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BIOE150. Introduction to Bio-Nanoscience & Bio-Nanotechnology

MIDTERM EXAMINATION (Oct 12, 2017)

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

TOTAL NUMBER OF POINTS = 110

1. Briefly define the terminology and describe the similarity and differences between the two terms: (**21**)

a) Phage Display and SELEX (6)

Both phage display and SELEX utilize directed evolution to identify a biomolecule with desired properties. Both begin with creating a large library (i.e. diversification), panning this library against a target of interest (i.e. selection), amplifying the binders (i.e. amplification), and repeating until a consensus of binders have been identified. (2)

Phage Display *– high-throughput screening process that identifies functional peptides. The process begins with a diverse library of bacteriophage that display peptides as fusion proteins on the phage coat surface. These bacteriophages are panned against a target of interest, washed to remove weakly-bound peptides/bacteriophages, eluted, amplified, and repeated until strong-binding peptide(s) have been identified. (2)*

SELEX *– a similar method to phage display with the exception of identifying RNA ligands (i.e. aptamers). SELEX (systemic evolution of ligands by exponential enrichment) begins with a randomized DNA library that is first transcribed to RNA, which forms unique structures that are capable of binding a target of interest. The RNA library is then exposed to target for selection. Loosely bound RNAs are washed away and bound RNA are eluted then enriched by reverse-transcribing the RNA binders to cDNA and amplifying using PCR. The selection step is repeated until consensus sequence(s) have been reached and strong RNA aptamer binder(s) have been identified. (2)*

b) AFM and STM (5)

Both AFM (atomic force microscopy) and STM (scanning tunneling microscopy) are subtypes of scanning probe microscopy techniques, which use a fine tip probe to scan across the surface of a sample that can then help generate an image of a nanoscale structure. (1)

AFM measures the electrostatic forces between the tip and the sample. (2)

STM measures the electrical current flowing between the tip and the sample. (2)

c) DNA Origami and DNA Brick Approaches (5)

Both DNA origami and DNA brick approaches can be utilized to self-assemble larger DNA structures (1).

The DNA brick approach employs short synthetic DNA strands that self assemble due to complementary sticky ends. (2)

DNA origami, on the other hand, uses short staple strands that are complementary to a scaffold template to fold a long single-stranded DNA into a desired shape. (2)

d) Bottom-Up and Top-Down Approaches (5)

Both bottom-up and top-down approaches can be used to make nanostructures. (1)

Bottom-up approaches rely on the arrangement of smaller components into a larger or more complex assembly (ex: self-assembly of DNA, self-assembly of peptide amphiphiles, nanowire growth, dip-pen lithography, etc.). (2)

Top-down approaches start from a bulk material and selectively remove sections until a desired smaller feature has been achieved (photolithography, E-beam lithography, Focused ion beam processes). (2)

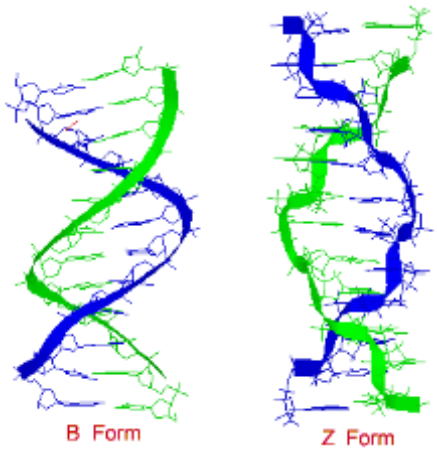
Your Name: SID: ( )

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. (**18**)

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

B-Form DNA:

- *Right-handed (1)*
- *Smooth phosphate backbone (1)*
- *10 bp/turn*
- *0.34 nm/bp*

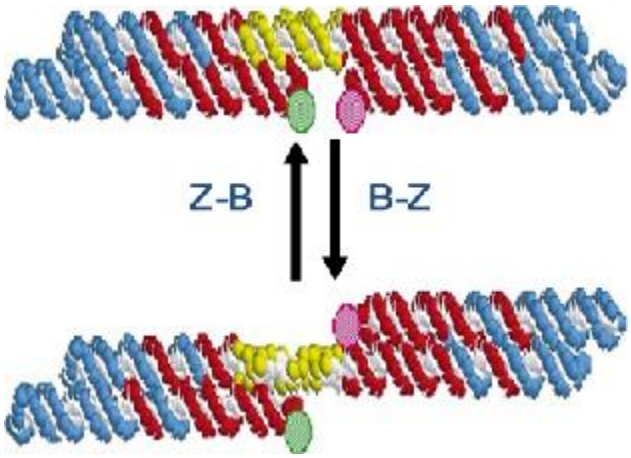


Z-Form DNA:

