Name with Last Name, First: **Student ID:**

BIOE111: Functional Biomaterial Development and Characterization
Mid-Term EXAM (March 23, 2017)

Question 0: Fill in your name and student ID on each page. (1)
Question 1: Briefly define the following terms (15):

a) SELEX (**3 points**)

b) SPR **(3 points**)

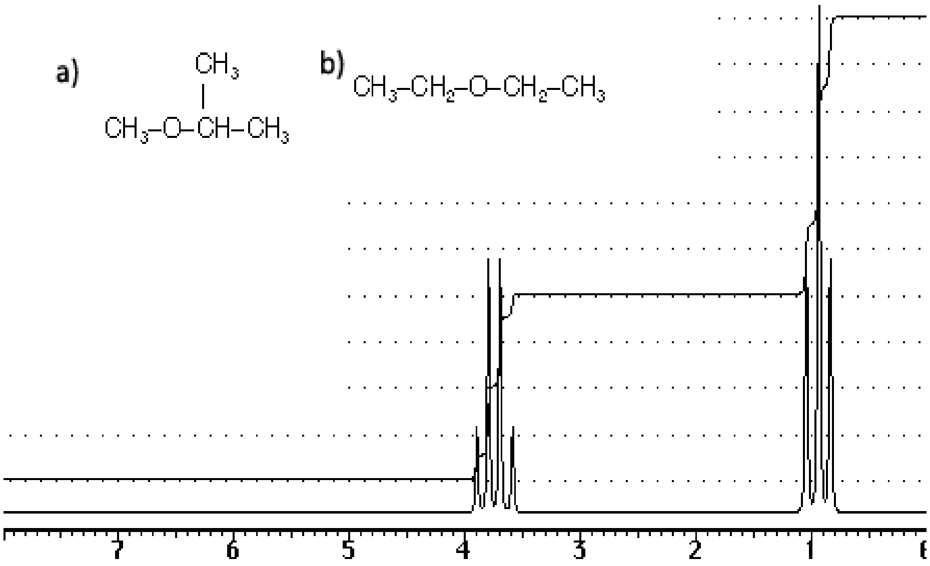
c) Shotgun DNA sequencing (**3 points**)

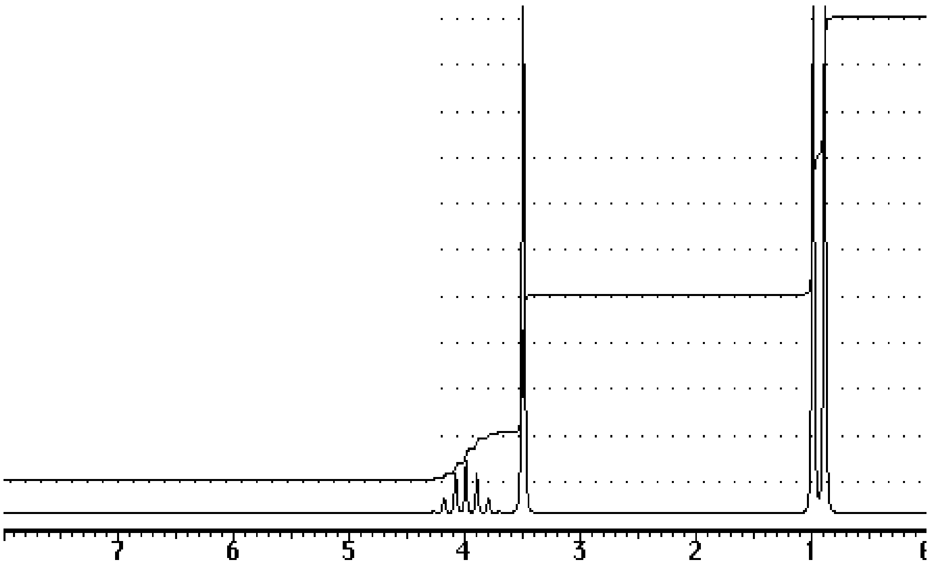
d) ITC (**3 points**)

e) FRET (**3 points**)

Question 2. NMR (Total 15 points)

a) Structures a and b were analyzed by H-NMR. Which structure is associated with which spectrum? Assign all proton in the spectrum (**6 points**)





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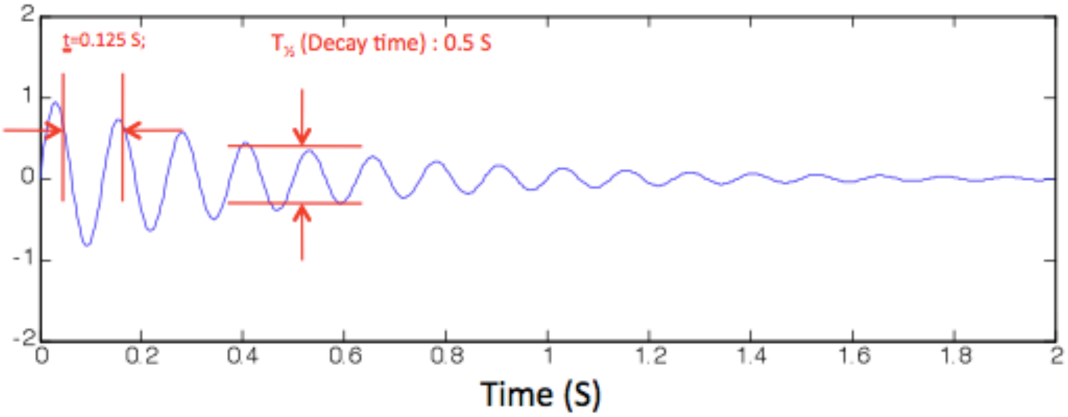
b) When we illuminate the frequency of the light corresponding to 3.4 ppm on the sample to exhibit the 2nd NMR spectrum in (a), what will be the expected changes of the NMR spectrum and explain why (**5 points**).

c) Sketch the frequency spectrum of the time-varying signal shown below. Label the frequency axis with units. Solve for the width at half-height of any peaks (**4 points**).



