



Biochemistry Lab I

Paper Instructions



Writing is easy. All you do is stare at a blank sheet of paper until drops of blood form on your forehead.

Gene Fowler

Brilliance has an obligation not only to create but also to communicate.

J.R. Platt

The trouble with most of us is that we would rather be ruined by praise than saved by criticism.

Norman Vincent Peale

INTRODUCTION: A major goal of this course is the development of effective technical writing skills. To help you become an accomplished writer, you will prepare several research papers based upon the studies completed in lab. Note that research papers are not typical "lab reports." The latter tend to be informal internal reports, or in a teaching lab, answers to a set of questions.

Required Reading: At The Bench About Writing - See web for pages.

Your experiments from the first day you worked with MGH to today will be reported in the format as prescribed by one of the leading biochemical journals, the *Journal of Biological Chemistry* (www.jbc.org).

BASIC INFORMATION/INSTRUCTIONS ABOUT YOUR PAPER:

Length of Paper – There is no minimum length nor is there a maximum length for the paper. This is likely to be about 8 to 12 pages with graphs. Longer papers will be more the rule than the exception. I do not grade based on length but rather completeness and the manner in which you communicate your work from the semester.

EACH STUDENT IS REQUIRED TO WRITE THEIR OWN PAPER. Failure to do so will result in an automatic F for all students involved.

Appearance:

- Manuscripts must be typed with **1.5 line spacing** throughout with at least one half -inch margins on all sides.
- The text must be typed in a font size of at **ten points** using **Arial Font**.
- Following the title authors and addresses, the remaining portion of the manuscript must be in **two columns** with the **figures imbedded** into the text. This will take time, but managing your format in this manner is an important soft skill.
- Include page numbers on all pages with the running title and last name of the first author in the header of each page after the first page. To do this, in Microsoft Word, click on the "Insert" menu and chose the page numbers option. In that window you will have the option to show numbers on the first page. Do not show numbers on the first page and then when you add a header in the second page, all subsequent pages will have the running title.

Arrangement - The manuscript is to be arranged in the following order: (a) title, author(s), and complete name(s) of institution(s); (b) running title; (c) summary; (d) introduction; (e) experimental procedures; (f) results; (g) discussion; (h) references; (i) footnotes; (j) figure legends; (k) tables; and (l) figures. Number all pages with the title page as page 1. Begin each section on a new page. Indicate by marginal notes the suggested location in the text of each figure and table.

Level of Detail in Your Writing – This should NOT read like a recipe, a lab book protocol, OR the protocols from the web. The best way to understand how these papers should look is to read the At the Bench, Writing about Biology AND read the JBC paper on purification. The paper is found on the lab website.

BAD - *A column of DEAE was run to purify MGH.* While correct, this way off writing is too terse and will NOT receive C grade. Instead details, background and supporting evidence are critical..

OK - *10 ml of lysates was applied to a DEAE column and the MGH was eluted with a pH change...* Much better than the last attempt, but this does not give enough information to help the reader know what and why you did the experiment, not does it communicate how you did it.

Better but not complete - After diluting 10 ml of lysate with 20 ml of 10 mM Tris-Cl, pH 8.0, 0.1 mM EDTA and 0.5 mM DTT (DEAE Wash Buffer), the sample was loaded onto a 15 ml DEAE-Sephacel column (1.0-5 cm) equilibrated with five column volumes (CV) of DEAE Wash Buffer at a flow rate of 1.0 ml/min. The column was then washed with two CV of DEAE Wash Buffer before eluting the MGH with a 10 CV gradient of 0-250 mM NaCl in DEAE Wash Buffer. Throughout the gradient, 2.5 ml fractions were collected... See the JBC paper for more examples and style.

Tips -

In general, past tense is used as an objective description of the results. Even in the methods section do not write instructions rather than a report of what was done. In other words, do not write Then add 1.0 ml of Bradford reagent. Rather 1 ml of Bradford reagent was added to each sample.

Avoid the use of first person I did not see an increase ... instead use the absorbance increased over time.

