Towards fluorescence-based probes of gas-phase protein structure

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Introduction:
Given the newfound prevalence of the soft electrospray ionization (ESI) method for analyzing biological molecules by mass spectrometry (MS), an important goal that has been placed at the forefront of the field has been on the development of new structural probes for studying protein structure in the gas phase [1-5]. MS analysis is ultimately done in vacuum, an environment which differs significantly from the crowded aqueous environment of a cell, or even the hydrophobic environment of a cell membrane. Thus, a necessary prerequisite to fully exploiting the very useful mass information provided by a mass spectrum is to have a solid understanding of the solvation–desolvation link. In this work, we extend efforts towards the development of an instrument which enables the use of fluorescence-based probes [5-7] for studying gas-phase protein structure.

Experimental:
A commercial 4.7 Tesla FT-ICR mass spectrometer (IonSpec Inc.) is currently being modified for fluorescence spectroscopy, using commercially available parts, and using components made in-house.

Results:
Instrument design considerations will be illustrated in depth, including those specific to robust laser beam alignment, fluorescence collection alignment, and maximization of measurement duty cycle. Gas-phase fluorescence experiments, including those on the bright class of rhodamine dyes, and their noncovalent complexes with host molecules, will be shown. For these model systems, we will show that gas-phase fluorescence measurements can be used to delineate the modulating photophysical effects of solvation. Current efforts are focused on improving the collection efficiency of the set-up, using state-of-the-art sensitive detectors, and an improved fluorescence collection alignment procedure. Time permitting, novel measurements of fluorescent molecules non-covalently bound to model proteins will be demonstrated. By comparison with measurements done in solution, gas-phase fluorescence measurements will aid in assessing the integrity of the dye’s binding site in the gas phase, thus shedding light on the structure of the noncovalent biomolecular complex in the gas phase.

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