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]

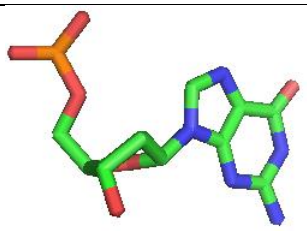
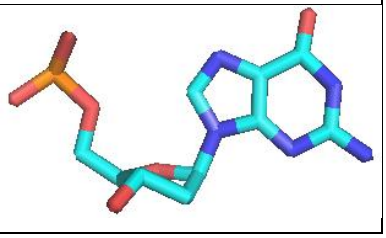
A. Basic structure and forms: Import two DNA structures, 1BNA and 440D, to pymol. Generate figures separately. For each figure, complete or answer the following exercises. To measure distances, you can use Wizard -> Measurement from PyMol menu. [total 10 points]

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]

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]

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
	

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]

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`

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
ii. 2OR1
iii. 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]
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]
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]

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]
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]
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]
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]

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]

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

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]

4. Nucleosome: Histones [15 points]

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

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]

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]

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]

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]

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]

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]