

UNIVERSITY of TORONTO
Faculty of Arts and Science

December 2012 EXAMINATIONS

BCH 425H1 F / JBB1425

Duration - 3 hours

Examination Aids: You may use non-programmable calculators, compasses, protractors and rulers (supplied by the student).

Approximate times for each question are given and are equivalent to the point value of the question. Therefore, the total number of points available is 180. Check your time for questions so that you evenly apportion time to each. This exam will count as 45 % of the final mark of this course.

There are 13 questions in the exam on 6 pages (including this one). There are two sections: (1) X-ray Crystallography and Electron Microscopy, and (2) NMR Spectroscopy and Fluorescence Spectroscopy & Scanning Probe Microscopy. **Please write your response to each section in a separate examination booklet (i.e. use at least 2 exam booklets).** You should write all your answers in the examination booklets and not on this exam sheet.

Be sure to print your **NAME** and **STUDENT ID** at the appropriate place on each examination booklet. Try to write clearly. If your answer cannot be read, it cannot be marked correctly.

Course Coordinator: John Rubinstein (john.rubinstein@utoronto.ca)

X-ray section (Prof. Howell)

PLEASE ANSWER QUESTIONS FROM THE X-RAY AND EM SECTIONS IN THE SAME EXAM BOOK(S)

(30 marks / 30 minutes)

Question 1. The following represents the **diffraction pattern** (ie the Fourier transform) of a one-dimensional crystal.



(a) What would the diffraction pattern look like of the same molecule in a crystal with double the unit cell length? **(6 Marks)**

(b) Explain the concept of the convolution with regards to the composition of a crystal and the diffraction pattern **(6 Marks)**

Question 2. The principles of diffraction apply to all forms of energy that have a wave character. List 4 differences between neutron and X-ray diffraction, and the application of the two techniques for structure determination. **(7 marks)**

Question 3. X-rays are high frequency electromagnetic rays that are part of the electromagnetic spectrum.

(a) Describe the minimum equipment required to generate X-rays using a lab generator. **(4 marks)**

(b) List the types of X-radiation that are produced by a Lab generator? **(2 marks)**

(c) For each type of radiation that you have listed – describe how they are produced and what characteristics they have? **(5 marks)**

EM Section (Prof. Rubinstein)

PLEASE ANSWER QUESTIONS FROM THE X-RAY AND EM SECTIONS IN THE SAME EXAM BOOK(S)

(30 marks / 30 minutes)

Question 4 (8 Marks).

In a computer, the amplitude and phase at every position of a Fourier transform are represented by a complex number of the form $A + Bi$.

a) How can a complex number be used to describe the amplitude and phase angle of a wave? Use a vector diagram to illustrate.

b) What are the amplitude and phase described by the complex number $2+2i$?

Question 5 (7 Marks).

Use one or two word answers for the following questions.

ANSWER IN THE ANSWER BOOK, NOT ON THIS QUESTION SHEET

a) What kind of interaction of an electron with the specimen causes radiation damage?

b) As the energy of an electron increases, it's _____ of interaction with the specimen decreases

c) Two images that are similar will have a high _____ coefficient

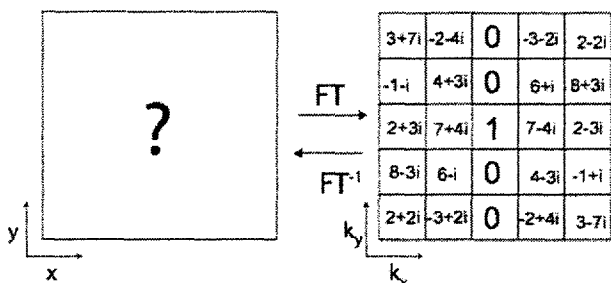
d) The image from an electron microscope is the projection of the specimen _____ with the point spread function of the microscope and corrupted by noise.

e) The Contrast Transfer Function is the _____ of the point spread function of the microscope.

f) In random conical tilting, two images of the specimen must be obtained: in the first the specimen is _____ and in the second it is _____.

g) The projection theorem states that the 2D Fourier transform of a projection of an object corresponds to a _____ through the 3D Fourier transform of the object.

Question 6.



The picture above shows an image, $f(x,y)$, and its (rather peculiar) Fourier transform, $F(k_x,k_y)$. We do not know what the image looks like (hence the big “?” in the middle of the image). The coordinate axes are shown for both the real space image and the Fourier transform.

(a) Draw the Fourier transforms of the two projections $\int f(x,y)dx$ and $\int f(x,y)dy$. (6 marks)

(b) Sketch and describe what the projection $\int f(x,y)dx$ must look like based on what its Fourier transform is. (6 marks)

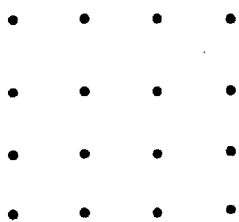
(c) (BONUS 2 marks): Do you notice an interesting symmetry in the 2D Fourier transform? The same symmetry is seen in the Fourier transform of $\int f(x,y)dy$. Describe the symmetry. This is a property of Fourier transforms of real functions (as opposed to complex functions) and images are, by definition, real functions.

Question 7 (3 Marks)

a) Given the function, $[f(x,y)]$



And the lattice $[g(x,y)]$



Draw the convolution of the function and the lattice $[f(x,y) \otimes g(x,y)]$.

