CHEM 4416/6416
Molecular Spectroscopy (Biochemical Mass Spectrometry, Protein/ Peptide MS)
Problem Set

1. Draw the structure for the following dipeptides (showing all atoms and all bonds, ensure that all functional groups are ionized assuming they are in low pH solution).
    a. Ala-Gly
    b. Gly Ala
    c. Cys-Lys
    d. His-Phe
2. Calculate the m/z for each of these dipeptides ($[M+H]^+$ and $[M+2H]^{2+}$ if possible)
3. Write a mechanism for the formation of a b-series ion for each of the dipeptides in question 1. Calculate the m/z for each fragment that should be detected in an MS/MS experiment.
4. Write a mechanism for the formation of a y-series ion for each of the dipeptides in question 1. Calculate the m/z for each fragment that should be detected in an MS/MS experiment.
5. Explain the method of protein mass finger printing. What kinds of Mass analyzers and ionization sources are used for this technique, and why?
6. Explain the method of peptide sequencing. What kinds of Mass analyzers and ionization sources are used for this technique, and why?
7. What are the advantages and disadvantages to the techniques in questions 5 and 6?
8. How do programs such as Sequest make use of genomic data to determine protein identity? What other steps are required in such an experiment?
9. How are tools such as endopeptidases (trypsin, chymotrypsin, pepsin etc) and chemical reagents such as cyanogen bromide used to identify protein structure?
10. Draw the structure for the following peptides. Calculate their appropriate m/z under acidic analysis conditions. Predict all b- and y-series ions.
    a. Ser-Phe-Tyr-Cys-Lys-Ala-Cys
    b. Cys-Ala-Lys-Cys-Tyr-Phe-Ser
11. Repeat question 10 but consider that the peptide was first alkylated with iodoacetamide.
12. Assume you have a protein with 4 Cys residues. You have reason to believe that two compose a disulfide linkage that is crucial for proper protein folding. The other two are suspected to be in the reduced form. How do you design an experiment to determine unambiguously which Cys are which?
13. Assume you are charged with determining the sequence of a novel protein from a rare slime mold newly discovered by science. All you have to work with is an LC-MS/MS system, any reagents typically required for protein peptide analysis by MS, and a freezer full of all known endopeptidases. Design your approach and explain how you will unambiguously determine the structure.
14. Repeat question 13 if you freezer full of endopeptidases has been replaced by a freezer full of all known exopeptidases. How does your approach change? Do you require different instrumentation?

15. Draw the structure and calculate the expected m/z for the peptide Ala-Ser-Gly-Met-Asp-Thr-Ala-Lys under acidic conditions. What peptides result from treatment of this peptide with cyanogen bromide? Write a mechanism for this reaction. Determine the b- and y-series ions for all peptide products.
16. Additional practice questions can be found embedded within the lecture material provide along with this topic.