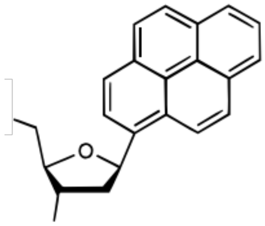
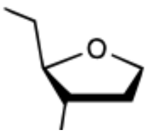
Discussion Worksheet – Winter 2024 - Week 3
Group 1:

Scientists have studied the effect of incorporating non-natural nucleosides on the Tm of DNA molecules. In particular they have used nucleotides where the base has been changed to a pyrene group (P), or nucleotides with no base (abasic = ϕ). The structure of these nucleosides is shown on the right.


P


ϕ

The Tm of 12 base-pair long DNA duplexes containing either a normal A-T base pair, or the chemically modified nucleotides in place of the A-T base pair are indicated below:
A-T = 43.2°C A-P=38.7°C A-ϕ = 22.2°C P-ϕ = 41°C

A- Compare the stabilities of the DNAs containing either A-T or A-P and propose an explanation for the impact of the Pyrene on the stability of double-stranded DNA

- The A-P base pair slightly destabilizes the DNA compared to an A-T base pair as shown by a small reduction in the Tm.
- This effect might be due to either loss of H-bonding since P cannot donate/accept H-bonds, or to a steric hindrance effect, as an adenine opposite to a P would be significantly larger than an A_T base pair (only one reason required for full credit).

B- Compare the stabilities of the DNAs containing either A-T or A-ϕ and propose an explanation for the impact of the presence of the abasic nucleoside ϕ on the stability of double-stranded DNA

- The A-ϕ base pair strongly destabilizes the DNA compared to an A-T base pair as shown by a strong reduction in the Tm.

- This effect might be due to the loss of stacking interactions to the base pair above and below the residue – since there is no base present, bases located above and below on that strand can no longer stack which leads to a strong destabilization.

C- Compare the stabilities of the DNA containing either A-T or P-ϕ and propose an explanation for the impact of the combination of the pyrene and of the abasic nucleoside ϕ on the stability of double-stranded DNA.

The P-ϕ base pair has a very modest impact on the stability of the DNA compared to an A-T base pair as shown by a very slight reduction in the Tm (2°C).

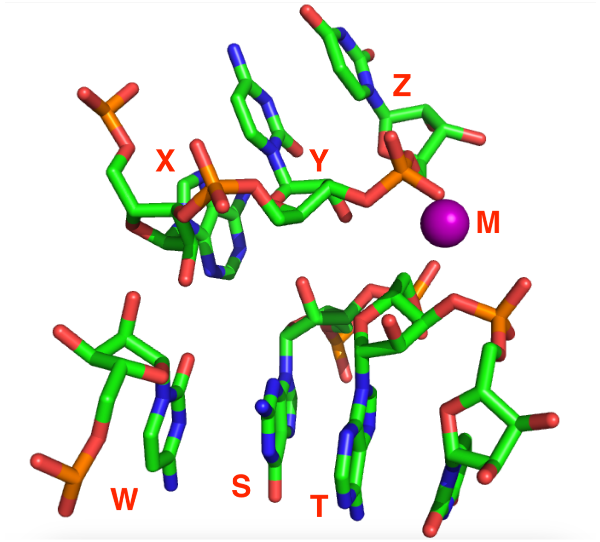
- Having a P opposite to ϕ results in having a single large aromatic group intercalated in one layer of the DNA instead of a normal base pair. This large aromatic group satisfies stacking with the bases above and below the modified nucleotide. Thus, despite the lack of H-bonding in the P-residues, the DNA stability is virtually similar, showing the importance of stacking interactions on the stability of the DNA.

D – How do these results provide experimental confirmation of the contribution of two specific type of interactions to the stability of double-stranded DNA?

The interactions that stabilize double-stranded DNA are stacking (major) and hydrogen bonding (minor). Here, we see a clear illustration that stacking is important as the A-ϕ base pair is very destabilized compared to A-T. We see that hydrogen bonding is a minor interaction to stability, illustrated by the only slight decrease in Tm with P-ϕ compared to A-T.

Group 2:

The structure of a nucleic acid is shown on the side. M is a positively charged ion.



A – Describe the main potential interaction between nucleotides S and T

The bases of S and T stack onto each other (or form a hydrophobic/van der Waals interaction; either answer gets full credit)

B – What is the primary interaction between nucleotides S and Y? There is a similar interaction between nucleotides W and X.

2’OH - 2’-OH hydrogen bonding (cannot tell the direction so either interaction is possible and given full credit; only one is shown on the structure)

C – From the interactions described in part B, what structural motif can you identify in this structure? Explain your answer in one sentence.

A ribose zipper is being made through interaction between nucleotides S and Y described above and X and W.

D – What is the role of M in this structure?

M is localized close to the interface between two phosphate groups; because it is positively charged it will neutralize the electrosatic repulsion between the two strands to allow the two strands to interact with each other.

Q3.

