Final Exam, Biology 555, Summer 2012

This exam, including the portions provided by Profs. Irving and Stark, is due at 5:00 pm on Tuesday 31 July 2012. It should be turned in electronically directly to the "Digital Dropbox" slot on Blackboard, or the "Assignments" slot. If you encounter difficulties doing that, please let Prof. Howard know no later than 5pm Monday that you are encountering difficulties so he can facilitate the submissions. If you submit the exam and then decide you want to change something, let Prof. Howard know that; he can clear your original submission and you can resubmit. Obviously that requires that you save a copy for yourself!

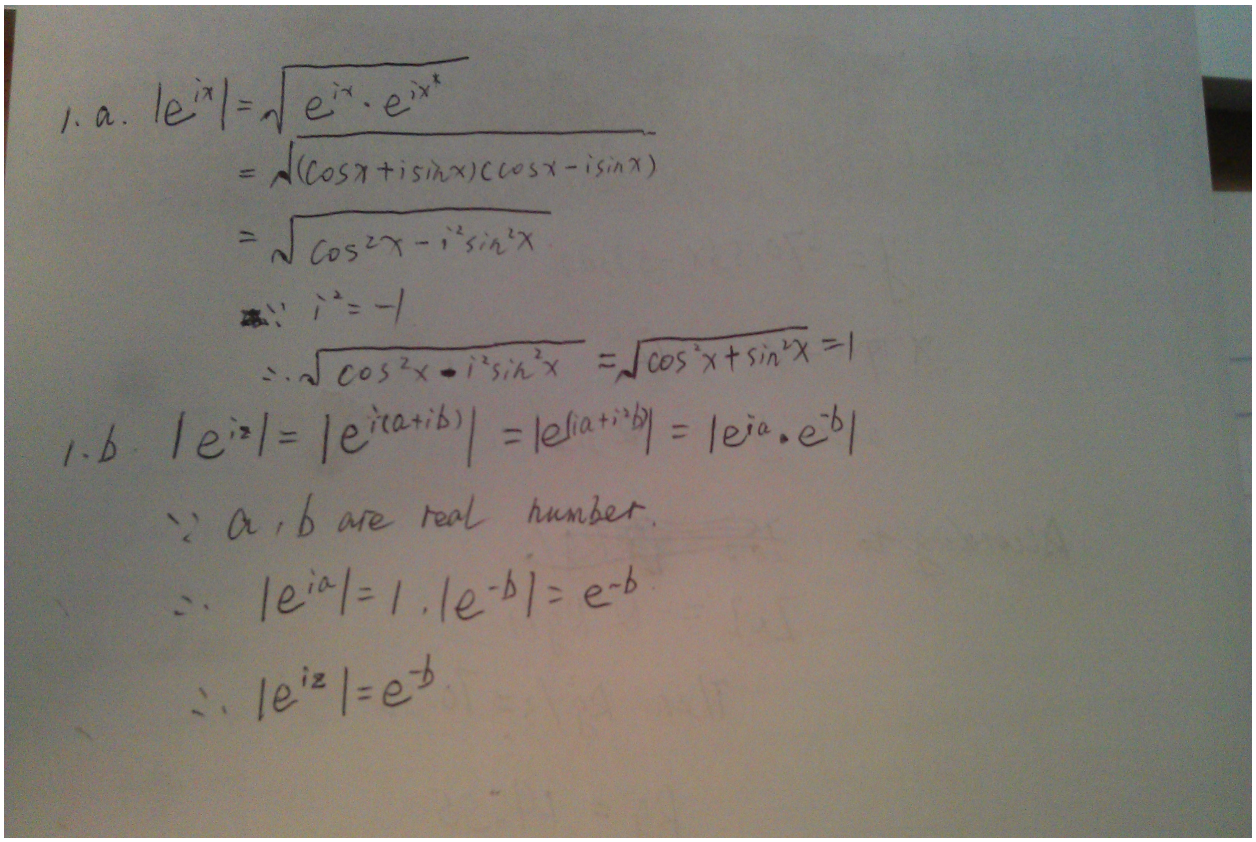
Remember that, although I will allow you to discuss this exam with your classmates prior to your submission, your final answers *must* be entirely your own work. Any strong similarities between textual answers from any two of you will be taken as evidence of copying and will result in zero credit for that question. Any answers that are demonstrably plagiarized from published or web documents will result in a zero score for the entire exam.

