Chapter 1. Introduction

1.1. Background

Non-covalent bonds play a critical role in determining the structure and behavior of biological molecules.[1] The tertiary structure of proteins is largely determined by non-covalent interactions, and double-stranded DNA is held together through non-covalent interactions between base pairs. This thesis examines reactions occurring in the gas phase between non-covalently bound reaction partners, with a focus on biologically relevant molecules. Typically these reactions are referred to as cluster phase reactions, since the species are not physically separated, but bound together through non-covalent interactions. This can be thought of as the gas phase counterpart to template-directed synthesis, which uses non-covalent interactions to enhance the rate and/or selectivity of chemical reactions.[2]

Reactions of gas phase molecular clusters are of intrinsic interest, as they provide an isolated system in which basic principles of reactivity can be studied. Clusters of solvated ions can provide a link to condensed phase solution reactions, while ionically bound clusters can resemble condensed phase salts and other solids. The rational design and assembly of small molecular aggregates holds significant promise for new scientific investigations, ranging from the preparation of novel photonic and electronic materials[3-6] to solid state chemical synthesis.[2,7] Finally, structural information may be obtained from reactions such as H/D exchange reactions, peptide backbone fragmentation reactions, and even ion solvation patterns. Such information can be used to more swiftly and accurately sequence peptides and proteins, to examine higher-order structure, and to distinguish between isomers.

Important differences exist between reactions in the gas phase and in solution. For example, consider the reaction diagram shown in Figure 1.1, which portrays the reaction of a doubly charged anionic species with a singly charged cationic species. (The polarities of these species can be reversed with no loss in generality.) The reaction begins with the two molecules at a large distance from each other. In the gas phase, the dianionic species is subject to Coulomb repulsion between the two charged groups, with a dielectric constant of 1. In solution, however, the Coulombic repulsion between the two similarly charged groups is mediated by solvation of the charges, resulting in a higher dielectric constant (80) and a significant overall stabilization of the system. As the reaction proceeds, and the positively charged species is brought closer to the dianionic species, the overall effect in the gas phase is that of mediating the repulsion between the anionic charges, typically in a salt-bridge configuration. The total energy of the gas phase system is lowered and a metastable adduct is formed. If the two molecules are able to undergo a reaction to neutralize the charge, resulting in a neutral species and a negatively charged species, these products will typically be energetically downhill from the adduct, as the Coulombic repulsion is completely removed when the reaction proceeds. However, in the solution phase, the approach of the positively charged ion results in partial desolvation of all charges, resulting in an energy *maximum* that is the barrier to reaction. The reaction products of such a reaction are often endothermic, as the stabilization energy of charge solvation is removed as the reaction proceeds.