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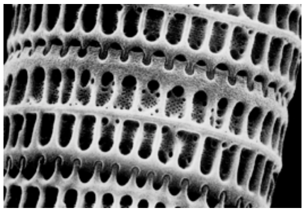


Figure 1. Picture of a diatom

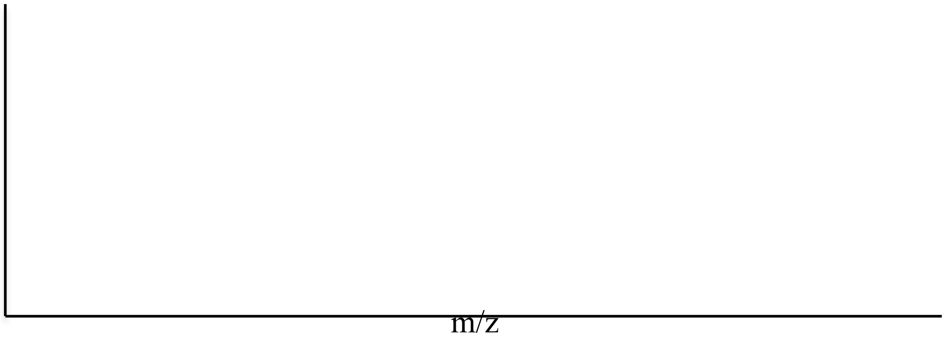
Question 3. Diatoms are a single-celled algae with characteristic rigid cell walls called 'frustules'. Frustules are composed of silicon oxide made through protein-templated biomineralization. The protein of interest was discovered and named "silaffin" Answer the following questions related to this system.
(Total 44 points)

a.) Name two methods that could be used to determine the DNA sequence and Protein sequence of silaffin. Briefly describe how they work. (**6 points**)

b.) You discover the DNA sequence for silaffin. You can extract the proteins from the diatoms but you also decide to express it recombinantly in E. Coli. Describe the methods how to express the desired sillaffin protein (**5 points**).

c) Suppose that you will use HPLC to purify the sillafin. Describe instrument setup for the HPLC and explain how it work. (**5 points**)

c.) Suppose that you obtain the proteins after the purification. The molecular weight of the expected silaffin is 31.8kilodaltons based on the primary sequences. Draw an expected MALDI-TOF Mass Spectrometer data with at least 3 peaks assuming it can be ionized multiple times (**3 points**).

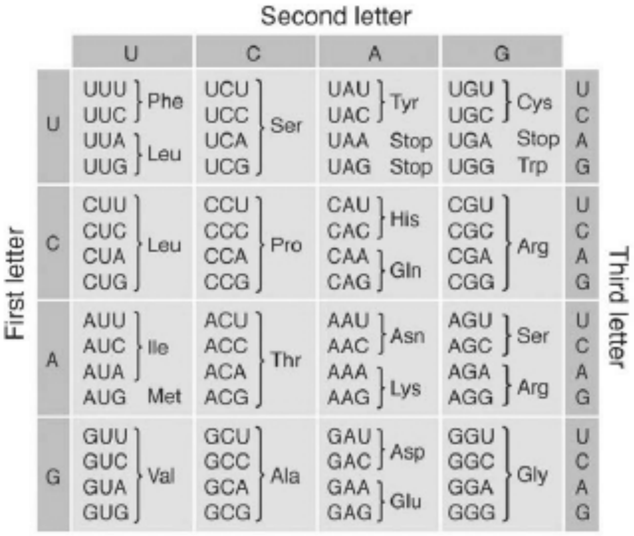


d.) Derive an equation for the transit time of an ion in a time-of-flight based mass spectrometer which has a drift tube length of length, L. Define the variables you use (**5 points**).

e.) We would like to use a peptide library to discover a synthetic silaffin like peptide which binds silicon oxide. Our library should be 5 amino acids in length and be able to incorporate the 20 natural amino acids as well as phosphoserine, a serine which is modified with a phosphate group. Describe how you would go about creating this library (**5 points**), and determining the sequence of the best binder. **(5 points),** and how to sequencing the resulting tendem mass (MS/MS) spectrometry (**5 points**)**.**

f.) Using the method you chose from part e, suppose the sequence you found from N-terminus to C-terminus is SKGRR. Calculate the probability of choosing this peptide at random from your library **(5 points)**.

Genetic Code



	Second letter								
First letter	U		C		A		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

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Question 4: Suppose that there is a single stranded DNA that can form a double helix with itself separated by a loop in the middle (hairpin). It exhibited the following melting temperature profiles measured with a UV spectrophotometer. (**25 points**)

Temperature (Celsius)	**A260**
10	0.66
20	0.66
30	0.70
35	0.78
38	0.88
40	0.99
42	1.10
45	1.20
50	1.26
55	1.30
65	1.32
75	1.32

(a) What is the approximate melting temperature of the double helix (**3 points**)

(b) What is definition of the equilibrium constant for the melting transition (**3 points**)

(c) What is approximate numerical value of the equilibrium constant at 50^{o}C (**3 points**)

(d) What quantifies could be plotted to obtain an estimate of ΔH and ΔS for the double helix to single stranded unfolded transition. Derive the equation. (**4 points**)

(e) Calculate of ΔH and ΔS value from the above transition (6 points).

(f) What is the meaning of the ΔH and ΔS for the above DNA melting process (6 points).