- *Left-handed (1)*
- *Zig-zag backbone (1)*
- *$(GC)_x$ repeats (1)*
- *Salt ions shield charged phosphate backbone (1)*
- *12 bp/turn*
- *0.37 nm/bp*

b) Using the B-Z DNA switch, we can design a molecular optical switch that can sense the salt ion concentration. Explain the principle behind this optical switch and how it operates in low and high ionic concentrations. (6)

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



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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^{o} 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 FRET acceptor and donor (1)

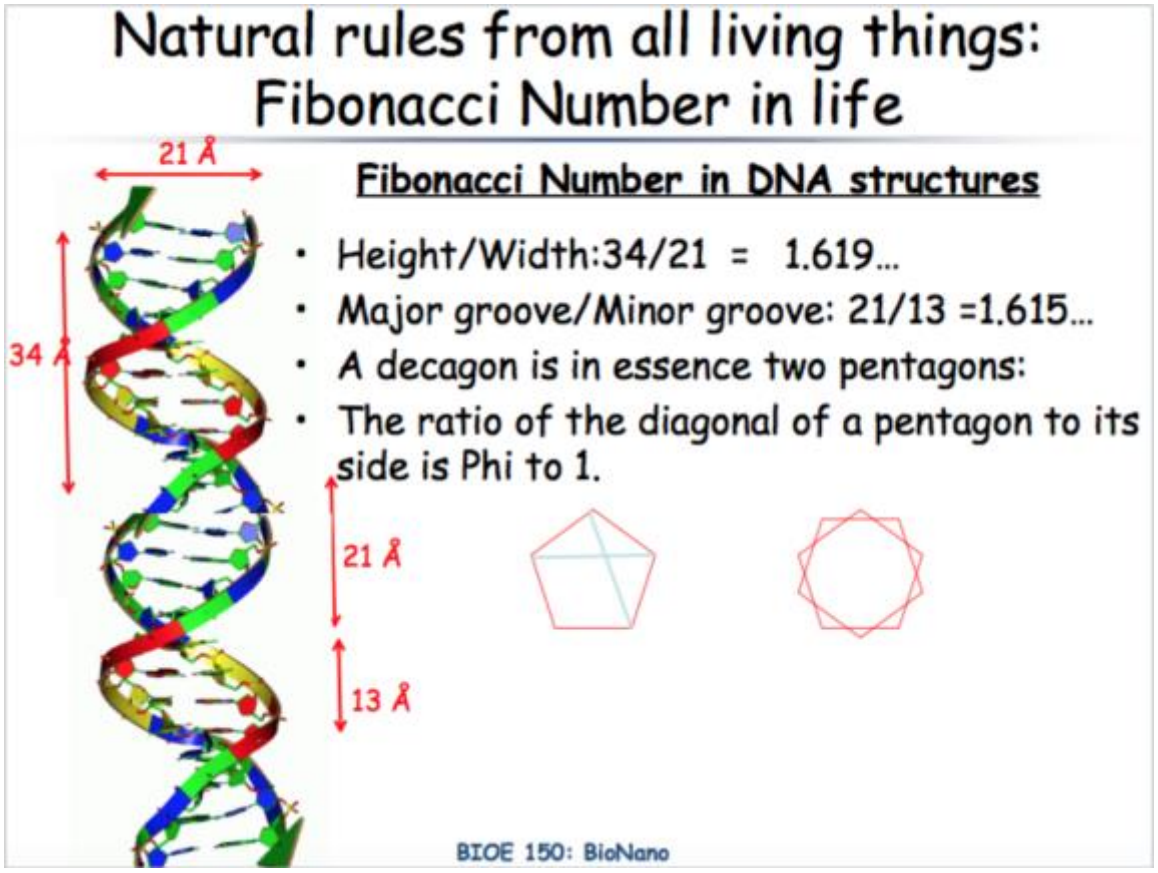
Turning of DNA/induced rotation in B-Z DNA transition (1)

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 (2)

Your Name: SID: ( )

c) Many biomaterials exhibit characteristic features of Fibonacci number in their structures. Draw brief schematic figures of side and cross sectional views of the DNA double helix and describe the features of Fibonacci numbers in B-form DNA (6).



