BMB 170 2016

Problem Set 3: Nucleic Acids (100 Points)

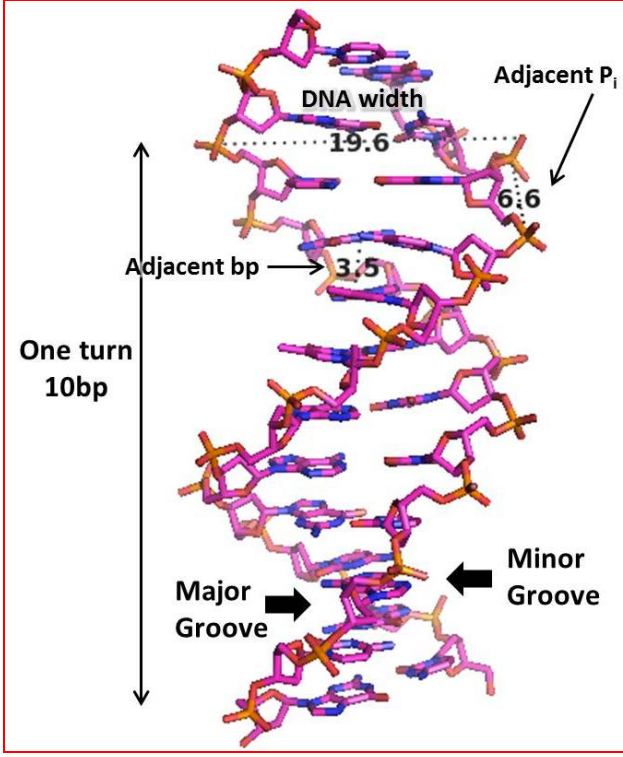
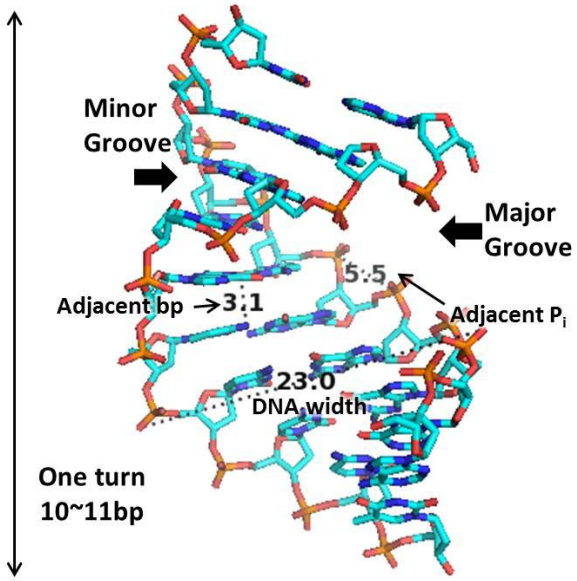
Due 11/07/2017 12:00pm

Please remember to use ray tracing (set ray_shadows, 0) to generate all PyMol figures.
Turn in as a HARD COPY or as a PDF FILE by 12:00PM 11/07/2017. Points will be deducted otherwise.
PS#3 office hours:
Monday 11/06, 4:00-6:00PM, SFL GSR 231
Send questions and PDF file to: icho@caltech.edu

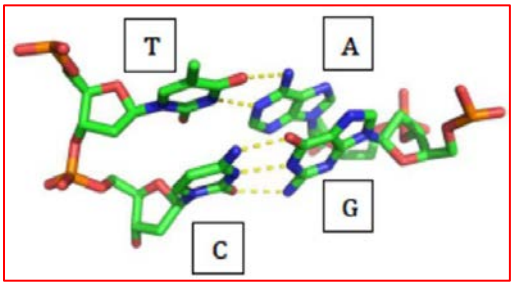
1. Basic Chemistry [27 points]

A. Basic structure and forms: Import two DNA structures, 1BNA and 440D, to pymol. Generate figures separately. For each figure, complete or answer the following exercises. To measure distances, you can use Wizard -> Measurement from PyMol menu. [total 10 points]

i. Label the major and minor grooves [2 points]
ii. Measure and label the distance between adjacent bases [2 points]
iii. Measure and label the distance between adjacent phosphates in the backbone [2 points]
iv. Measure and label the width of the DNA molecule [2 points]
v. Which structure corresponds to A-DNA and which to B-DNA? [2 points]