1. *Structure math:*

a. The magnitude or absolute value of a complex number is defined as the square root of the product of the complex number and its complex conjugate: That is, for a complex number $z = a + ib$, with a and b real, we define its complex conjugate z^* as $a - ib$ and $|z| \equiv \sqrt{(zz^*)}$. Use the Euler formula ($e^{ix} = \cos x + i \sin x$) to show that the absolute value of e^{ix} is one, provided that x is real.

b. Let the complex number $z = a + ib$, with a and b real. Show that $|e^{iz}| = e^{-b}$.

Answer:



2. *Crystallography:* Space group P21212, International Tables number 18, has the following equivalent positions:(x, *y, z)* ; (-x, *-y, z);* (-x + *1/2*, *y* + *1/2*, *-z);* (x + *1/2*, *-y* + *1/2*, *-z).*

a. Is this space group triclinic, monoclinic, orthorhombic, tetragonal, trigonal /hexagonal, or cubic?

b. In which direction do you find a 2-fold axis, and in which directions do you find two-fold screw axes in this space group?

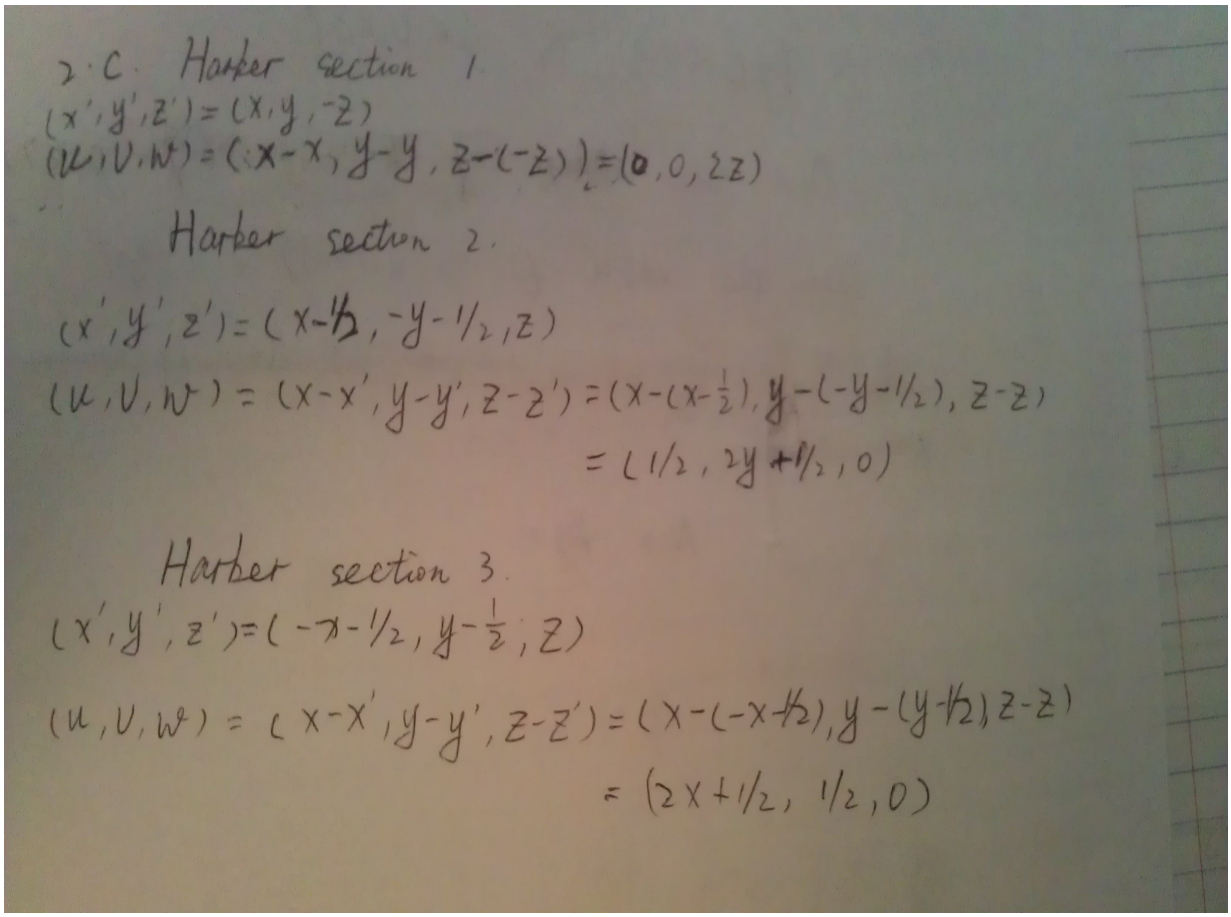
c. What conditions on *u, v,* and / or *w* will define the Harker sections in this space group? Show your work. Hint: There are three Harker sections. If your knowledge of Patterson syntheses is incomplete, you may find it

