Your Name: MID-TERM ANSWER SHEET SIN: ( )

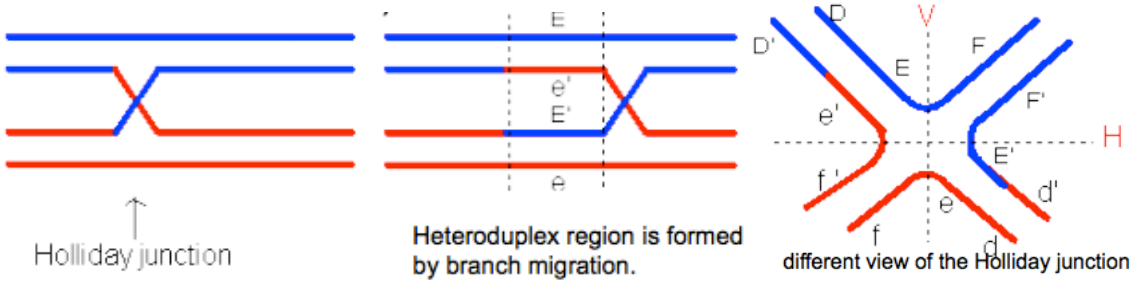
MIDTERM EXAMINATION (March 22, 2012)
BIOE150. Introduction to Bio-Nanoscience & Bio-Nanotechnology
Spring Semester, 2012

0. Write down your name and the last 4 digits of your SIN in all the pages (1)

1. Define the following terms briefly: (12)

a) Holliday DNA junction (3):

Genetic **recombination** of 2 **homologous DNA** strands line up and cross over. Because of sequence symmetry at branch points, **branch migration** forms a **heteroduplex** region.

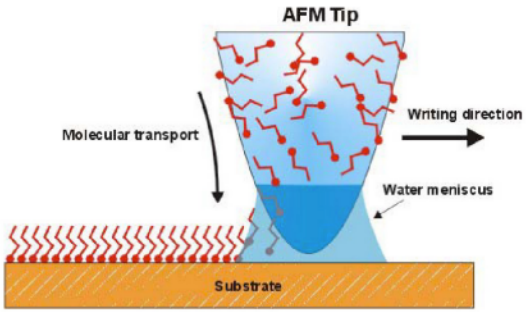


b) Surfactant number (3):
Expression to predict the structure that a surfactant will self-assemble into.

$$N_s = \frac{V}{a_0 l}$$

V = volume of tail group
a_0 = area of head group
l =hydrocarbon tail length

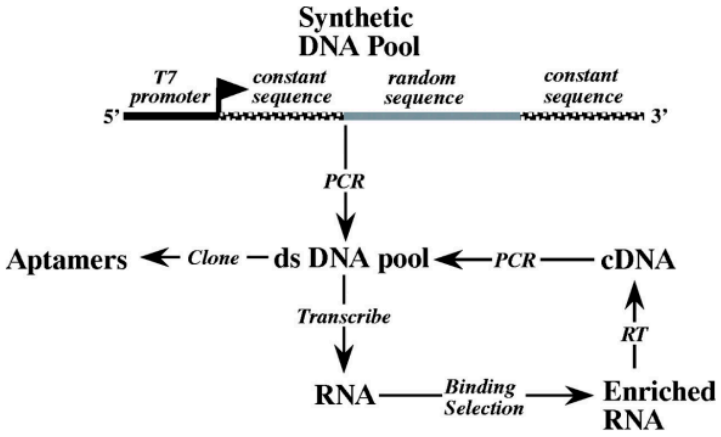
c) Dip Pen Lithography (4)



DPN is a direct-write scanning probe-based lithography. An AFM tip is used to deliver chemical reagents (peptides) to nanoscopic regions of a target substrate where they can self assemble. Desired chemistry is carried out exactly where it is needed.

d) SELEX (4)

Systematic Evolution of Ligands by Exponential Enrichment (1)
A method that relies on the process of evolution (variation -> selection -> replication) where a pool of RNA completely randomized at specific position is subjected to selection for binding. The selected RNA's are amplified as double-stranded DNA through *in vitro* transcription. This newly transcribed RNA is enriched for better binding sequences and is then subjected to selection for next cycle. Multiple rounds of enrichment result in exponential increase of best binding ligands. Discover specific binding aptamer through high-throughput screening.