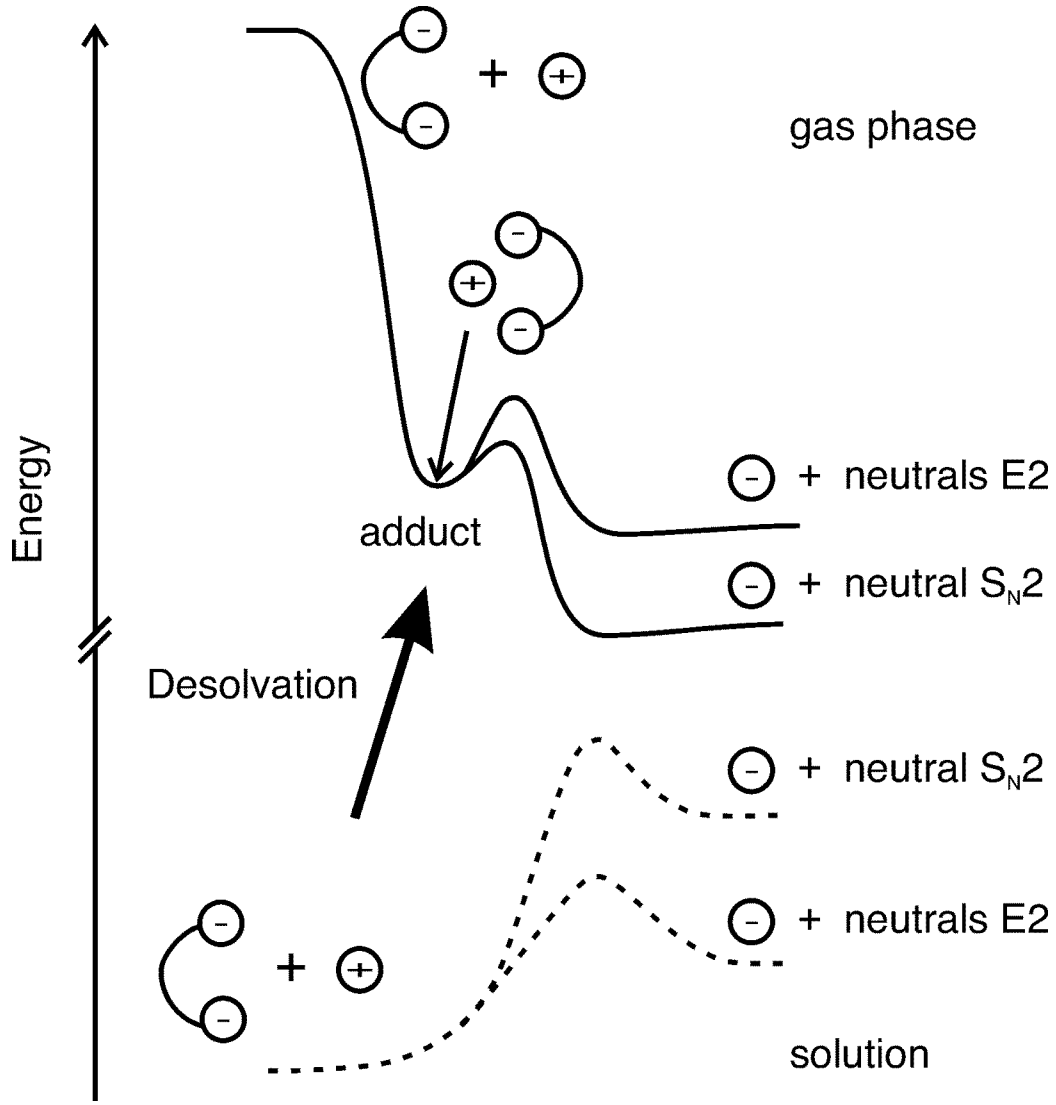


Figure 1.1. Schematic energy diagram illustrating the differences between solution phase reactions (dotted lines) and gas phase reactions (solid lines). Note that the energy scale is discontinuous.

All of the experiments presented in this thesis rely on the technique of electrospray ionization to generate ions in the gas phase. Electrospray ionization is a gentle process in which reactants are gradually completely desolvated.[8,9] In the experiments presented in Chapters 2 – 4, the reactants are initially in solution. During the process of electrospray ionization, the reactants are gradually completely desolvated and

detected as metastable adducts in the gas phase. The gentle nature of the electrospray ionization process allows the formation of metastable clusters that do not have enough internal energy to overcome the energy barrier to product formation, as shown in Figure 1.1. Collisional activation of such clusters can result in dissociation or, if the cluster components are strongly bound, in reaction of the cluster components to break and form covalent bonds.

In Chapter 5, the electrospray process allows the transportation of an azo compound into the gas phase. The azo compound is reactive enough that it fragments before any other bonds in the molecule, forming a reactive free radical species that can then be isolated and dissociated. Chapters 6 and 7 examine ions that have not been fully desolvated during the electrospray process. The evaporation dynamics as a function of time and the patterns observed in the mass spectrum of solvated ions can reveal information about their structures.

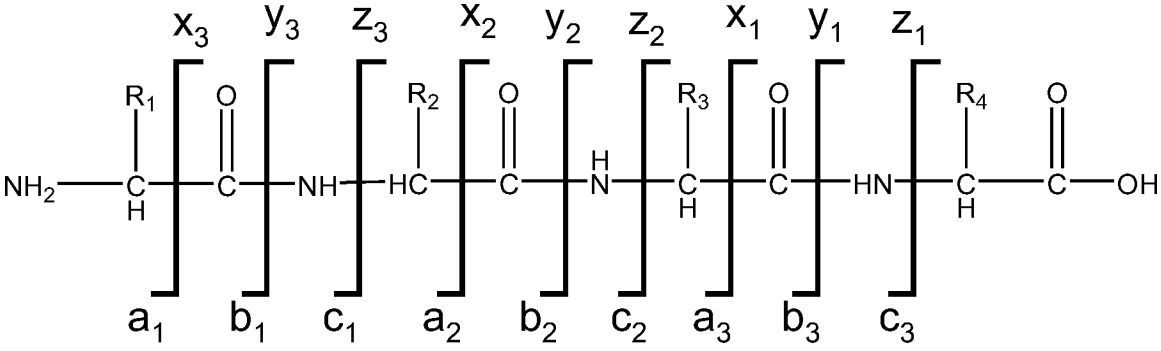


Figure 1.2. Fragmentation nomenclature used throughout the thesis.

