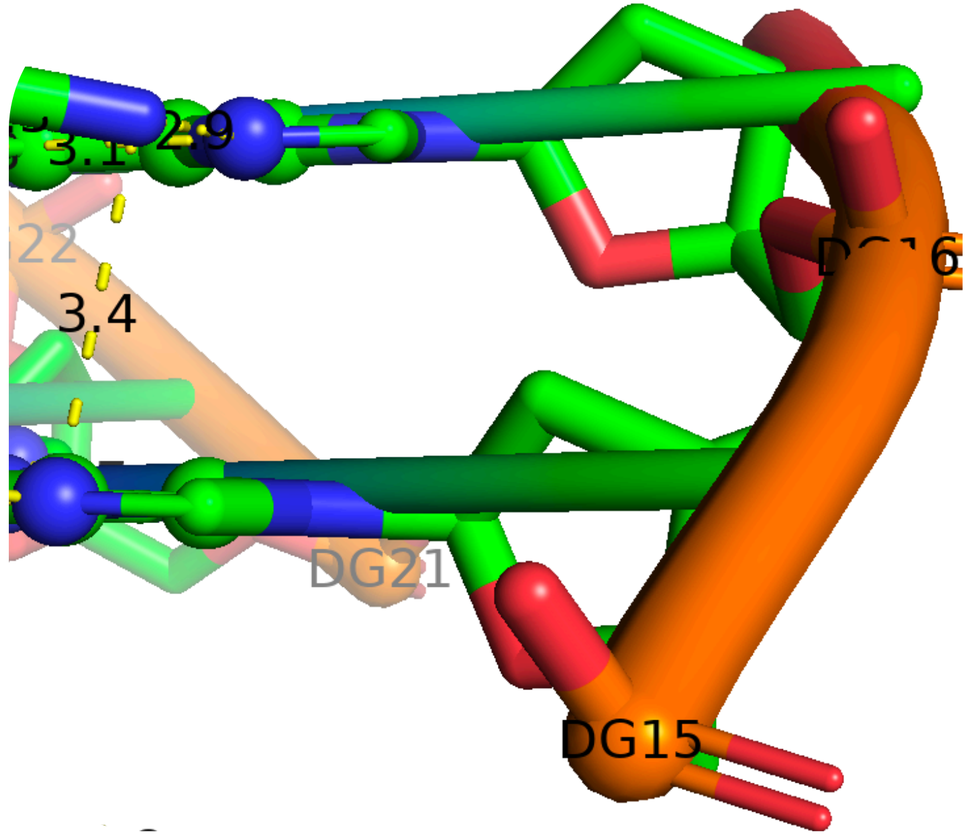
↓

ion-dipole interactions

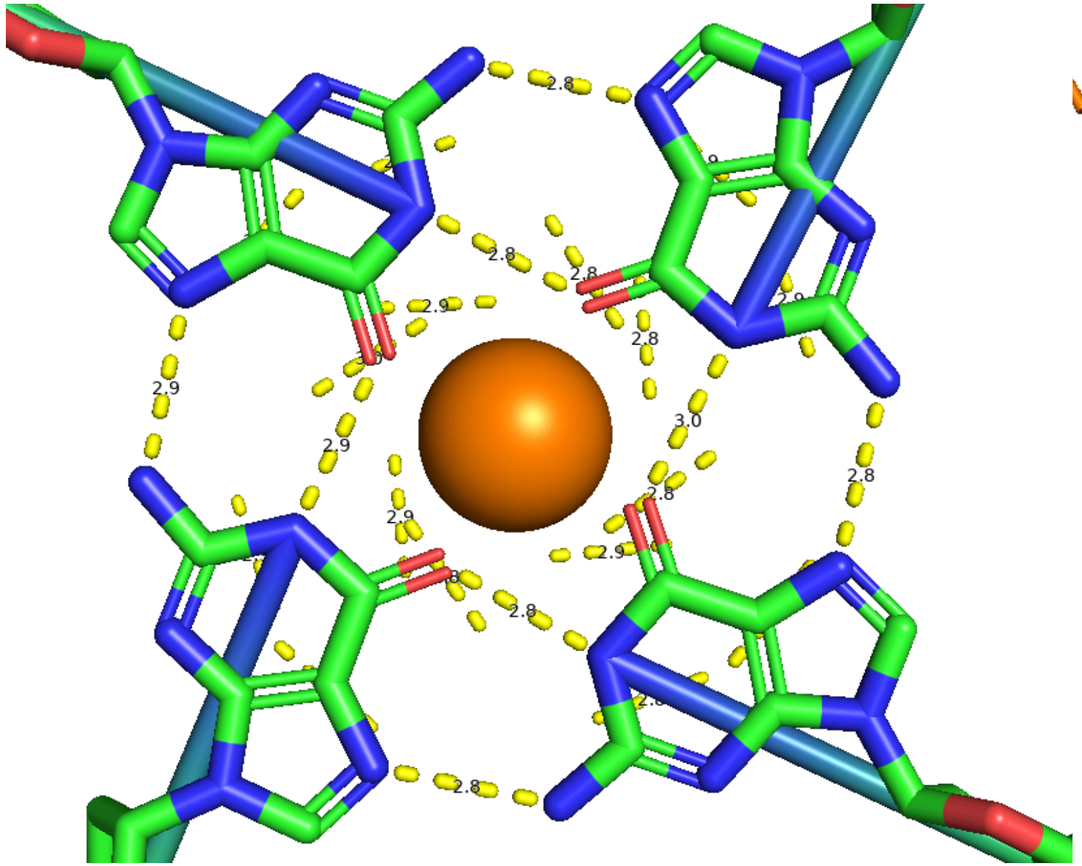
G-QUADRUPLEX

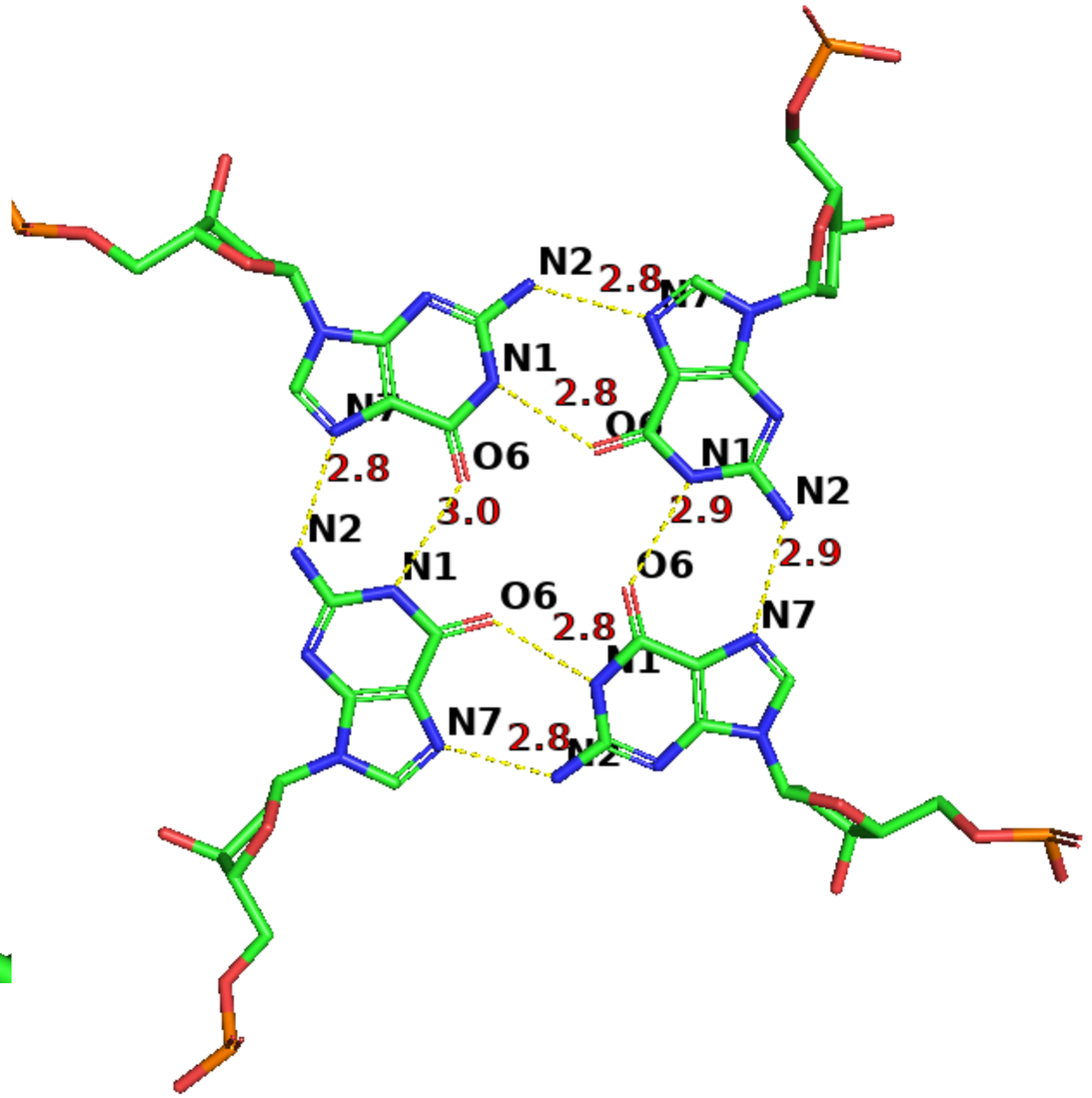
- K^+ ion
- π stacking between G-tetrads



