## *Preparation for the exam 2*

**Chem112L, Spring 2013** **Exam dates: June 10, 4 PM; June 12, 7:30 PM**

This exam focuses on mass spectrometry, protein-ligand interactions, and crystallization / diffraction analysis of macromolecules. I expect that you know the basic material from the three previous experiments as well. I intend to have a mix of knowledge-showing essay-type, problem-solving, and multiple-choice questions. Knowledge of the following helps you in preparing for the exam:

1. Physical principles behind each of the molecular process
    a. Protein ionization and its relevance to mass spectrometry
    b. Ligand binding to macromolecules
    c. Protein crystallization

2. Physical principles behind each of the observation/detection methods
    a. X-ray diffractiometry
    b. UV absorption spectroscopy, design of a UV-Vis spectrophotometer
    c. Separation of molecules based on *m*/*z* values; protein identification
    d. Identification of structure of peptides and metabolites via mass spectrometry

3. Theoretical description of biochemical processes such as ligand binding
    a. Estimation of thermodynamic parameters from experimental data
    b. Dissociation constant in relation to equilibrium concentrations
    c. Rate constants in relation to the equilibrium constant
    c. Relationships between free energy, enthalpy, entropy and heat capacity

4. Structural and functions concepts pertaining to ligand binding, protein function and metabolites
    a. Biological function and catalytic mechanism of lysozyme
    b. Structure of peptides, proteins, and nucleic acids
    c. Fragmentation of peptides by collision-induced dissociation
    d. Fragmentation of cation radicals during electron impact ionization
    e. Fragmentation of cations and anions during electrospray ionization
    f. Electronic structure of molecules, molecular orbitals
    g. Electronic $\pi \rightarrow \pi^*$, and $n \rightarrow \pi^*$ transitions
    h. Solvatochromic shifts in UV-Vis spectra

5. Instrumentation
    a. Basic design and operation of a X-ray diffractiometers and synchrotrons
    b. Basic design and operation of a dual-beam UV spectrophotometer
    c. Ionization/vaporization methods for small molecule MS
    d. Ionization/vaporization methods for macromolecule MS
    e. Basic design and operation of a ESI mass spectrometer
    f. Basic design and operation of MALDI mass spectrometer
    g. Comparison of different mass analyzers
    h. Operation of a SLR digital camera for image acquisition

6. Broader applications of methods covered; other approaches to study these phenomena
    a. Using mass spectrometry to study ligand binding
    b. Other UV spectrophotometry to study structure and ionization of binding

c. Comparison of NMR and crystallography for determination of protein structures
d. How to apply crystallography to other problems in biochemistry
e. Applications of mass spectrometry in proteomics
f. Application of mass spectrometry in metabolite analysis

7. Practical aspects of each of the experiments and computations
    a. Why such wavelengths and cuvettes
    b. Why such concentrations, pH, salts, buffers, etc

8. Data analysis.
    a. Understand why we used such model equations for fitting
    b. Understand the meaning of each of the fitting parameters
    c. Understand the measures of quality of data and fitting
    d. Understand the workflow of scientific data analysis programs such as *Mathematica*
    e. Understanding the workflow of a crystallography programs such as Coot
    f. Understand how to interpret 2D X-ray diffraction data
    g. Understand how to interpret 3D electron density maps
    h. Different ways to plot and analyze ligand binding data
    i. Different ways to plot ligand binding data
    j. Analysis of binding data when ligand and protein concentrations are similar
    k. Analysis of first order irreversible kinetics
    l. Analysis of first order consecutive reaction kinetics

9. Miscellaneous
    a. How to derive equations for association/dissociation equilibrium
    b. Fluorescence as a method to study ligand binding
    c. Assumption of high ligand and low protein concentration
    d. How to visually estimate fitting parameters
    e. How to derive equations that describe temperature-dependence of equilibrium constants

**Answers to many of the questions require substantial thinking. Memorizing all the material may not be the best way to study for this exam.**