Your Name: Last digit of your SID: ( )

MIDTERM EXAMINATION (October 15, 2009)
BIOE150. Introduction to Bio-Nanoscience & Bio-Nanotechnology
Professor Seung-Wuk Lee
Fall Semester, 2009

0. Write down your name and the last digit of your SID on all the pages (1)

1. *Briefly* define the following terminology ()

a. Nanometer (1):

10^{-9} meters

b. Surface Probe Microscope (3):

Nanoscale microscopy technique whereby a surface probe tip rasters across a surface while the interactions between the surface and tip are monitored to create an image

c. Surfactant Number (3):

Expression to predict the structure that a surfactant will self-assemble into:

Surfactant number = (volume of the headgroup)/(area of the headgroup)x(length) (3)

d. Beta Helix (2):

Peptide super-secondary structure where beta sheets separated by turns form a helix stabilized by beta-sheet type hydrogen bonds

e. FRET (3)

Förster/fluorescence resonance energy transfer. Can be used to determine when two objects are interacting/in proximity of each other. When certain chromophores are in close proximity the excitation of one (donor) causes the emission of the other (acceptor)

2. Peptide self-assembly (15)

DAR16-IV peptide with sequence NH_2-(DADADADARARARARA)-COOH can switch between alpha helical and beta sheet secondary structures depending on pH, ionic strength, and temperature.

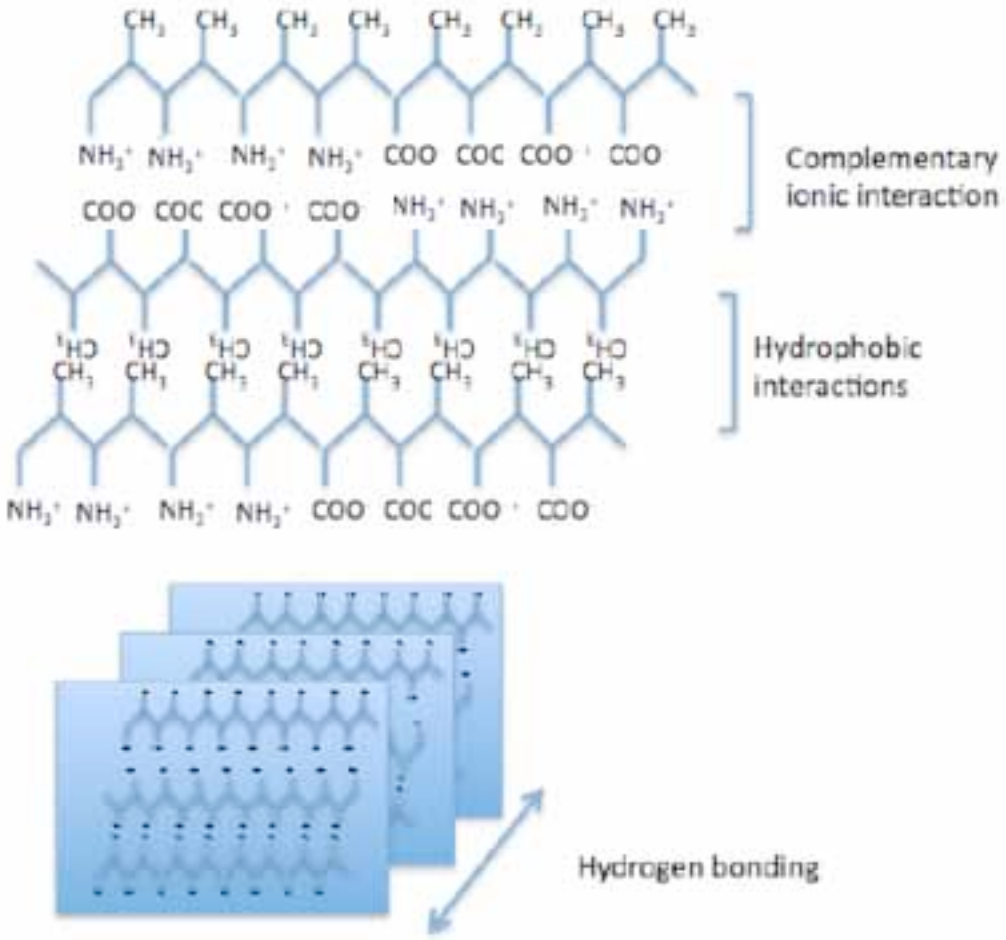
a. Circular dichroism (CD) is a method to determine the secondary structures of peptides or proteins. We can use CD spectra to follow the peptide conformational switch above. Describe the principle of how CD works? (4)

