

National Exams May 2015

04-Bio-B10, Analytical Biochemistry

3 hours duration

NOTES:

1. If doubt exists as to the interpretation of any question, the candidate is urged to submit with the answer paper, a clear statement of any assumptions made.
2. This is a CLOSED BOOK EXAM.
Any non-communicating calculator is permitted.
3. FIVE (5) questions constitute a complete exam paper.
The first five questions as they appear in the answer book will be marked.
4. Each question is of equal value.
5. Most questions require an answer in essay format. Clarity and organization of the answer are important.

Q	Mark
1	/20
2	/20
3	/20
4	/20
5	/20
Total	/100

Marking Scheme:

Question 1: (20 marks total) Affinity Purification and SDS-PAGE

- a. (4 marks)
- b. (7 marks)
- c. (9 marks)

Question 2: (20 marks) Polymerase Chain Reaction (PCR)

- a. (5 marks)
- b. (5 marks)
- c. (5 marks)
- d. (5 marks)

Question 3: (20 marks total) Infrared/Fourier Transform Infrared Spectroscopy (IR/FTIR)

- a. (8 marks)
- b. (4 marks)
- c. (4 marks)
- d. (4 marks)

Question 4: (20 marks total) Atomic Force Microscopy (AFM)

- a. (8 marks)
- b. (4 marks)
- c. (8 marks)
 - i. (3 marks)
 - ii. (5 marks)

Question 5: (20 marks total) Flow Cytometry

- a. (10 marks)
- b. (10 marks)

Question 6: (20 Marks Total) High Performance Liquid Chromatography

- a. (15 marks)
 - i. (3 marks)
 - ii. (7 marks)
 - iii. (5 marks)
- b. (5 marks)

Question 1: (20 marks total) Affinity Purification and SDS-PAGE

You are purifying a 30 kDa polypeptide containing no cysteine amino acids from solution. You decide to use an antibody-based affinity capture methodology that uses antibodies, which have very specific affinity for your polypeptide, linked to magnetic nanoparticles. Once the capture step is complete, the magnetic nanoparticles are removed from the original solution (using a magnet) and after several wash steps, you attempt to release your protein from the antibody. To check the capture and purity of the polypeptide you decide to run the sample that you suspect now contains the polypeptide on SDS-PAGE. You find two bands, one at 30 kDa and the other at 150 kDa. You suspect the second band to be the antibody.

- a) (4 marks) What would you use to visualize the proteins in your gel? Explain.
- b) (7 marks) Sketch what the gel would look like with the two bands. Include the loading wells in your sketch as well as where you would expect the anode and cathode to be located. How did you determine the size of the proteins in the two bands? If possible add how you determined the size in your sketch.

You decide that adding dithiothreitol (DTT) or β -mercaptoethanol and heating your sample before adding it to the gel may give you a better indication if the second band was indeed an antibody.

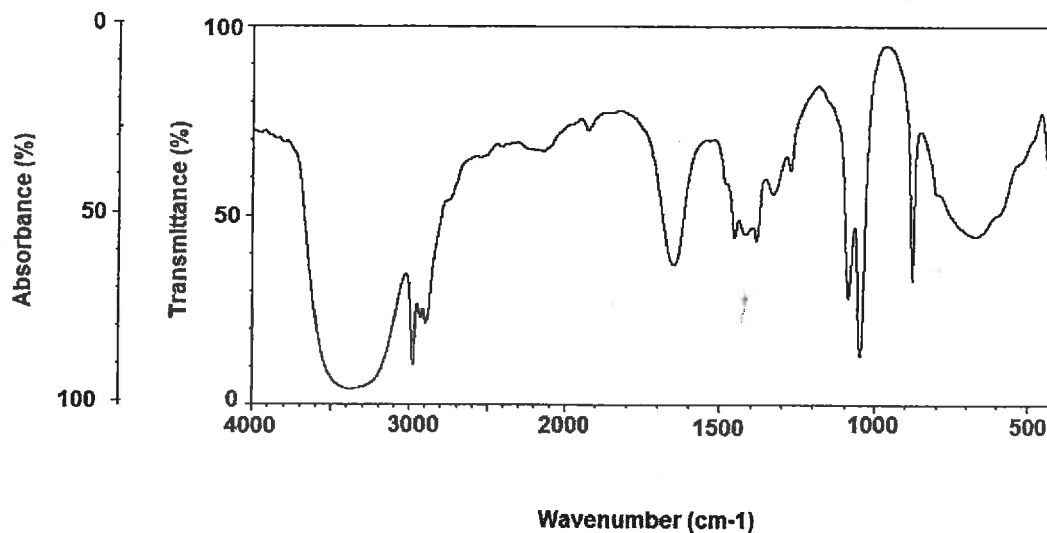
- c) (9 marks) Sketch and explain what the gel would look like after running the samples containing either DTT or β -mercaptoethanol. Include the loading wells in your sketch. Label any and all bands that might appear in this new gel.