Grading schematic:

Describe Fibonacci sequence (2)

Draw B-Form DNA, both side (1) and cross-sectional views (1)

Relate DNA features to Fibonacci sequence/golden ratio (2)

Your Name: SID: ( )

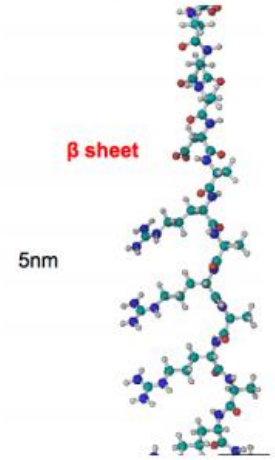
3. The following molecule (DAR16-IV) is a peptide that exhibits both alpha-helix and beta-sheet structures depending on pH and temperature. pK_a of Asp and Arg side chains are 3.90 and 12.48, respectively. (30)

DAR16-IV: DADADADARARARARA

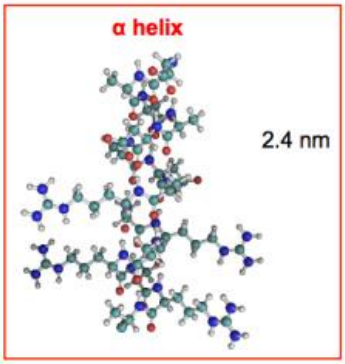
a) Draw the schematics of alpha helix and beta sheet structure of DAR16-IV. Indicate the possible charge of N-terminal and C-terminal and their charged side chains at pH 7.5 and indicate what is the major force to make them stabilize the alpha helix and beta sheet structures (6)

beta sheet here **alpha helix here**

beta sheet here



alpha helix here



Grading schematic:

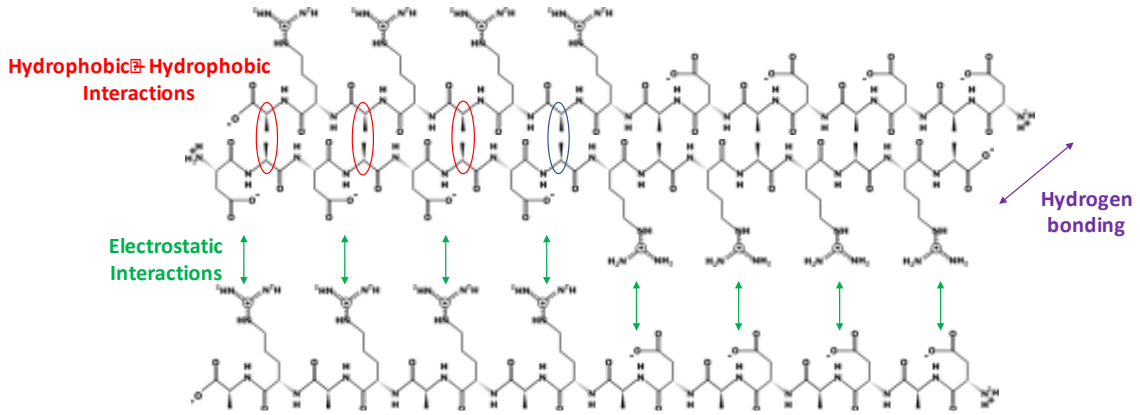
Draw correct peptide structure (1)

Draw beta sheet (2)

Draw alpha helix (2)

Identify intermolecular vs intramolecular hydrogen bonding (1)

b) When beta sheet structure is stabilized at certain conditions, draw the schematic diagram of three weak interactions (H-bonding, hydrophobic-hydrophobic interaction, electrostatic interaction), leading the DAR16-IV to the expected self-assembled structure. (6)



Grading schematic:

Identify electrostatic interactions between charged side groups (2)

Identify hydrophobic-hydrophobic interactions (2)

Identify hydrogen bonding between sheets (2)

c) Urea is a well-known chaotropic agent that blocks hydrogen bonding in aqueous solution. When you add high concentration of the urea to the above self-assembled solution, what is the expected change of the self-assembled structures and draw a schematic diagram (4).

