

MARZLUFF, ELAINE M. (1967) Associate Professor. A.B., 1989, Harvard University; Ph.D., 1995, California Institute of Technology; Research Associate and Postdoctoral Fellow, 1995-1996, Howard Hughes Medical Institute and California Institute of Technology; Visiting Assistant Professor, 1996-1997, Pomona College. Assistant Professor, 1997-2003, Grinnell College. Visiting Professor, Oxford University, 2004-05. PHYSICAL CHEMISTRY. Research Interests: Investigation of protein and peptide structures and dynamics using mass spectrometry and NMR; gas phase ion molecule chemistry. (641) 269-4314. MARZLUFF@GRINNELL.EDU

My group uses mass spectrometry combined with hydrogen/deuterium (H/D) exchange and computational chemistry to characterize the molecular interactions and dynamics in macromolecules (specifically amino acids, peptides and proteins). Mass spectrometry is emerging as an effective probe macromolecule structure in both solution and the gas phase. The long-term goal of these projects is to take advantage of these techniques to develop a greater understanding of non-covalent interactions and molecular dynamics of large biological molecules in both solution and the gas phase. This summer we will focus primarily on studies in the gas phase using mass spectrometry.

Characterization of the gas phase structure of proteins is important for understanding the degree to which interactions with water play a role in determining three dimensional protein structures. In the early 1990's Fred McLafferty showed that proteins appear to adopt compact structures in the gas phase.¹ An accompanying commentary by Peter Wolynes discusses how in the absence of solvent "the stability of the native protein is not compromised... the classical secondary structures should be even more thermodynamically stable." He goes on to say "Definitively establishing the degree of structural similarity between the compact structures of McLafferty and co-workers and the native solution structure thus become a very high priority, both to understanding the microscopic forces of folding and perhaps to understanding evolutionary constraints on protein folding kinetics."² This summer we will focus on hydrogen deuterium exchange of peptides, as understanding the exchange of simple systems is necessary for complete analysis of proteins.

The mass spectrometry part of these studies is carried out using a Finnigan Electrospray Ionization Ion Trap Mass spectrometer.³ (ESI-ITMS). Electrospray ionization allows the volatilization of large molecules for study by mass spectrometry. H/D exchange of labile protons in a molecule (typically the hydrogen attached to nitrogen and oxygen) results in a mass shift by 1 amu for each hydrogen exchanged and is easily followed by mass spectrometry. This summer I plan to have 2-3 students working with me on different aspects of the projects described below. No previous experience is necessary; students will learn techniques involved in studying structures of large and small molecules using mass spectrometry. Interested students might also be able to apply these techniques in solution as a comparison to the gas phase data.

Hydrogen/Deuterium exchange of peptides.

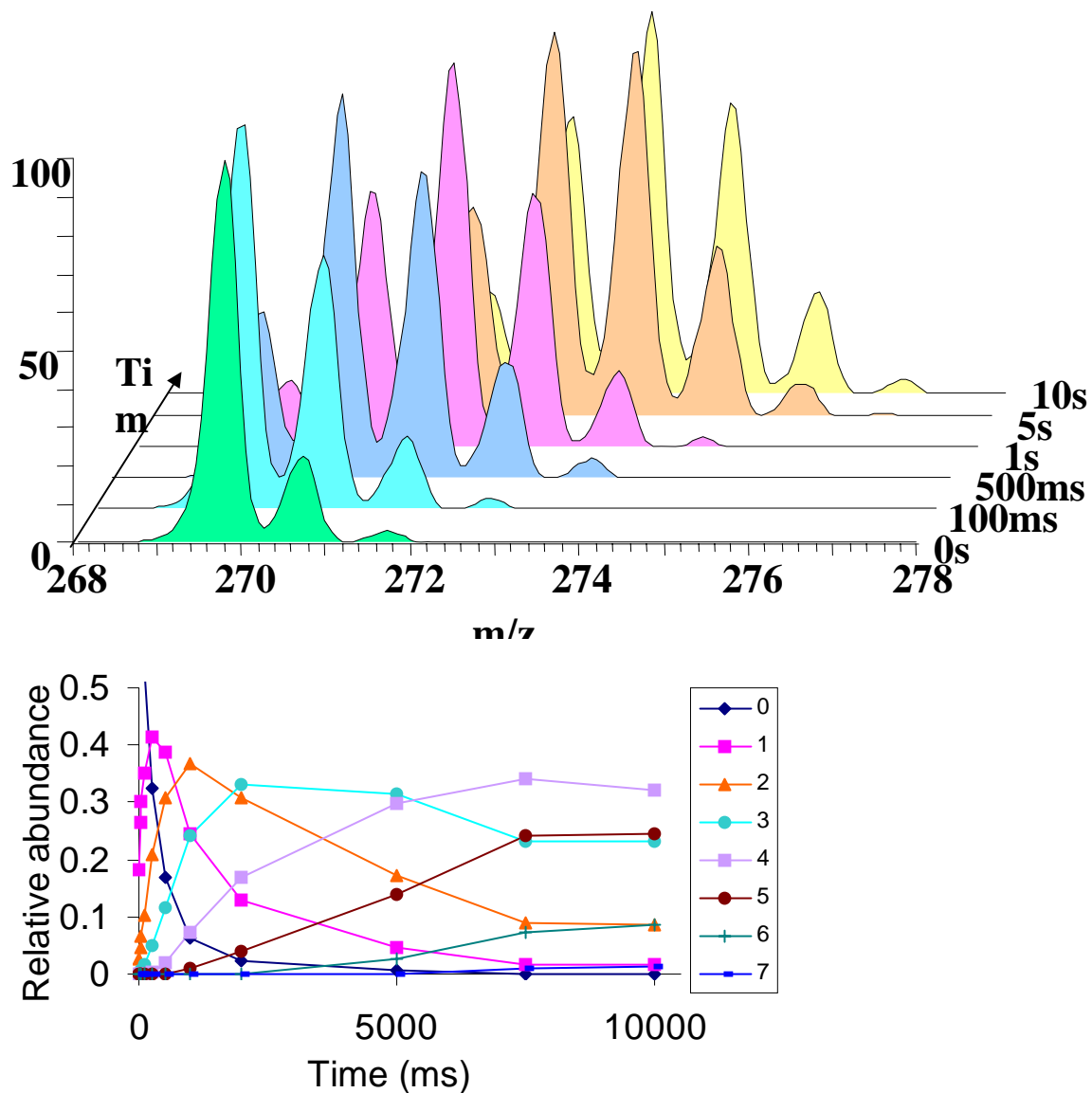
Hydrogen/Deuterium exchange mass spectrometry studies have not been limited to solution but have also been used to probe gas phase structures of proteins and peptides.^{4,5} To assist analysis of protein H/D exchange, a thorough understanding of the mechanisms and intrinsic rates of exchange are necessary. Figure 1 shows sample data of H/D exchange with time for the peptide gly-his gly. The number of exchanges increases with time, and from this data rate constants for each exchange can be obtained. Figure 2 shows the peptide gly-gly with the exchangeable protons in grey. Even in this simple peptide, there are 3 different chemical environments for the protons. Marshall and co-workers analyzed the site-specific kinetics of gly-gly and showed that the amine protons exchanged fastest and the carboxylic acid protons exchanged slowest.⁶ Last summer we investigated the rates of exchange of small di and tri peptides with exchangeable side chains. We use solid state synthetic techniques to create custom peptides, and computational chemistry to determine the possible low energy conformations of the molecules. These combinations of techniques have allowed us to start to investigate the