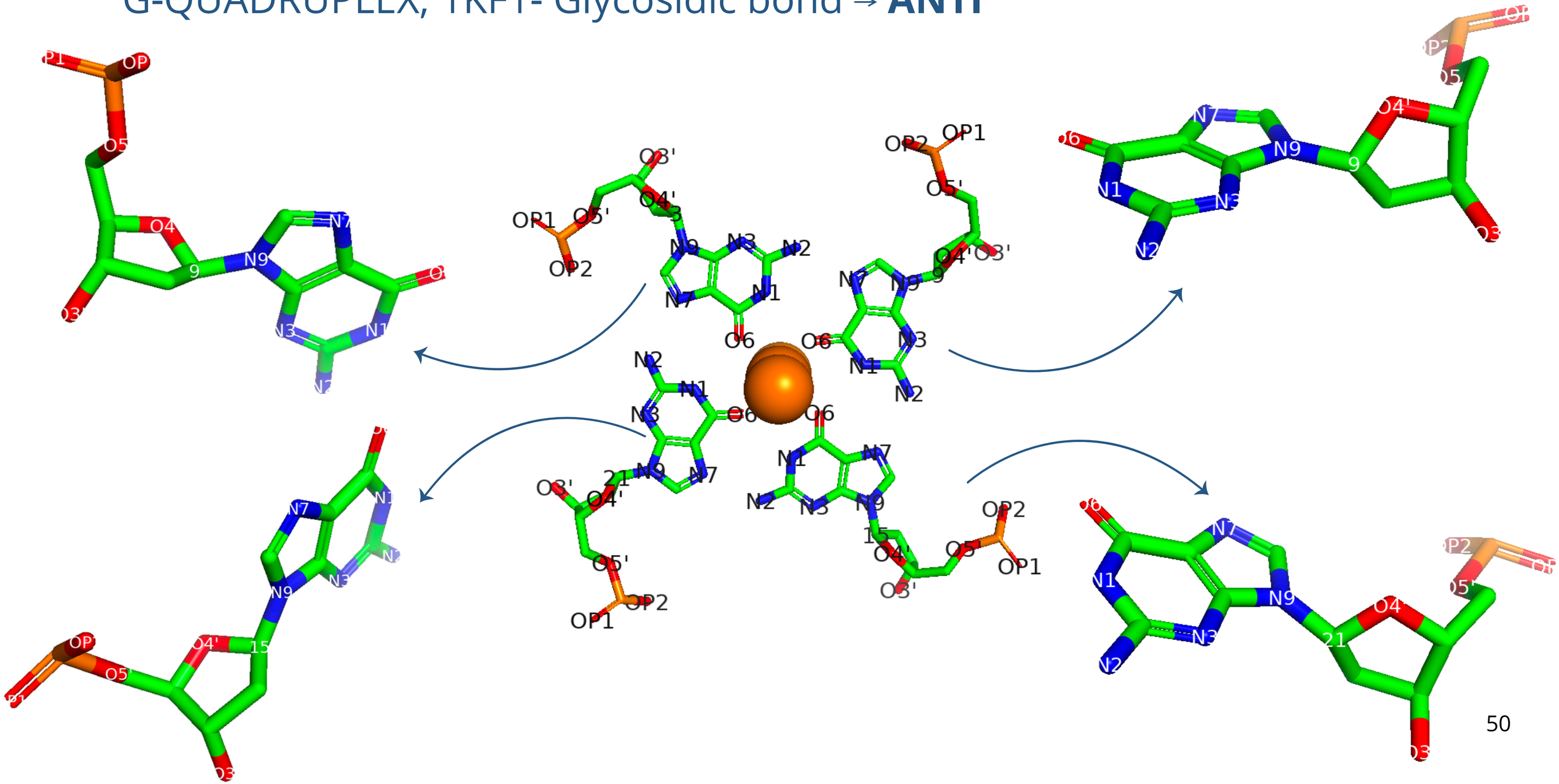


BASIC UNIT: G-TETRAD

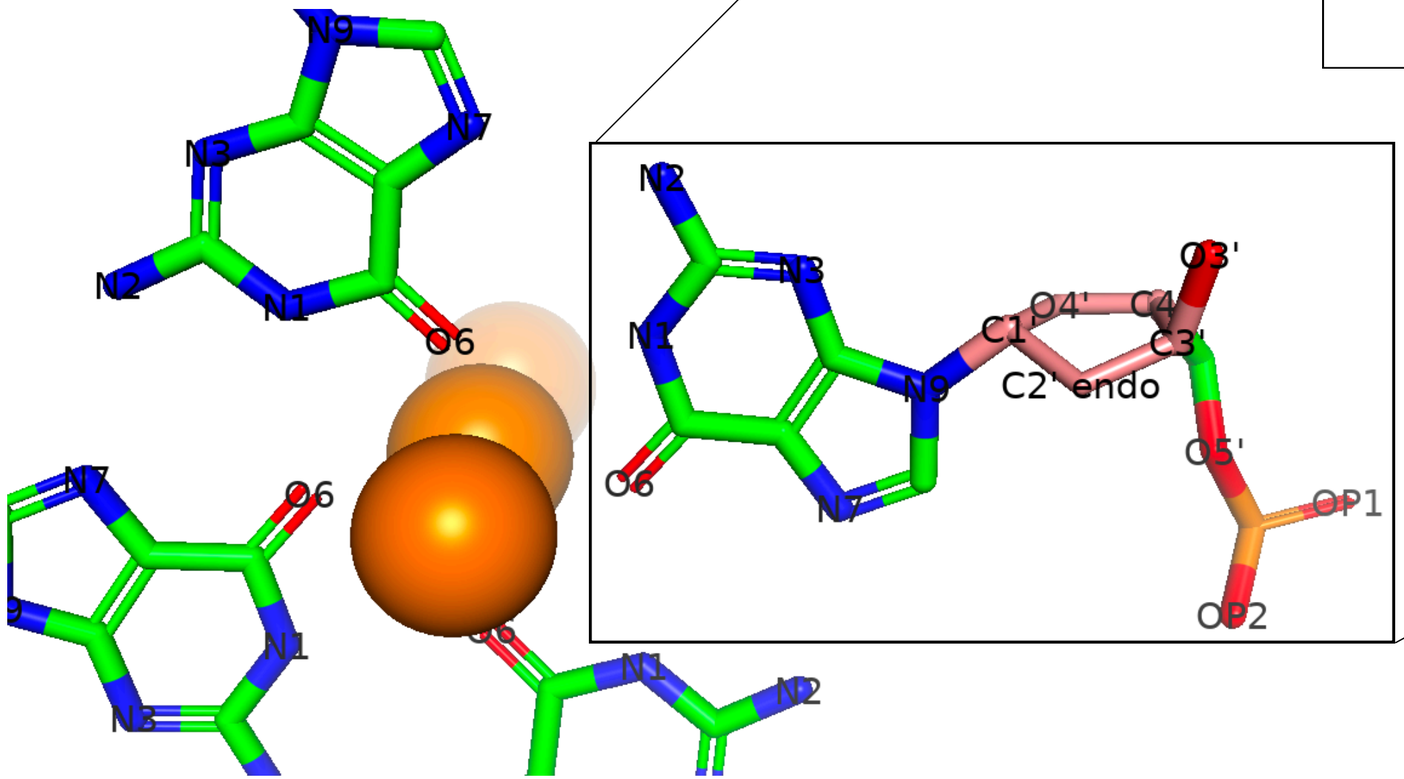


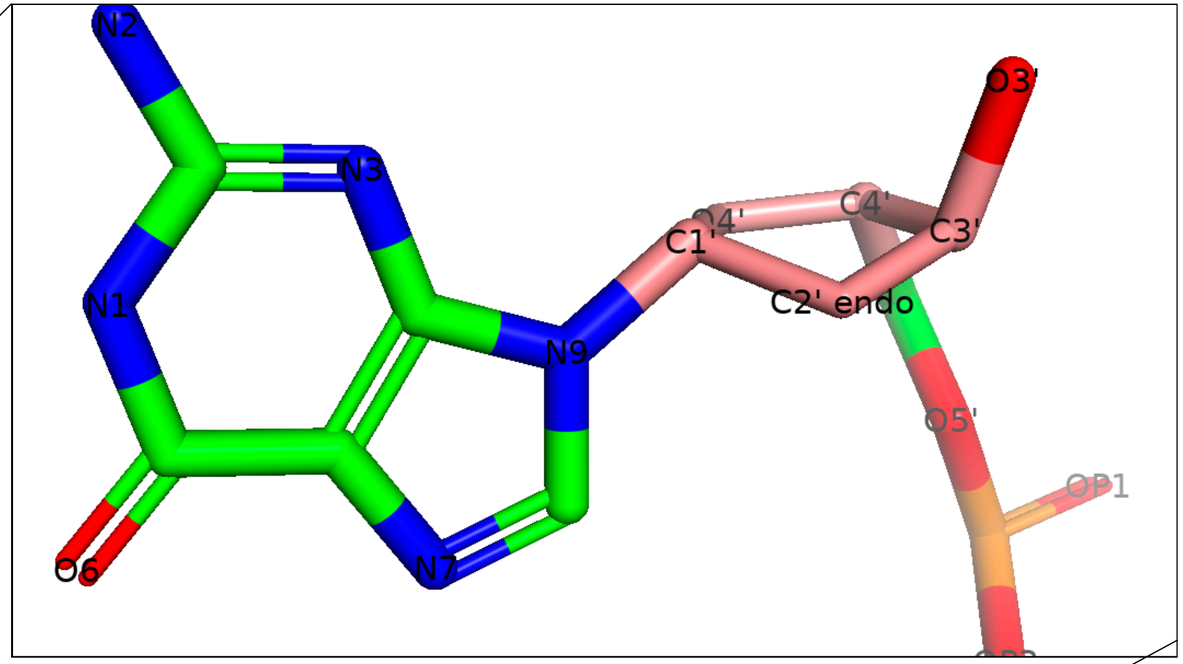


G-QUADRUPLEX; 1KF1- Glycosidic bond → **ANTI**



Sugar Pucker - C2' endo

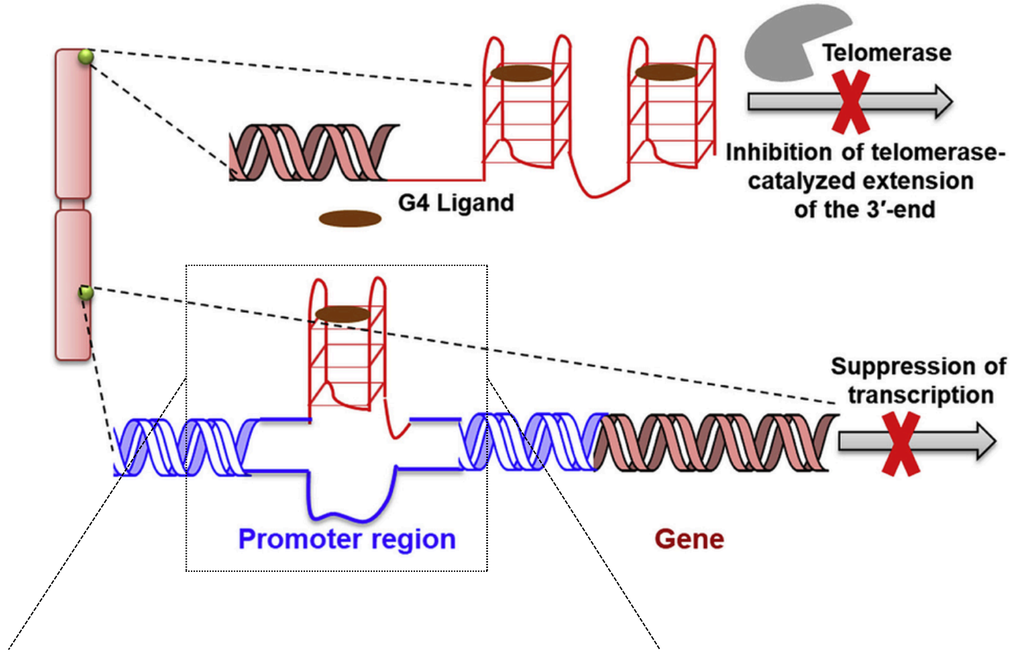




THERAPEUTICS

