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

MIDTERM EXAMINATION (October 23, 2008)
BIOE150. Introduction to Bio-Nanoscience & Bio-Nanotechnology
Professor Seung-Wuk Lee
Fall Semester, 2008

0. Write down your name and the last digit of your SIN in all the pages (2)

1. Define the following terms briefly:
Nanometer (2):

Unit of length equal to 1 x 10^{-9} m

Staple DNA (3):

In DNA orgami, a staple strand is a single stranded DNA sequence which hybridizes to sections of the scaffold DNA to physically connect the two sections.

B-DNA to Z-DNA switch (4)

Conformational change from typical B-DNA (right handed) structure to Z-DNA (left handed) structure which occurs for certain sequence (high G-C pairs) when its solution's ionic strength is increased.

Peptide amphiphile (4)

Amphiphilic amino acid containing molecule composed of a hydrophobic portion such as an alkyl chain and a hydrophilic peptide head self-assembles by formation of non-covalent bonds

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2. Explain the difference between a natural Holliday DNA junction and a synthetic DNA junction used to build DNA nanostructures. Specifically, why are synthetic junctions stable (5)?

Natural Holliday junctions occur during homologous recombination and have sequence homology at branch points allowing for branch migration. However, migration is prevented in synthetic junctions by avoiding sequence symmetry.

3. Explain the difference between top-down and bottom-up nanofabrication (give one example each) (6):

Top-Down: construction by selective removal of pieces of a larger material (2)

(ex: etching for photolithography process). (1)

Bottom-up: Construction by assembly of individual building blocks into a larger material (2)

(ex: self-assembling peptide amphiphiles, nanowire growth using gold nanoparticles…) (1)

4. What are the similarities and differences between the transmission electron microscope (TEM) and the standard light microscope? What are the characteristics of a sample that make it appropriate to examination by the TEM? (7).

Light microscopes are lower resolution and can be viewed by eye but TEM requires a phosphorescent screen or CCD camera.

Light microscope utilizes photons and optical lenses to image the objects.

TEM use electrons and magnetic lenses to image the objects.

TEM samples have to be thin and dried for imaging in high vacuum

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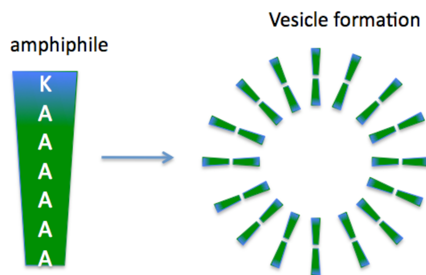
Table I. Sequence of peptides in single letter code.

1. RADARADARADARADA
2. DADADADARARARARA
3. AEAEAEAEAKAK
4. AAAAAAD
5. AAAAAAK
6. VVVVVVD
7. RADSAAAC
8. FEFKFEFK

5. Among the peptide sequences shown in the table 1, which sequence will form vesicles or nanotubes with positively charged surfaces in aqueous solution. Explain, briefly by drawing a schematic diagram (5).

Sequence #5: AAAAAAK

AAAAAAK possess surfactant-like properties with non-polar $(Ala)_6$ and (+)-charged lysine head group forms bilayer vesicles or tubes like as below.



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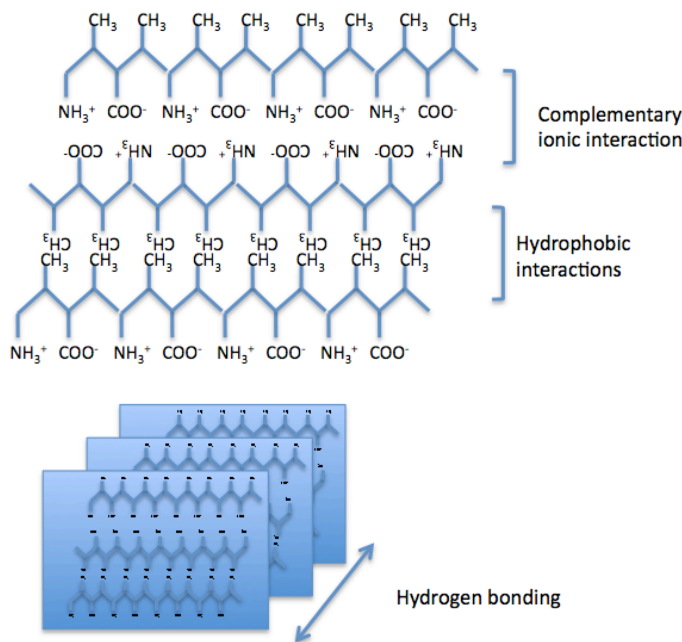
6. Among the peptide sequences shown in Table 1, which sequence will form a self-assembled hydrogel network structure, which can be used to culture various cells (4). How does this sequence form the networks (e.g. what secondary structure does it adopt, what type of bonds hold it together) (6)?

