Biochemistry 465 **Your Printed Name:**_____
Section I and only
April 15, 1999 **Your SS#:**_____
Exam #2
Prof. Jason D. Kahn **Your Signature:**_____

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise.

You will not need a calculator for this exam, and no other study aids or materials are permitted.

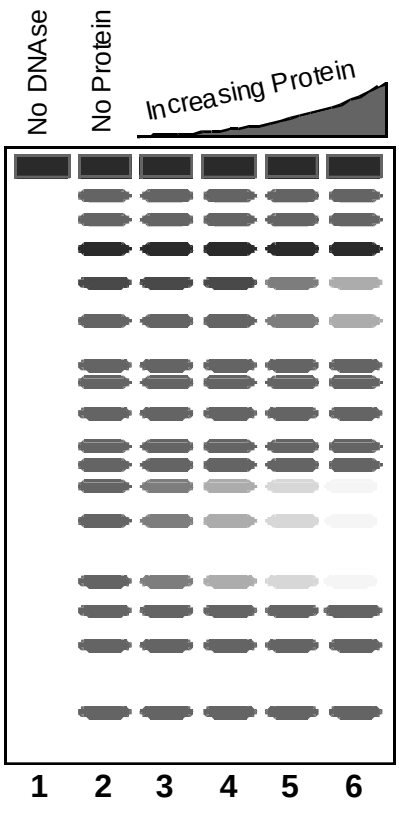
Useful equation:

$\Theta = P / (P + K)$

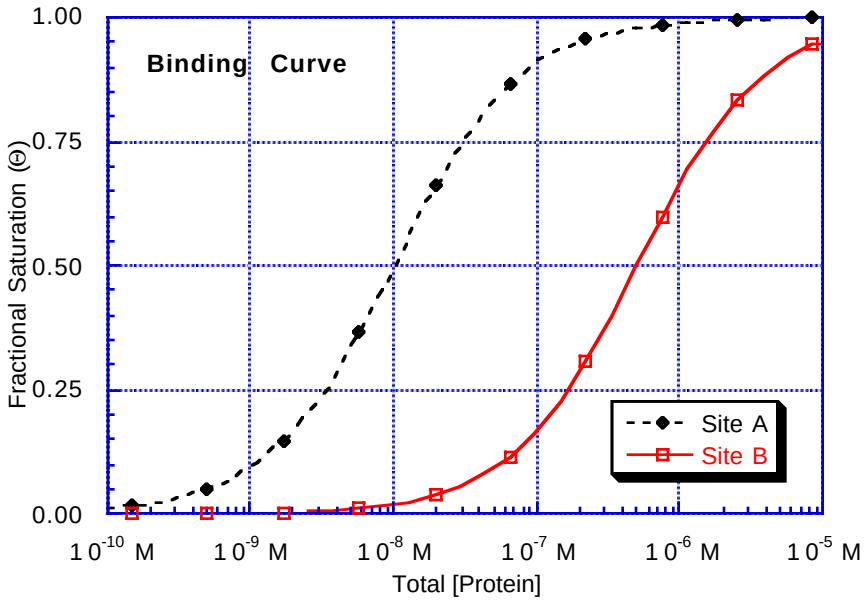
1. (20 pts) Footprinting and protein-DNA binding

The autoradiogram on the left below shows DNAse I footprinting of a sequence-specific DNA binding protein to a labeled restriction fragment. The four lanes at the right show increasing total protein concentrations, with each lane having about 5 times as much DNA as the one to its left. Some of the data on the binding curve at the right came from the experiment on the left.

(a; 6 pts) Indicate in the box in the center below where sites A and B are on the gel. Explain your reasoning briefly:



Answer for (a): ⇓



Note that the gel lanes do not necessarily correspond to particular points on the curves above.

(b; 4 pts) Using the binding curve above, determine numerical values for the protein's dissociation constants (K_d's) from sites A and B.

(c; 4 pts) How would the curves above change (qualitatively: which direction would they shift?) if the experiment were done at higher salt concentration? Why?

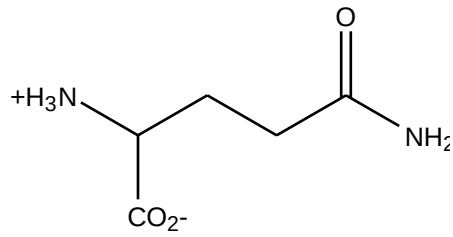
(d; 6 pts) The gel mobility shift or EMSA experiment is often used very qualitatively to identify the presence of a low-abundance sequence-specific DNA binding protein in a complicated mixture like a nuclear extract. Suggest why the EMSA is a better assay for this purpose than a DNAse I footprint (hint: there's very little of the protein of interest in the mix). Why is it often necessary to include a source of non-specific DNA (e.g. poly d[A-T]) in the reaction mix?

