Syllabus and Term Paper Instructions  
BIOCH 6820: Mass Spectrometry / Proteomics

Class Meetings  
8:00 a.m. to noon, and 1:00 p.m to ~ 5 p.m.  
Monday-Friday, August 11-15, 2008

Instructor  
Dr. Steve Hartson  
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Course Description  
This workshop will provide a thorough introduction to the field of proteomics. Lectures will introduce participants to a full spectrum of technologies and strategies. Hands-on exercises will teach participants to operate OSU's MALDI-TOF mass spectrometer, to manipulate and interpret mass spectra, to perform protein trypsinolysis, and to use this technology to ID unknown proteins via database searching. Participants should have previous life sciences knowledge equivalent to that of an advanced undergraduate or a graduate student (e.g., basic knowledge of proteins, PAGE, GenBank), but no previous knowledge of mass spectrometry or proteomics is assumed.

Required Materials  
You will receive a course manual at the beginning of the first meeting, and no other materials are required except writing implements and a calculator. You are encouraged to bring and use your personal laptop for the bioinformatics unit, or one will be provided.

Policies  
Please turn off cell phones and texting devices during lectures.

Due to the short nature of this course, students must attend each of the four lectures and must complete each of the five lab exercises. Additionally, at least two drafts of a term paper are required for students taking the workshop for course credit (see the attached instructions).

Points can be earned as follows:  
- attendance of each lecture: 40 pts (10 pts per lecture x 4)  
- completion of each lab: 50 pts (10 pts per lab x 5)  
- first draft of term paper: 10 pts  
- final draft of term paper: 20 pts  
- total: 120 pts

Grading Scale:  
>114 pts = A, >104 pts = B, >94 pts = C, > 84 pts = D  
Please note that each missed 10-point assignment equals a one-letter-grade reduction, the final draft of the term paper equals two letter grades, and together the two drafts of the term paper equals three letter grades. To earn an "A," you'll need perfect attendance, and you'll need to do well on the graded draft of the term paper.
Deadlines:
Lab exercises must be completed by Friday, 5:00 p.m. August 15, 2008 (but see Make-ups, below).

Term Papers
August 22, 5:00 p.m., first draft
August 29, 5:00 p.m., final draft

Late term papers will be penalized 10% of earned points per day late.

Make-ups
Missed laboratory exercises may be completed by appointment through August 29, 2008. Students with extraordinary circumstances must meet with the instructor to arrange an "Incomplete," and will subsequently pursue missed assignments during a later semester. Please note, however, that these lectures are offered only on an annual schedule. See http://osu.okstate.edu/acadaffr/aa/adv-AcaReg-Ind.htm for more information on Incompletes.

Revisions of the Final Draft of the Term Paper
You will be provided with clear detailed guidelines regarding how to prepare your term paper. Simply turning in the first draft will earn you the full 10 pts. The instructor will critique this initial draft, noting areas needing improvement.

The final draft of the term paper will be graded using an objective grading key. In order for you to earn an "A" for this course, your final term paper must capture 55 individual objective points listed on this key. Moreover, the Instructor's critiques of your drafts (and your responses), are not guaranteed to reconcile perfectly with the key.

However, you have the opportunity to pursue multiple revisions. You may submit your term paper drafts early, get a critique, and revise further up until the final deadline of 5:00 p.m. August 29, 2009. After the deadline, your last submitted draft will constitute your grade for the term paper. Simply put, you can revise your way toward an "A" until the final deadline.

Academic Honesty
Term papers should be a "de novo" effort on your behalf. You are explicitly forbidden to utilize legacy materials from previous workshop participants as templates, and you are not permitted to work with another student on this term paper. You may utilize style manuals, "instructions for authors" from scientific journals, and any other materials, except a previous or current student's term paper. You are encouraged to crib heavily from the examples provided in the instructor's instructions and critiques, but not from a previous or current student's term paper. If you use published work to facilitate your thinking, you should reference that work. In this case, your report's prose should not be a mere revision of previously published prose (don't just shuffle the words around), but should instead represent wholly new sentences written by you.

Violation of these policies will result in an "F-shriek" for this course.

Given the extensive guidelines you will be given to prepare your report, the opportunities generated by the revision policy, and the value of your integrity and reputation, you should be eager to pursue your term paper independently. 'nuff said.
Overview

The purpose of this paper is to reinforce the knowledge that you have gained on the subjects of mass spectrometry, peptide mass fingerprinting, and proteomics identifications. A secondary goal is to provide experience with the scientific method and with technical writing.

At least two sequential drafts will be required. The first draft will be critiqued and returned to you for revision. After revision, you will re-submit additional drafts for critique. After the deadline, your last draft submitted will serve as the basis for the final term paper grade.