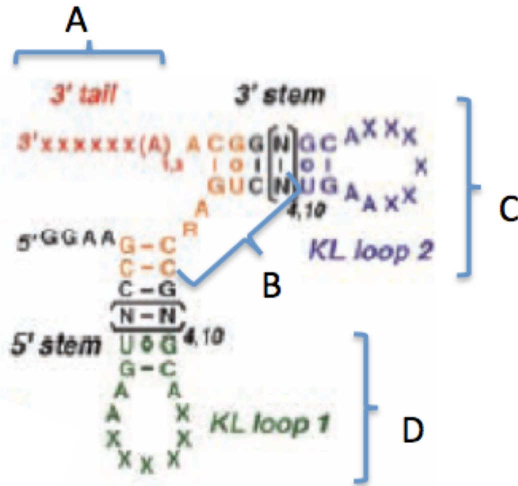
$(RADA)_4$-sequence #1 for cell culture due to similarity to RGD (4)

Adopts a beta-sheet structure held together by ionic bonds between Arg (NH3+) and Asp (COO-) and hydrophobic interactions between Ala (CH3). H-bonding occurs between layers

(6)



7. Label the four components of the RNA below which is used in the formation of RNA Jigsaw Puzzle nanostructures. Explain the role of each compoents A-D in the formation of nanstructures eand draw schematically how four such strands can form a rectangle. How can the lenghs of the rectangle sides be tuned?



A. Sticky end tail used to assemble RNA squares into larger ordered structures through specific recognition of complementary sequences.

B. Right angle motif constrains the angle between helices to 90 degrees

C and D: Hairpin kissing loops used to bind RNA components together through specific recognition of the complementary loop sequences.

Tune side lengths by altering 5' and 3' stem lengths

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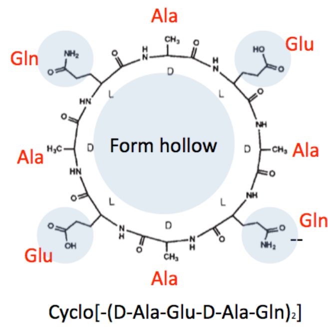
8. Cyclo[Ala-Glu-Ala-Gln- Ala-Glu-Ala-Gln] (the underlined amino acids correspond to the D form and the rest of them are the L form) is known to form a tubular nanostructure by peptide ring stracking based on circular beta sheet structures. These peptide rings can stack together to self-assemble peptide-nanotube.

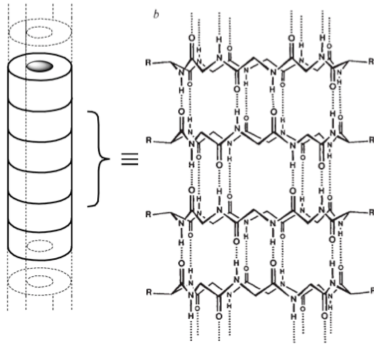
Draw a schematic diagram of the circular peptide (4).



Cyclo[-(D-Ala-Glu-D-Ala-Gln)$_2$]

Draw a schematic diagram of stacked circular peptide. What is the driving force for stacking. In the case of Cyclo [Ala-Glu-Ala-Gln- Ala-Glu-Ala-Gln] why does self-assembly occure only in the present of acid (7)

Driving force is intermolecular backbone-backbone hydrogen bonding as below:

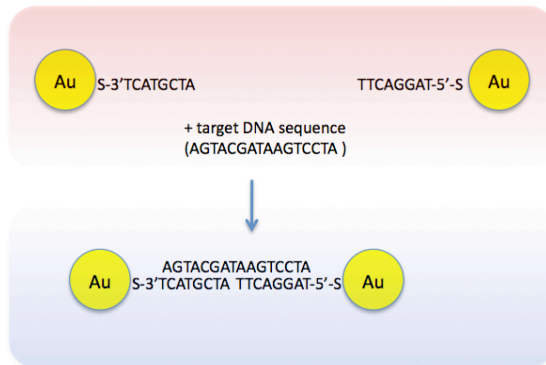


As pH decreases Glu side chains become protonated rather than negatively charged. Thereby, decreasing coulombic repulsion.

