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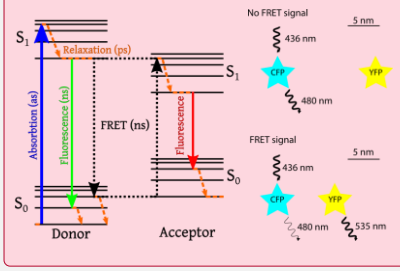
MIDTERM EXAMINATION (October 6, 2016)
BIOE150. Introduction to Bio-Nanoscience & Bio-Nanotechnology
Fall Semester, 2016

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

1. Define the following terms briefly: (each 4, total 20)

a) FRET

Commented [SL1]: Fluorescence Resonance Energy Transfer (1)
FRET is a mechanism describing energy transfer between two chromophores.
A donor chromophore, initially in its electronic excited state, may transfer energy to an acceptor chromophore through nonradiative dipole–dipole coupling.



b) DNA brick

short synthetic DNA strands that self assemble

c) Peptide amphiphile

Commented [SL2]: Amphiphilic molecule typically composed of a hydrophobic alkyl chain and a hydrophilic peptide head self-assembles by formation of non-covalent bonds

d) STM

information is acquired by monitoring the current as the tip's position scans across a surface

e) Staple DNA
oligonucleotides complementary to a scaffold template, designed to fold DNA into complexes

Name: SID: ( )

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. Structural transition between B- and Z-DNA can occur based on the ionic concentration of the environment making these structure transitions useful for molecular switch function. Explain how the transition works (4)?

Commented [SL3]: 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.

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

Commented [SL4]: Top-Down: construction by selective removal of pieces of a large (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)

Name: SID: ( )

Table I. Sequence of peptides in single letter code.

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

4. 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).

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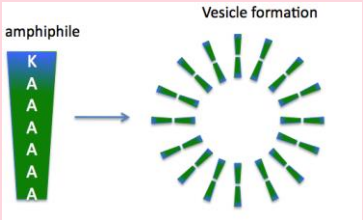
Commented [SL5]: Sequence: AAAAAAK
AAAAAAK possess surfactant-like properties with non-polar $(Ala)_6$ and (+)-charged lysine head group forms bilayer vesicles or tubes like as below.

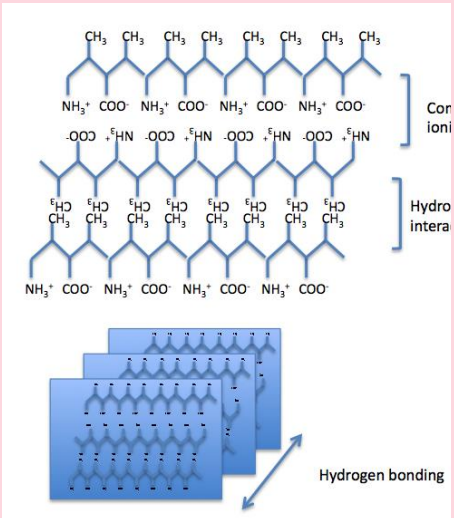


Name: SID: ( )

5. 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)?

Commented [SL6]: (RADA)4-seuqnece (4)
Adopts a beta-sheet structure held together by ionic bonds between Arg (NH3+) and Asp (COO-) and hydrophobic interactions between Ala (CH3).

(6)



Name: SID: ( )

6. In nature, many of biologically growing self-templating materials have a characteristic to grow in a very specific shape and pattern which can be explained by Fibonacci sequences. Using an example of abolone shell, explain how the sea shells can grow into spiral shape by drawing golden rectangle and golden spiral, and Fibonacci sequence (6).

Draw rectangle correctly
Write out Fibonacci sequence correctly

Using a golden rectangle, write down an equation related to golden ratio (phi) and solve the equation to obtain the phi value (4)

Commented [SL7]: From the golden rectangle, we know that: $\frac{(a+b)}{a} = \frac{b}{(a-b)}$
$\therefore a^2 - b^2 = ab$
$\therefore \frac{a}{b} - \frac{b}{a} = 1$
Assuming $(a/b) = \varphi$,
$\varphi - \frac{1}{\varphi} = 1$
$\therefore \varphi^2 - \varphi - 1 = 0$
Plug this into the quadratic formula and you get,
$\varphi = \frac{1 \pm \sqrt{5}}{2}$
Since the golden ratio is a positive number,
$\varphi = \frac{1+\sqrt{5}}{2} = 1.618\ldots$

Name: SID: ( )

7. Systematic evolution of ligands by exponential enrichment (SELEX) is an in vitro selection or in vitro evolution process, which is a combinatorial chemistry technique in molecular biology for producing oligonucleotides of either single-stranded DNA or RNA that specifically bind to a target ligand or ligands, called aptamers.

a) Describe the basic principle of the directed evolution by comparing with natural evolution (4).

