28 Sep 2017

Welcome to the Second Exam!

BNFO 300: Molecular Biology Through Discovery

The primary purpose of this exam is to serve as an educational tool. With this in mind, do not be surprised if some of the problems go beyond your present abilities and try to connect things you may not yet have connected. Still, almost all of the elements have been drawn from the problem sets and study questions. If you ask yourself, "***Have I seen something like this before?***" the answer is generally yes. Answer what you can and go as far as you can (and further) with the rest.

Don't allow yourself to get stuck! There are always a way of getting through or around a problem. It exists. *Find it*. If you think you're going in circles, stop and ask for directions.

RULES OF THE GAME

- **The Prime Directive:**
  Be honest! Speak in your own voice. No quotes, no paraphrases. Say what you've found to be true. Qualify what you do not know to be true.
  Be transparent! Explain how you have come to your decisions. No assertions without thought processes and sources.
- **Available resources:** This is an open book exam. It is an open notes exam. It is an open web exam. Most important, it is an open brain exam.
- **Unavailable resources:** …but not an open people exam. If you disagree with my admonition, at least show your conviction by listing those with whom you had contact and why.
- **Major exception - Consultations:** Jared, Thomas, and I ***are*** open resources for this exam. We would be delighted to consult with you any time you feel the need. In fact, you and I will schedule a time to meet – that's part of the exam.
- **Ask questions that help me answer them:** If you say "*I don't know what is going on*" I may respond "*I'm sorry to hear that.*" Instead, describe the problem you've encountered, the steps you have taken to overcome it, and what you feel you need to make further progress.
- **How to consult:** E-mail, telephone, walk-in... all work, within the limits of my schedule. To the contact information on the web (click on *Who we are*), add the home phone 285-2447, open for business until 10:30 PM, every day including weekends. I'm often at VCU on weekends during the day.
- **Where to find the exam and related files referred to in the exam:** In your e-mailbox.
- **When to submit:** Your responses are to be submitted in two parts:
  - By 5:00 PM **Wednesday, October 4**, you will respond to an on-line survey that asks for each question what, if anything, prevents you from answering the question – e.g., what factual information you see you are lacking. I will respond to each stated need as soon as I can.
  - By 7**:00 AM, Tuesday, October 10**, not later, all your responses to the questions must be submitted Why then? (1) You will have had ~160 hours to work on the exam (2) We need to move on. (3) We will be rehashing the exam in class. (4) It is a kindness to have closure to projects that could otherwise go on forever
- **What to submit:**
  - In general, text-based electronic submission is preferred (not scans, unless absolutely necessary). Embed figures into the word-processor document.
  - If you send one or more attached files, ***be sure the filename begins with your last name*** (so I don't get a mailbox full of files named "Exam.doc"). **Any file submitted whose file name does not begin with the last name of the submitter will have its spirit extracted and placed inside a doll, which I will then stick pins in.**

Preliminaries

1. If you have neither received nor given aid regarding this exam (instructor excepted) (I could always use some aid), then **sign/provide your name**. Otherwise sign/provide someone else's name.

2. Have you read the instructions on page 1 of this exam? If you have, have you understood them? If you have not understood them, have you sent an e-mail to me describing how you are confused and requesting elucidation? Have you completed the online survey, asking about your first pass through the exam? In doing any of this, have you heeded the lesson of Question 3 below?

Scientific Questions

3. Suppose that someone has asked you for help in solving a problem, reaching out to you in one of the ways listed below. You really want to be of service. Which way would most inspire/ enable you most to provide effective help?

A. "*I wanted to find the length of the genome sequence of Anabaena variabilis, so using CyanoBIKE, I brought down LENGTH-OF and put in its box SEQUENCE-OF Avar. I executed the function, expecting to get a number of some millions of nucleotides, but instead I got the number 5! I tried COUNT-OF SEQUENCE-OF Avar and got the same answer. I looked at the sequence of Avar using SEQUENCE-OF Avar, and the top of the screen says "anabaena variabilis atcc29413 b - Chromosome (1 -> 6000 of 6365727)". I went to the end of the sequence, and indeed the last nucleotide* ***does*** *have the coordinate 6365727. Why did my first attempt give 5?*"

B. "*I tried to get the length of a genome and it didn't give me the right answer. Why?*"

C. "*I'm sooooooooo confused! Please help!*"

D. "*I have your dog and I know where you live.*"

4. Go to the course web page and read (or re-read) the quote at the bottom taken from Xenophanes. Which of the following best captures its meaning?