9. Suppose that "AGTACGATAAGTCCTA" is a marker DNA sequence related to bacterial food poisoning in frozen food. Using gold nanoparticles, design a colorimetric DNA sensor which can detect the target bacterial DNA (5). Explain how this colorimetric sensor works (5).

Assuming you have single stranded "AGTACGATAAGTCCTA".

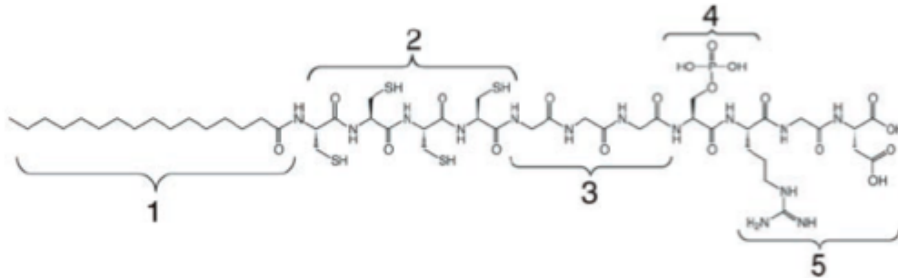
Take advange of colorimetric shift between freely soluble gold nanoparticles and aggregated gold nanoparticles (Red=> blue (or purple)). Use thiolated single stranded DNAs complementary to the target sequence to assemble on the nanoparticles surface.



Example shows two type of nanoparticles with DNA bound which is complementary to the two halves of the target sequence. The particles assemble, changing the light scattering properties of the solution resulting in a color change

(Can also use strategy involving disassembly of pre-aggregated particles)

10. The following molecule is called a peptide amphiphile. It can form a nanofiber-like structure in solution. The final fibrillar nanostructures also can induce formation of various minerals or regulate the growth patterns of various cells.



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

1. Hydorphobic alkyl chain forms the core of the nanofibers

2. Cysteine residues for disulfide bond formation

3. 3Gly: provides regular H-bonding for beta sheet formation

4. phosphoserine provides charge repulsion to make assembly responsive to pH and ionic strength. Also for nucleation of mineral by attracting positively charged metallic ions.

5. RGD motifs to promote integrin mediated cell binding

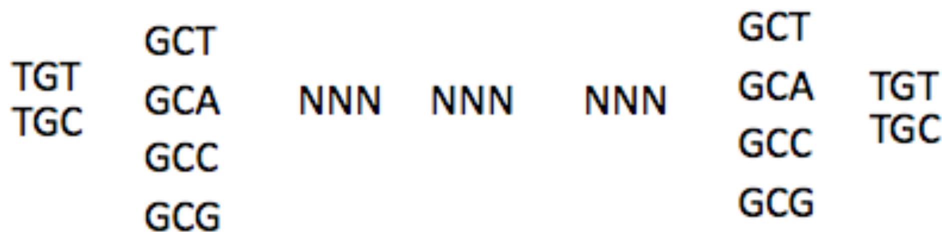
Explain how it forms nanofiber structures (5)

The hydrophobic tails aggreagate together due to the hydrophobic effect when the repulsion between the head groups are sufficiently screened. The mid-section amino acids form a beta sheet structure. The polar head group is selectively displayed on the surface, exposed to solvent.

11. 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 tri-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-Ala and Ala-Cys as below:

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

Design a DNA sequences which will express the target library sequences (label as N if it is a random nucleotide) (5).



Biotin-streptavidin binding is one of the well-established molecular conjugation pairs which are commonly used in biology. Using the phage library constructed above, suppose that we identified the specific binding motif sequence against streptavidin which can replace the function of biotin. The resulting sequence is Cys-Ala-His-Pro-Gln-Ala-Cys. Based on the assumption that we can construct a 100% unbiased peptide library through randomized gene insertion into the phage genome using the above approach, what is the probability to have the Cys-Ala-His-Pro-Gln-Al-Cys sequence emerge from the library (5).

$$\left(\frac{2}{64}\right)\left(\frac{4}{64}\right)\left(\frac{2}{64}\right)$$

$=1/16384=6.1 \times 10^{-5}$

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Table: genetic Code

First letter	Second letter: U		Second letter: C		Second letter: A		Second letter: G		Third letter
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G