In weakening/blocking the hydrogen bonding, you prevent the peptide from assembling into the z direction. (2)

Therefore, the peptide will assemble as a nanosheet in one plane but not assemble into another plane. (2)

d) At pH 1 condition, what are the expected charge changes in the DAR 16-IV molecules from pH 7.5. As a result, what structure is expected to form? (4).

DAR16-IV: DADADADARARARARA

At pH = 7.5,

$NH3^+$ - DADADADARARARARA – COO^-

- - - - + + + +

At pH = 1,

- *you lose the negative charge of the aspartic acid (D) (1)*
- *C terminus is protonated (1)*
- *peptide no longer can assemble via complementary electrostatic interactions between D and R (1)*
- *Positive charges will result in repulsion -> might be more favorable to form alpha helix (1)*

e) At high temperature (higher than 60°**C**), what structure is favored? Explain why. (4)

At high temperature, the alpha helix conformation is favored. (2)

*The increase in thermal energy disrupts **intermolecular** hydrogen bonding, favoring **intramolecular** hydrogen bonding. (2)*

(f) RAD16-I and RAD16-IV are known to support tissue growth on the surface of self-assembled peptide nanostructures. When DAR16-IV is self-assembled into the fibrillar structure, can we expect them to support tissue growth? Compare the properties of RAD16-I and RAD16-IV with DAR16-IV and describe the reason for the possibility of your answers. (6)

RAD16-I: RADARADARADARADA

RAD16-IV: RARARARADADADADA

DAR16-IV: DADADADARARARARA

Grading schematic:

Write out correct peptide sequence (1 point for each x 3)

Identify and explain why RAD is similar to RGD -> charged residues and A -> G (2)

DAR16-IV will most likely not interact with cells as it has a negatively-charged half and a positively-charged half. There is only 1 RAD/RGD resembling sequence BUT the N and C terminus are also switched. (1)

Your Name: SID: ( )

4. The following **molecule I** is called a peptide amphiphile developed by Dr. Samuel Stupp for the purpose of biomedical engineering. This peptide amphiphile is able to form a fibrous nano-scaffold, which is able to direct the growth of neural stem cells. (**16**)

Molecule 1: $\underline{CH_3(CH_2)_{15}}$GGGAAAAEEEEIKVAV (underline is alkyl chain and all other letter is representing amino acid in a single letter code)

a) Explain the role of each component labeled 1-5 below (4).

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

Hydrophobic alkane chain (1)

2. GGGAAAA

Flexible spacer and making a shape control (1)

3. EEEE

Depending on the charge controlled by pH, they can assemble and dissemble the nanostructure (i.e. tunable feature). (1)

4. IKVAV

Hydrophilic head group/cell-adhesion peptide (1)

b) The molecule I is self-assembled at a certain pH and ionic concentration. Describe the expected interaction between the molecules and their self-assembled structure when in deionized water vs. a 150 mM NaCl solution at pH 7.5 (4).

At pH 7.5, 4E have (-) charges, and the molecules repulse each other in deionized water. (2)