A. We approach truth (but never reach it) by weaving together observations whose interconnections give us increasing confidence.

B. By careful application of the scientific method, we can proceed in the course of time from observation, through conjecture, to confirmed scientific truth.

C. Nature is secretive and treacherous, weaving webs that ensnare us in doubt.

D. "I think I think, therefore I think maybe I am, sort of… Geez, I don't know."

5. If a researcher has agreed to serve as your mentor for your research proposal, respond to Question 5. Otherwise, respond to Question 6.

5a. What is the name of your mentor? ***Provide the name.***

5b. ***Write a paragraph*** justifying your research proposal. The paragraph/justification should take the following form:

- Provisional title: Don't be too concerned about getting it exactly right as you'll have many opportunities to change it later, but as best you can encapsulate the specific, experimentally accessible question you intend to address.
- First sentence: Begin the paragraph with a broad view of the larger question encompassing your topic, in terms that your colleagues in class will understand and appreciate. The sentence should include a reference to an appropriate article that reviews the field.
- Last sentence: Clearly state the question you intend to address by experiment. The question should be answerable by a single experiment (or perhaps a tight cluster of highly related experiments).
- Intermediate sentences: Make a logical connection between the first sentence and the last sentence. To the extent possible, make that connection by citing experimental results that have already been published. If you can't do this yet, then indicate what sort of experimental results you hope to find in the literature.

5c. Provide an exhaustive bibliography that encompasses the highly focused topic of your research proposal. ***The bibliography (in a separate document) should consist of several complete references[1] to research articles. A URL for each would also be nice. Provide also the search strategy (including database and specific terms) you used to find the articles.*** If you've already sent in a bibliography and you're happy with it, just say so.

5d. Choose one experiment from one article (probably one of the articles in your bibliography). The experiment should be central in some way to your research proposal. ***Identify the article (with a full citation) and the experiment*** briefly in words and by indicating the figure or table containing the results of the experiment. ***Why is this experiment central to your proposal?***

5e. If you understand the principle behind the key technique in the experiment, briefly explain it. If you don't (that's OK), then identify the technique and describe what you don't understand. ***Either way, a couple of sentences should do it.***

6. (Respond to this question only if you did not answer Question 5)

6a. Who is your top candidate for a faculty member to serve as your mentor for your research proposal? What steps have you taken thus far to obtain a faculty mentor? ***Provide the name and description of the steps.***

6b. What articles have you read authored by your candidate? Any problems? ***Provide citations for the articles and any specific problems you may have encountered ("I didn't understand it" is not a specific problem – what didn't you understand?).***

6c. If you have written to your candidate, provide the e-mail you sent (unless you've already copied me on it). If you have not yet written to your candidate, provide your best draft of a message, in light of suggestions given in How to Find a Mentor. ***Provide the message.***

6d. Describe how the topic of your proposal fits in with the research of your mentor and how the topic is molecular in nature. ***A sentence or two plus a reference from your mentor's lab should do it.***

[1] Complete references should contain authors, title, journal, volume, pages, and (if possible) a URL.

6e. Provide a citation to a review article related to the topic of your research proposal. ***Provide a full citation, including authors, title, journal, volume, page numbers, and URL (if available) in any convenient format, plus a brief description of how you found it.***

7. Almost half of you indicated in the survey you submitted in January that you were aiming at a career in some medical profession. We haven't dwelt on health issues much, but let's take a detour in that direction now. It's necessarily fiction, but I've tried to make it as close to reality as possible.

