MASS SPECTROMETRY CHARACTERIZATION OF NOVEL SYNTHETIC MATERIALS

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MASS SPECTROMETRY CHARACTERIZATION OF NOVEL SYNTHETIC MATERIALS

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ABSTRACT

This dissertation focuses on the characterization of novel synthetic materials by mass spectrometry approaches. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS) are utilized to perform single-stage mass spectrometry experiments, providing mass \( (m/z) \) information. ESI-MS coupling with ion mobility spectrometry has been achieved to determine molecular structures and conformations. The compounds examined in this dissertation are polyhedral oligomeric silsesquioxanes (POSS) diadducts, self-assembled supramolecules (two-dimensional and three-dimensional), including metallo-triangles, a terpyridine-based tetramer, bistertpyridyl o-carborane-based metallosupramacrocycles, and a tristerpyridine-based nanosphere.

Chapter IV describes the isomeric architectures of POSS adducts, including those diadducts synthesized \textit{via} thiol-ene chemistry and azobenzene functionalized POSS adducts. The compositions of POSS diadducts were quantified, identifying the \textit{para} isomer, as the major product, in which steric hindrance is minimized.

Chapter V discusses the effect of counterions on the collision cross-sections (CCSs) of self-assembled metallo-triangles. Molecular structures of triangles in different charge states were simulated and the CCSs were calculated theoretically for comparison with experimentally derived values.
Chapter VI concerns supramolecular architectures. Conformers of supramolecules and their fragments were analyzed by ion mobility mass spectrometry. The corresponding fragmentation mechanisms were also elucidated. Ion mobility mass spectrometry combined with gradient tandem mass spectrometry (gMS²) is shown to be ideally suitable for probing the structures and intrinsic stabilities of metallo-supramolecules.
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CHAPTER I

INTRODUCTION

During the past decades, mass spectrometry has advanced significantly, becoming increasingly important in chemical analysis by enabling the separation and characterization of ions in the gas-phase based on their mass to charge ratio (called $m/z$), which is the basic parameter measured by mass spectrometry. Mass spectrometry is widely applied to determine the molecular weight of chemicals, the structures of novel compounds and the compositions of materials.\textsuperscript{1,2} The invention of soft ionization techniques, such as ESI (electrospray ionization)\textsuperscript{3} and MALDI (matrix-assisted laser desorption ionization),\textsuperscript{4,5} has expanded the application of mass spectrometry, by making possible the formation of intact molecular ions in the gas-phase from synthetic polymers and supramolecules.\textsuperscript{6-12}

For novel synthetic materials with complex compositions, molecular weight information (\textit{i.e.}, single-stage mass spectrometry) is not sufficient for deducing molecular structures. In such cases, tandem mass spectrometry (MS/MS) can be employed to determine the structures of materials.\textsuperscript{2,13-15} In MS/MS, ions with a single or very narrow mass distribution (a few Da) are isolated, accelerated into high kinetic energy and collided with neutral molecules (helium, nitrogen or argon) to form fragment ions, in a process called collisionally activated dissociation (CAD).
Single-stage mass spectrometry and tandem mass spectrometry separate molecular and fragment ions based on their mass (specifically, their \( m/z \) ratio), but generally do not provide conformation information or in many cases, the connectivity information needed to distinguish isomers. Isomers are defined as molecules with the same molecular formula but different chemical structure.\(^\text{16,17}\) Organic molecular isomers may display distinct physical and chemical properties.\(^\text{18,19}\) Isomers are divided into two classes, structural isomers and stereoisomers. Structural isomers contain the same atoms but different chemical bonding; \( e.g. \), 1,2-, 1,3- and 1,4-dichlorobenzene, which are also named ortho-, meta- and para-dichlorobenzene. Stereoisomers contain the same chemical bonding but different geometry in space, such as cis- and trans-azobenzene (called cis/trans isomers), or cyclohexane with boat and chair conformation (called conformers). Conformers can easily interconvert, whereas structural isomers cannot.

