BCHM 444  SPRING 2010  Biochemistry and Molecular Biology Methods

Prerequisites: CHEM 311/312 plus BCHM 340 and/or 441 (preferred).

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Office hours: by appointment

Text: None. We will be reading original papers and there will be some lab protocols posted on the web site. It is your responsibility to download, print and read the protocols before class, as well as to bring them or write the protocol in your notebook.

Mon & Wed 3:10-6:00 PM (ending time is approximate; be prepared to start on time, and hopefully we won’t finish late too often)

Description
This class will consist of about 1 hour of lecture and discussion; the remaining 5 hours per week will be laboratory work. We will study the enzyme alkaline phosphatase from Escherichia coli. You will read the published papers about this enzyme and study its 3-dimensional structure using computer graphics, then develop a hypothesis concerning a structure/function relationship in the protein and test it by mutating the gene, cloning and sequencing it, expressing the protein, isolating & purifying the enzyme, and comparing its activity to the wild-type enzyme and the mutants made by your fellow students. Lab work will be done in teams of two, but exams, notebooks, and the final paper will be individual.

Presentation – 10%
Each student will be assigned a presentation generally consisting of a 20 minute report using powerpoint slides on a background research article, a current research article or a technique article related to the coursework. The talk will be followed by a question period in which class participation will be noted. To facilitate class participation, relevant papers will be made available to the class in advance of the presentation. Slides will be posted after the presentation.

Midterm – 25%
The Midterm exam will consist of a series of questions requiring discussion of the background, hypotheses, methods, and results to date for this project and other material covered in the class.

Final Paper - 25%
The Final Exam will consist of a research article describing the work done by the entire class, in a form suitable for publication in a major scientific journal. It will be due at the beginning of Finals Week.

Assignments and Quizzes - 15%
There will be certain assignments you will work on outside of class and turn in. Each student must turn in their own work, but you may help each other and/or share data. These include: 1) A one page paper describing the mutation you propose to make and your hypothesis concerning the predicted effects of this mutation on the structure and function of the protein, complete with references supporting your analysis and predictions. 2) The design of the primers you propose to use for the mutagenesis, along with the computer analysis of their properties. 3) Purification data charts with calculations of total activity and specific activity from every step of the purification for both wild-type and mutant enzymes. 4) Calculations of $K_M$, $V_{max}$, and other parameters for both mutant and wild-type enzymes.

There may also be a short quiz at any time during a lab period, covering the procedures to be done that day or any aspect of what we have learned to date.

**Notebook** - 25% (including lab performance)
The notebook MUST BE PERMANENTLY BOUND, not a ring binder nor spiral bound.
- Pages must be numbered consecutively and none may be ripped out.
- Work should be written in pen.
- Mistakes are to be crossed out but left on the page.
- No writing on loose sheets of paper, paper towels, backs of hands, or anywhere but the notebook while you are in the lab.
- Experiments should have
  - a title,
  - a short purpose statement,
  - a brief protocol written before class (work to be done)
  - a clear, succinct record of what you did, to be written down in lab while work is being performed
  - comments, observations and additional information discussed in class
  - drawings, photos or printouts of results as they appear
  - calculations and graphs (if these were done in a spreadsheet, printouts and sample calculations or formulas should be in the notebook)

Notebooks are of prime importance in this course; the notebook, together with lab performance, will comprise **25% of the course grade**. Certain assignments, such as the mutation hypothesis, primer analysis, and calculations will be turned in on separate sheets or electronically and graded separately (see above), but they must also be included in, or attached to, the notebook when it is turned in. Notebooks will be examined once or twice without prior warning during the semester, as well as turned in at the end of the semester together with the final paper.