Suppose that you are a physician twenty years ago and that you are visited by a representative from a pharmaceutical company. Such visits are generally welcome, because they often give out free samples that you can pass on to your patients, thereby saving them a bundle. This visit plays out like so:

Rep: *Get any patients with chronic back pain?*
You: *All the time.*
Rep: *What do you do for them?*
You: *Well, I like to direct them to physical and psychological therapies. It's the best we have.*
Rep: *Uh-huh… and does insurance pay for it?*
You: *No, but…*
Rep: *So do your patients do it?*
You: *No,… Where are we going with this?*
Rep: *I have something you might be interested in. A new drug that can restore the morale of your patients who are broken by pain, allowing them the dignity of going back to their jobs. Here, let me show you…*
You: *What's the drug?*
Rep: *It's a formulation of oxycodone…*
You: *An opioid? You want me to prescribe an opioid for back pain???*
Rep: *Pain is a disease. Oxycodone has been prescribed to fight this disease for years…*
You: *Yes, in late stage cancer patients.*
Rep: *Actually, things have progressed. The World Health Organization has encouraged physicians to consider opioids for the management of long term pain in general.*
You: *I'm not going to put more opioids out there…*
Rep: *This is a special formulation of oxycodone,… it's time-release, completely abuse-resistant. You don't even get a buzz off it.*
You: *How do I know…*
Rep: *Look. Any drug I or any other sales rep offers has to, by law, get by the FDA. The full weight of the US government is behind any claim I make. We have to submit every brochure, every video -- anything -- to the FDA before it sees the light of day.*
You: *OK, but I'm still kinda worried about addiction… has there been any research…?*
Rep: *We couldn't get FDA approval without a ton of basic research. It's been shown repeatedly that opioid analgesics are safe and effective as treatment for chronic pain so long patients have no prior history of drug abuse. There's Portenoy and Foley (1986). They're from Sloan-Kettering. And there's Porter and Jick (1980).*

That last one was published in The New England Journal of Medicine, perhaps the most prestigious medical journal in the U.S.

You: *Do you happen to have copies of these articles?*

Rep: *I don't carry them in my back pocket, but of course I can get them to you. You'll hear from me. In the meantime, I'm going to leave you two dozen starter coupons that will get your patients a 30 day supply for free. Need any hats or coffee mugs?*

You: *No, that's all right. I'm good.*

With some additional prodding, you do eventually get the two articles that the rep mentioned (Portenoy and Foley (1986) was put online, behind the usual username/password combination because it's not freely available. You're on your own for Porter & Jick (1980)):

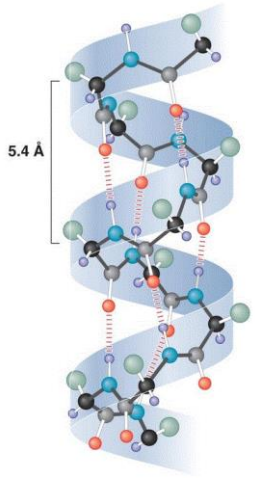
Porter J, Jick H (1980). Addiction rare in patients treated with narcotics. New Engl J Med 302:123.

Portenoy RK, Foley KM (1985). Chronic use of opioid analgesics in non-malignant pain: Report of 38 cases. Pain 25:171-186.

The ball is now in your court. You have patients who are in pain. Do you prescribe or not? ***Provide an analysis of the two articles, citing specifics, that leads you to your decision.***

8. How wide is a biological membrane? By that I mean, what is the cross-sectional distance from one phospholipid head group to the one on the other side? You can readily find the answer to this question on a variety of web sites, articles, text books, etc., and you're invited to gain inspiration from whatever you find in these sources. However, the justification for your answer to this question will make use only of the following pieces of information:

- Proteins that span membranes generally do so as alpha-helices.
- Each turn of an alpha-helix is associated with a rise of 0.54 nm
- The backbone of each alpha helical turn contains about 11 atoms
- In addition, you may use any information you find in material for this course (Notes, problem sets,…)



Protein alpha helix
[Pearson Education (2008)]

8a. From this information, calculate the width of a biological membrane? I don't care much what answer you get, so long as you ***provide a reasonable calculation and justify each step.***

8b. Proteins that bind to DNA (e.g. transcription factors) often do so using a helix-turn-helix structural motif conserved over much of life. When the protein and DNA bind, does the DNA helix fit between the turns of the protein helix, or does the protein helix fit within the turns of the DNA helix? Using the sources of information listed above, ***argue for one of these two answers, providing specific numbers and perhaps calculations.***

9. The online *Science Encyclopedia* covers an enormous range of science topics. Suppose that you've been asked by them to participate in a review of their articles, aimed at quality control. Specifically, you've been asked to review the article on DNA palindromes, in recognition of your acknowledged expertise in the area. ***Critique the article, identifying what's good about it and what (if anything) is wrong with it. You can copy the article into a word processor and interpolate comments or otherwise indicate what phrase/sentence you are referring to.***

