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

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MIDTERM EXAMINATION (Oct 20, 2015)
BIOE150. Introduction to Bio-Nanoscience & Bio-Nanotechnology
Fall Semester, 2015

Write down your name and the last 4 digits of your SID on all the pages (1)

1. Define the following terms briefly: (16)

a) Phage display (4)

Phage display is a high-throughput, combinatorial screening process that identifies peptides with binding affinities to a target sequence by selecting from random peptide libraries expressed as fusion proteins on the phage coat surface.

Idea of screening from a larger peptide library/large number of variants (2)
With certain binding affinity (1)
Peptides expressed as fusion proteins on the surface(1)

b) Dip Pen Lithography (4)

Dip Pen Lithography is a direct-write scanning probe lithography technique which uses an atomic probe microscope "pen" coated with a molecule or material-based "ink" to create nanoscale patterns on a surface.

Direct-write scanning probe lithography technique(1)
Uses an AFM tip as an application tip (2)
To coat inks that are chemicals of interest (1)

c) Surfactant number (surfactant packing parameter) (4)

The surfactant packing parameter is a measure of the shape of the surfactant molecule. This parameter is the volume of the hydrophobic chain divided by the product of the cross-sectional area of the hydrophilic head and the length of the hydrophobic chain. (If an equation is shown, terms need to be defined).

Measure of shape (1)
Explanation of 3 terms in the packing parameter equation (3, 1 for each)

d) Solid phase peptide synthesis (4)

Solid phase peptide synthesis is the standard laboratory technique for the in vitro synthesis of peptides, with the peptides being added in a C-terminal to N-terminal direction. This method involves immobilizing peptide chains covalently onto small solid porous beads via functional linkers, and adding new amino acids via repeated cycles of coupling, washing, followed by deprotection and washing.
Idea that peptide chains are attached onto solid substrate (beads) (1)
Peptides synthesized in C-N direction (1)

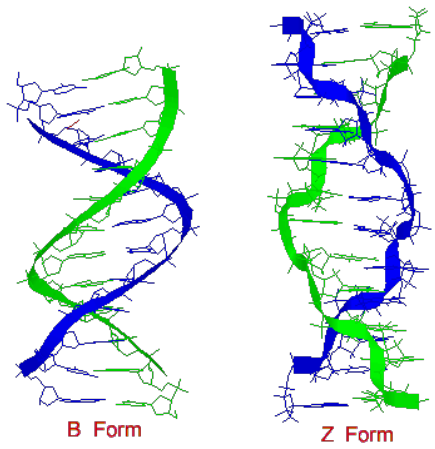
Coupling, washing, deprotection and washing cycles that are repeated (2)

2. B-DNA is the most stable form of the DNA double helix structures discovered by Watson and Crick in 1953. Z-DNA is an additional DNA structure discovered by Alexander Rich in 1979. (13)

a) Describe the major structural differences between B-DNA and Z-DNA and draw their respective schematic structures (6).

Feature	B-DNA	Z-DNA
Handedness	Right	Left
Backbone	Smooth helix	Zigzag
Bp/turn	10.5	12
Vertical rise/bp	3.4	3.7
Diameter	21	18
Repeating unit	1	2 ($(GC)_2$ repeats)
Major and minor groove length	Different	Same

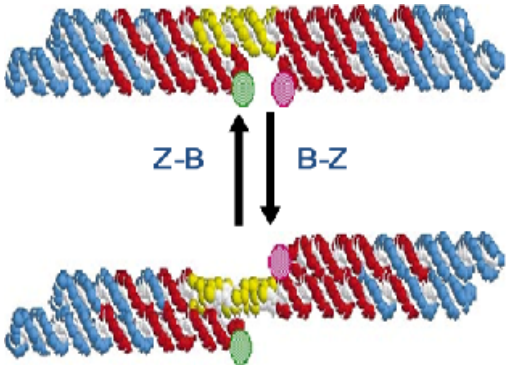
2 point per difference, up to maximum of 4 points



2 points for figure of B- and Z-DNA

b) Using the B-Z DNA switch, we can design a molecular optical switch that can sense the salt ion concentration. Explain the principle how this optical switch can operate in low and high ionic concentration. (7)

The B-Z switch consists of 2 DNA double crossover molecules connected with 4.5 turns of DNA between nearest crossover points.



2 fluorescent dyes (fluorescein and Cy3) are attached to the free hairpins near the middle of the molecule. At the center

of the connecting helix is a 20 nucleotide region of proto-Z DNA $d(GC)_{10}$ in the B-DNA conformation. When there is high ionic strength, a B to Z DNA transition takes place, approximately 128° for each GC dinucleotide, resulting in a total twist change of 3.5 turns, increasing the distance between the donor (fluorescein) and acceptor (Cy3) FRET pair. The distance increase results in a lower donor energy transfer, as monitored through fluorescence readout. The B-Z transition occurs because of high salt ions electrostatically shielding the negatively charged DNA backbone phosphates in B-DNA from each other, allowing the phosphates to adopt a more compact structure.

