Lecture 3:
Introduction to Nucleic Acids

Question 1:

Which of the following statements are TRUE, and which are FALSE? Why?

a) Nucleobases are termed bases because they can accept protons (or donate electrons) due to the presence of amino and imino groups, giving them basic character.

b) Complementary single-stranded DNA molecules spontaneously assemble into a double-helix in water. This is driven exclusively by the hydrogen bonds formed between G-C and A-T base pairs.

c) A double-stranded DNA molecule in distilled water may be stabilized by addition of monovalent (Na^+; K^+) or divalent (Ca^{2+}; Mg^{2+}) cations. This results in shielding of the negative charge of the sugar-phosphate backbone and lowers the energy of the system.

d) The 2' hydroxyl group of the ribose is missing in DNA, but present in RNA. This is the only chemical difference between DNA and RNA molecules.

e) The nucleobase cytosine may spontaneously undergo deamination, which produces thymine. This results in a mismatched base pair in double-stranded DNA and can lead to mutations.

f) The presence of methyl goups on thymine and cytosine (upon methylation) changes the Watson–Crick hydrogen-bonding pattern.

g) GC-rich DNA regions are more stable compared to AT-rich regions due to greater hydrogen bonding capacity.

Question 2:

a) Draw the complete chemical structure of deoxythymidine triphosphate (dTTP).

b) Indicate within your structure which part corresponds to:

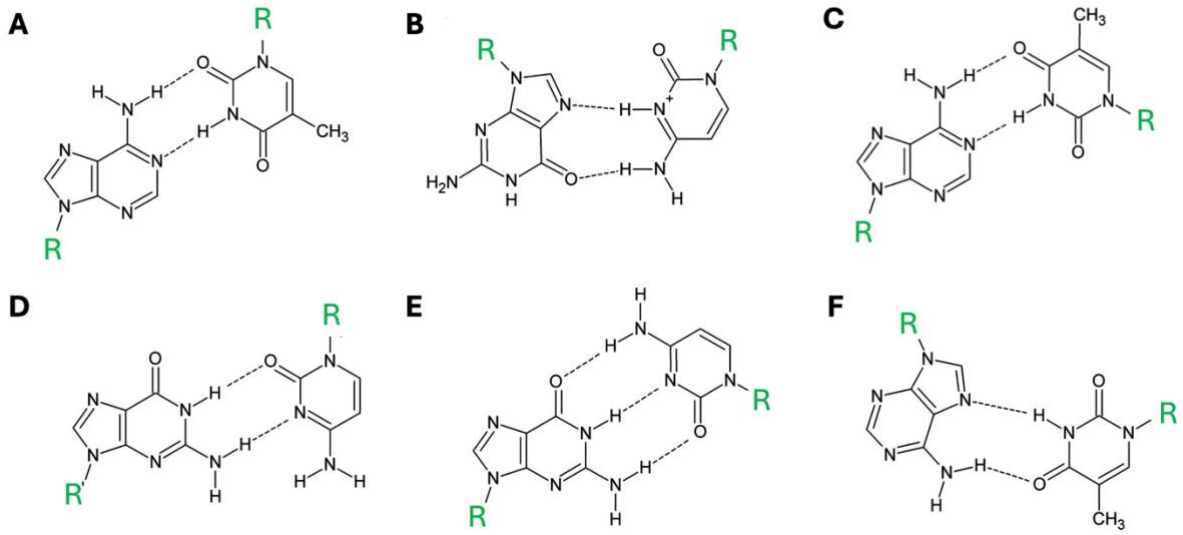
- the nucleoside
- the nucleobase
- the ribose

c) During DNA replication, a nucleoside triphosphate is added to the 3′ end of the growing DNA strand via a phosphodiester bond. From which chemical bonds in dTTP does this energy derive?