10. Suppose we lived in a universe where proteins are not linear arrays of amino acids but instead branched arrays of amino acids. Suppose, specifically, that the beta chain of insulin has the following structure:

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     ERGFFYTPKARREVEGPQVGALELAGGPGAGGLEGPPQKRGIVEQCCASVCSLYQLENYCN [end]
FVD<
     HLCGSHLVEALYLVCG [end]
```

How would this structure change the results of Sanger and Tuppy (1951) [Biochem J 49:463-481]?[2] There are a lot of possible changes to consider… for the sake of your sanity, confine your attention to Table 5 of the article. ***Go through each line of that table and indicate whether the result is compatible with the branched structure given above. If it isn't compatible, indicate why and, if possible, modify the result for that line so that it is compatible. A response in the form of a table would make my life easier.***

11. OpenStax College shares online educational material so that faculty members can cobble together modules to make free textbooks or other classroom material. Their collection of biology modules contains one devoted to a relatively standard account of DNA replication and the Meselson-Stahl experiment. Go to that module, and consider Figure 1, which displays three models of DNA replication. The last, dispersive replication, may be the least familiar to you.

11a. Starting with a diagram of the proposed mechanism of dispersive DNA replication (which you will find elsewhere), modify the diagram, labeling it with orientation (5', 3') and perhaps other helpful labels or graphical conventions to show clearly how that mechanism relates to the graphic of dispersive replication shown in Fig. 1. ***Provide the modified diagram and a short narrative to go along with it. Any figure you construct should not exceed 0.5 Mbytes.***

11b. Meselson and Stahl showed that after one generation of growth in ^{14}N-medium, DNA banded at a certain density. Does this result distinguish between the semi-conservative and dispersive models of DNA replication. Why or why not? ***A couple of sentences should do it.***

11c. Meselson and Stahl didn't realize it, but their experiment subjected *E.* coli DNA to shear forces, causing extensive fragmentation of the DNA. Does this knowledge influence your answer to Question **11b**? ***A couple of sentences should do it.***

12. The human population carries many dozen variants of normal adult hemoglobin, some of them causing physiological problems. After determining the structure of horse hemoglobin, Max Perutz and his group turned to these variants to assess why the mutations they carry might affect the functioning of hemoglobin. The following article contains much of this work:

Perutz MF, Lehmann H (1968). Molecular pathology of human haemoglobin. Nature 219:902-909.

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[2] One obvious way branched proteins could change their results is by causing Sanger and Tuppy to no longer exist,… but disregard that possibility.

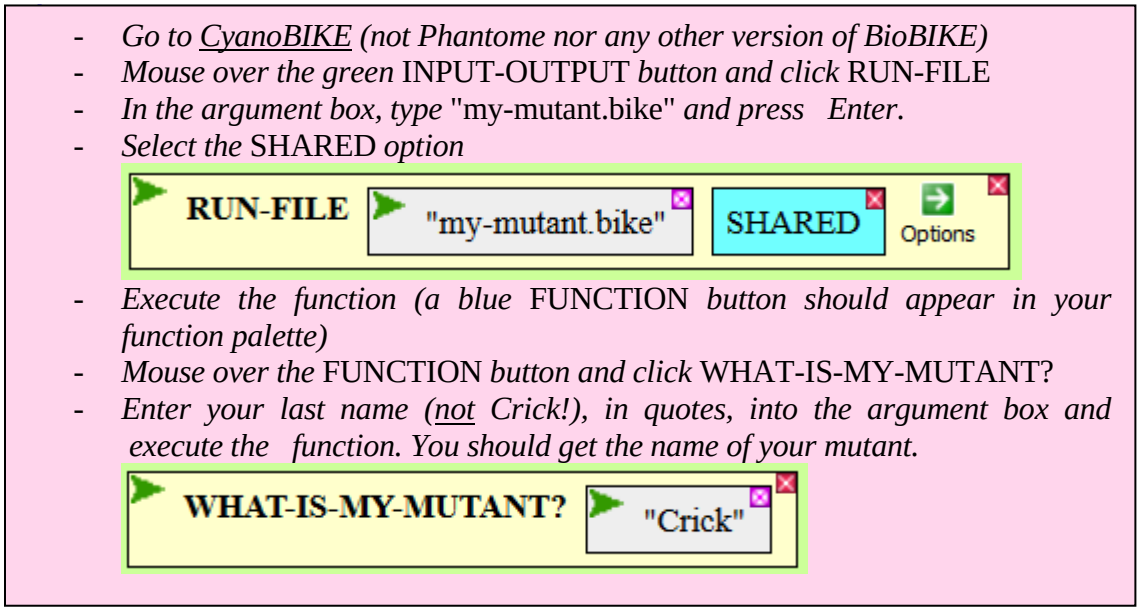
Get the full text of this article. You'll notice that much of the article is taken up by Tables 1 through 3, list hemoglobin variants. There's a lot of information in these tables. Here's an example of how to read them, using as an example the first line of the first table:

Table 1. RESIDUES IN CONTACT WITH HAEM GROUPS

Reference	Designation	Residue No. Position	Replacement From	Replacement To	Variability	Clinical symptoms	α-Chain Abnormal properties	Structural effects of replacement	Illustration