Instructions

Your term paper should represent a mock manuscript that might be submitted for publication in a peer-reviewed journal. It should describe the experimental characterization of one or more unknown proteins by peptide mass fingerprinting. You may choose any of the protein sample(s) utilized in the workshop, ideally, one or more of the unknown protein bands that you characterized in Sample Preparation (Unit 4) and Your Samples (Unit 5). However, if you feel that your experimental data are too poor to justify this effort, you may choose the "digital" protein that was the focus of the Explorer and Peptide Mass Fingerprinting exercises (Units 2 and 3, respectively). No penalties will be levied for choosing one protein over another. **The exercise will be greatly simplified if you execute the Workshop with this paper in mind.**

Two to three pages of double-spaced text, excluding Figures, are anticipated. Specific individual sections with appropriate content are required, and are described in more detail below. Each Figure should have a simple title and a simple legend. As a whole, your paper should build up to an argument that "the unknown protein(s) were characterized by this technique, resulting in such-and-such data, and were identified on the basis of these criteria." Succinctly address these goals and points while limiting "window dressing" and fluff. Focus on the scientific method and utilize the scientific style, as described in more detail below.

Do not write a review or a generalized essay. Do not summarize or discuss the irrelevant workshop exercises. Don't present generalized theoretical scenarios that are not essential to your persuasive arguments.

Within each paragraph, try to structure your prose using the scientific method of Intro (why), Method (how), Result (what), Discussion (interpretation). Use topic sentences, wherein the first sentence of each paragraph nicely summarizes the paragraph's message, and all sentences in the paragraph fit this topic. Avoid "run on" sentence styles, where you try to cram multiple ideas into one sentence. As a rule of thumb, one sentence should contain only one idea. If you find that your sentence contains several ideas, you are "running on," and you need break up the ideas. Your word processor has plenty of periods: don't be afraid to use them.
Details

Below, specific guidelines are given for the preparation of manuscripts in general. Some of these guidelines will not be relevant to our exercise, but are provided for your information only. More guidelines can be found in "Instructions for Authors" for individual journals and in style manuals such as that by Day, Robert A. (1995) How to Write and Publish a Scientific Paper 4th ed Cambridge UP.

Your paper must have six sections:

Abstract or Summary
This section appears first and should serve as a stand-alone summary. What is the question under study and why is it important? What did you do and find? What do you conclude, and what is the significance of this conclusion. It is important to realize that the abstract will be the most-read section of the manuscript. Although guidelines vary, I suggest writing the abstract last, by assembling the topic sentences from all major paragraphs, then polish it up.

Note that the abstract is not an introduction! Don't try to use it as one. Don't try to write it first. Don't be general or philosophical. Your abstract should be short, sharp, concise, and precise summary of the whole paper. It will contain each of the themes from the scientific method for your study [Intro (why), Method (how), Result (what), Discussion (interpretation)]. It should not contain generalized theory, philosophy, or educational statements that don't describe, or derive from, your findings.

Introduction:
General-
Typically, "Introductions" include arguments as to why the question was worth asking, what is known already, and what the goal of the research was. Intro's should be pointed and specific versus generalized or unfocused literature reviews. Typically, they are written primarily in the present or present perfect tenses: things that are happening now ("The protein binds…") or a past action viewed from the present ("The protein has been shown to bind…").

Typical manuscript introductions include a final "teaser" or summary statement that tells the readers why they (as your research peers) should go on to spend precious time reading your latest results. Typically, teasers state what you concluded. Why a teaser? If your work doesn't get read, it won't get cited; then you'll be sad.

For this term paper-
However, this aspect of scientific writing is not readily applicable for our exercise. Instead, use the stylistic approach that "we wished to identify a specific unknown protein(s) with reasonable confidence on the basis of its peptide mass fingerprint." For your intro, write 3-6 sentences describing the general niche of MS in systems biology, how peptide mass fingerprinting works (in general, not methods), and compare and constrast the advantages/limitations of PMF v.s. MS/MS.

Materials and Methods:
Anything that describes "exactly how you did the expts" is M+M. The preferred verb form is past tense.
The goal is to allow "reasonably skilled peers" to duplicate your work while conserving precious journal page space. Typically, research publications describe the techniques in general terms and cite previous papers that have already described the techniques in detail. In contrast, new protocols are described in exhaustive detail. Standardized techniques such as non-analytical PCR, traditional cloning, DNA sequencing, etc. are typically omitted. However, new analytical assays, new protein purifications, and other new methods that are "non-routine" should include detail. Reagent sources need be included only if the supplier is difficult to identify or the specific supplier is crucial to the effort (you can skip "Tris from Sigma," but "monoclonal antibody X from company Y" needs to be included). Intentionally hiding a crucial detail is unethical.