In many of the experiments detailed here, peptides are fragmented to obtain structural information about the location of modifications or to determine what fragmentation pathways predominate. The nomenclature used for peptide ion fragments is that described by Biemann and shown in Figure 1.2.[10] Singly negatively charged

peptide fragments, $[M - H]^-$ are two mass units lower than their singly positively charged counterparts, $[M + H]^+$, and each additional charge corresponds to a change in mass of two Daltons.

1.2. Thesis Content

1.2.1. Cluster Phase Reactions

Chapters 2 – 4 discuss work on cluster phase reactions. Cluster phase reactions are reactions occurring between two components of a gas phase cluster. In order to facilitate a cluster phase reaction, rather than a dissociation of cluster components, the cluster must be held together strongly, and proton transfer must be inhibited. In Chapter 2, these principles are set forward and used in a study of the reactions of a series of alkylammonium ions with triphosphate. It is also shown that DNA can be alkylated by alkylammonium ions in the gas phase, and peptides with carboxylate residues, such as Asp and Glu, are also alkylated using tetraalkylammonium ions.

Chapter 3 studies cluster phase reactions of negatively charged methyl phosphate clusters, held together with either protons or sodium ions. While in Chapter 2, proton transfer was inhibited by studying systems without labile protons, the methyl phosphate clusters in Chapter 3 include both sodium ions and labile protons that can be transferred between methyl phosphate groups. Series of clusters in which protons are sequentially replaced by sodium ions are studied. The cluster phase reactions observed are also studied in terms of the cluster size. While sodium ions typically stabilize the cluster and allow for more condensation reactions between phosphates to occur in salt clusters, at large cluster sizes, salt clusters are no longer sufficiently strongly bound to undergo condensation reactions in any significant abundance. This may impose a natural limit on

the utility of small molecular clusters for directed chemical synthesis. The end product of a series of collisional activations is a fully condensed methyl polyphosphate species to which sodium cations are bound. Figure 1.3 shows the structure of methyl triphosphate with two sodium cations. As the reactant cluster size increases, the structure of the polyphosphate obtained from collisional activations more closely resembles that found in the condensed phase.

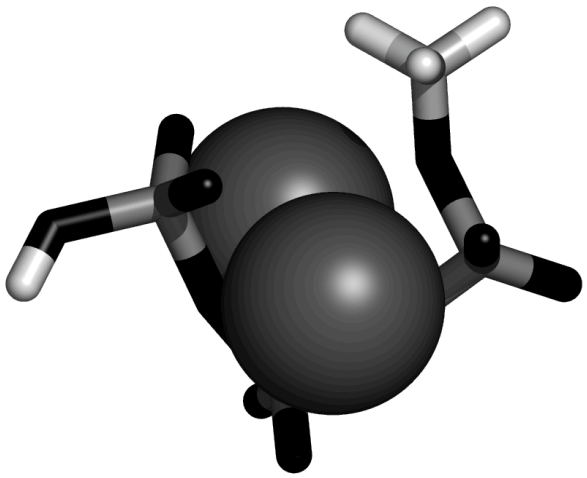
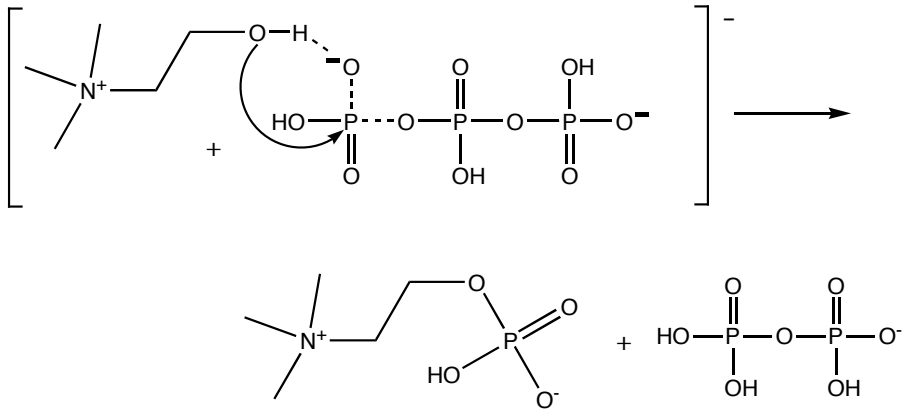


Figure 1.3. Triply negatively charged methyl triphosphate complexed with two sodium cations.