Grading scheme:

2 DNA double crossover molecules connected by Proto-Z DNA/ GC dinucleotide repeats (1)

Double crossover molecules connected to acceptor and donor(1)

Turning of DNA/induced rotation in B-Z DNA transition (1)

Donor fluorescence decrease because of increased donor/acceptor distance, lower FRET(1)

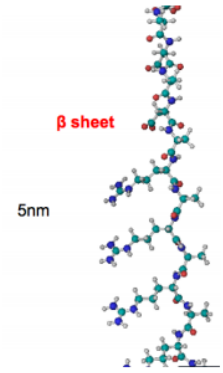
Salt ions electrostatically shield phosphate residues on DNA backbone, inducing more packed conformation (3)

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)

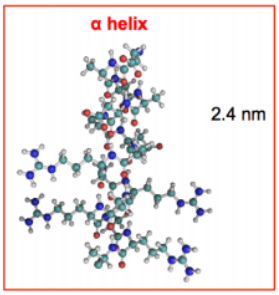
a) Draw the schematics of alpha helix and beta sheet structure of DAR16-IV. Indicate the possible charge of N-terminal and C-terminal and their charged side chains at pH 7.5 (4)

beta sheet here alpha helix here

beta sheet here



alpha helix here



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

Alpha helix

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

The increase in thermal energy induces intramolecular rather than intermolecular hydrogen bonding

1. AAAAAAAAAAAAAAK
2. AAAAAAAAAAAAAAD
3. GGGGGGGAAAAAAAK
4. GGGGGGGVVVVVVD
5. RGEGGGGGGGGGC
6. RGDGGGGGGGGGC
7. RADARADARADARADA

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).

7. does not form a surfactant.

5,6 have large headgroups and can be ruled out.

Looking at sequences 1-4, headgroups are approximately the same size and V_{tail} is the distinguishing factor. Sequence 3 has the smallest V_{tail} and therefore the smallest surfactant number. It assembles into nanovesicles or nanotubes.

5. The following molecule is called a peptide amphiphile developed by Dr. Samuel Stupp for the purpose of directing biomineralization for bone tissue engineering. This peptide amphiphile is able to form a nanostructured fibrous scaffold reminiscent of collagen fibers, and this is able to direct the growth of hydroxyapatite crystals, similar to the interaction within bone. (10)

CH3(CH2)15CCCCGGG(PS)RGD

PS = phosphoserine

a) Explain the role of the each component labeled 1-5 below (5).

1. CH3(CH2)15

Hydrophobic alkane chain

2. CCCC

Crosslinker (disulfide bonds from Cysteine residues)

3. GGG

Flexible spacer

4. PS

Recruitment of calcium ions for hydroxyapatite crystal formation

5. RGD

Hydrophilic head group/ cell adhesion peptide

(1 point per explanation)

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

The surfactant number is between 1/3 and ½ (2 pts), which means that the shape of the surfactant is a truncated cone (1 pt). This means that the nanofiber structure assembled is a cylindrical micelle.

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 the formation of bilayers instead of nanofibers. (2)

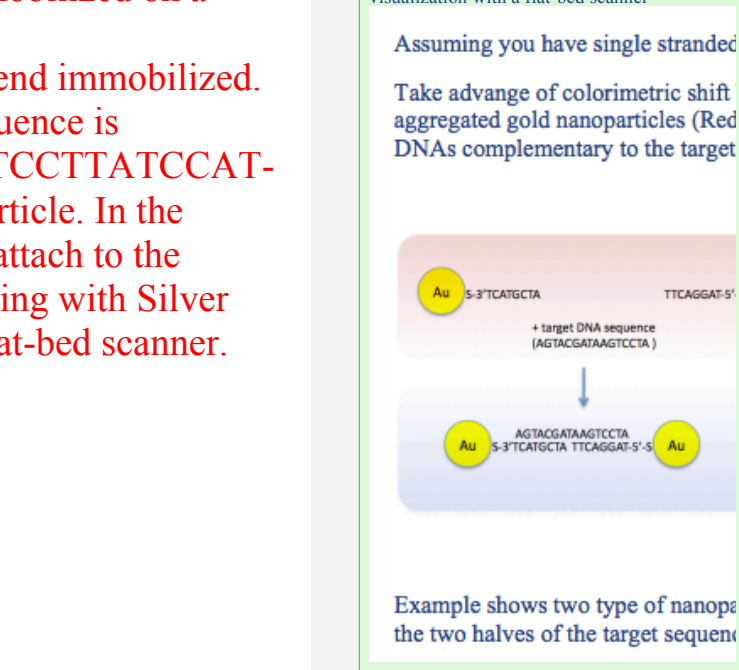
Increase V_{tail} such that the surfactant number increases to ½-1. We can do this by adding on alkane tails or increasing the bulk of the tail groups by adding on amino acids with large side groups.