Ion mobility spectrometry is a technique, which can separate gas-phase ions based on their size, shape, and charge. Combined with mass spectrometry, these two techniques provide complementary information about synthetic materials (molecular weight and conformation), which is particularly useful for differentiating conformers and structural isomers. Separation is achieved by allowing the ions, formed in the ion source (\( e.g. \) by ESI), to drift under the influence of an electric field against the stream of a buffer gas (nitrogen).\(^\text{20-35}\) IM-MS (ion mobility mass spectrometry) has been applied to the architecture characterization of polymers,\(^\text{36}\) surpamolecules,\(^\text{37-43}\) proteins,\(^\text{44-51}\) peptides,\(^\text{52-57}\) and nanoparticles.\(^\text{58-61}\)
In this dissertation, analytical methodologies have been developed that couple mass spectrometry with ion mobility spectrometry and/or tandem mass spectrometry for the characterization of novel synthetic materials.

Chapter IV focuses on the characterization of polyhedral oligomeric silsesquioxane (POSS) diadducts by mass spectrometry and ion mobility mass spectrometry. In the past decades, silsesquioxane chemistry, which is based on compounds with the RSiO$_{3/2}$ repeat unit, has grown increasingly. T8 polyhedral oligomeric silsesquioxanes (POSS) carrying octacyclic inorganic silicon and oxygen frameworks with the formula (RSiO$_{3/2}$)$_8$ have been widely used as versatile building blocks for the construction of various macromolecular architectures, polymer cross linking systems, and polymer crystals. Appropriate functionalization of the POSS cage’s arms (mono-, multi- or heterofunctionalized) allows for its incorporation into organic systems (such as polymers) without modification of existing manufacturing processes. Structural isomers are possible due to the multi-reaction points on the POSS cages. Photo-responsive polymers have also received considerable attention in the past few decades, especially azobenzene based polymers, which can be used to form functionalized micelles, vesicles, and thin films. Azobenzene functionalized POSS nanoparticles are able to form photoresponsive cis/trans isomers. Structural isomers in POSS with different substituents (styryl and epoxy phenyl) were investigated by Bowers and co-workers by MALDI-IM-MS.\textsuperscript{62} In Chapter IV, the compositions of structural isomers in POSS diadducts were determined and quantified by ESI-TWIM-MS.

In Chapter V and Chapter VI, self-assembled metallo-supramolecular systems are investigated using ESI-TWIM-MS. Self-assembled metallo-supramolecules are
composed of organic ligands and metal ions based on coordination interactions. Traditional analytical methods face problems in the characterization of such supramolecules. NMR is used to elucidate the chemical environment of atoms based on their chemical shifts. Traditional NMR is not able to unveil specific structural features of highly symmetrical analytes.\textsuperscript{63,64} Meanwhile, for compound mixtures, NMR spectra often show complex, superimposed signals that are hard to separate. For symmetrical supramolecules, chemical shifts may be quite similar making it hard to determine the structures in detail. Generally, highly concentrated and purified sample solutions or solids are necessary for NMR measurement. X-ray crystallography is also employed in supramolecules structure analysis. X-ray crystallography can unveil structural information at the atomic level directly. The difficulty in generating suitable single crystals prolongs supramolecular analysis times (time-consuming). For giant supramolecular systems, the complexity of X-ray diffraction patterns is a further key issue; in addition, the high energy of X-rays may also damage crystal structures with weak interactions.\textsuperscript{65,66}

ESI-MS has enabled the characterization of supramolecules in minutes. Nevertheless, if the noncovalent binding in a supramolecule is weak, ESI-MS will induce fragmentation easily. Symmetrical supramolecular assemblies composed of the same units, such as a trimer composed of three ligands and three metal ions and a tetramer composed of four ligands and four metal ions, will show the same $m/z$ ratios in ESI-MS, although with different masses and charges. Their isotope distributions will overlap making it hard to distinguish them even with MS/MS. Coldspray ionization (CSI) mass spectrometry has been developed by Yamaguchi and co-workers for the analysis of labile organometallic
supramolecules. It reduces the internal energy gained in the ion source, leading to little or no fragmentation due to the liquid nitrogen cooling used in the ion source.\textsuperscript{67,68} However, CSI-MS is not commercially available and thus difficult to apply. High resolution mass spectrometry, such as Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry, can be employed to detect analyte ions with high mass accuracy and well-resolved isotope patterns, but still cannot deconvolute overlapped isotopes of isomers or conformers.\textsuperscript{69,70}