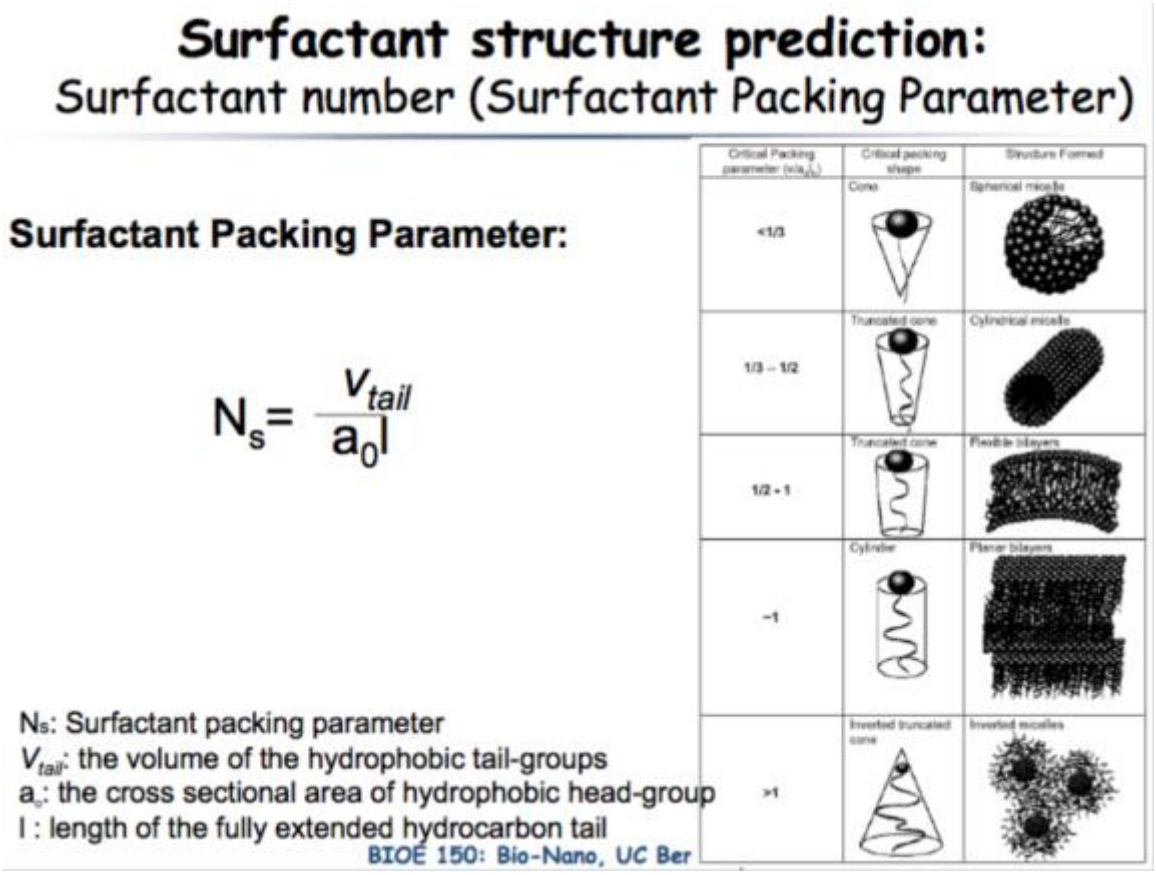
In the 150 mM NaCl solution, the charge is shielded by Na+ ions, and the molecules begin to self assemble into the fiber structures (2).

c) Using surfactant number, explain why it assembles into nanofiber structures. (4)

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

Grading schematic:

1. *Equation for calculating surfactant number (1)*
2. *Nanofiber structure => cylindrical micelle (1)*
3. *Cylindrical micelle => surfactant shape is a truncated cone (1)*
4. *Truncated cone => surfactant packing parameter = 1/3 – ½ (1)*



d) 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? (4)

Increase V_{tail} such that the surfactant number increases to ½-1. (2)

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. (2)

Your Name: SID: ( )

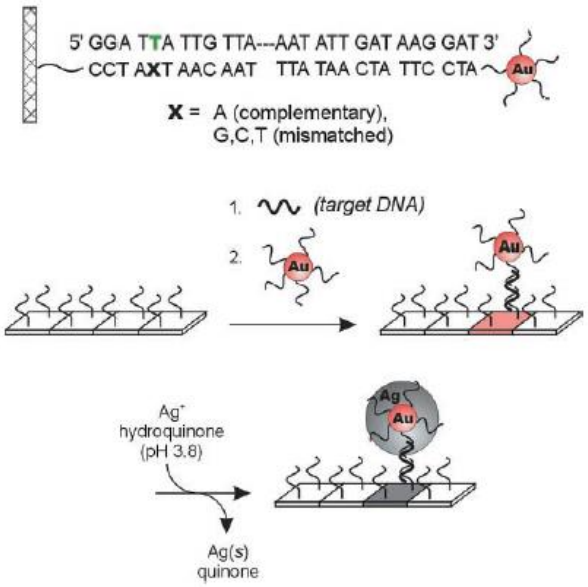
5. Suppose that "GGC UUA UUG UUA AAU AUG GAU AAG GAU" is a unique RNA sequence to distinguish Zika virus. Suppose that you will design **RNA-based sensor system**. However, your budget for developing the sensor is limited. You only can purchase **RNA sequences, gold nanoparticles or plates, some chemicals.** In addition, you will use your recent iPhone, which can read out the high-resolution contrast in 16 bits. Using the conditions given above, design a sensor that allows you to detect and quantify the Zika virus and explain how it works. Suppose that the above sequence does not form any secondary structures (10).