NMR Section (Prof. Sharpe)

**PLEASE ANSWER QUESTION FROM THE NMR AND FLUORESCENCE SECTIONS
IN THE SAME EXAM BOOK(S)**

(80 marks / 80 minutes)

Question 8 (9 marks).

- (a) What type of electromagnetic radiation is used for NMR spectroscopy?
- (b) Why is NMR a relatively insensitive technique, as compared to other forms of spectroscopy?
- (c) List 2 factors in the experimental setup that play a role in the sensitivity of the NMR experiment, and describe how they could be altered to improve the sensitivity.

Question 9 (8 marks) .

Distinguish between T_1 and T_2 relaxation.

- (a) In terms of the physical basis for these phenomena.
- (b) In terms of their effect on observable NMR magnetization.

Question 10 (14 marks).

You are planning to determine the structure of a newly discovered 30 kDa protein using NMR spectroscopy.

- (a) List 4 types of NMR data that can be used as sources of structural constraints.
- (b) For 3 of these, BRIEFLY describe the physical basis for how this data reports on protein structure.

Question 11 (15 marks).

As the first step in studying the structure of this novel 30 kDa protein by NMR, you have optimized the experimental conditions to give a good ^1H - ^{15}N HSQC spectrum.

- (a) Give an example pair of NMR experiments that could be used to obtain backbone resonance assignments for this protein.
- (b) Indicate the magnetization transfer pathways for these two experiments, within adjacent amino acids. How does this facilitate sequential resonance assignments?
- (c) Give an example of an NMR experiment that can be used to obtain assign side chain resonances.

Question 12 (9 marks).

- (a) What is meant by 'resolution' when referring to NMR-derived structures?
- (b) How does this differ from the resolution reported for structures determined by X-ray crystallography?
- (c) What factors can generally affect the resolution of an NMR structure?

Question 13 (15 marks).

You are investigating three proteins in equilibrium between their free and ligand-bound states.

(i) For protein 1, addition of ligand leads to the appearance of a second set of peaks in the ^1H - ^{15}N HSQC, with intensities that grow as the peaks for the free state diminish.

(ii) The HSQC of protein 2 is well resolved in the free state and in the fully ligand-bound state, but exhibits significant peak broadening at many sites as ligand is titrated into the sample.

(iii) For several sites in protein 3, the well-resolved and sharp HSQC cross peaks exhibit a ligand-dependent change in ^1H and ^{15}N chemical shift.

(a) Which regime of chemical exchange is exhibited by each of the protein-ligand complexes for proteins 1,2 and 3?

(b) For protein 1, how can you determine the binding site for the ligand?

(c) For protein 3, describe how to determine the equilibrium constant for the ligand binding from the NMR titration data.

Question 14 (10 marks)

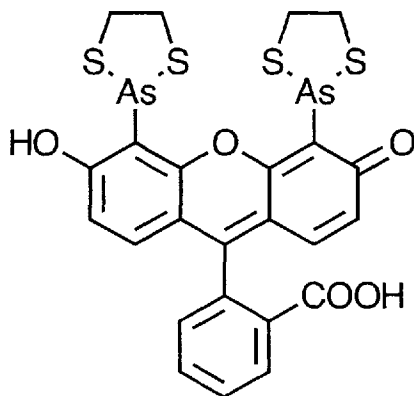
NMR is a powerful tool for studying the global and internal dynamics of molecules.

a) Suggest three measureable NMR parameters that are sensitive to dynamic processes.

b) Briefly describe how two of these parameters can be used to probe the local motions occurring in a protein.

Fluorescence Spectroscopy and Scanning Probe Microscopy (Prof. Yip)
PLEASE ANSWER QUESTIONS FROM THE NMR AND FLUORESCENCE SECTIONS
IN THE SAME EXAM BOOK(S)
(40 marks / 40 min)

Question 15. The following fluorescent compound: fluorescein arsenical hairpin binder (FlAsH) has been shown to be particularly useful for mapping protein regions and structures in living cells and membranes (**Total: 26 marks**)



- Describe and explain in detail two aspects of this molecule that make it appropriate for this purpose (**10 marks**)
- FlAsH is said to increase its fluorescence quantum efficiency by $\sim 50,000$ to 0.5 upon binding to a protein. Provide a definition of quantum efficiency and a reasonable explanation for this dramatic increase upon binding. (**8 marks**)
- Propose an experimental strategy for using FlAsH to map out the structure of G-protein coupled receptors (GPCR), which are membrane proteins with 7-transmembrane spanning α -helices. Your answer must clearly state the principles that you are applying in your strategy and how you will analyse the resulting experimental data. (**8 marks**)

Question 16. Scanning probe microscopy has emerged as a particularly powerful strategy for biophysical analysis of molecular and cellular structures and dynamics. (**Total: 14 marks**)

- Describe in detail 3 key advantages that it has over conventional tools in structural biology. (**10 marks**).
- SPM, along with other techniques such as optical tweezers, can be used to measure the inter- and intra-molecular forces associated with protein unbinding and unfolding. There are a number of experimental challenges underlying these single molecule force spectroscopy experiments. In class, we considered the case of linking affinity constants with unbinding forces. Describe in detail another key challenge with these single molecule force spectroscopy experiments and a means for addressing it. (**4 marks**)