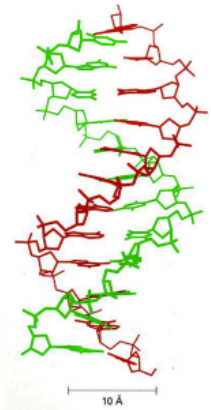


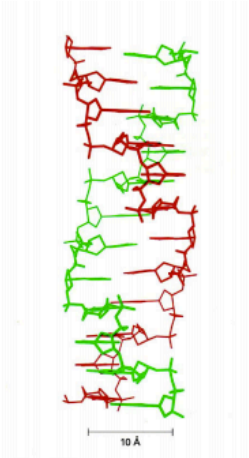
2. B-DNA is the most stable form of the DNA double helix structures discovered by Watson and Crick in 1953. There is additional DNA structure discovered by Alexander Rich in 1979 called Z-DNA. (10)

a) Explain the major structural difference between B-DNA and Z-DNA by drawing a schematic structure (6).

B-DNA (3) Z-DNA (3)

B-DNA:	Intermediate	Z-DNA:	Narrowest
•Most stable •Right handed •Hydrated form	3.4 Å	•Left handed •Short DNA has alternative purine and pyrimidine ring (G-C) can form Z-DNA. •Look like a zig-zag form the side view	3.8 Å
	23.7 Å		18.4 Å
	Right-handed		Left-handed
	anti		*anti* for C, T *syn* for G
	10.4		12
	35.4 Å		45.6 Å
	1°		9°
	Wide and quite deep		Flat
	Narrow and quite deep		Very narrow and deep





b) Structural transition between B- and Z-DNA can be occurred in an ionic concentration dependent manner. Therefore, these structure transitions can be used for molecular switch function. Explain how it works (4)?

Due to the high G-C pair, hydrogen bonding between two DNA chains are very strong. Z-DNA has a closely spaced phosphate backbone, which is unfavorable due to charge repulsion unless screened by ions in solution. Therefore, at high ionic strength, B-DNA transitions to Z-DNA.

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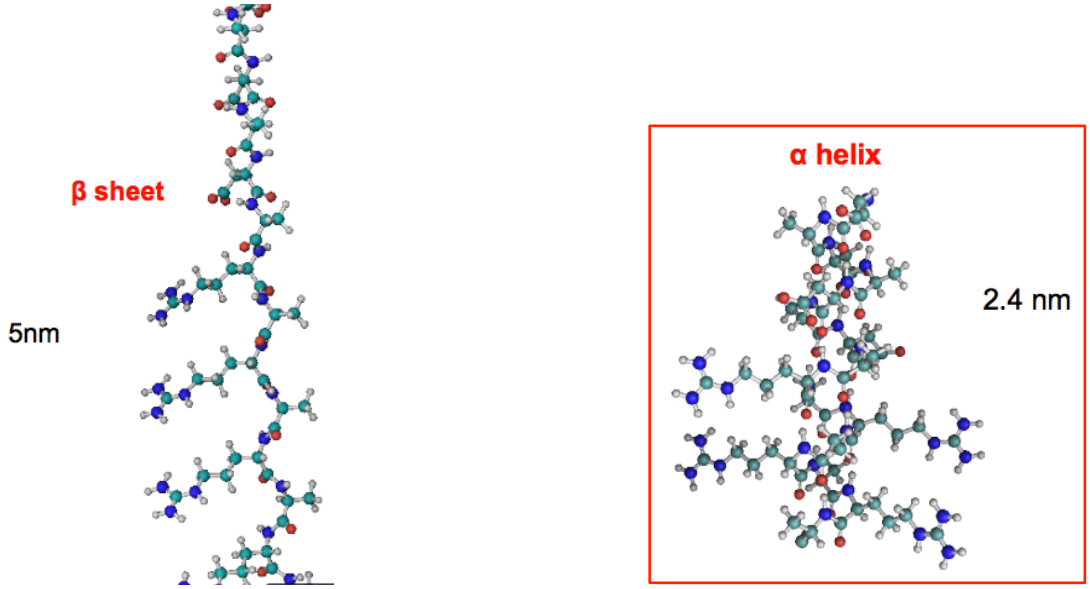
3. DAR16-IV is a peptide exhibits both alpha-helix and beta sheet structures depending on pH and temperature. pK_a of Asp and Arg is 3.90 and 12.48 respectively. (10)

a) Draw the schematics of alpha helix and beta sheet structure of DAR16-IV. Indicate the possible charge of N-terminal and C-terminal and their charged side chains at pH 7.5 (4)

beta sheet here alpha helix here



N-terminal (Aspartic acid – D, negative) neutralizes the helix dipole; C-terminal (Arginine – R, positive).

b) At high temperature (higher than 60**°C**), which structure is favored? Mark a circle on the above drawing. (3)

c) Explain why it favors the structure that you choose (3)

At high temp, alpha helix is favored because thermal energy can induce intramolecular hydrogen bond rather than intramolecular interaction.