CD measures the difference in absorption of left and right circularly polarized light as a function of wavelength which occurs when linearly polarized light goes through an optically active sample. The spectra can be used to determine the presence of various secondary structures

b. RAD16-IV NH_2-(RARARARADADADADA)-COOH is composed of the same amino acid composition with DAR16-IV but with a different sequence ordering. However, RAD16-IV does not switch into an alpha-helix. Explain why. (3)

It is unfavorable for RAD16-IV to form an alpha helix compared to DAR16-IV because of charge repulsion between the Arginine and the N-terminus and between the C-terminal and Aspartic Acid. It is more favorable for DAR16-IV to form an alpha-helix because the N-terminal Aspartic acid helps neutralize the helix dipole

c. Draw a schematic of the self-assembled structure of RAD16-IV and label the intermolecular forces taking part in holding the structure together (5).



d. RAD16-I NH_2-(RADARADARADARADA)-COOH peptide has been known to form hydrogels which cells can be cultured within. For example, the RAD16-I peptide has been demonstrated to regenerate neuronal functions in hamster brain by "Nano neuro Knitting". Although DAR16-IV has the amino acid composition, its self-assembled structure shows significantly less cell adhesion. Explain why this might be (3).

RAD16-I has RGD like sequences throughout its backbone. However RAD16-IV possesses only one RGD like sequence.

3. Molecular Carpet (10)

a. RGDAAAAAC is a peptide that we can use in "molecular paint/molecular carpet" applications. What are the three basic components of this peptide's sequence and what are their roles. (6)

1. RGD: Bioactive peptide which can promote cell binding
2. AAAAA: Spacer peptide
3. C: Linking amino acid for self-assembly onto certain substrates

b. Using Dip Pen Nanolithography (DPN), we can fabricate arbitrary nanoscale peptide patterns on gold surfaces using our molecular carpet peptides. Explain the concept behind how DPN works. (4)

DPN is a direct-write scanning probe-based lithography method. A DPN tip coated in peptide containing solution can be directed to touch a surface in certain defined regions, depositing peptide onto the surface in the process where it can self assemble.

4. The following molecule is called a peptide amphiphile. 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. (14)

$CH_3(CH_2)_{15}$AGAGAGAEEEEIKVAV

a. Explain the role of the each component labeled 1-4 below (8).

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)

Two form nanofibers it must be a cylindrical micelle and therefore have a surfactant number between 1/3 and 1/2

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)

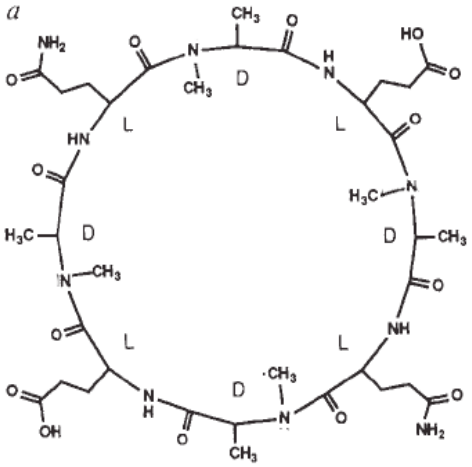
Need to increase the surfactant number to between ½ and 1 for example by using a two tailed alkane chain or by decreasing the size of the hydrophilic region

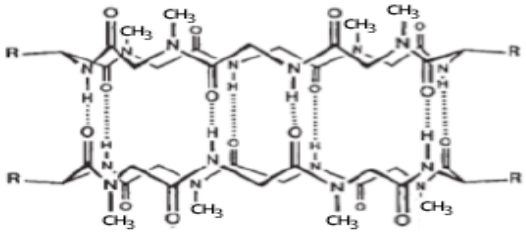
5. Various cyclic peptides such as Cyclo[(D-Ala-Glu-D-Ala-Gln)$_2$] are known to form ring-shaped nanostructure. These peptide rings can self-assemble by stacking to form nanotubes in the presence of acid. (10)

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

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).





It may form a dimer because the methyl groups will prevent H-bonding to further peptides

6. **Dr. Nadrian Seeman developed a high resolution DNA self-assembly approach to form periodic structure of the DNA crystals using synthetic DNA junctions. The Figure (a) is a schematic diagram of DNA crystal segments with 36 nt length corresponding to 12.6 nm in length (14).**

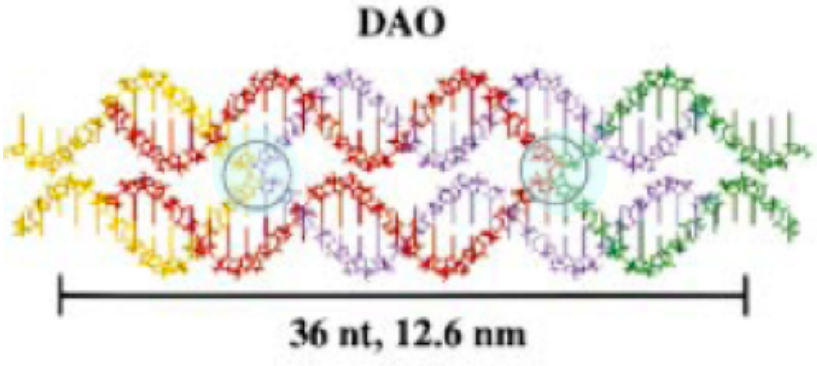


Figure (a) schematic diagram of DNA crystal segments

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 (4).