Question 3:

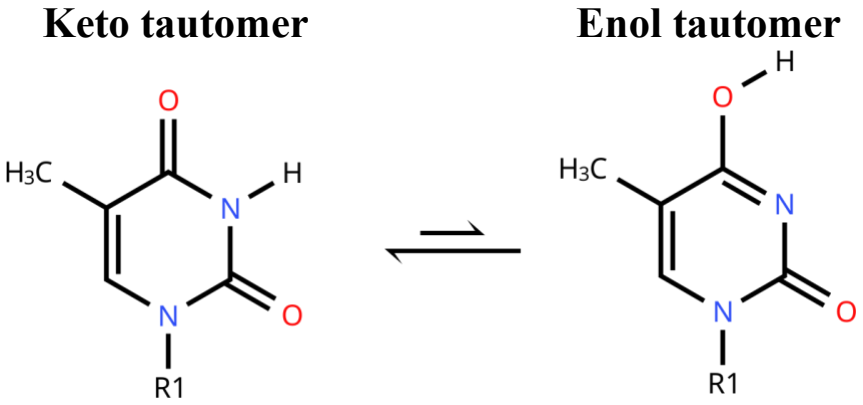
Non-canonical base-pairing occurs when two nucleotides form hydrogen bonds in a manner distinct from the patterns defined by Watson and Crick. They are particularly common in RNA due to additional flexibility and lack of complementary strands (in most cases). Some examples include Hoogsteen and Wobble pairing.

a) In the 6 examples listed below, identify the bases comprising each pair and assign whether they assemble in a canonical (i.e., Watson-Crick) or non-canonical manner?
b) What would be the effect of non-canocical base-pairing on the phosphodiester backbone of the poly-nucleotide chain? How would it impact helical assembly? (hint: look at the relative location of the ribose "R" group)?
c) In terms of hydrogen bond count, how do the interactions compare between non-canonical base pairs and the standard A:T/G:C pairs? Which hydrogen bonding network is stronger and why?



Question 4:

The nucleobase thymine can undergo keto–enol tautomerism. In the diagram provided, R1 represents the sugar-phosphate backbone.



Drawn using Ketcher v2.25.0 (moldraw.com)

a) For both the keto and the enol tautomer: Identify which functional groups can act as hydrogen bond donors and which can act as hydrogen bond acceptors in base pairing.

b) Different tautomeric forms of thymine may contribute to mutations during DNA replication. Explain how keto–enol tautomerism could lead to the misincorporation of an incorrect base (i.e., mutation).

c) Assumung that enol form of thymine would pair differently from keto form, what would be the most suitable partner (=complementary base). Draw it and indicate hydrogen bonds.

Question 5:

A bacterial DNA polymerase replicates DNA at a rate of approximately 1000 base pairs per second. The polymerase holoenzyme is about 110 Å (angstroms) in length.

a) How many times its own length does the polymerase move forward along the axis of the DNA double helix in 5 seconds? We can assume standard geometry of the DNA helix in B form and that the DNA remains stationary.

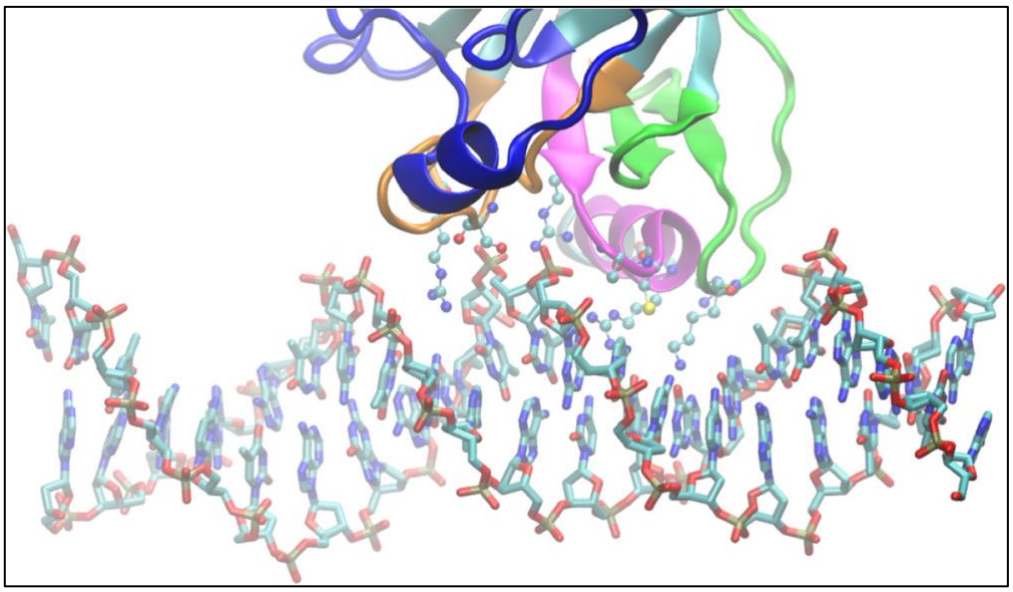
b) If the DNA molecule were held fixed and the polymerase had to “corkscrew” around the helix as it moved forward, how many complete rotations would it make during this time?