Chapter 4 extends the principles of cluster phase reactions to examine the reactions of triphosphate with molecules containing hydroxyl substituents. The reaction of choline is used as a model system. In this case, a labile hydrogen on the hydroxyl substituent is available for transfer to a negatively charged phosphate residue. However, proton transfer is accompanied by an attack by the hydroxyl oxygen on a phosphorus atom, resulting in the phosphorylation of the hydroxyl residue. The positively charged site on the choline increases the acidity of the alcohol group and facilitates the reaction

shown in Scheme 1.1. Similar reactions are reported for peptides containing residues with hydroxyl groups, such as serine and threonine.



Scheme 1.1. Reaction of positively charged choline with doubly negatively charged triphosphate.

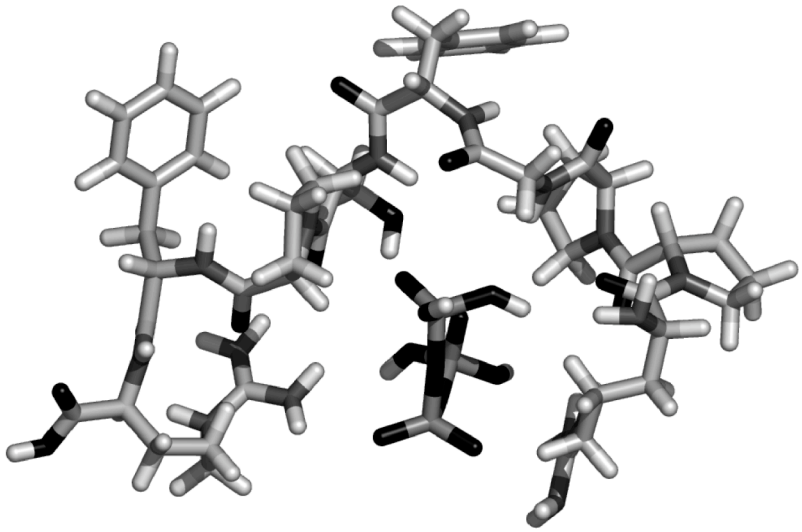


Figure 1.4. PM5 optimized structure of doubly positively charged bradykinin and negatively charged triphosphate.

These unique reactions represent the first gas phase phosphorylation of alcohols. In addition, the observed reaction occurs at a site (the hydroxyl moiety) remote from

charged functional groups in the same molecule, which offers the possibility of extending the versatility of cluster phase reactions for chemical synthesis. The interaction of positively and negatively charged sites may lead to alignment in a pair of molecules and facilitate a desired transformation. Figure 1.4 shows how this might occur for bradykinin, which is observed to be phosphorylated by triphosphate. In this instance, the protonated guanidinium groups hold the triphosphate anion in close proximity to the serine residue. Additionally, the ease of phosphorylation of choline by triphosphate in the gas phase may be useful in understanding prebiotic syntheses of membrane lipid components.[11,12]

1.2.2. Free Radical Peptide Sequencing

Chapter 5 details results of experiments using free radicals to initiate peptide cleavage in the gas phase. Free radical initiated peptide sequencing, or FRIPS, is accomplished by covalently attaching an azo free radical initiator (Vazo 68) to a peptide in solution phase using standard peptide coupling techniques. While the free radical initiator is covalently bound, reaction occurs when the radical interacts with the rest of the peptide through noncovalent interactions. Multiple collision-induced dissociation steps result in backbone fragmentation products. While collision-induced dissociation of a standard peptide yields *b*- and *y*-type fragment ions, *c* and *z* fragments are most commonly observed with FRIPS. These fragments are also commonly observed in electron capture dissociation experiments, in which radical peptides are collisionally dissociated. The selectivity of the fragmentations depends strongly on the reactivity of the free radical initiator. By changing the nature of the azo species conjugated to the peptide, the reactivity of the conjugated complex could be enhanced or diminished. Such

efforts may lead to the synthesis of a true gas phase enzyme, which would cleave only at specific sites in a protein.

1.2.3. Solvated Ions

Solvated ions can be thought of as occupying a phase space between the gas phase and solution phase. Such clusters are observed under high-vacuum conditions, and correspond to a micro-solvated species. While they can serve as useful models for solution phase phenomenon, the chemistry observed in water clusters is distinct from both solution phase and gas phase chemistry. Such clusters are typically weakly bound in that water molecules are more likely to evaporate from the cluster than react within the cluster, although in some cases (see the *t*-butyl chloride discussion below) covalent bonds can be broken and formed within a cluster.