1. AAAAAAK
2. AAAAAAD
3. GGGAAAK
4. VVVVVVD
5. RGEGGGC
6. RGDGGGC

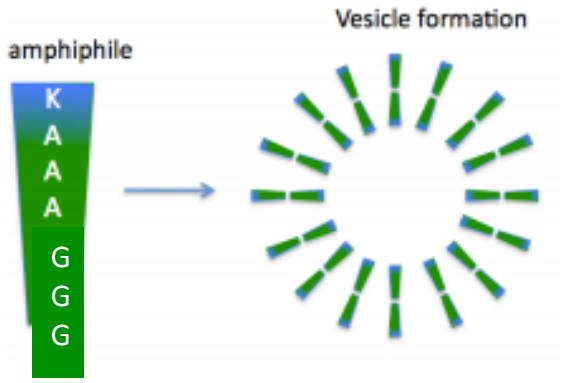
Table I. Sequence of peptides in single letter code.

4. Among the peptide sequences shown in the table 1, which sequence is expected to possess the smallest surfactant number and draw the schematic diagram of the resulting supramolecular structures (5).

Sequence #3: **GGGAAAK** (1)

Based on initial assessment, sequences #1-4 have similarly sized head groups and same hydrocarbon length. Thus the determining factor is the volume of the tail group and sequence #3 has the smallest tail group volume because glycine (G) is the least bulk amino acid compared to alanine (A) or valine (V). (2)

With positive K on the exterior and hydrophobic A and G inside, the amino acid sequences will self-assemble into either a nanovesicle or nanotube. (2)



5. Among the peptide sequences shown in the table 1, which sequence can be ideal to fabricate the gold substrates to make cell attachment favorable and explain why (5).

Sequence #6 **RGDGGGC** (1)

RGD is a bioactive peptide that promotes cell growth because it is responsible for integrin-mediated cell adhesion. (1)

C provides SH bond that can be used to conjugate with gold substrates. (1) GGG spacer peptide. (1) This peptide sequence can attach to gold substrates with RGD domain that serve as a cell adhesion component to promote cell growth. (1)

6. Suppose that "GGA TTA TTG TTA AAT ATT GAT AAG GAT " is a marker gene sequence for prostate cancer protein. Therefore, if we can detect this gene sequence in the body, we can diagnose prostate cancer early enough to save the life. Suppose that you will fabricate DNA-based sensor system. However, your budget for developing the sensor is limited. You only can purchase DNA sequences, gold nanoparticles or plates, some chemicals. In addition, you will use an off-the-shelf scanner in your office to quantify the DNA sensing. Using the condition given above, design the sensor that you can quantify the prostate cancer and explain how it works (10).

Half the complementary sequence is immobilized on a surface for example the sequence 3`-CCT AAT AAC AAT -5` with the 3` end immobilized. The other half of the complementary sequence is immobilized on gold nanoparticles 3`-TTA TAA CTA TTC CTA-5` with the 5` end attached to the thiol linker and gold nanoparticle.

Using thiolated single stranded DNAs complementary to the target sequence, the nanoparticles will attach to the surface when the DNA hybridizes.

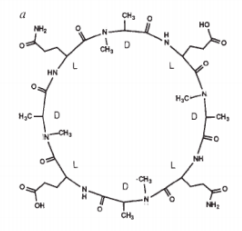
Staining with silver ions, the particles will assemble and light scattering properties of the solution will result in a color change and can be visualized with a scanner.

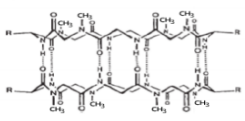
7. Various cyclic peptides such as cyclo[(D-Ala-Glu-D-Ala-Gln)$_2$] are known to form ring-shaped nanostructure. These peptide rings can self-assemble by stacking to form nanotubes in the presence of acid. (10)

a. What intermolecular force holds stacked rings together (3)?

Intermolecular backbone-backbone hydrogen bonding between the peptide bond (Nitrogen and Oxygen groups).

b. Suppose we replace all the **hydrogens** on the amino groups of the D-Alanines with **methyl** (CH_3) groups in the cyclo[(D-Ala-Glu-D-Ala-Gln)$_2$] peptide as shown below. Draw a schematic diagram of what structure these modified peptides will form and explain why they form such structures (7).





Hydrogen bonding interaction cannot be formed in every other amide bond because of methyl groups. Therefore it forms a dimer.