7	Torino	43 CD1	phe	val	I	Inclusion body anaemia	Unstable at 50° C	Substitution removes the contact of the haem group with Cε and Cζ of phe, leaving a gap at the surface of the haem pocket. $C\gamma_2$ of val makes a short contact with Cβ of the phe CD4(46) which would disturb the conformation of the CD segment	Fig. 1 of this article, and Fig. 2 of ref. 4

1 2 3 4 5 6 7 8 9 10

1. T



affects the alpha chain of hemoglobin (also known as the A chain).

2. You may learn more about this variant from reference 7 at the back of Perutz and Lehman (1968).
3. The common name for this variant is Hemoglobin Torino.
4. The relevant mutation is at the 43rd amino acid, counting from the N-terminus.
5. The 43rd amino acid lies in between the C and D alpha helices, as defined by Perutz et al (1965).
6. The amino acid normally appearing at the 43rd position is phenylalanine.
7. The amino acid appearing in Hemoglobin Torino at the 43rd position is valine.
8. The mutant hemoglobin has been observed to denature (fall apart) at 50° C.
9. The authors imagine that the structure of Hemoglobin Torino would be affected as described.
10. You can find a picture of the relevant region of hemoglobin in the indicated figures.

You have been assigned one of the mutants in Tables 1 through 3. To find out which one, do the following:

12a. Find the mutant given to you by CyanoBIKE's WHAT-IS-MY-MUTANT? (see above), and in either Table 1, 2, or 3 of Perutz and Lehman (1968). Identify whether the mutation is in hemoglobin chain A or chain B. Get the amino acid sequence of normal human hemoglobin, chain A or B, as appropriate, from an appropriate source, and find the position of the mutation in the sequence. ***Report a five-amino acid sequence (two***

amino acids to the left of the variant site, the normal amino acid at the variant site, two amino acids to the right of the variant site) and briefly describe how you found the sequence.

12b. Use Protein Explorer to display normal hemoglobin, and modify the image to highlight the mutated amino acid and any other relevant features (critical helices, amino acids, or atoms). Use the image you have created in Protein Explorer to illustrate an argument as to why the mutation in the amino acid sequence might produce the abnormal property and/or structural effect given in Perutz & Lehman's table. ***Provide the picture of hemoglobin, fully labeled as called for by your argument, draw insights from it, and use them in a brief argument that employs language understandable to you and your colleagues. Any figure you construct should not exceed 0.5 Mbytes.***

See the course Links page for help in using Protein Explorer

13. This exam has generated at least two files (the responses to the questions and the bibliography, preferably in Docx or RTF format, but I'll take anything). ***Have you named each file beginning with your last name?***

14. Now that you are at the end of the exam (do this question last!), consider responding to the *Post-Exam II Questionnaire*, which will become active March 16 (available also through the calendar entry for March 17). Unlike previous questionnaires, this one is anonymous and is intended to solicit your views on the course thus far. ***Have you submitted your response to this questionnaire?*** I hope the answer is yes, but if it is very very late and you are very very wrecked, please at least promise to submit the questionnaire within the next 24 hours. I will count the number of yes's + promises, compare that sum to the number of questionnaires I receive, and base my view of human righteousness on the degree to which the two are similar.