Target: 5' - GGC UUA UUG UUA AAU AUG GAU AAG GAU – 3'

We are given that the target sequence does NOT form any secondary structure -> NOT a RNA aptamer -> need to design a sensor that is specific to sequence

Complementary: 3' – CCG AAU AAC AAU UUA UAC CUA UUC CUA - 5'

Half the complementary sequence is immobilized on a surface. The other half of the complementary sequence is immobilized on gold nanoparticles with the 5`end attached to the nanoparticle by incorporating a terminal thiol group. In the presence of the Zika virus 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. (Below is example figure from class)



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

What is the target (1)

Design of strands correctly (1 point each x 2)

Mention that half are immobilized on surface (1)

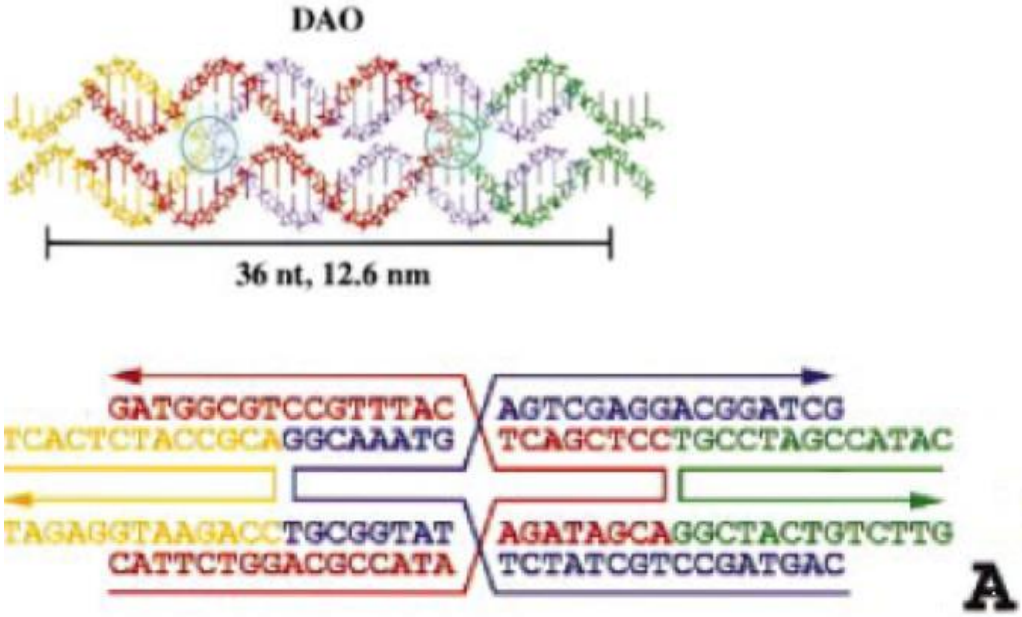
Half are immobilized on Au NPs using gold-thiol chemistry (1)

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

Staining with silver ions allow for visualization with flatbed scanner (2)

Your Name: SID: ( )

6. 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 a 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 (1)

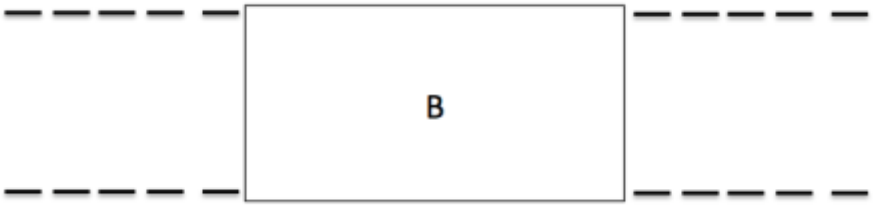
A: Dyad axes anti-parallel to the helical axes (1)

O: Odd number of half-turns between crossovers (1)

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

No sequence dyad symmetry flanking the branch point (3)

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)**



5' GTATG (1)……..AGTGA 3'(1)

3' AGAAC (1)……..ATCTC 5'(1)

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).**

Note: there are a variety of different answer that would work

Possible answers:

- *1. Use a DNA hairpin loop itself as a defining feature (incorporate into B DNA segment sticky end)*
- *2. Create a hairpin loop that can bind a complementary sequence conjugated to a gold nanoparticle/contrast agent*
- *3. Incorporate a fluorescent tag on one of the right sticky ends to B DNA segment*

Grading schematic:

- *Contrast strategy (3)*
- *Place contrast agent on B DNA segment (2)*