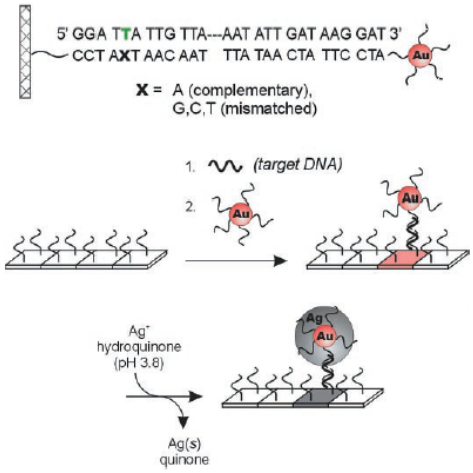
6. Suppose that "GGC TTA TTG TTA AAT ATG GAT AAG GAT " is a **marker gene** sequence for pancreatic cancer protein. Therefore, if we can detect this gene sequence in the body, we can diagnose pancreatic cancer early enough to save the life. Suppose that you will fabricate **DNA-based sensor system**. 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 pancreatic cancer and explain how it works (10).

Half the complementary sequence is immobilized on a surface. For example the sequence 5`-ATTTAACAATAAGCC-3` with the 3` end immobilized. The other half of the complementary sequence is immobilized on gold nanoparticles 5`-ATCCTTATCCAT-3` with the 5` end attached to the nanoparticle. In the presence of target the nanoparticles will attach to the surface when the DNA hybridizes. Staining with Silver ions will allow for visualization with a flat-bed scanner.

Seung-Wuk Lee 10/15/2015 3:23 PM

Comment [1]: Half the complementary sequence is immobilized on a surface for example the sequence 5`-ATCGTACT-3` with the 3` end immobilized. The other half of the complementary sequence is immobilized on gold nanoparticles 5`-TAGGACTT-3` with the 5` end attached to the nanoparticle. In the presence of target the nanoparticles will attach to the surface when the DNA hybridizes. Staining with Silver ions will allow for visualization with a flat-bed scanner





(Example from lecture provided, replace DNA strands with those designed above)

Design of strands correctly (2 points each)

Mention that half are immobilized on surface (1)

Half are immobilized on Au NPs(1)

In presence of target sequence the NPs will attach to said surface (1)

Staining with silver ions allow for visualization with flatbed scanner (3)

7. (a) Various cyclic peptides such as cyclo[(D-Ala-L-Glu-D-Ala-L-Lys)$_2$] are known to form ring-shaped nanostructure. These peptide rings can self-assemble by stacking to form nanotubes. Draw a schematic diagram of the self-assembled structure of the above sequence and indicate the possible weak interactions (8)

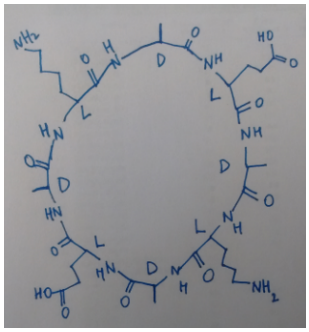
Antiparallel beta sheet stacking

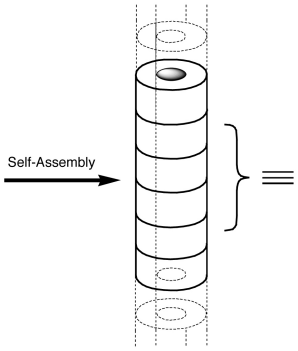
Ring structure (4)

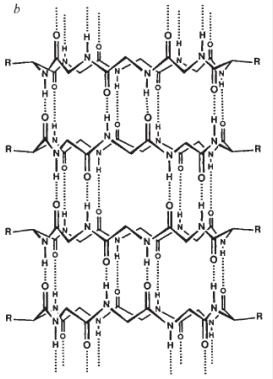
D/L not indicated (-1)

Stacking of peptides (4)

No h bonds (-1)







Any structure not present (-2 for each)

(b) The following table shows the sequences of the cyclic peptides as antibiotic therapeutic efficacy depending on the sequence. Each number shows IC_{50} value, amount of drug dosage to kill the 50% of the target organism. Suppose that we will choose the one of the cyclic peptide sequences, which might be most promising to treat human patients who are infected by MRSA. Explain why (5)?