8. Dr. Nadrian Seeman developed a high resolution DNA self-assembly approach to form periodic structure of the DNA crystals using synthetic DNA junctions. Suppose that we will design a DNA based nanofibers (or nanowires) through the similar approach. The below figure (A) is a schematic diagram of DNA crystal segments with 36 nt length corresponding to 12.6 nm in length. (15)

a) What does "DAO" mean in the above DNA structure? Explain briefly. (3)

D: Two double crossover

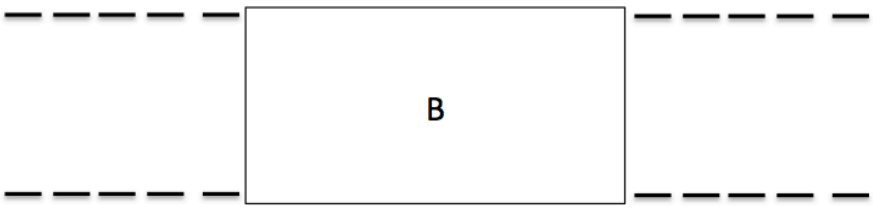
A: Dyad axes anti-parallel to the helical axes

O: Odd number of half turns between crossovers

b) What are the sequence requirements for creating a stable synthetic DNA junction (3).

No sequence dyad symmetry flanking the branch point.

c) Design a counter part (B) that can self-assemble DNA nanowire. **Design the specific DNA sticky ends in the B segment using the below template that assembles the A and B DNA segment in a linear manner (B) (4 points)**



GTATG… AGTGA

AGAAC… ATCTC

c) Suppose that we will fabricate a DNA ruler by extending the above resulting structure to measure the nanometer scale distance. In this DNA ruler, we will mark every ~25 nm through incorporation of a contrasting region. Explain the strategy to generate such markers and how it works (5 points).

Au + phosphine + dsDNA → endonuclease (XhoI)

Attach dsDNA to Au nanoparticles with thiol linker and add specific restriction enzymes to cleave DNA (XhoI @ 25nm rule).

9. The following molecule is called a peptide amphiphile developed by Dr. Samuel Stupp. It is freely suspended in solution at pH 7.4. When this solution is mixed with a solution containing enough cations, nanofiber-like structures form. The final fibrillar nanostructures form hydrogels which can promote differentiation of neural stem cells, therefore they can be useful in the regeneration of damaged neural cells. (10)

$CH_3(CH_2)_{15}$AGAGAGAEEEEIKVAV

a) Explain the role of the each component labeled 1-4 below (4).

1. $CH_3(CH_2)_{15}$

Hydrophobic alkane chain

2. AGAGAGA

Beta-sheet forming region

3. EEEE

pH/ion responsive hydrophilic region

4. IKVAV

Bioactive cell signaling peptide motif to stimulate neural cell sprouting

b) Explain why it assembles into nanofiber structures using surfactant number (3)

To form nanofibers it must be a cylindrical micelle and therefore have a surfactant number between 1/3 and ½.

c) Suppose that you want to design bilayer membrane structures by changing the components of the peptide amphiphile discussed above. How can you alter the design to induce formation bilayer formation instead of nanofibers. (3)

Need to increase the surfactant number to between ½ and 1 for example by

- using a two-tailed alkane chain
- by decreasing the size of the hydrophilic region.

10. Phage display is a method to identify functional peptide motifs by exploiting phage biology and bacterial metabolism. Using the phage display approach, we plan to construct a loop-shape penta-peptide library, which is known to have a stable conformational structure and enhance binding affinity. The loop-shape library can be designed through franking the amino acid sequences between Cys- and -Cys as below: (14)

Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Cys (Xaa=random amino acid residue)

a) Design the nucleotide sequences that can express the above library (3)

RNA sequence:

UGU NNN NNN NNN NNN NNN UGU

UGC UGC

DNA sequence:

ACA NNN NNN NNN NNN NNN ACA

ACG ACG

b) How big is the size of the random amino acids generated by the above library construction (3).

20^5

c) Suppose that we will use the above library for *in vivo* kidney cancer target peptide through *in vivo* phage display process for the mouse. Describe actual *in vivo* phage display screening procedures in mice (4).

1. Introduce the phage library into the mice

2. Harvest the kidney tumors

3. Wash away weakly bound phage

4. Elute the bound phage from the tumors

5. Amplify and Sequence the eluted phage

6. Check for a consensus sequence, otherwise repeat the process using the eluted phage

d) Suppose that, after *in vivo* phage screening with the above library against the kidney tumors we identify a peptide with the sequence sequences GRGDS. Calculate the theoretical probability of finding the GRGDS sequence from the original library (4).

G – 4/64; R – 6/64; G – 4/64; D – 2/64; S – 6/64

GRGDS – (4/64)*(6/64)*(4/64)*(2/64)*(6/64)= **1.07x10^-6**

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Table: genetic Code