Normal cells → INACTIVE telomerase

Cancer cells → REACTIVE telomerase

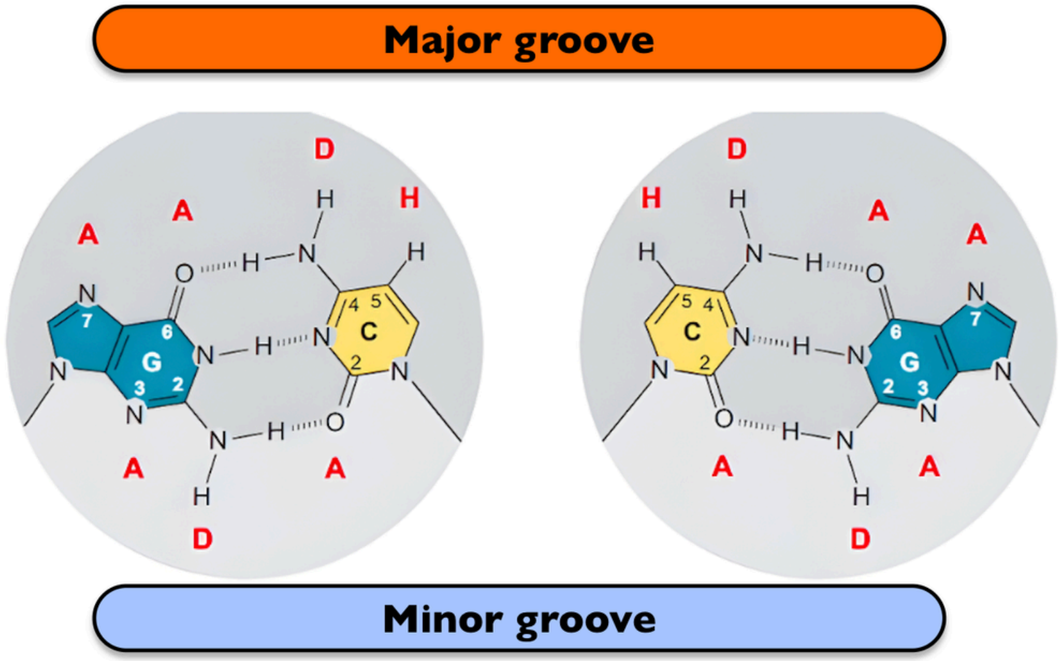


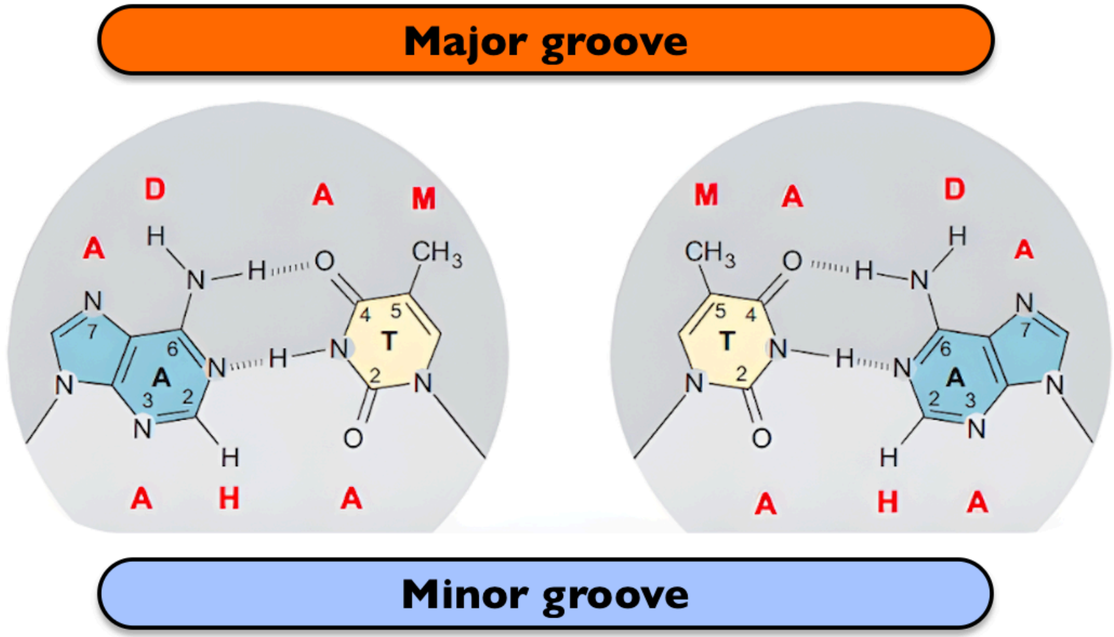
TARGET: **oncogenic promoters**

- ~40% of human gene promoters contain potential G4 sequences.
- In cancer-related genes, the prevalence is >60%.