2. (20 pts) Specificity and Structure

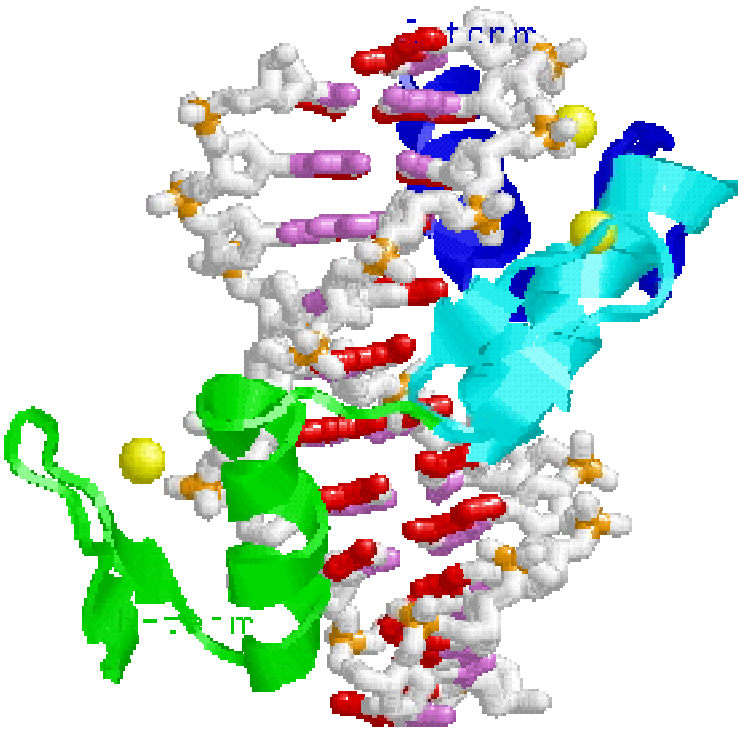
(a; 2 pts) What is the single most common general structural motif for a protein-DNA interaction?

(b; 3 pts) Why do sequence-specific RNA binding proteins never recognize fully double-stranded RNA?

(c; 5 pts) Draw a hydrogen bonding interaction between the glutamine side chain and a nucleic acid base to which it can make two hydrogen bonds when the base is in a Watson-Crick duplex.



(d; 5 pts) Why are zinc fingers especially suitable for designing artificial DNA binding domains with user-defined specificity? Why are they always found to be at least dimeric if not multimeric DBD's? (The answers are related).



(e; 5 pts) What do we mean by the hydrogen bonding matrix that a protein "sees" upon approaching DNA? Give a specific example of how this allows discrimination between two different base pairs.

3. (20 pts) DNA replication

(a; 8 pts, 2 each) In the fundamental mechanism for nucleotide incorporation by DNA polymerases, what reaction provides proofreading (removal of a misincorporated base)? Why is it important for this mechanism that extension using the 3′ end of a misincorporated bases is slow? It what way does this process spend energy? Why is coupling error correction to an irreversible process essential?

(b; 5 pts) What are the important players in the active site of T7 DNA polymerase and many other phosphoryl transfer enzymes? What do they do (how do they activate the reaction)?

(c; 4 pts) Sketch a replicating bacterial chromosome theta structure as seen during exponential growth, assuming a 20 min doubling time and 40 min required for chromosome replication. (Any time point during the cell cycle will do.)

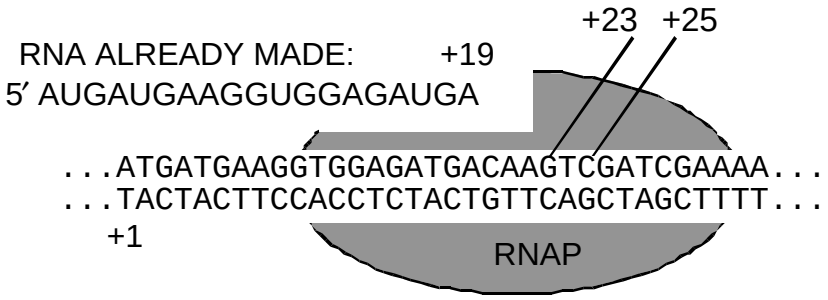
(d; 3 pts) Why is the replicative DNA polymerase III a dimer?

4. (20 pts) Transcription

(a; 3 pts) Phage T7 RNAP is a single 100 kD protein that synthesizes RNA quite well. E. coli RNAP has at least four different subunits and a mass of about 450 kD. Eukaryotic RNAPs ~2000 kD. What's all the rest of the mass of cellular RNAPs for, if not chemistry?

(b; 6 pts) Sketch the nucleic acid components of the transcription complex during elongation. Why do RNA polymerases absolutely have to be processive? What's the opposite of processive?

(c; 6 pts) How could we "walk" an RNA polymerase ternary complex stalled at position +19 on the template sequence below to position 23? What is the advantage in having a His_6 tag or other affinity label on the enzyme if we want to walk it further, e.g. specifically to position 25 and not further?



(d; 5 pts) Phage T4 encodes its own sigma factor and inactivates the host sigma factor. What effect would this have on cellular transcription, and what advantages does it confer for the phage?

5. (20 pts) Miscellaneous: Chromatin, telomeres, genomics

(a; 4 pts) The measured ΔLk induced by RNA polymerase binding is about -1.7 (18 bp), and yet it seems to unwind only about 12 bp. A nucleosome wraps about 1.7 turns of DNA around itself but induces a ΔLk of only -1. Give brief possible explanations for these two puzzles.

(b; 6 pts) Give a brief sketch and explanation of how shotgun sequencing of genomes works.

(c; 7 pts) Why do organisms with linear chromosomes (like us) need telomerase? What does telomerase use as a template? Why is telomerase under study as an anti-cancer target?

(d; 2 pts) When we speak of a "rotationally positioned nucleosome" what is or is not rotating?

(e; 1 pt) What creature from science fiction of the late 1960's is brought to mind by bacterial DNA replication?

Do Not Write Below This Line

Score:

Question 1: _____ out of 20: Footprinting and protein-DNA binding

Question 2: _____ out of 20: Specificity and Structure

Question 3: _____ out of 20: DNA replication

Question 4: _____ out of 20: Transcription

Question 5: _____ out of 20: Miscellaneous: Chromatin, telomeres, genomics

Total: _____ out of 100