Chapter 6 details experiments involving solvated cluster ions. First, a novel method of differentiating between diastereomers is demonstrated. The water clusters around enantiomeric ions are shown to have reproducibly differing intensity ratios. While the dipeptide examined did not show evidence of having any "magic number" clusters of unusual stability, this method might be used in other contexts to examine how diastereomers locally organize solvent molecules. Results of experiments on water clusters of tetraalkylammonium ions are also presented. These molecules are used as structure-directing agents in zeolite formation, and the ordering of water around the alkylammonium ions may play a key role in directing zeolite synthesis. A series of such ions is examined, including doubly charged quaternary ammonium ions. A regular oscillation in the water cluster spectrum of a doubly charged quaternary ammonium ion is attributed to sequential solvation of each charge site.

Chapter 6 also includes a study of the reaction of *t*-butyl chloride with water to lose HCl in water clusters. Such S_N1 reactions are quite difficult to study in the gas phase, as they require dissociation of one reactant to occur before the reaction takes place. To bypass this difficulty, the solution phase is mimicked by allowing the reaction to occur within the micro-solvated environment of the water cluster. The *t*-butyl chloride is taken up in the electrospray plume, and the water clusters produced via electrospray can be examined. The most persistent species formed is $(H_2O)_2C_4H_9^+$, which remains after all other peaks disappear from the trap. This indicates that at some point during the evaporative process, HCl is lost from water clusters and the reaction of *t*-butyl chloride with water occurs. The reaction seems to take place predominantly in medium-sized water clusters, containing between 39 and 60 water molecules. These results open up the possibility of studying as-yet inaccessible reactions in solvated systems. Results from such experiments can be compared to calculations utilizing only a finite number of water molecules, rather than necessitating a continuum model as solution phase reactions do.

1.2.4. H/D Exchange Dynamics

Isotope exchange experiments are often used to probe the conformation of a molecule, in both solution and the gas phase. Typically, a reagent molecule exchanges hydrogen for deuterium at exposed labile sites on the target species. Information about molecular structure is inferred from the rate of exchange and number of exchanges that occur. The cluster composed of the target species and the exchange reagent is formed in the gas phase, and although it can be strongly bound, the binding energy is present within the complex, so the complex dissociates before it is observed.

In Chapter 7, the exchange behavior of sodiated glycine oligomers (Gly_1 to Gly_5) with ND_3 is studied. The observed H/D exchange behavior does not always correlate to the minimum-energy structure, or the structure determined via other methods, of the gas phase sodiated glycine oligomers. The solvation energy provided by the exchange reagent allows the ion-molecule complex to access intermediates structurally distinct from the parent ions. For example, Figure 1.5 shows the H/D exchange behavior of sodiated diglycine with ND_3. Initially, sodiated diglycine is in a charge-solvated conformation. However, the complexation of ammonia to the sodiated diglycine provides enough energy to allow the complex to rearrange to a zwitterionic conformation, where the N-terminus of the dipeptide is protonated and the C-terminus is deprotonated. The exchange behavior observed (one fast exchange and two slow exchanges) is characteristic of the zwitterionic conformation.