Table 2 *In vitro* activity of D,L-α-amino acid cyclic peptides

	Peptides	*S. aureus* (MRSA)* MIC (μg ml^{-1})	*E. coli*† MIC (μg ml^{-1})	Haemolysis‡ HD_{50} (μg ml^{-1})
1	(SKSWLWLW)	10	100	30
2	(THSWLWLW)	100	100	100
3	(RGDWLWLW)	100	100	100
4	(KQRWLWLW)	**6**	**80**	**45**
5	(KQRWLWLW)	8	80	40
6	(RQRWLWLW)	18	90	25
7	(KQKWLWLW)	45	100	50
8	(KSKWLWLW)	**5**	**40**	**100**
9	(SHKWLWLW)	10	100	35
10	(SKHWLWLW)	15	100	20
11	(SHHWLWLW)	20	100	20
12	(EKHWLWLW)	100	100	>100
13	(KKKWLWLW)	**8**	**70**	**50**
14	(RRKWLWLW)	**6**	**15**	**50**
15	(KRKWLWLW)	10	40	50
16	(RRRWLWLW)	10	50	35
17	(HKHWLWLW)	12	15	25
18	(KHKWLWLW)	10	80	30
19	H_2N-WKKKKWLWL-COOH	60	–	100
20	H_2N-KKKWLWLW-$CONH_2$	50	–	100
21	Ac-KKKWLWLW-$CONH_2$	80	–	100
22	(KKLWLW)	**10**	**17**	**80**
23	(KHLWLW)	10	100	25
24	(KSLWLW)	75	100	90
25	(RRLWLW)	**35**	**5**	**90**

An ideal peptide would have the lowest concentration needed to kill MRSA while having the highest concentrations needed to induce hemolysis. This would mean the lowest S. aureus IC_{50}/HD_{50} ratio. Peptide 8 is therefore the best choice, with this ratio being the lowest at 0.05.

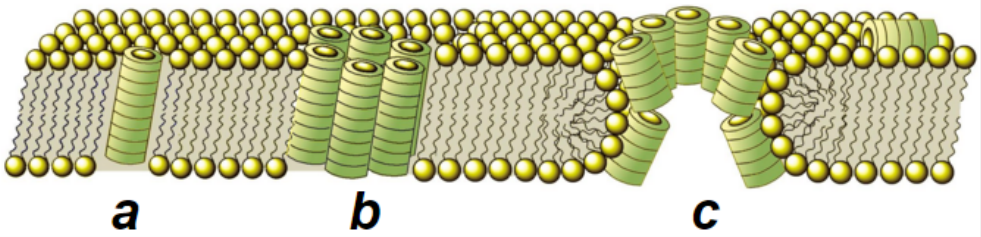
Correct selection (3)

Explanation (2)

(c) When we apply the selected cyclic peptide from the above table against the target cells, what can be a possible structures on the cell surfaces and how to kill the bacterial cell. Drawing possible interaction on the surfaces schematically (7).

They associate with the bacterial membrane bilayers and form pores, affecting membrane integrity and ion gradient necessary for bacterial life (1 point)

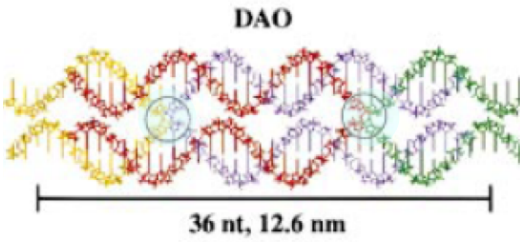
Possible structures:



a. *Through Pore*
b. *Barrel Stave*
c. *Carpet-Like*

2 points per structure (1 point for stating structure, 1 point for actual drawing)

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 crossovers

A: Dyad axes anti-parallel to the helical axes

O:Odd number of half-turns between crossovers

1 point per term

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

No sequence dyad symmetry flanking the branch point

Seung-Wuk Lee 10/15/2015 4:02 PM
Comment [2]: D: Two double crossover
A: Dyad axes anti-parallel to the helical axes
O: Odd number of half turns between crossovers

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)**

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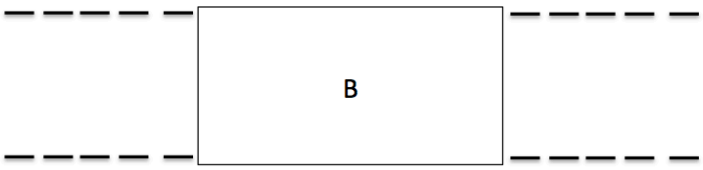
Comment [3]: No sequence dyad symmetry flanking the branch point.



5’ GTATG… AGTGA 3’

3’ AGAAC… ATCTC 5’

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).**

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Comment [4]: 5’ GTATG… AGTGA 3’
3’ AGAAC… ATCTC 5’

Many possible explanations, for example:

1. Inclusions of DNA hairpins that project of the plane of the helices
2. Conjugating DNA to gold nanoparticles, and then attaching them to the DNA at restriction-digested sites