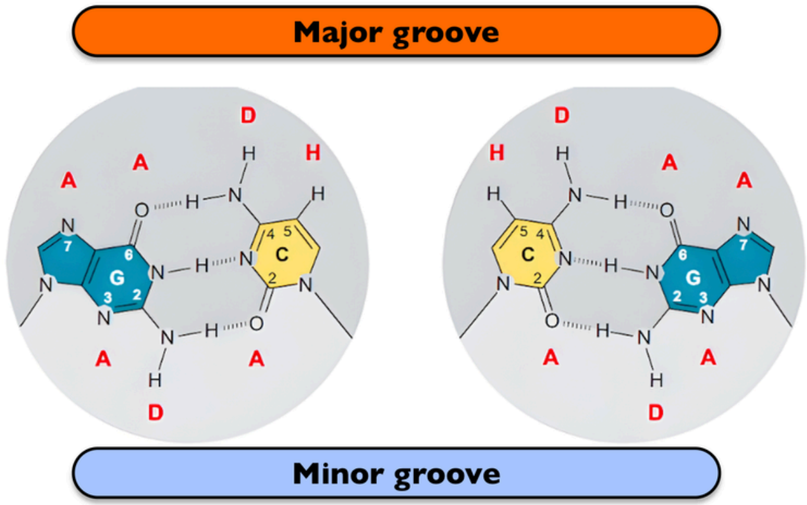
Mangesh H. Kaulage, Basudeb Maji, Sanjeev Pasadi, Asfa Ali, Santanu Bhattacharya, K. Muniyappa, Targeting G-quadruplex DNA structures in the telomere and oncogene promoter regions by benzimidazole–carbazole ligands, European Journal of Medicinal Chemistry,2018