useful to consult a document I've made available: http://csrri.iit.edu/~howard/harker.html.

Answer: a. It's orthorhombic.

b. 2-fold axis is z; 2-fold screw axis are x and y.

c.



3. *Multiple methods:* Search the Protein Data Bank (www.rcsb.org) for a protein whose structure has been determined by two different methods. The two methods probably would be NMR and X-ray crystallography, but they could instead be NMR and cryoelectron microscopy, or X-ray and neutron crystallography, or some other permutation. Summarize in a three-paragraph (roughly 15-sentence) essay the similarites and differences between

the two structures. You should reference published papers about these structures as well as the PDB submissions themselves. Cite your sources.

Answer:

Myosin is well studied with the methods of X-ray and NMR. Both of these two methods can be used to provide large structure information. They can detect structure on atomatic level.
However there are some differences among these two methods. X-ray provides static structural information, but NMR offers a dynamic structure. The motions of atoms are incorporated into the atomic model by a B-value. There is no B value demonstrated on PDB file of NMR structure of myosin, because NMR structures are dynamic.
NMR is often used for small structure, but X-ray often used to detect large structures.

References:

1. Crystal structures of the GCaMP calcium sensor reveal the mechanism of fluorescence signal change and aid rational design. J Biol Chem. 2009 Mar 6;284(10):6455-64. Epub 2008 Dec 18.

2. Crystal structure of a phosphorylated light chain domain of scallop smooth-muscle myosin. Biophys J. 2011 Nov 2;101(9):2185-9. Epub 2011 Nov 1.

3. Asymmetric mode of Ca^{2+}-S100A4 interaction with nonmuscle myosin IIA generates nanomolar affinity required for filament remodeling. Structure. 2012 Apr 4;20(4):654-66. Epub 2012 Apr 3.

4. *Structure refinement:* Suppose an X-ray crystal structure has been correctly determined, except that one amino acid's side chain has been completely mispositioned.

a. Would you expect this error to affect the calculated structure amplitudes derived from thisalmost-correct structure: would a small number of structure amplitudes be completely wrong whereas the others are correct—or would a large number of structure amplitudes be slightly wrong? Explain briefly.

b. How would you expect to identify this error during the structure refinement process?

Answer:

a. No. The small errors can be refined by refinement methods unless the overall structure amplitudes are correct.

b. When processing, you can take 5% to 10% of the structure amplitudes out and use the rest to fit the data. Do it over and over.