Avoid the use of pronouns. Overuse of words such as those, this, them, and so on, simply muddy up your writing style. Remember your assignment is a formal paper and writing needs to be clear.

Style - READ the assigned pages in at the bench and writing about biology AND read the JBC paper. You can NOT read the assignments and the JBC paper enough times! Look for style in each section. DON'T try to write "fancy". Write in a similar manner that you speak, just more formally. Again - look a the JBC paper for your guidance.

Oops - If something didn't work (such as a gel) be aware of it, but don't belabor the point. This is not an opinion piece. Therefore your paper should not include your feelings about if you liked or didn't like something. Critiquing that you would do something again to be certain doesn't belong in this kind of a paper either.

Proofing - read the paper out loud a paragraph at a time. If what you are hearing doesn't sound like what you meant, then you need to edit each line. This is an easy but effective method in checking your structure and readability.

Abbreviations can be used for words used many times after first spelling it out then followed by the abbreviation. "protein kinase C (PKC)" Exceptions to the rule are widely used abbreviations such as NaCl, ATP, NADH and so on. A rule of thumb is if it a common chemical then you don't need to spell it out if you don't know it off of the top of your head then spell it out and introduce the abbreviation

SECTIONS OF THE PAPER

In all sections of your paper

- Stay focused on the research topic of the paper
- Use paragraphs to separate each important point (except for the abstract)
- Present your points in logical order
- Use present tense to report well accepted facts - for example, 'the grass is green'
- Use past tense to describe specific results - for example, 'When weed killer was applied, the grass was brown'
- Avoid informal wording, addressing the reader directly, and jargon or slang terms
- Avoid use of superfluous pictures - include only those figures necessary to presenting results



Biochemistry Lab I

Paper Instructions



Title - The Title of the manuscript should be as short and informative as possible. It should not contain non-standard acronyms or abbreviations nor exceed two printed lines. The title page should also give the names of all authors and their complete mailing addresses.

The title page should also include the name, the telephone and fax numbers, and the E-mail address of the author to whom all correspondence about the manuscript, including proofs, will be sent.

The Running Title to be printed at the top of each page of a published paper cannot exceed 60 characters and spaces.

Abstract - This will be a brief description of the project and the results. In the abstract you will include the specific aim or purpose of the experiment. The abstract should be less than 200 words. Despite its brevity, all the important results of the work should be noted. *The abstract is sometimes the hardest part to write and I found it is easiest to write it after the rest of the report has been written.*

Introduction - The introduction should be a succinct statement of the state of knowledge of the project at the time of its inception, the purpose of the research, and the approaches taken. Statements of previous knowledge in the introduction should include supporting citations. Simply put – this is the background section that fills the reader on your protein or system and the current knowledge of the science.

- Your introduction will include information on each of the following (each major portion/experiment will have two or three paragraphs on the background of that technique):
 - Enough information to guide your reader to what MDH, GFP and a his tag are. Include more than a description for each protein.
 - Explain what a fusion protein is and how they are used in biochemistry.
 - Include at least one current example of how GFP is used in science/biochemistry.
 - Transformation
 - Plasmid DNA purification – include a bit about cell strain selection. Also include different types of DNA purification technique and the theory about DNA purification (w/pros and cons of the various styles of DNA purification).
 - One or two paragraphs about chromatography in general – column selection, buffer use, other general issues to be concerned about when running any chromatography
 - One to three paragraphs about the type of chromatographies you selected. (Include a bit on important properties – chemical – of the resin and important considerations when doing that kind of chromatography. Be certain to have specifics. i.e. don't just say a compound is important, explain chemically or mechanistically why something is important.
 - Background on SDS PAGE (what it is, what it does, and the biochemistry of how it works)
 - Background on WesternBlot – see above.
 - For EACH technique/method include EACH of the important components of the buffers or solutions and indicate the role each plays with in their work. For example: tween 20 in a western blot is a weak detergent that decreases the non-specific hydrophobic interactions between proteins and proteins with the blotting paper. Another example might be the DTT in lysis buffer, which acts as a reducing agent for the protein at lower concentrations. DTT assists in stopping the oxidation of disulfide bonds of a protein, thus keeping the protein in a native state... This kind of information is found in your handouts, your class textbook, *At the Bench*, or on many websites...
- LIMITING YOUR TEXT TO THE INFORMATION IN THE HANDOUTS WILL RESULT IN A MINIMAL GRADE FOR THIS SECTION.
- Style – Use past tense for most of Introduction section. When discussing established facts you can then switch to present tense. Use returns to increase readability.