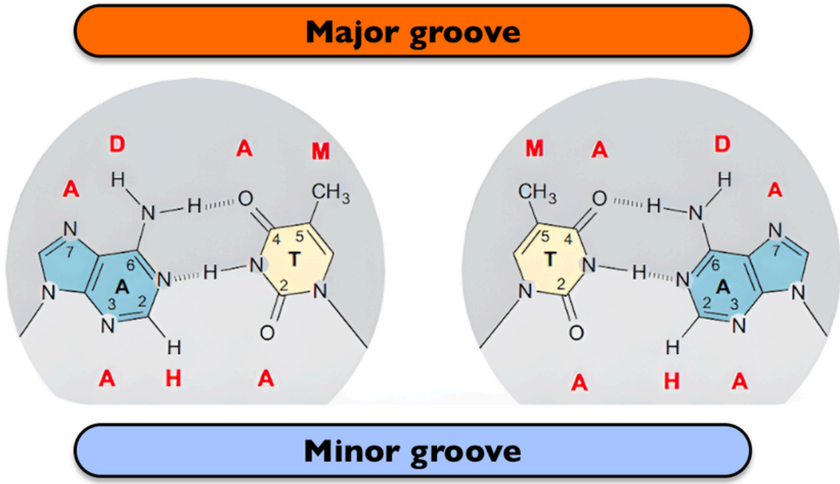
DNA-PROTEIN RECOGNITION: SPECIFIC VS. NON-SPECIFIC RECOGNITION





DNA-PROTEIN RECOGNITION: SPECIFIC VS. NON-SPECIFIC RECOGNITION



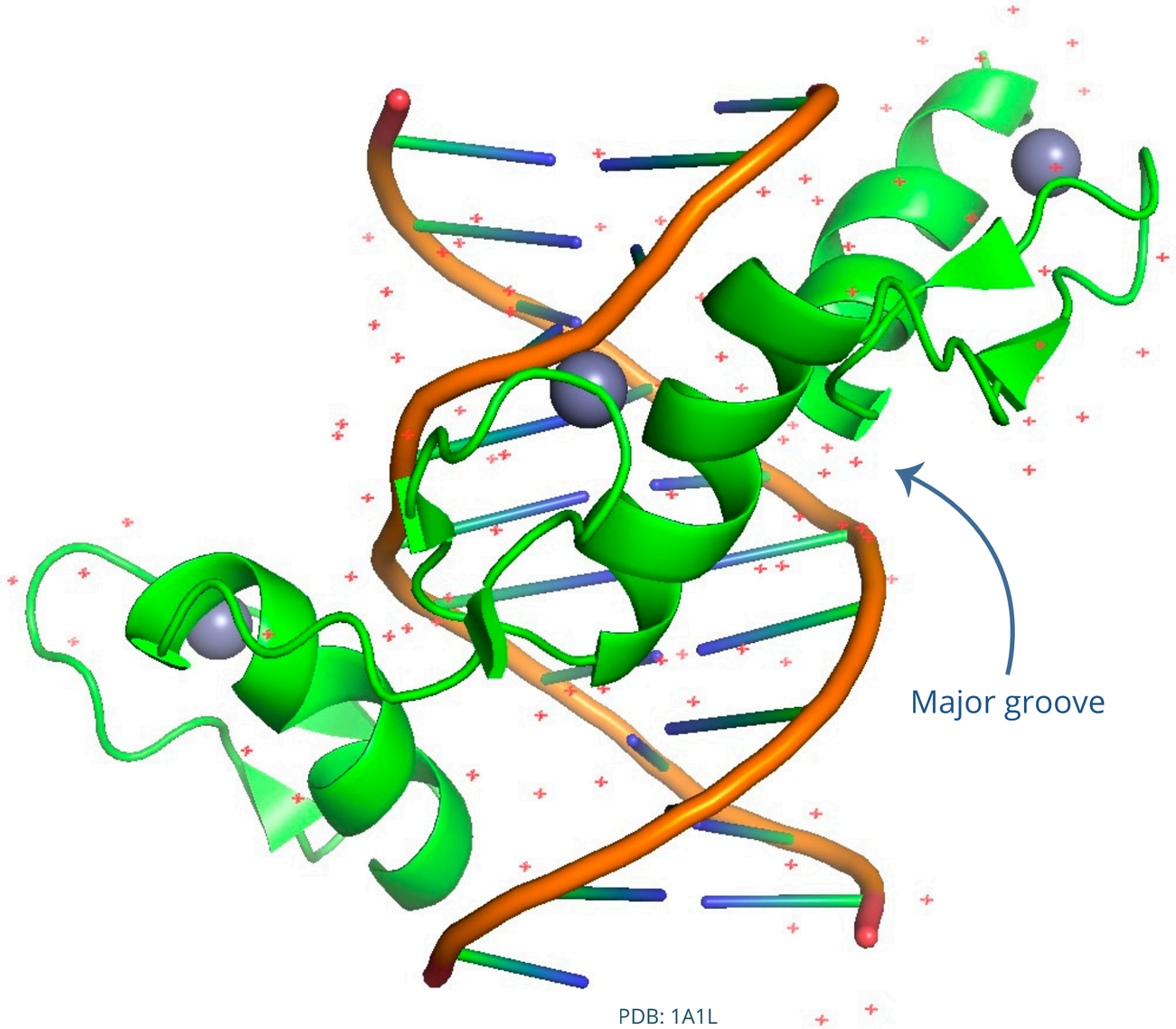


Aminoacids that tend to interact with DNA

- Asparagina (Asn)
- Arginine (Arg)
- Alanine (Ala)
- Serine (Ser)

DNA-PROTEIN RECOGNITION:

INTERACTION EXAMPLES: ZINC FINGER-DNA COMPLEX



PDB: 1A1L

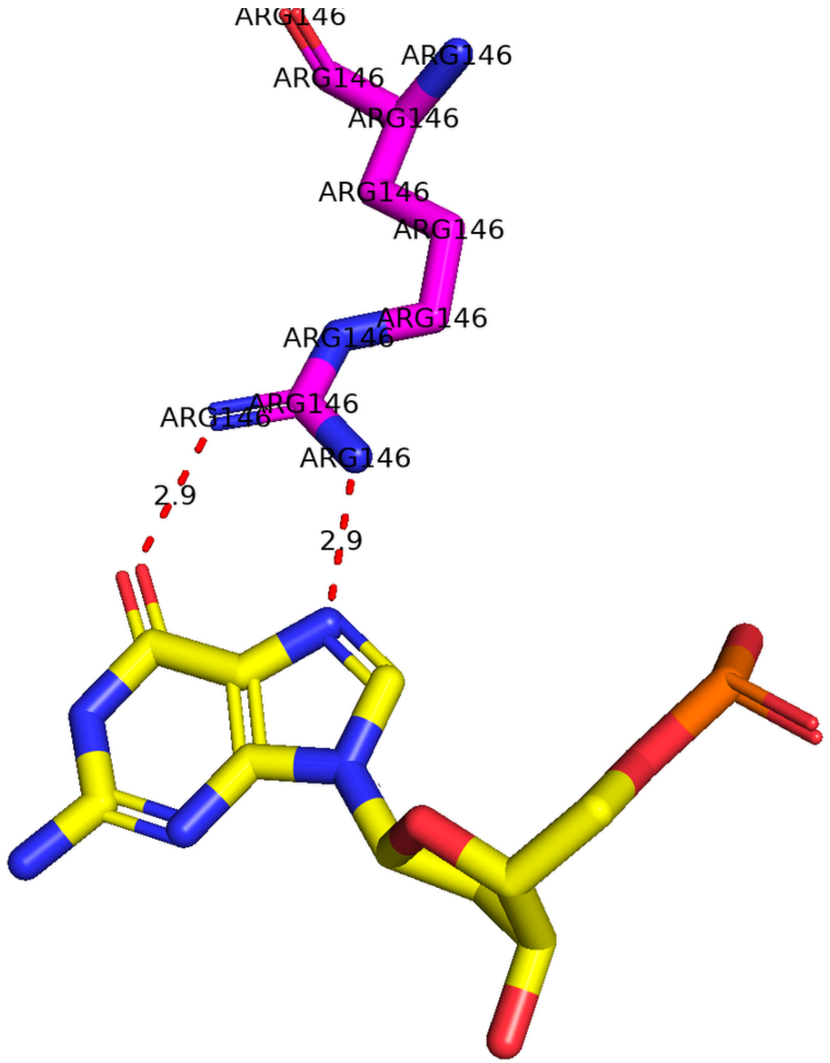
DNA-PROTEIN RECOGNITION:

INTERACTION EXAMPLES: ZINC FINGER-DNA COMPLEX

Hydrogen bonds XML

##	Structure 1		Dist. [Å]	Structure 2	
1	B:DG	2[N7]	2.73	A:ARG 180[NH2]
2	B:DG	2[O6]	2.88	A:ARG 180[NH1]
3	B:DG	4[OP1]	2.86	A:HIS 153[ND1]
4	B:DG	4[O3']	3.37	A:ARG 142[NH2]
5	B:DG	4[N7]	3.01	A:ARG 174[NH1]
6	B:DG	4[O6]	2.84	A:ARG 174[NH2]
7	B:DG	6[OP2]	2.92	A:SER 145[OG]
8	B:DG	6[N7]	2.64	A:HIS 149[NE2]
9	B:DG	7[OP2]	2.90	A:HIS 125[ND1]
10	B:DG	7[N7]	2.88	A:ARG 146[NH1]
11	B:DG	7[O6]	2.86	A:ARG 146[NH2]
12	B:DG	8[OP1]	3.18	A:ARG 103[NH2]
13	B:DG	8[OP1]	3.02	A:ARG 103[NH1]
14	B:DG	8[N7]	2.77	A:ARG 124[NH2]
15	B:DG	8[O6]	2.99	A:ARG 124[NH1]
16	B:DC	9[OP2]	2.91	A:ARG 118[NH1]
17	B:DC	9[OP2]	2.97	A:ARG 118[NH2]

EMBL-EBI. (s/f). PDBe < PISA



PDB: 1A1L

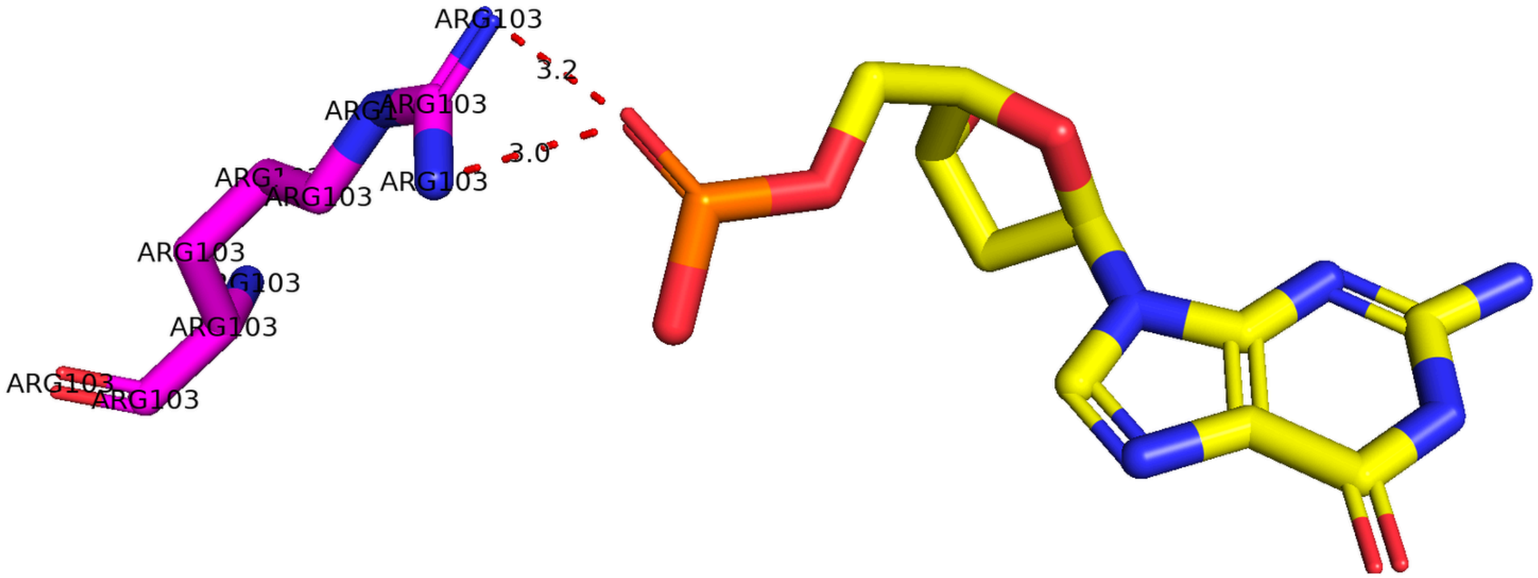
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INTERACTION EXAMPLES: ZINC FINGER-DNA COMPLEX

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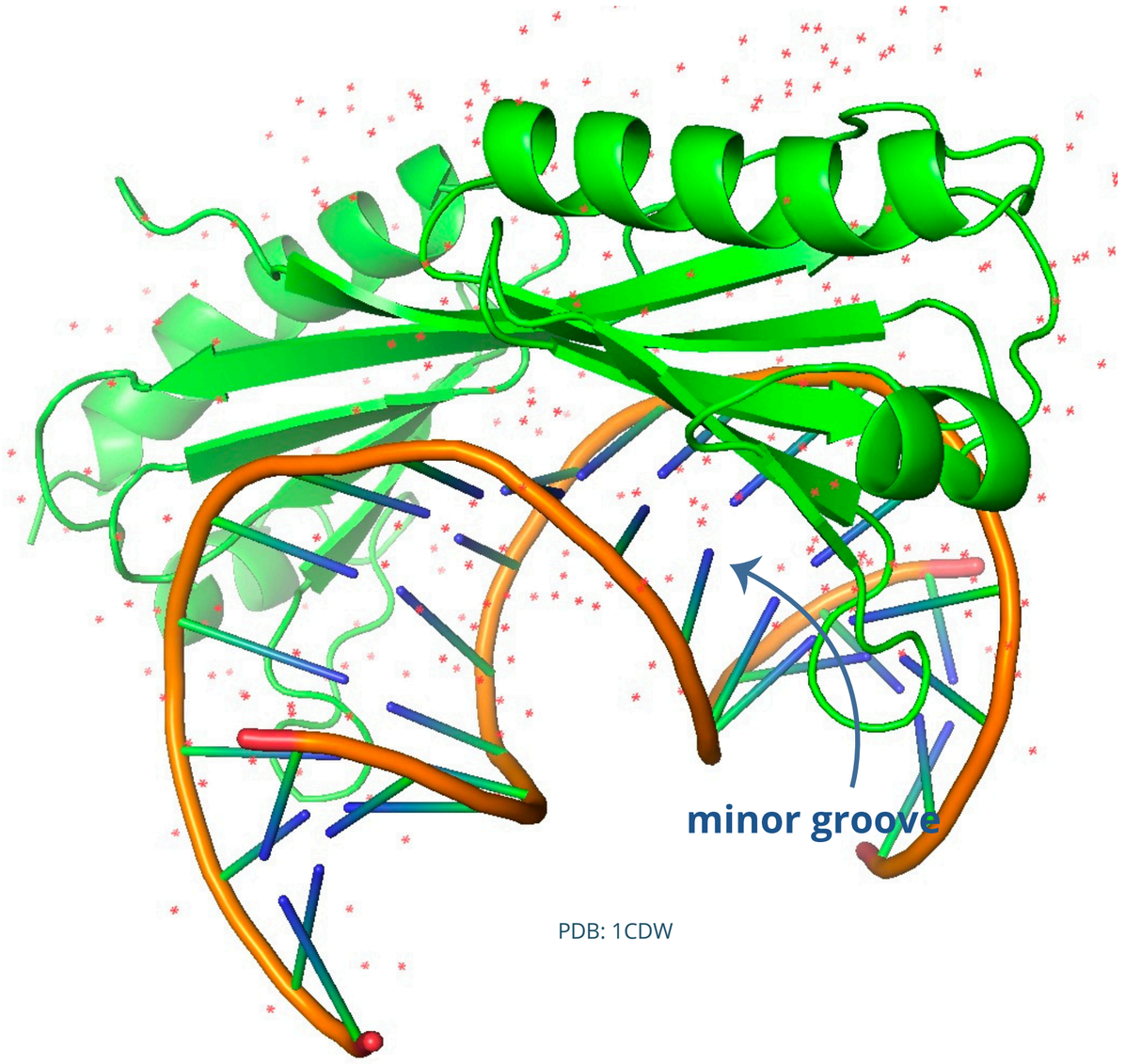
EMBL-EBI. (s/f). PDBe < PISA