No sequence dyad symmetry flanking the branch point

c. Suppose that you will design a self-assembled DNA structure using A and B segments as shown in Figure (b). We already have the sequences that compose the A segment as shown in Figure (c) below. Design a set of sequences which can be used to create the B segment (7).

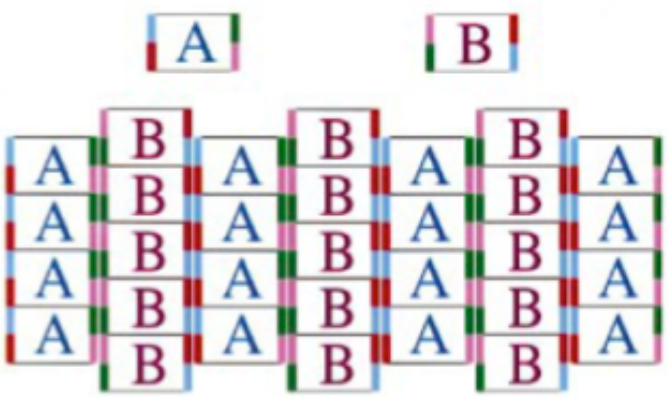
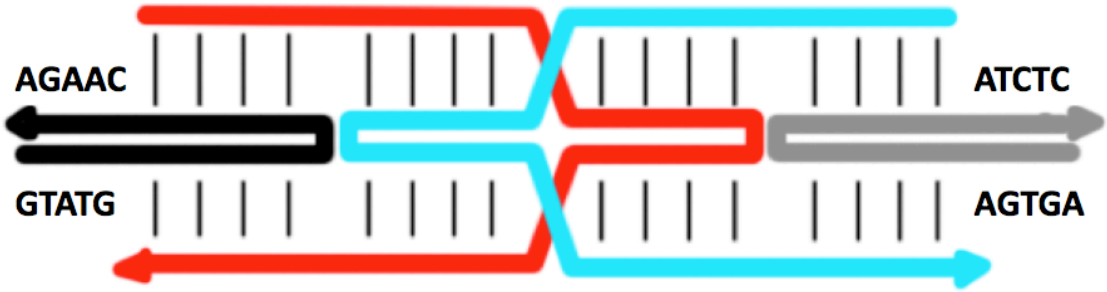


Figure (b) Self-assembled DNA crystals

Figure (c) Sequence design for A segment.



7. B-DNA is the most stable form of the DNA double helix structures with right handed conformation discovered by Watson and Crick in 1953. When B-DNA possesses large numbers of GC pair, a left-handed DNA double helix structure can be induced, which is called Z-DNA. By controlling ionic strength of suspension, we can switch right and left handed conformation between B-DNA and Z-DNA. Explain why ionic concentration plays a critical role in inducing the transformation between these two DNA structures. (3)

Z-DNA has a closely spaced phosphate backbone which is unfavorable due to charge repulsion unless screened by ions in solution.

8. Suppose that 5`-AGTACGATAAGTCCTA-3` is a DNA sequence from a bacteria associated with food poisoning. Using gold nanoparticles, design a scanometric DNA sensor which we can use for detection of the bacterial DNA using a flat-bed scanner. Explain how it works (8):

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

9. Phage Display (13)

Phage display is a combinatorial process to identify specific binding peptides against various targets using genetically engineered viruses such as M13 bacteriophage (phage). It can be used to identify specific recognition peptides which can be used to deliver therapeutics.

a. **Suppose we construct a phage library where each phage expresses a random seven amino acid (7-mer) peptide. What is the theoretical number of unique sequences in our library? (3)**

20^7

b. **Suppose we have a set of mice with tumors in their kidneys. Outline the procedure you would use to identify a specific peptide sequence which can bind to the kidney tumors. (6).**

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

c. **Suppose that, after *in vivo* phage screening with a 7-mer library against the kidney tumors we identify a peptide with the sequence sequences YIGSRFP. Calculate the theoretical probability of finding the YIGSRFP sequence from the original library (4).**

(2/64)(3/64)(4/64)(6/64)(6/64)(2/64)(4/64)

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Table: Genetic Code