ESI-MS coupling with IM-MS has overcome the mentioned problems and enabled the mass spectrometry analysis of isomeric architectures. Isomeric molecular ions are now readily separated if they differ in shape.

The shape of molecular ions determines their collision cross-section (CCS). In simple hard sphere collision theory, CCS is defined as the area of the circle with radius equal to the collision diameter. When a particle (analyte molecular ion) collides with another particle (nitrogen buffer gas), the CCS of the analyte molecule is the area with a radius equal to the sum of the radii of these molecules. CCSs can be calculated from drift times measured by IM-MS. Traditional drift time ion mobility mass spectrometry (DTIM-MS) is the only IM-MS technique that can render CCSs directly from the measured drift times. In DTIM-MS, ions are introduced into a drift tube to which a constant, low electric field is applied and which is filled with a drift gas (helium or nitrogen).\textsuperscript{71,72} In contrast, traveling wave ion mobility mass spectrometry (TWIM-MS) employs a constantly changing electric field, which prohibits derivation of the CCS directly from the measured drift time. Here, drift time calibration is conducted using standards with known CCSs, and preferably, similar chemical and physical properties with the analyte.\textsuperscript{73,74} The
standards are analyzed by IM-MS at the same setting as the analytes; the three most critical parameters that must be kept constant are the traveling wave height, traveling wave velocity, and the buffer gas flow rate.

The CCSs of standards are measured by traditional ion mobility mass spectrometry or X-ray crystallography. The library of supramolecular standards is very limited. Fortunately, proteins of comparable molecular weight have been well studied by ion mobility mass spectrometry in the past decades and are available to serve as CCS standards for supramolecular systems.

In TWIM-MS, experimental CCSs are obtained from calibration curves based on the corresponding drift times. Additional structure information is gained by comparison of the experimental CCSs with theoretical CCSs. Theoretical CCSs are generated by computational molecular dynamics simulations. The most commonly used models for calculating CCSs from energy-minimized (computationally) structures are the projection approximation (PA), the exact hard-sphere scattering (EHSS) and the trajectory method (TM). In the PA method, the ion is modeled by a collection of overlapping hard spheres with radii equal to hard sphere collision distances and the calculated CCS is an average of projection areas from all possible collision geometries. In the trajectory method, ions are considered as a collection of atoms and the long-range interactions between the analyte ions and buffer gas are taken into account. The trajectory method is time consuming although it is more suitable for large and especially nonspherical molecules. Chapter V focuses on the effect of counterions of self-assembled metallo-triangles, by comparing experimental CCSs with theoretical CCSs calculated by different methods. CCS trends are extensively discussed in Chapter VI, which describes the IM-
MS characteristics of metallo-supramolecular structures and the isomeric architecture arising by the dissociation of thermally excited supramolecular assemblies.
CHAPTER II

MASS SPECTROMETRY BACKGROUND

2.1. Introduction

Mass spectrometry is widely applied for the characterization of a variety of chemical compounds, including small molecules in pharmaceuticals, peptides and proteins involved biological processes, industrial polymers and supramolecules. A mass spectrometer separates molecular ions in the gas phase by their mass-to-charge ratio ($m/z$). The typical mass spectrometer contains five components: sample inlet system, ion source, mass analyzer, ion detector and data system (as shown in Scheme 2.1).\footnote{1}

![Scheme 2.1. The typical components of a mass spectrometer.]