PDB: 1A1L

DNA-PROTEIN RECOGNITION:

INTERACTION EXAMPLES: HUMAN TBP CORE DOMAIN COMPLEXED WITH DNA



PDB: 1CDW

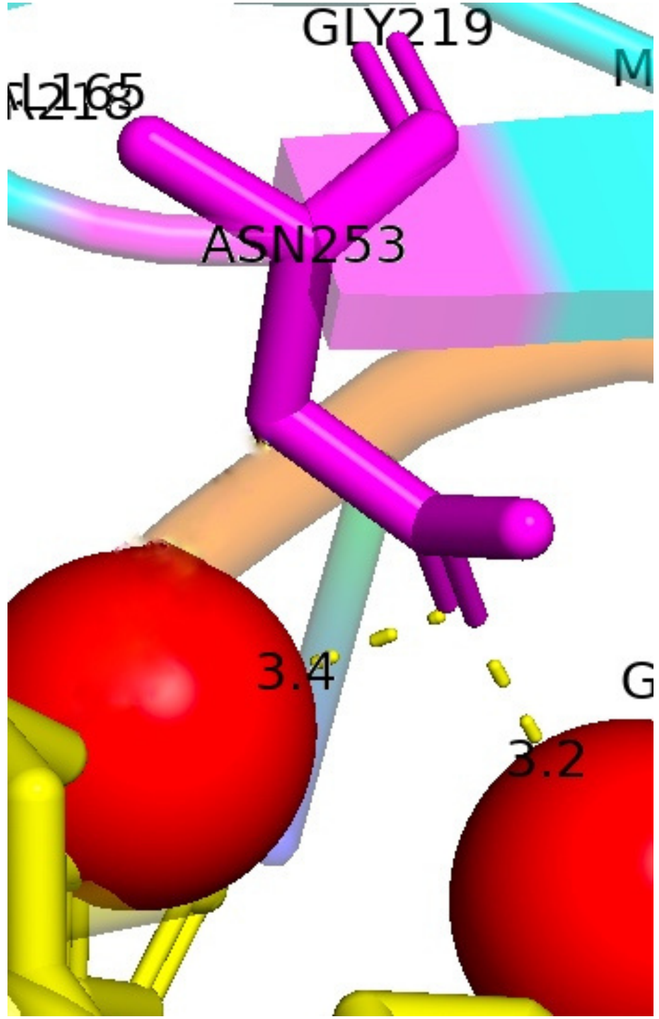
DNA-PROTEIN RECOGNITION:

INTERACTION EXAMPLES: HUMAN TBP CORE DOMAIN COMPLEXED WITH DNA

Hydrogen bonds XML

##	Structure 1	Dist. [Å]	Structure 2
1	B:DA 8[N3]	3.19	A:ASN 253[OD1]
2	B:DA 8[N3]	2.94	A:THR 309[OG1]
3	B:DA 9[N3]	3.37	A:ASN 253[OD1]
4	B:DG 12[OP1]	2.69	A:SER 212[OG]
5	B:DA 8[OP1]	2.51	A:ARG 290[NH1]
6	B:DT 7[OP1]	2.81	A:ARG 290[NH2]

EMBL-EBI. (s/f). PDBe < PISA



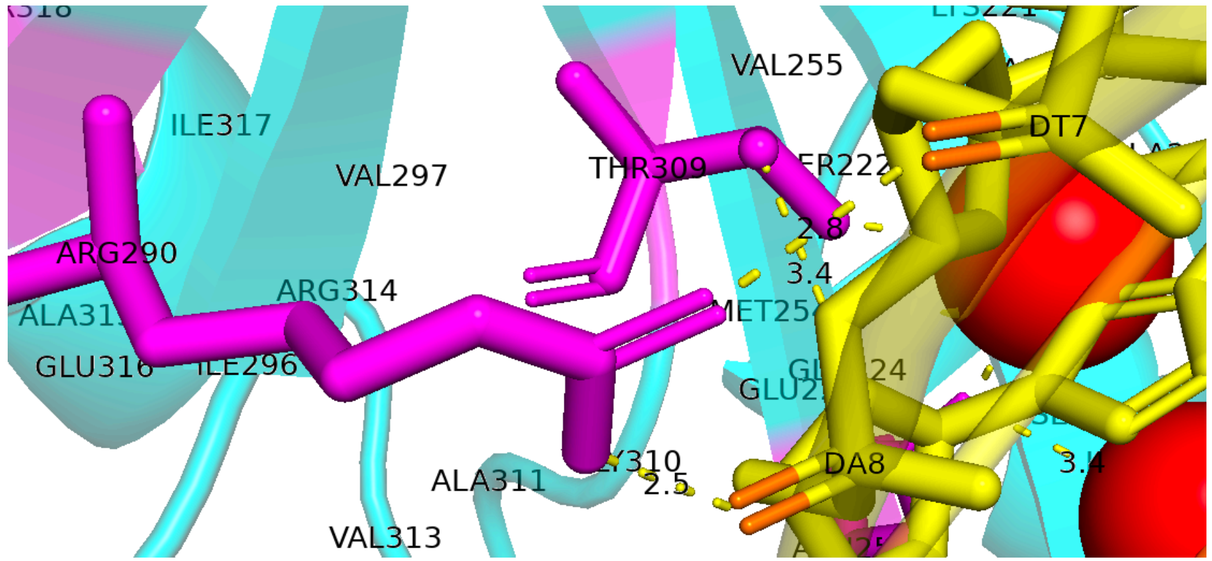
DNA-PROTEIN RECOGNITION:

INTERACTION EXAMPLES: HUMAN TBP CORE DOMAIN COMPLEXED WITH DNA

Hydrogen bonds XML

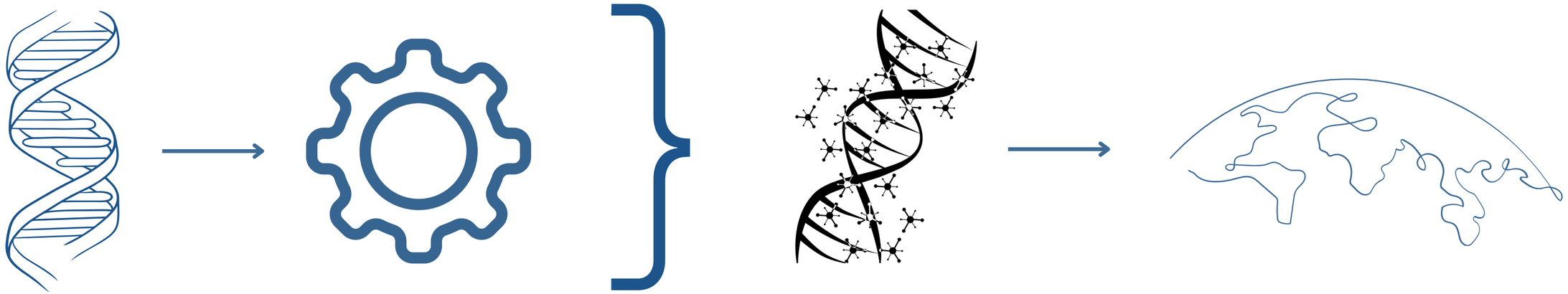
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EMBL-EBI. (s/f). PDBe < PISA

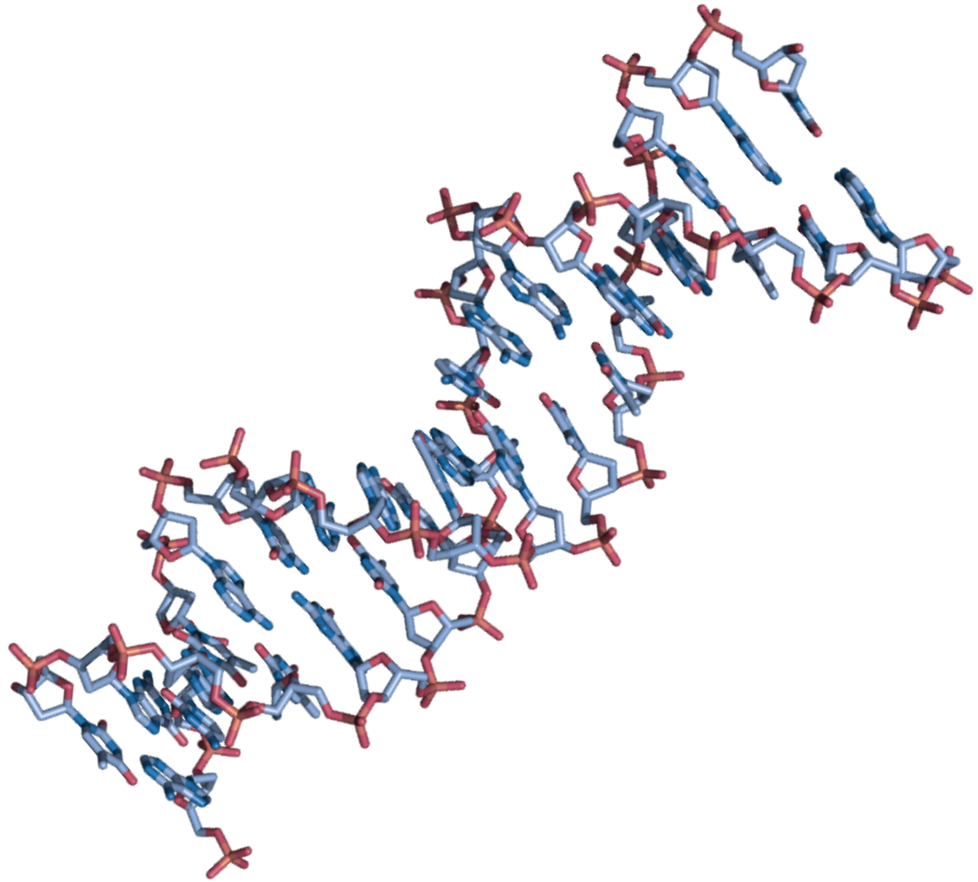


Take Home Message

"Structure determines function, but dynamics determines life"



Thank you for your attention



Júlia Masip, Mar Oropesa, Sara Rica & John Torres

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PEM Questions

1. Regarding the N-Glycosidic bond, which of the following statements are correct?

1. It links the C1' of the sugar to the N1 of pyrimidines.
2. It links the C1' of the sugar to the N9 of purines.
3. This bond is rigid and does not allow any rotation.

A) Only 1 is correct.
B) Only 2 is correct.
C) 1 and 2 are correct.
D) 1, 2, and 3 are correct.
E) None are correct.

2. Which of these descriptions correctly matches the Sugar Pucker of B-DNA and A-DNA?

a) B-DNA uses C3'-endo; A-DNA uses C2'-endo.
b**) B-DNA uses C2'-endo; A-DNA uses C3'-endo.**
c) Both B-DNA and A-DNA use C2'-endo.
d) B-DNA uses a flat ring; A-DNA uses a pucker.
e) B-DNA uses C1'-exo; A-DNA uses C4'-endo.

3.What is the telomeric "3' overhang"?

a) An RNA sequence that protects the end of the chromosome.
b) A single-stranded DNA sequence rich in Guanines that protrudes at the end of the telomere.
c) A double-helix region rich in AT pairs.
d) The site where DNA polymerase binds to initiate replication.
e) A protein that wraps around the centromere.

4.What does the acronym TFO stand for in the context of Triple Helix DNA?

a) Transcription Factor Organizer.
b) Triplex-Forming Oligonucleotide.
c) Thymine-Free Oligomer.
d) Triple-Strand Functional Organelle.
e) Targeted Fiber Optic.

5. Which statement accurately describes the Pyrimidine Motif in Triplex DNA?

The third strand is rich in T and C.
The third strand binds in a parallel orientation.
It requires the protonation of Cytosine C^+ for stability.
a) Only 1 is correct.
b) 1 and 2 are correct.
c) 2 and 3 are correct.
d) 1, 2, and 3 are correct.
e) None are correct.

PEM Questions

6.Under conditions of low humidity or dehydration, DNA tends to adopt the following form:

a) B-DNA
b) Z-DNA
c) A-DNA
d) Triple helix
e) G-quadruplex

7.In the 1KF1 (unimolecular parallel) structure, the TTA loops are called:

A) Lateral loops.
B) Diagonal loops.
C) Helical loops.
D) Propeller loops.
E) Hairpin loops

8. What is the typical Sugar Pucker configuration in a Triplex structure?

a) All three strands are C2'-endo.
b) All three strands are C3'-endo.
c) The Duplex stays in C2'-endo, while the TFO (third strand adopts C3'.endo.
d) The Duplex is C3'-endo, while the TFO is C2'-endo.
e) The sugars become flat to accommodate the third strand.

9.Which of the following statements correctly describes recognition in the major groove of DNA?

a) It does not allow discrimination between A–T and T–A due to symmetry in chemical patterns.
b) It is mainly based on the overall shape of DNA rather than specific functional groups.
c) It provides a unique chemical pattern for each base pair, allowing discrimination of both type and orientation.
d) It is primarily driven by the entropic release of ordered water molecules.
e) It occurs only when the double helix is partially unwound.

10.Which of the following best describes protein recognition in the minor groove of DNA?

a) It allows clear discrimination of base pair orientation (A–T vs T–A).
b) It mainly recognizes the shape and structural properties of DNA rather than specific base identities.
c) It requires opening the double helix to read the bases.
d) It depends exclusively on bidentate hydrogen bonds with guanine.
e) It contains more chemical information than the major groove.