For our efforts, provide a brief overview of what you did, and then reference the protocols in the manual for details. For example, "Proteins were excised from 1-DE SDS-PAGE gels, diced, and digested with trypsin (as described in Exercise S1 in the manual)." If you/we deviated (intentionally or by accident), note the alteration.

In contrast, you should include the details that are frequently variable, yet crucial (e.g., trypsin concentration, peptide tolerances, etc.).

Results:
In this section, your goal is to give the reader a clever guided tour of your data, pointing out everything that you want them to notice. The past tense is the preferred verb form.

For this term paper, your figures should include:
- Fig. 1: your un-deisotoped spectrum, with each ID'd peak annotated with the peptide's sequence;
- Table 1: the first layer of your Mascot result;
- Table 2: the second layer of your Mascot result.

I want you to spend your efforts on thinking and writing, not on transcribing and graphic arts cut-n-paste. Thus, just print out the results described above, give each a meaningful name and methodology legend, and staple them to your paper.

For each figure, data set, or experiment, you'll write at least one paragraph resembling a "micro-paper." (1) Very very briefly, what were you hoping to demonstrate or generate in this experiment ("intro" or "why")? (2) Very very briefly, how did you do so ("methods" or "how")? (3) In some detail, what did you see ("results" or "what")? You might wish to supply some details to call the readers attention to specific features, controls, or the general quality of the data. (4) Very very briefly, what do you conclude (a minor conclusion only).

As a rule of thumb, if you find yourself referring to two different figures in one paragraph, you may be "discussing" in the "Results" section. This can lead to sloppy thinking on your behalf. In this vein, always differentiate, in your mind and in your gut, the difference between data and a conclusion (in your work and in that of your competitors!). Failure to do so is a classic weakness in scientific writing and thinking. When your competitors make this error, they may create great opportunities for you. You may wish to look up the word "dogmatic" in the dictionary.

In the Figures, we show and refer to the essential, best, and most convincing representative examples of our data. Data should be accurate representations (if you did the expt 10 times, and only saw the "desired" result once, well...). Digital images should be minimally manipulated to best resemble the original data. Be wary of omitting primary data in favor of derived data (e.g. the actual gel images are preferred v.s. the densitometry): your peers would much rather see at least one example of the
actual data. Each Figure needs a Title and a simple brief Legend outlining the experimental strategy, i.e., how the data was generated.

As an example of Results: "To obtain spectra representing the peptide mass fingerprint of Sample #1 (Intro), the trypsinolytic peptides of proteinX were subjected to time-of-flight analysis (M&M). Several good spectra were collected, each well-populated with 8-9 strong peaks (3e4 counts) as well as numerous minor peaks (<3e2 counts). Isotopic species for each peak were well resolved (R=xxx), blah blah blah...". (Results). The masses of 25 individual ions were extracted from the de-isotoped spectrum, and these ion masses represented our experimental trypsinolytic peptide mass fingerprint (minor conclusion).

Discussion:
Discussion sections in scientific papers should include clear specific statements of conclusions with very brief references to the data that compel these conclusions. Present and present perfect verb tenses are preferred.

For our exercise, you should build an argument that the identity you will provide is correct. First, decide upon an ID, then try and convince me that the ID is correct. What criteria might bolster your arguments? Consider the points covered in the worksheets for Unit 3 (also provided in your workshop appendix). Also cover any contradictory data: unmatched peaks and possible explanations. You may want to include a brief paragraph regarding what kind of experiments would be required or bolster or finalize the ID.

For example:

"We conclude that Sample #1 is actin on the basis of its peptide mass fingerprint. This identification is consistent a Mascot score that exceed the threshold for statistical significance (### v.s. the significance threshold of ##; see Table 2)...."

Figure Legends
Legends appear under each Figure in a published paper, but enjoy a separate page(s) in a rough manuscript. The purpose of Legends is to make each Figure a "stand alone" entity such that readers can skip everything else and just study your data (indeed, you should learn to read scientific papers this way). Legends very briefly describe how the data were generated. I suggest two-three sentences. Use the past tense verb form. Remember that your Tables need Legends also.

Example:
"Figure 1: Representative Spectrum of Trypsinolytic Peptides from Sample One"
The protein sample was excised and digested with trypsin, and the resultant peptides were analyzed by MALDI-TOF mass spectrometry as described in Methods."

DON'T FORGET TO GIVE YOUR TERM PAPER A TITLE AND AN AUTHOR

"My Excellent Term Paper"
Jane Doe

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If you have any questions regarding the report (or other subjects), please see me for clarification.