The inlet system introduces samples into the ion source of the mass spectrometer by a direct probe, injection valve or chromatography.\footnote{81} A direct probe or target is employed in MALDI (matrix-assisted laser desorption ionization). Conversely, a syringe pump is
employed as an injection valve in ESI (electrospray ionization). LC (liquid chromatography) is usually combined with ESI as an inlet system.

2.2. Ion sources

A mass spectrum can only be obtained when the sample is ionized into the gas phase. Therefore, the first step for MS analysis has always been to ionize the sample molecules and produce charged species. The sample molecule can be ionized by electron capture, electron ejection, protonation, deprotonation, or cationization of neutral species in the ion source. There are basically two categories of ionization methods, viz. hard and soft. Hard ionization methods include EI (electron ionization), CI (chemical ionization), FI (field ionization), FD (field desorption) and FAB (fast atom bombardment). Soft ionization methods include APCI (atmospheric pressure chemical ionization), ESI and MALDI, which enable the formation of intact molecular ions with no or little fragmentation. ESI and MALDI will be discussed in detail since they were the two ionization methods employed in this dissertation.

2.2.1. Electrospray ionization

Electrospray ionization (ESI) has become an important and widely used ionization technique in modern mass spectrometry. Multiply charged ions can be formed in the ESI source. The typical electrospray process is described schematically in Figure 2.1.
Figure 2.1. Schematic diagrams of electrospray sources, using heated nitrogen gas flow (top) or heated capillary for desolvation (bottom). Reproduced from reference 1 with permission.

The sample solution (analyte) is introduced into the ion source under atmospheric pressure through a metallic capillary; a strong electric field (3-6 kV) is applied between the metallic capillary and the counter-electrode (labeled as "Lens" in Figure 2.1). The high electric field induces charge accumulation on the surface of the sample solution, which breaks into highly charged droplets at the end of the capillary. As the sample solution is sprayed, a heated nebulizing gas (sheath gas), usually nitrogen gas (which is inert to most chemical reactions), is used to assist the droplet formation process. As the
charges accumulate on the analyte droplet at the capillary exit due to solvent evaporation, the repulsive forces between the charges on the droplet become higher than the surface tension and the droplet changes to a Taylor cone shape and releases smaller droplets ("Coulombic explosion"), forming a spray. The charged droplets decompose at the Rayleigh limit, at which the repulsive force between the charges is equal to the surface tension. Further evaporation of solvent molecules is assisted by the heated nitrogen gas flow. Repeated Coulombic explosion and solvent evaporation will ultimately lead to single ions (charge residue mechanism). When the charge density on the droplet surface becomes high enough, single ions may also be desorbed from the droplet (ion evaporation mechanism). The resulting ions are able to carry multiple charges and can be used to determine molecular masses. The ions are sent to the mass analyzer and subsequently to the ion detector for analysis of their m/z values.

2.2.2. Matrix-assisted laser desorption ionization

In MALDI, the sample is first dissolved into a proper solvent and then mixed with small organic molecules (called matrix) at the molar ratio of analyte : matrix of 1 : 1000 ~ 10000. These small molecules have strong absorption at the laser wavelength; they absorb laser power and evaporate, taking with them the analyte molecules into the gas phase. This process protects the sample molecules and prevents them from breaking apart by the high laser power. The excess amount of matrix molecules also helps to disperse the analyte molecules, preventing the formation of analyte clusters. A small droplet of the sample/matrix solution is deposited onto a target plate and dried. After the solvent evaporates, a solid solution of the sample in the matrix is obtained, which is introduced
into the vacuum system and bombarded by laser light. The principle of MALDI is described schematically in Figure 2.2.

Figure 2.2. Schematic diagram of a matrix-assisted laser desorption ionization source (MALDI). Reproduced from reference 1 with permission.