1BNA: B-DNA	440D: A-DNA
	

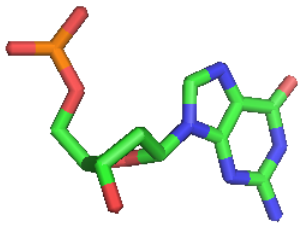
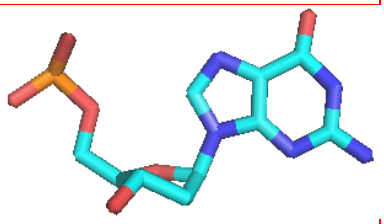
B. Base pairing (i): For structure 1BNA, hide everything. Show bases 4, 5, and their complementary bases. Label the identity of each base (A, T, G, C) and highlight the base pairing interaction. [2 points]



C. Base pairing (ii): Neither base pair 4 nor 5 are exactly perpendicular to the direction of helix. What kind of twist are they (in terms of DNA parameter)? What stabilizes the DNA structure other than the hydrogen bond between base pairs? [3 points]

Base pairs 4 and 5 correspond to propeller twist. Base stacking stabilizes the DNA structure.

D. Structural difference between A- and B-DNAs: Look at a figure of guanine with ribose and phosphate backbone, from both 1BNA and 440D. Look at the ribose ring of each bases. Describe the difference in ribose conformation in 1BNA and 440D. [4 points]

1BNA	440D
	
C2 is above the plane that O, C1, C3, C4 forms.	C2 is below the plane that O, C1, C3, C4 forms.

E. RNA form: Unlike DNA, RNA has a hydroxyl group attached to C2. How does this restrict the form that RNA can take, compared to DNA? Among A-form and B-form, what does RNA adopt? [4 points]

The 2-OH that RNA additionally has introduces a steric hindrance between it and 3-OH, restricting the form that RNA can take. As this steric hindrance makes B-form more unstable than A-form, RNA takes A-form rather than B-form.

F. Melting temperature: Consider two short, double stranded DNA sequences. Which one is more likely to have a higher melting temperature? Why? [4 points]

i. ATG CGT CCA GCT TGT CGG TAA CCC GTC TCC TGC
TAC GCA GGT CGA ACA GCC ATT GGG CAG AGG ACG

ii. TGT TTC GTG TCC CGC CGG TAA GAA TGT AAT AAT
ACA AAG CAC AGG GCG GCC ATT CTT ACA TTA TTA

Double stranded sequence (i) will have higher melting temperature, because Tm depends on G+C content, and (i) has higher G+C content of 20/33 compared to 14/33 of (ii).

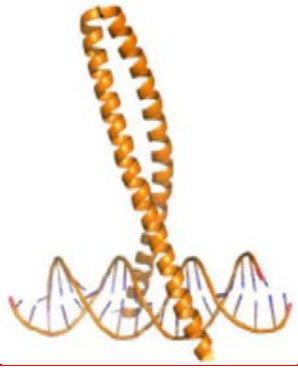
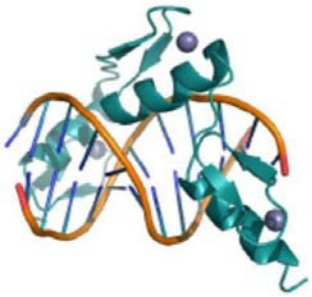
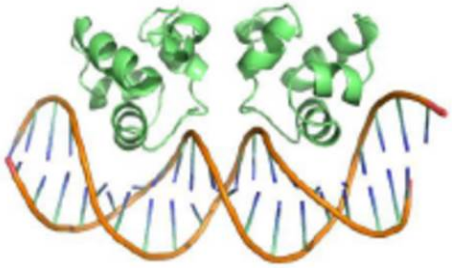
2. DNA binding interactions [32 points]

A. Binding motifs (i): Generate a figure and identify the motif that is interacting with DNA for each of the following PDB codes. [6 points]

i. 1FOS Leucine Zipper

ii. 2OR1 HTH

iii. 1ZAA Zn finger

1FOS	2OR1	1ZAA
		

B. Binding motifs (ii): DNA-binding proteins utilize simple secondary structures to interact with DNA. [Total 9 points]

i. What form of DNA do the binding motifs in question 2A interact with? [2 points] B-DNA

ii. What secondary structures of proteins are complementary to the DNA form mentioned in 2B(i)? Describe which part of DNA each secondary structure fits into. [4 points]

A-Helix fits into the major groove of B-DNA. Two-stranded antiparallel B-sheet can fit into the minor groove of B-DNA.

iii. Why are the DNA binding motifs mentioned above not able to bind an RNA, even when the RNA takes the double-stranded form? [3 points]

Double stranded RNA takes A-form, whereas the DNA binding motifs mentioned above are binding motifs to B-form.