c) Consider 3 common forms of DNA: A, B and Z and the differences in helical parameters they feature. If polymerase adds nucleotides at the same rate (1000 bp/s) in each form, in which DNA form would the enzyme move forward the fastest *along the helix axis*? Briefly explain your reasoning.

Question 6:

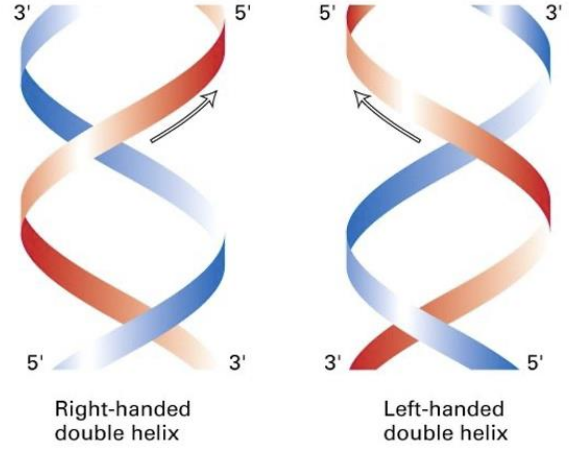
In the image below you can see a representation of a complex between DNA (stick representation) and the protein p53 (cartoon representation). This protein is a well-known tumor suppressor that regulates the cell cycle and helps prevent cancer by binding to DNA and activating genes that repair damaged DNA. Many DNA-targeting proteins such as p53 contain cationic domains (=enriched in positively charge amino acids).

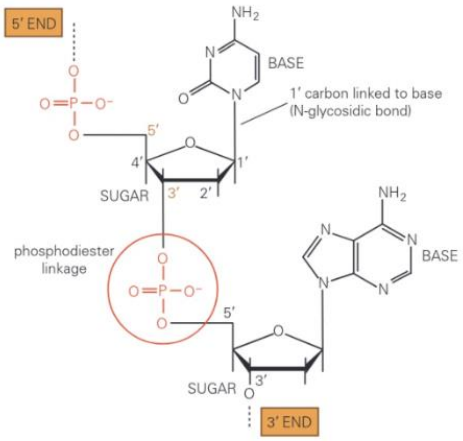
a. Explain why these cationic domains are important for DNA-binding proteins.
b. Would the interactions via cationic domains be sufficient to specifically recognize different nucleotide sequences within the DNA?
c. DNA-binding proteins can be covalently modified with a phosphate group within the DNA-targeting domains. How would this impact DNA binding? What do you think is the underlying purpose of such modification inside the cell?



Question 7:

DNA replication always take place in the 5′ → 3′ direction, assembling a right-handed helix in the case B-DNA form.





a) What aspects of nucleotide chemistry cause this to be the preferred direction?

b) What would you need to change on the nucleotide to invert the direction to 3′ → 5′?

c) What part of the nucleotide would you modify to change from right- to left-handed helix? Please explain.