Question 2: (20 marks total) Polymerase Chain Reaction

- a) (5 marks) Explain two characteristics that should be considered when designing primers for PCR.
- b) (5 marks) What is the Taq enzyme and what makes this enzyme “somewhat” unique?
- c) (5 marks) Why is $MgCl_2$ added to a polymerase chain reaction mix containing Taq enzyme?
- d) (5 marks) If a PCR is run for 35 cycles, and the initial copy number of your template DNA was 12, how many strands of DNA would you expect to have (assume perfect efficiency)?

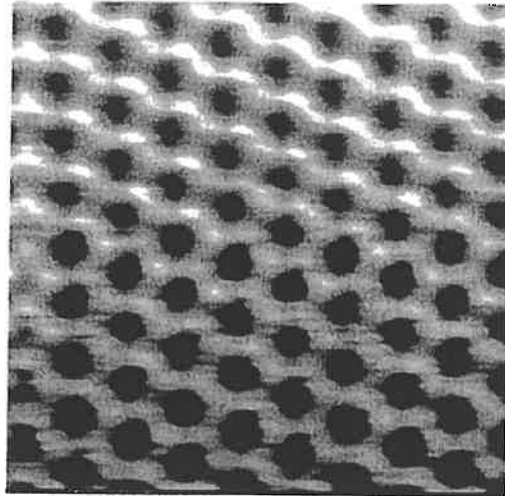
Question 3: (20 marks total) IR/FTIR

- a) (8 marks) What is FTIR used for and why is it called an absorption technique?
- b) (4 marks) Does N_2 have an infrared spectrum? Why?
- c) (4 marks) What is an interferogram?
- d) (4 marks) What type of molecule does the following IR spectrum represent? What makes you draw that conclusion?

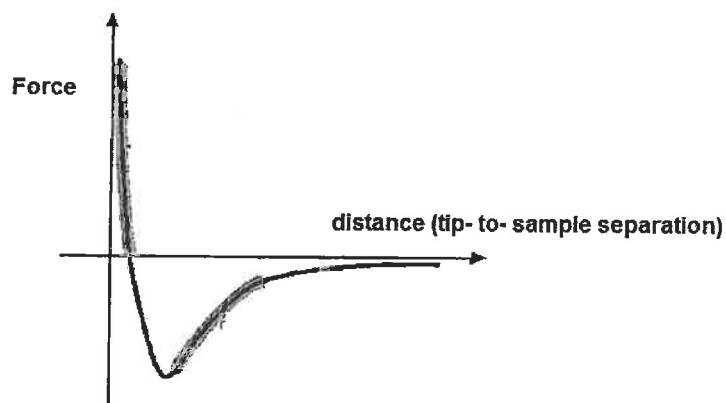


Question 4: (20 marks total) Atomic Force Microscopy

- a) (8 marks) Sketch a surface, an AFM probe (cantilever tip) and the detection system (laser/photodetector).
- b) (4 marks) In the AFM image below, explain how you would interpret light and dark regions.