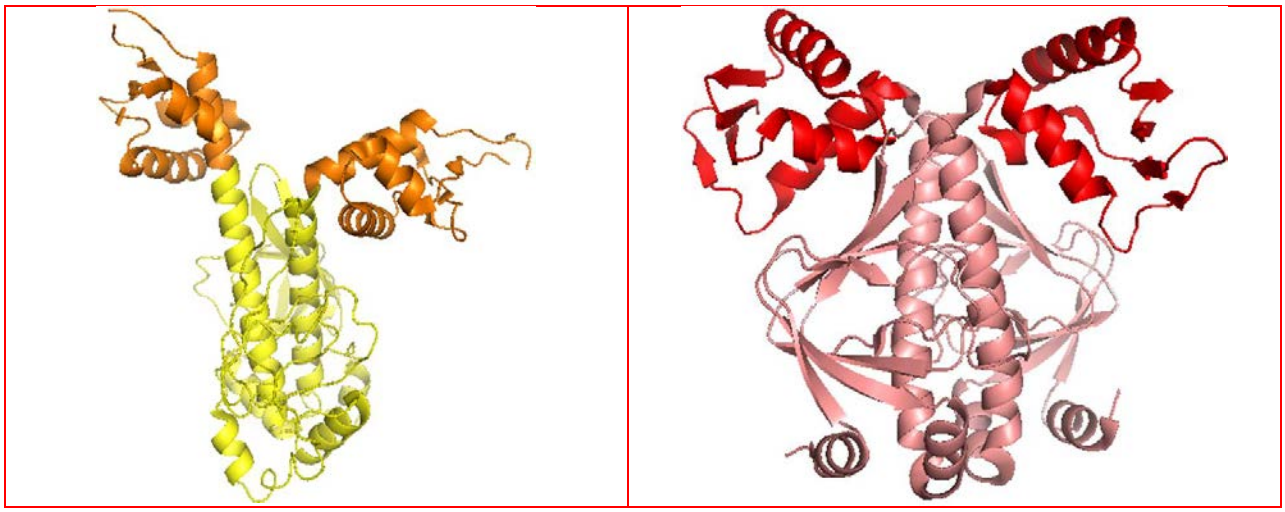
C. Catabolite activator protein (CAP): CAP, also known as CRP (cAMP receptor protein), is a transcriptional activator that interacts with DNA. A member of the CAP family, CooA has adapted a heme for site-specific DNA recognition. Lanzilotta et al. compares the structure of CooA with CRP (doi: 10.1038/82820). [Total 17 points]

i. What is the role of heme in CooA? [3 points]

The heme acts as a CO sensor that, upon binding CO, mediates changes in CooA structure and activates the protein.

ii. Import the structure of CooA (1FT9) and the structure of CAP (1G6N) independently. Mark or highlight the DNA binding motif in both structures. What motif is it? [6 points]

■ 1FT9 ■ HTH motif of 1FT9	■1G6N ■HTH motif of 1G6N



iii. Now, align 1FT9 and 1G6N. Highlight the heme molecule incorporated to chain A of the CooA. Based on the sequences near the location of CooA heme, what residues in CAP should be deleted if CAP were to accommodate a heme instead of cAMP? [4 points]

Residues 72-82 of CAP block the location where CooA incorporates a heme. If CAP were to accommodate a heme instead of just cAMP, residues 72-82 should be deleted.

iv. Select residues 2-107 for CooA and copy (or extract) to an object. Select residues 9-111 for CAP and copy (or extract) to an object. These are the effector binding domains for CooA and CAP. Align, and comment on the similarity. What does the RMSD value implies about the similarity? [4 points]

Effector binding sequences are highly conserved for CooA and CAP. RMSD, a value that tells the overall coordinate difference between two aligned structures, is very low for res 2-107 for CooA and 9-111 for CAP.

3. Ribozyme [11 points]

A. Spliceosome: Ribozymes are RNA molecules that function as enzymes, catalyzing biochemical reactions. Spliceosome is a ribozyme that removes introns from pre-mRNAs. Refer to a paper that explored the splicing mechanism (doi: 10.1126/science.aac8159). Summarize the 2 steps of nucleophilic attack reactions in the spliceosome-mediated pre-mRNA splicing. [4 points]

(i) 2'-OH of adenine attacks on the 5'-end intron guanine nucleotide

(ii) 3'-OH of the 5'-exon attacks on 5'-end of 3'-exon

B. Absence of Deoxyribozyme: Considering the mechanism in question (a), why there are no naturally occurring catalytic DNAs in comparison with ribozymes? [3 points]

DNA lacks the 2'-hydroxyl group that is present in RNA, and has different conformational states compared to RNA. Therefore DNA finds it hard to do the first step of nucleophilic reaction described in part A.