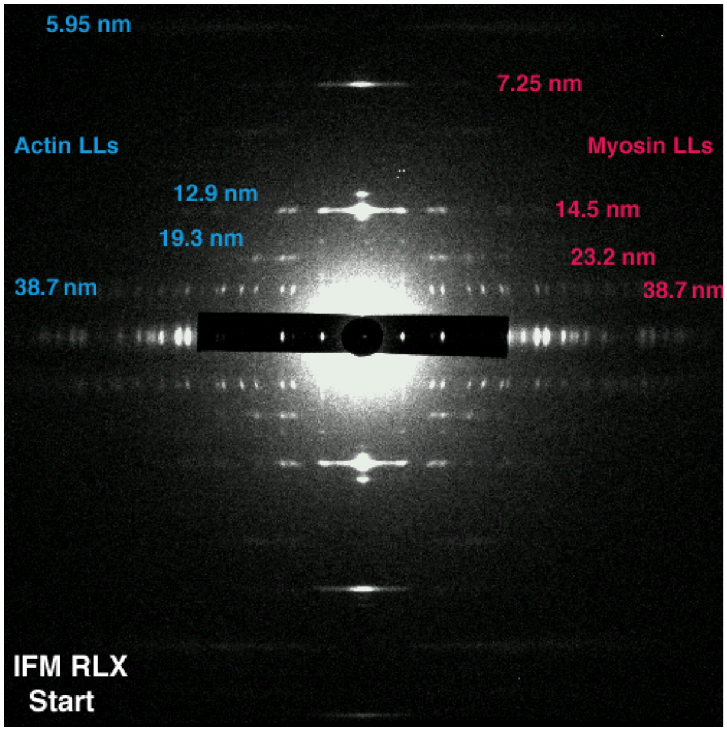
5. *Crystallographic structure determination:*Suppose the structure of a particular protein (which we will call protein M) is already known, and you are interested in determining the structure of a protein (which we will call protein N) whose sequence is 58% identical to that of protein M. You successfully crystallize protein N. You can now proceed to determine the structure of protein N either by experimental phasing (using MIR or MAD), or by molecular replacement using protein M as a search model. In ten sentences or so, describe the advantages and disadvantages of those two approaches.

   Answer: For MR, you don't need to get crystal, so the method is easy. Also, the model is easy to make, because it is little or no chain tracing required. But error from MR is greater than experimental phasing. For MR, you have to also tell the orientation and location of the model. For MAD and MIR, the Friedel pair difference can be used to extract starting phase if the AD is large. By using MAD, the lack of isomorphism is more often.

6. *Nucleic acid structure:* What are 12 different ways in which the parameters of two adjacent base pairs in a B-DNA double helix can vary from the average B-form structure of DNA?

Answer: Shift, slide, rise, shear, stretch, stagger, buckle, propeller, opening, tilt, roll, twist

7. *Muscle diffraction:* Examine this diffraction image:



The layer lines have been labeled in this diffraction pattern from relaxed insect flight muscle. They can be indexed as orders of a single long-spacing repeat. What is this repeat distance? Where does this come from? How might this diffraction pattern look different if it was from contracting muscle? Explain your answer. It may help you in answering this question to examine the paper describing this structure, which is available on the course Blackboard site.

Answer: The distance is 38.7nm. The layer lines are from the diffraction of actin and myosin. No difference could be found when the muscle contract. When contracting happens, actin and myosin will slide. However, the helical shapes of them won't change. According to Bragg's Law, layer lines get no change.

8. *Computational structural biology:* Summarize the types of computational tools that can be usefully employed at various time scales in characterizing biological structures. Explain how the results at the faster time scales help to inform the results derivable at the longer time scales.