Methods - The materials and methods section should succinctly describe how the work was performed. For our paper, we will skip the Materials Section. The Methods Section is a critical element of the paper. The

methods section will not be a re-write of the instructions of the lab handout. An example of the style of method expected is here:

If for example protein concentration was determined by the Bradford method, you need not write out the method. It would suffice to say "protein was measured by the method of Bradford (2)" with reference to the original protocol. Most times there are small changes and then the method would be written "protein was determined by the method of Bradford (2) with the following changes: ..." or .. by the method of Bradford (2), briefly, 20.0 μ l of samples were added to 2 ml of Bradford reagent and the absorbance at 595 nm determined.

This section is not a recipe or a step-by-step description of everything you did. Nor should the methods section be a copy of any handouts. Instead it should give the reader enough information on how you conducted your experiment.

- When describing reactions, assays or buffer composition volumes are rarely used. Instead use the final concentration of a buffer e.g. **BAD**- "we added 10 ml of 1M Tris and adjusted the pH to 7.0 with HCl to make running buffer". **GOOD**- " 10 mM Tris-Cl, pH 7.0 (Running Buffer)..."
- When describing a complex mixture, such as a chromatography buffer, list each of the components in the buffer followed by a parenthesis with the abbreviation.
- For each chromatography the writer should include the volume of the column, the inside diameter of a column, the buffer the resin was equilibrated in, flow rates, volumes of wash and elution and fraction size. If the column was run by gravity, then just state that the column was performed by gravity flow. See the JBC handout for examples.
- Be certain to include temperatures, times pH of solutions, incubation times for all methods.
- Generalize - report how procedures were done, not how they were specifically performed on a particular day. For example, report "samples were diluted to a final concentration of 2 mg/ml protein;" don't report that "135 microliters of sample one was diluted with 330 microliters of buffer to make the protein concentration 2 mg/ml." Always think about what would be relevant to an investigator at another institution, working on his/her own project.
- Each technique or experiment will have its' own method. Give each technique – experiment it's own paragraph with a header as seen in the JBC handout.
- **Style** – In the methods section, it is best to avoid first person (i.e. I added two microliters.. We then did...). You will write in a passive past tense voice for most of this section. Doing otherwise would focus the attention on the investigator instead of the method. Avoid lists and recipes and use complete sentences.

Results - The results section has the data that were obtained. These results are often shown as graphs or photographs. Do not put in tables of raw data if the report can be better represented as a graph. Tables of data should have a heading or title. Each graph and table will be numbered as Fig 1 or table 1. Use figure legends that describe the experimental conditions and nature of the experiment. IF you show a graph of some enzyme in a timed assay then the figure legend would say something about the specifics of the experiment. How many mg of protein was assayed for how long. what concentration of inhibitor, detergent or any other condition that is different than the standard assay described in the methods section. Try to avoid extensive discussion in the results section.

- Do not include the raw data. Do not include tables of data that have been graphed.
- Figures, images and tables should all be included in the body of the paper. If a table is too big then either add a section break in the column format of the paper, or included the table at the end of a paper.
- **Style** – For the results section, use past tense.

Discussion - The discussion section is where you talk about the significance of the results. For example, if an experiment failed, you might want to discuss what you think went wrong. The discussion is the appropriate place to go over the theory that is supported by or refuted by your data. Limitations in the data should be clearly noted, This is the appropriate place to answer the purpose of the experiment. If you want to compare four different methods of protein concentration assays, then here is where you would say what you found what it means and any comments for future experiments.