subtle effects of amino acid position on exchange. This summer we will continue these investigations. These studies will combine synthesis, mass spectrometry, kinetic fitting and computational chemistry.

Intermolecular Interactions:

One of the advantages of the gentle electrospray ionization technique is that it permits the study of complexes held together by intermolecular interactions. In past summers we studied the energetics of dissociation and H/D exchange of amino acid clusters. One particularly interesting question involves looking at the possibility of peptides and amino acids

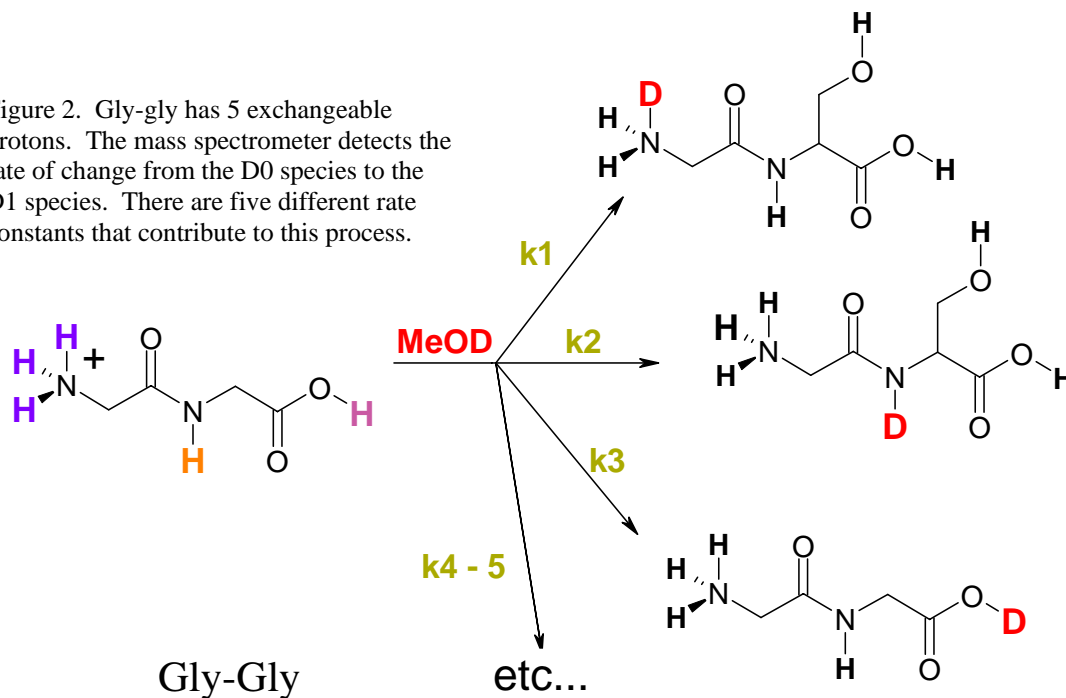
Figure 1: H/D exchange with time of the peptide gly-his-gly which has 7 exchangeable protons. A) Mass spectra as a function of time. B) Intensity as a function of time.



adopting a zwitterionic conformation in the gas phase. The results for arginine, for example, show significantly enhanced rates of exchange in clusters compared to in its isolated form. These enhanced exchange rates indicate a dramatic structure change, and are attributed to one of the arginines adopting the zwitterionic form.

Questions that may be investigated this summer include looking at interactions between peptides and potentially between ligands and protein mimics.

Figure 2. Gly-gly has 5 exchangeable protons. The mass spectrometer detects the rate of change from the D0 species to the D1 species. There are five different rate constants that contribute to this process.



Students interested in these projects should start by consulting references 3c and 3d and 4b as background reading. These are readily available through the Grinnell College Library

References

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