C. Presence of Ribozyme: Compared to proteins, RNA has building blocks with pKa far from physiologically relevant pH. RNA has less rigid backbone, and its tertiary structure makes the side chains to face inside rather than outside for efficient recognition. It seems that using RNA as an enzyme is very inefficient when proteins can be used. What else than the bases and side chains does RNA utilize for efficient catalysis? [4 points]

RNA incorporates numerous structural catalytic metal ions such as magnesium and manganese ions. RNA actively utilizes these metal ions to catalyze reactions, stop catalyzing reactions, and revive the catalytic ability.

4. Nucleosome: Histones [15 points]

A. Interaction: List four types of interactions histone makes with DNA. [5 points]

(i) Helix-dipoles form alpha-helixes in H2B, H3, and H4 cause a net positive charge to accumulate at the point of interaction with negatively charged phosphate groups on DNA

(ii) Hydrogen bonds between the DNA backbone and the amide group on the main chain of histone proteins

(iii) Nonpolar interactions between the histone and deoxyribose sugars on DNA

(iv) Salt bridges and hydrogen bonds between side chains of basic amino acids (especially lysine and arginine) and phosphate oxygens on DNA

(v) Non-specific minor groove insertions of the H3 and H2B N-terminal tails into two minor grooves each on the DNA molecule

B. H2B ubiquitylation: Fierz et al. studied the effect of histone H2B ubiquitylation on local and higher-order chromatin compaction (doi: 10.1038/nchembio.501). How do the authors conclude that chromatin compacts through heterogenous intermediates instead of homogenous intermediates? [4 points]

The authors applied computational modeling and reconciled with their fluorescence data. Calculated traces were generated for two compaction models: (i) a homogenous model in which chain parameters were gradually changed from decompacted to compacted values, and (ii) a heterogenous model In which local inter-nucleosomal contacts were randomly introduced and extended along the chains. The authors were only able to reproduce the predictions of heterogenous model with reasonable levels of FRET.

C. Ubiquitylation and acetylation: H2B ubiquitylation and H4 L16 acetylation are both histone post-translational regulations. How do they affect chromatin compaction? Are the effects additive? [2 points]

Both H2B ubiquitylation and H4 L16-acetylation inhibit chromatin compaction. The effect is not additive, as the inhibitory effect of H4 L16 acetylation overrides the effect of H2B ubiquitylation.

D. Specificity: How do the authors demonstrate that fiber disruption is a specific property of ubiquitin, not a simple consequence of added steric bulk? [4 points]

As a control experiment, the authors added a similar steric bulk, Hub1, a ubiquitin-like protein in yeast which shares 23% sequence and similar fold but has very different surface residues. Linking Hub1 instead of Ub by disulfide coupling strategy at position 120 had marginal effect on chromatin fiber compaction.

5. Ribosomes [15 points]

A. Cryo-EM for ribosome (i): A structure of human 80s ribosome was reported (doi: 10.1038/nature14427). This structure was solved using single-particle cryo-electron microscopy (cryo-EM). Describe why ribosome is such a suitable target for cryo-EM characterization rather than characterization by crystallography. Also, describe why biological EM could be challenging. [5 points]

The 4.3 MDa macromolecular complex contains ~5866 nucleotides and ~11590 amino acids. Ribosome contains lots of flexible regions and is heterogeneous and dynamic, making it extremely difficult to crystallize at all.

In biological, sample preparation methods such as sectioning and staining often damage the native structure of the sample. Long electron beam exposure can also destroy samples, posing dose (exposure) limitations.

B. Cryo-EM for ribosome (ii): Briefly describe the method Chen et al. (doi: 10.1016/j.str.2015.04.007) used to monitor the conformational changes of the ribosome on millisecond scale. [4 points]

They used mixing-spraying cryo-EM to visualize ribosomal subunit association on millisecond timescale and observed 3 populations of ribosome conformations (non-rotated, non-rotated with small subunit head swivel, and rotated).

C. Metal ions and ribosome: How many Mg^{2+} ions are present in this structure? What structural roles does Mg2+ play in nucleic acids? Compare the Mg^{2+} ions in this structure to the 741 Mg^{2+} ions in the structure of the bacterial 70s ribosome from Thermus Thermophilus (PDB 2J01 AND 2J02). Based on these structures, explain the differences in Mg^{2+} dependence between eukaryotic and prokaryotic ribosomes. [6 points]

There are 239 magnesium ions, which are important to stabilize charged phosphate groups of the nucleotide backbone and ensure rRNA folding. The authors observed 2'-OH groups of rRNA sugar play key role in ribose-ribose interaction through hydrogen bonds, rather than magnesium-mediated phosphate-phosphate electrostatic interactions; eukaryotic ribosomes depend less on magnesium ion than prokaryotic ribosomes.