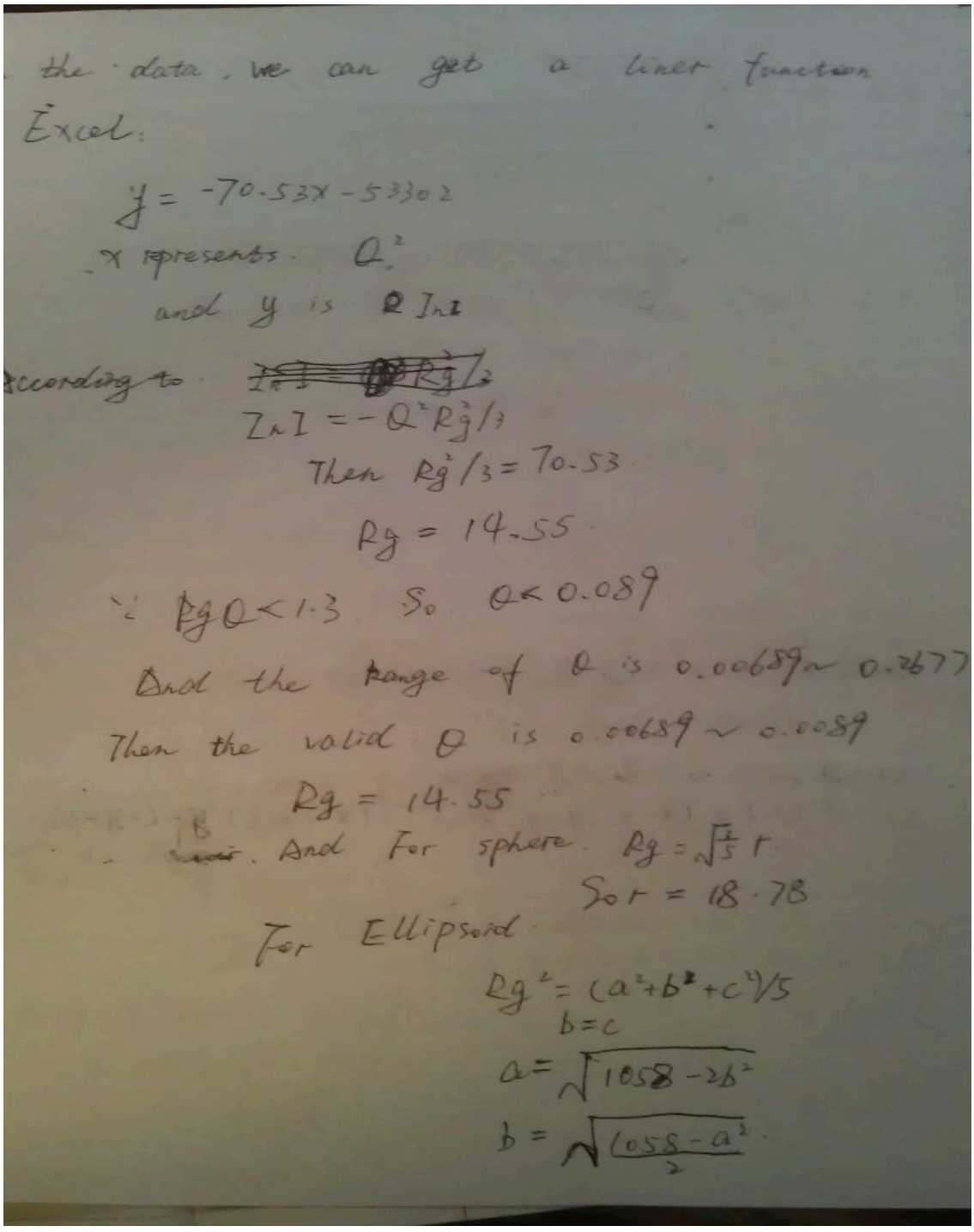
Answer: Molecular Dynamics, Langevin Dynamics, Brownian Dynamics, Monte Carlo, Energy Minimization. The faster time scales can provide the potential values for longer time scales. When the result from longer time scales is far away from the result from faster time scales, then we should get rid of the result.

9. *X-ray Scattering:*We have uploaded a spreadsheet called "SAXS data file" to Blackboard. It has 3 columns of numbers. The first is Q ($4\pi\sin\theta/\lambda$) in Å-1. The second column is the scattering (intensity) from the buffer alone and the third is the scattering from the protein dissolved in buffer (buffer+protein). Taking these data and, using a Guinier plot, calculate Rg for this molecule. Assuming a globular particle, calculate the radius of the particle. Assuming a prolate ellipsoid, calculate the principal axes a and b. In answering this question bear in mind that the Guinier approximation is only valid at low angles and will diverge at high angles. This should be apparent from the data. In answering this question bear in mind that the Guinier approximation is only valid at low angles and will diverge at high angles. This should be apparent from the data. There is a "rule of thumb" for deciding the appropriate range of data for calculating Rg:

$Rg \times Qmax < 1.3$

This implies that it might take a couple of iterations to get the range right. In your answer explain how you decided what range of Q to use to calculate Rg.

Answer:



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