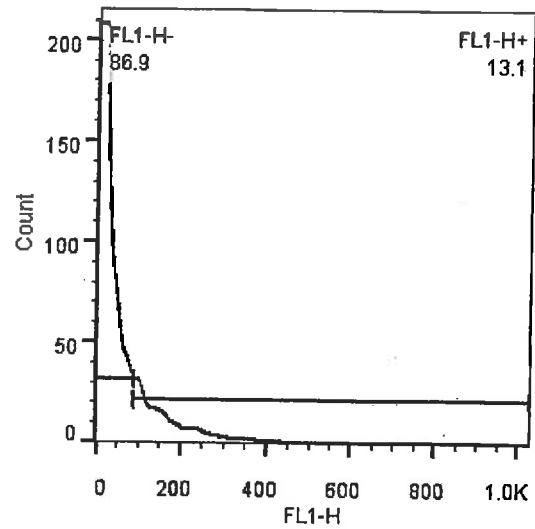
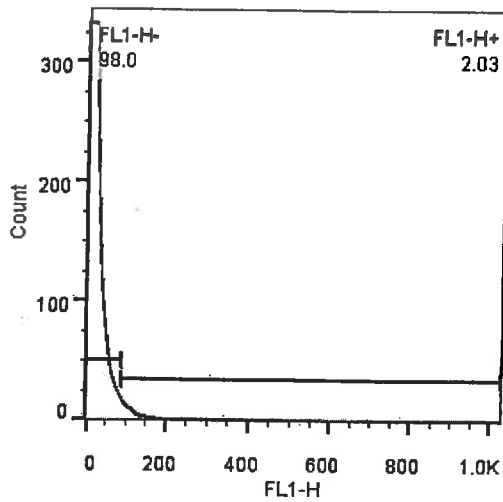


- c) (8 marks total) Van der Waals forces are said to be one of the predominant forces at play in atomic force microscopy.
 - i. (3 marks) Explain van der Waals forces.
 - ii. (5 marks) Explain the following graph representing the forces at play on the AFM tip in terms of attractive and repulsive forces.

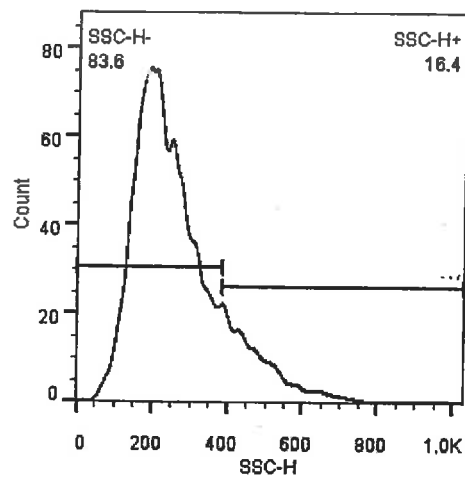
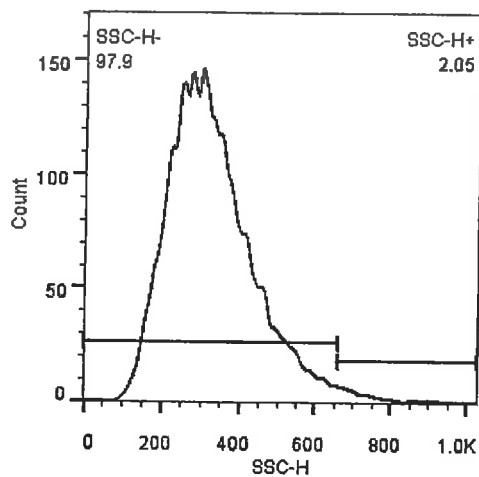


Question 5: (20 marks total) Flow Cytometry

- a) (10 marks) The two histograms below represent the same population of cells before (left) and after (right) treatment (transfection of plasmid DNA containing a GFP gene under the control of a strong constitutive promoter). How effective was the transfection? Explain.



- b) (10 marks) The graphs below represent the same cells as those in a) (pre- and post- treatment – left and right respectively); however, the graphs below are looking at the side scatter of the cells. Explain what is meant by side scatter i.e. how is side scatter information collected and what does side scatter represent in terms of a cellular characteristic.



c) Question 6: (20 Marks Total) High performance liquid chromatography (HPLC)

- a) (15 marks total) Ion exchange chromatography is one of the most utilized methods in high performance liquid chromatography.
- i. (3 marks) What is meant by a “weak” ion exchange matrix?
 - ii. (7 marks) Upon observing the chromatogram obtained by eluting protein from a cation exchange column (UV detection at 280 nm), it was seen that many peaks were overlapping. What strategy could be used to make sure the peaks did not overlap?
 - iii. (5 marks) Your boss wants you to change your elution profile from a linear gradient to a step gradient. What will be the consequences of this change?
- b) (5 marks) What is reverse phase chromatography?