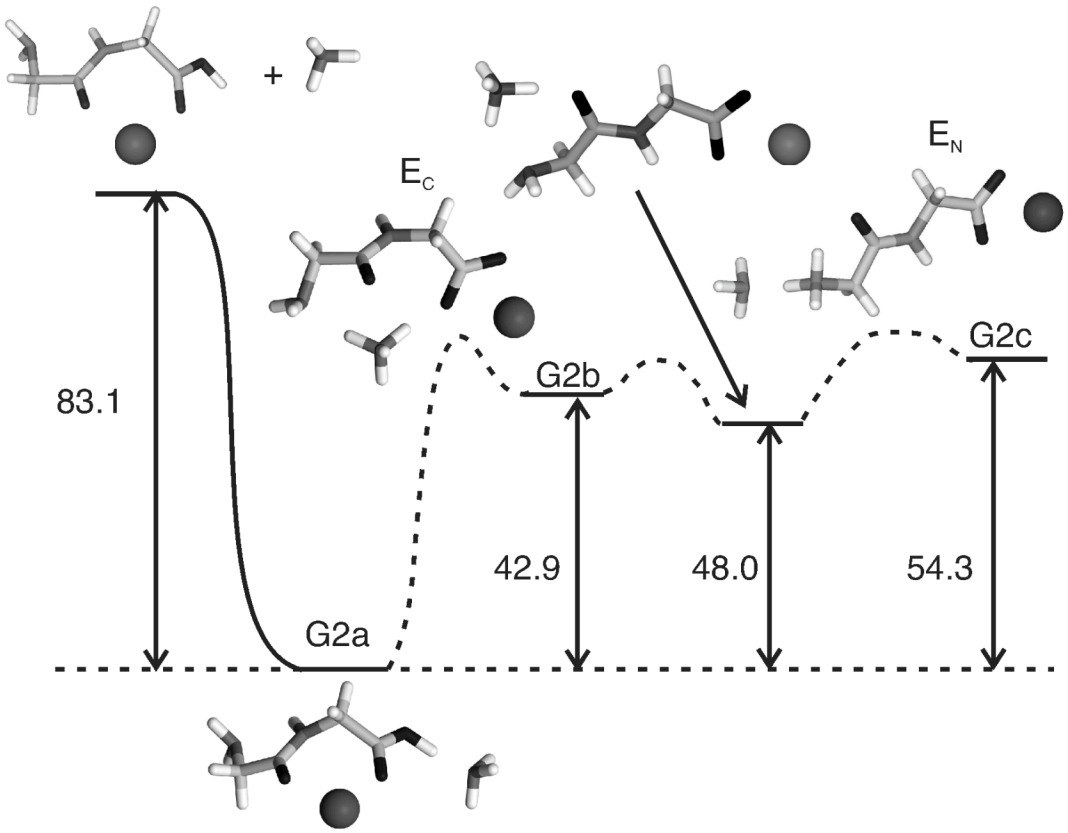


Figure 1.5. H/D exchange behavior of sodiated diglycine and ND_3.

Since molecules are able to access structurally distinct intermediates from the parent ion when complexed with an exchange reagent, the utility of the H/D exchange method as a probe of gas phase structure is questionable. The probe species (or observer) changes the structure, which is the measurable quantity of interest, and any attempt to assign structural information based on exchange behavior should be supplemented with a detailed examination of possible exchange mechanisms. This work helps to clarify the discrepancies between the structural information obtained using ion mobility spectrometry[13] and that obtained using H/D exchange dynamics.

1.3. Conclusion

Reactions occurring in small clusters can exhibit novel properties, distinguishable from both the solution and the gas phase. Important properties of biological molecules, such as structural information or higher-order structure, can be studied in small clusters, and these results may aid in understanding reactions within proteins and the origins of biological molecules. Covalent bonds may be broken and formed in both strongly and weakly bound clusters.

1.4. References

(1) Desfrancois, C.; Carles, S.; Schermann, J. P. *Chem. Rev.* **2000**, *100*, 3943.

(2) Prins, L. J.; Reinhoudt, D. N.; Timmerman, P. *Angew. Chem. Int. Ed.* **2001**, *40*, 2382.

(3) Alivisatos, A. P.; Barbara, P. F.; Castleman, A. W.; Chang, J.; Dixon, D. A.; Klein, M. L.; McLendon, G. L.; Miller, J. S.; Ratner, M. A.; Rossky, P. J.; Stupp, S. I.; Thompson, M. E. *Adv. Mater.* **1998**, *10*, 1297.

(4) Gu, Z.-Z.; Hayami, S.; Meng, Q.-B.; Iyoda, T.; Fujishima, A.; Sato, O. *J. Am. Chem. Soc.* **2000**, *122*, 10730.

(5) Murray, C. B.; Kagan, C. R.; Bawendi, M. G. *Annu. Rev. Mater. Sci.* **2000**, *30*, 545.

(6) Trindade, T.; O'Brien, P.; Pickett, N. L., *Chem. Mater.*

(7) Etter, M. C. *J. Phys. Chem.* **1991**, *95*, 4601.

(8) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64.

(9) Fenn, J. B. *Angew. Chem. Int. Ed.* **2003**, *42*, 3871.

(10) Biemann, K. *Methods Enzymol.* **1990**, *193*, 886.

(11) Ellison, G. B.; Tuck, A. F.; Vaida, V. *J. Geophys. Res.* **1999**, *104*, 11633.

(12) Tuck, A. *Surveys Geophys.* **2002**, *23*, 379.

(13) Wyttenbach, T.; Bushnell, J. E.; Bowers, M. T. *J. Am. Chem. Soc.* **1998**, *120*, 5098.