Commented [SL8]:
Directed evolution – This is a relatively fast laboratory technique comprising diversification, selection, and amplification to find a biomolecule or an organism with desired properties. The process begins by creating a large library of gene variants and their mutagenesis during amplification, and ends with selection of a product with the best characteristics.

Natural evolution – This is a natural process comprising of variation, inheritance, and natural selection. All modern life forms have evolved from simple biological systems over millions of years of genetic mutations, inheritance of genes and survival of the fittest organisms.

b) Explain a working principle how SELEX can identify RNA aptamer sequences for a desired target (4).

Explain SELEX in detail including how the RNA library is established

c) Suppose that we will identify aptamers for AMP. Design an experimental procedure to discover selective AMP binding aptamers using SELEX by using ATP and ADP during the selection process (4).

+2 for general explanation

+2 for explaining that ATP/ADP serve as negative controls that aptamers shouldn't be able to bind to

Name: SID: ( )

d) Suppose that we will design more than a trillion of different kinds of diversity using SELEX process. What is the minimum length of combinatorial RNA sequence to cover a trillion diversity of RNA (4).

4^n = 10^12

N = 20

e) Suppose that the following is the AMP binding aptamer sequence for the AMP that was discovered though the SELEX process.

AGUACGAUAAGUCCUAAGUACGAUAAGUCCUA

Using gold nanoparticles, design a colorimetric DNA sensor which can detect the target AMP (~~5~~4). Explain how this colorimetric sensor works (~~5~~4).

+4 for correctly writing the sequences of the 2 sensors (as DNA, not RNA – RNA sensor got only 3 points)

+4 for correct explanation of how aggregation will cause color change of red → blue

Name: SID: ( )

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

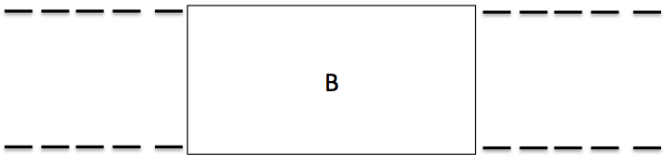
a) What does "DAO" mean in the above DNA structure? Explain briefly. (3)

Commented [SL9]: 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)

Commented [SL10]: No sequence dyad symmetry flanking the branch point.

c) Design a counterpart (B) that can enable self-assembly of DNA nanowire in coordination with (A). 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)



Commented [SL11]: GTATG… AGTGA
AGAAC… ATCTC

Name: SID: ( )

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).

Commented [SL12]: There can be many different answers:

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

2. Design a specific sequence to form loop every 25 nm (with GGGGG like sequence) and label this sequence with other complementary sequence (CCCC like) which is attached with gold nanoparticle or other contrasting reagent.

9. Suppose that we will design a DNA origami approach to design a DNA ruler that has a 100 nm in length and 20 nm in height. We will label every 25 nm with a mark with 5-nm gold tethered with stable DNA.

a) What can be a minimum length of single stranded DNA to design 100 nm x 20 nm based on the Dr. Paul Ruthemund design principle. Explain why (5)

100 nm/5.3 nm = 19

You have 19 1.5 turns in the x direction

19 * (16 nuc per 1.5 turn) = 304 nuc

3.1x + 2.1 = 20, x ~ 6, total number of row is 7

304 * 7 = 2128 nuc

Credit was given for math and numbers close to this

Name: SID: ( )

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(b) Describe your design in detail and justify how you will achieve the 100 nm x 20 nm DNA origami structure. Write down the first three staple DNA sequences (10 points).

+5 for explanation

+5 for correct staples

Name: SID: ( )

atagctaaac aggttattga ccatttgcga aatgtatcta atggtcaaac taaatctact
cgttcgcaga attgggaatc aactgttaca tggaatgaaa cttccagaca ccgtacttta
gttgcatatt taaaacatgt tgagctacag caccagattc agcaattaag ctctaagcca
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