Biochemistry Lab I

Paper Instructions



MSUM Biochemistry

- For your paper, you will need to reference the blot, SDS-PAGE and chromatographs and discuss the results appropriately. Compare your results to other similar projects (this might be difficult for your paper – but, look up other MDH purification papers for comparison – this is required). DON'T forget to reference accordingly.
- Finish with a paragraph that discusses the future avenues of research. For this paper – you must include what you will do with the protein now that you've purified it. Be creative here...
- DO NOT BE SUPERFICIAL in your discussion or simply restate the results.
- I will specifically be looking for questions on purity for each step, relating the purification results obtained with the gel and the purification table as well as the kind of questions found on the westernblotting handout.
- Purification table goes in this section. Refer to the table in discussion section.
- **Style** – In the discussion section, refer to your studies in the present tense and other data in the past tense. When referring to commonly accepted scientific facts or widely known observations/behaviors, use the present tense.

Reference – You will reference according to the JBC style (see below). A declarative sentence should be referenced. DO NOT reference a website (except for our lab's webpage).

- Each paper MUST include a minimum of 5 references on MDH, GFP and or other background info.
- Most of your references will be in the introduction section with a few more showing up in the discussion section.

References for journals and books should be in the following styles:

1. MacDonald, G. M., Steenhuis, J. J., and Barry, B. A. (1995) J. Biol. Chem. 270, 8420-8428
2. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

All abbreviations used in the text must be defined in a single footnote inserted in the text immediately after the first abbreviation is cited. The abbreviations of some important biochemical compounds, e.g. ATP, NADH, DNA, and amino acids in proteins, need not be defined. Phrases such as "central nervous system" or "red blood cells" should not be abbreviated. Names of enzymes are usually not abbreviated except in terms of the substrates for which there are accepted abbreviations, e.g. ATPase and RNase.

See next page for grading rubric.

Biochemistry Lab I grade analysis

Student: _____

Title page and general neatness (5 pts)
Follows guidelines from handout

Total(____)
1 2 3 4 5

Introduction (56 pts)

Total(____)

- MDH, GFP & His - General intro
- Use of GFP in science
- DNA Purification - Purification methods
- Pros and Cons
- Strains
- Transformation
- Chromatography intro.
- Chromatography I
- Chromatography II
- SDS-PAGE - What is it
- Determination of Size
- Western Blot

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Methods (38 pts)

Total(____)

- DNA Purification
- Transfection
- Chromatography I
- Chromatography II
- SDS-PAGE
- Western Blot

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Results (68 pts)

Total(____)

- DNA Purification - Did they show results
- Was it correctly labeled
- Figure legend for Gel
- Transfection - Some table of results
- Chromatography I
- Combined graph of RFU and Bradford
- Figure legends
- Writing of Results
- Chromatography II
- Combined graph of RFU and Bradford
- Figure legends
- Writing of Results
- SDS-PAGE - Gel
- Standard curve
- Legend
- Western Blot - Blot
- Legend
- Purification Table

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Discussion (25 pts)

Total(____)

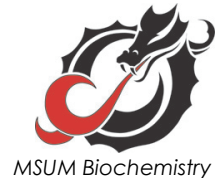
- DNA Purification
- Yield and purity
- Transfection
- Overall results
- Effects of antibiotics on plate growth
- Why transform in amp presence?
- Chromatography
- notice of resolution
- comparison with other data

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SDS-PAGE		
- Size determination by SDS PAGE	1 2 3 4	
- Contaminants?	1 2 3 4	
- Presentation of Gel and fig legend	1 2 3 4	
- Comparisons between intensity and bands	1 2 3 4	
Western Blot		
- Correlation with gel and blot	1 2 3 4	
- Purity	1 2 3 4	
<u>References (5 pts)</u>		Total(____)
- Style/format	1 2 3 4	
- Additional citations	1 2 3 4	
<u>Overall Writing Style (10 pts)</u>		Total(____)
Comment:		