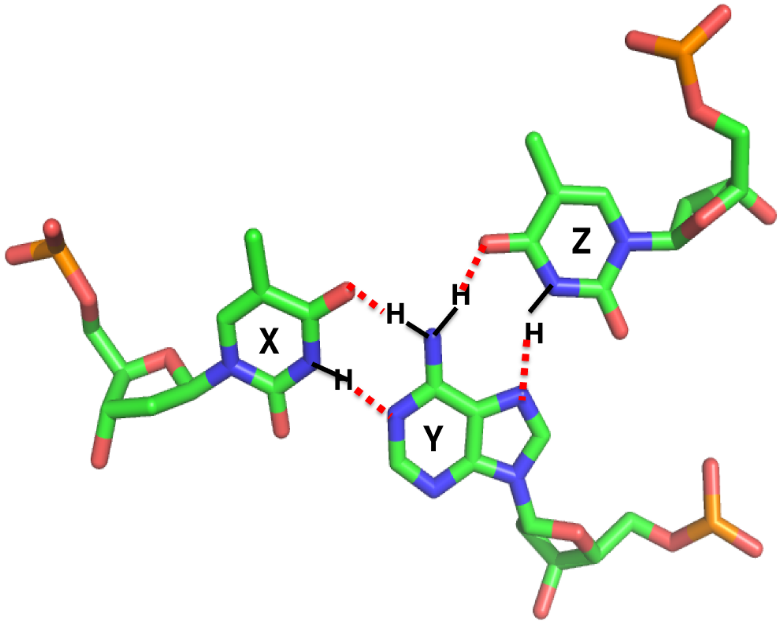
A – What type of structure is formed by this nucleic acid? Explain your answer.

Two strands form a double helical structure and a third strand follows the double helical path = this is a triple helix.

B – From the picture at the bottom, what interactions exist between nucleotides X, Y and Z. Specify the type of interactions and atoms involved.

X and Y = standard Watson-Crick interaction

Y and Z = N6 NH_2 donates a H bond to the carbonyl O4 of Z; N3 of Z donates a H bond to the N7 of Y.

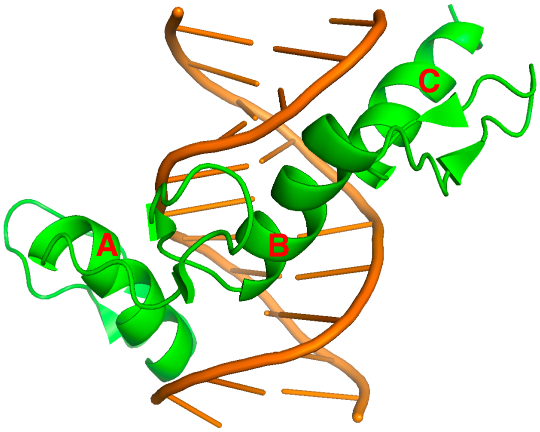


C – A similar structure has been studied in class. Explain below two major differences between the structure shown here and the structure studied in class.

The triple base pairs showdeoxynucleotides instead of ribonucleotides with a T-A-T interaction instead of a U-A-U => This is a DNA triple helix instead of an RNA triple helix.

The structure shown here, a strand of Ts interacts with a “standard” double helix with the Watson-Crick T-A base pair. This is the opposite of the RNA triple helix studied in class where a polyA strand interacts with an “internal loop” made of polyUs.

Group 4:
Scientists determined the structure of a sequence-specific DNA binding protein (shown in green) to better understand how it recognizes its target DNA (orange). The protein is comprised of three alpha-helical domains, which are labeled A-C on the upper image.

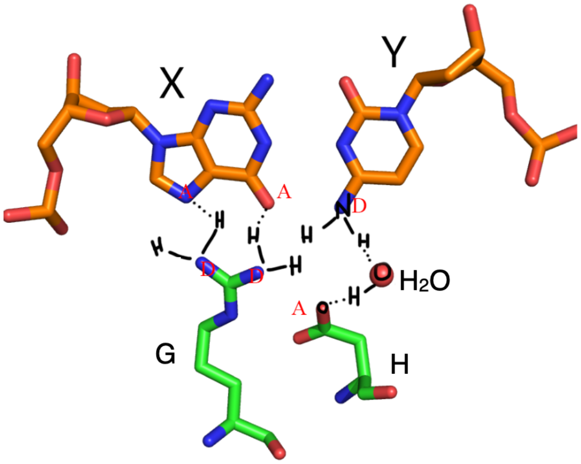


A. Which groove of the DNA is this protein predominantly engaging to identify its target sequence? List three structural features that led you to this conclusion.

This is B-DNA based on 1) smooth phosphate backbone, 2) very little tilt seen with bases, and 3) grooves are of similar depth. From this, we know the larger of the two grooves where A-C are interacting with DNA are through the major groove.
(Noting that we see the contacts below and they interact through the major groove is also fine)

B. An atomic view of domain B interacting with its target DNA sequence is shown in the lower image. How does residue G of the protein interact with base X of the DNA? Mark all hydrogen bond donors (as D) and acceptors (as A), draw any hydrogens, and explain in one sentence.

See image; amino groups in this arginine act as H-bond donors to N7 and carbonyl of base X.



C. In the lower image, how does residue H of the protein interact with base Y of the DNA? Mark all hydrogen bond donors/acceptors, draw any hydrogens, and explain your answer in one sentence (hint: think about the properties of water)

Here water acts as a bridge between residue H of the protein and base Y, with the amino group on base Y donating a hydrogen to the water molecule and residue H accepting a hydrogen from the same water.

D: Do the interactions shown in the lower image likely contribute to the affinity or specificity of the protein for its target DNA sequence? Explain your answer in one sentence.

This drives specificity – the protein is reading the DNA through the major groove in a manner that would read out the identity of the base pair. H-bonds have a relatively small contributions to overall stability of protein-DNA interactions