The rapid heating of matrix crystals on the target plate by laser irradiation causes ablation and vaporization of a portion of the crystals and expansion of the matrix into the gas phase; the matrix plume formed this way contains intact analyte molecules and matrix ions. Analyte molecules are ionized by proton transfer from the matrix ions. For analytes that do not protonate easily, a proper salt solution is added into the analyte and matrix mixture deposited onto the target plate. For example, Ag⁺ is usually added to ionize polymers containing aromatic rings, like polystyrene, while Na⁺ and K⁺ are usually added to ionize polymers containing oxygen.

The matrix is chosen depending on the sample properties. Typical matrices for synthetic polymers are 1,8,9-trihydroxyanthracene or dithranol (DIT) and trans-2-[3-(4-
tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) whereas α-cyano-4-hydroxycinnamic acid (CHCA) is a commonly used matrix for peptide and protein ionization. The structures of these matrices are shown in Figure 2.3.

![Chemical structures of common matrices: a). dithranol (DIT); b). trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB); c). α-cyano-4-hydroxycinnamic acid (CHCA).](image)

2.3. Mass analyzers

The ionized sample molecules are accelerated into mass analyzer and separated by their mass-to-charge ratio \( (m/z) \). Common mass analyzers in modern mass spectrometry are the quadrupole (Q), quadrupole ion trap (QIT), time-of-flight (ToF) tube, quadrupole time-of-flight (Q/ToF) analyzer and orbitrap. The quadrupole (Q), time-of-flight (ToF) and quadrupole time-of-flight (Q/ToF) analyzers will be discussed in this dissertation.

2.3.1. Time-of-flight mass analyzer

Time-of-flight mass analyzers measure the flight time of ions from the ion source to the ion detector, as shown in Figure 2.4. Ions from the ion source are accelerated by a
potential $V_s$ to gain kinetic energy and fly through a field-free region of distance $L$ before reaching the detector.

![Diagram of LToF mass spectrometer](image)

Figure 2.4. Principle of a LToF (linear time-of-flight) mass spectrometer equipped with a MALDI source. Reproduced from reference 1 with permission.

The electric potential energy $E_{el}$ supplied to the ions upon acceleration is equal to their charge ($q = ze$) multiplied by $V_s$. $E_{el}$ is converted to kinetic energy ($E_k$) when the ions leave the ion source as described in Equation 2.1. After exiting the source, the ions travel into a field free region at a constant velocity $v$ for a distance $L$ to arrive at the detector. The corresponding flight time is calculated by Equation 2.2. Combination of Equations 2.1 and 2.2 gives Equation 2.3, which describes the relationship between $m/z$ and the measured flight time $t$. 

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A quadrupole mass analyzer contains four cylindrical rods, and the rods must be perfectly arranged parallel to each other. Ions are accelerated along the z axis to enter the space between the quadrupole rods. A positive ion entering the space will be drawn to a negative rod. If the potential changes to opposite mode before the ion discharges on the rod, the ion will change its direction. The motion of the ions flying through the quadrupole rods is controlled by the DC (U) and RF (Vcosωt, ω is the angular frequency) potentials applied onto the rods. One pair of rods (opposite to each other) are supplied with the potential U-Vcosωt. The other pair of rods are subjected to the negative potential -(U-Vcosωt). The ion motion in a quadrupole mass analyzer is shown in Figure 2.5. As the sign of the RF voltage changes, the ions will move in the opposite direction, resulting in a zigzag motion. A quadrupole mass analyzer with RF only potential acts as focusing lens to transfer all ions to the detector, whereas a quadrupole mass analyzer with DC and RF potential acts as a mass filter by allowing a selected mass range to pass, while ions out of this mass range hit the rods and discharge themselves.

\[
E_{el} = qV_s = \frac{mv^2}{2} = E_k \hspace{2cm} \text{Equation 2.1}
\]

\[
L = vt \hspace{2cm} \text{Equation 2.2}
\]

\[
m/z = \frac{2t^2eV_s}{L^2} \hspace{2cm} \text{Equation 2.3}
\]
The motion of ions through a quadrupole mass analyzer is mathematically described by the Mathieu equation (Equation 2.4).\textsuperscript{1,84}

\[ \frac{d^2 u}{d(\omega t/2)^2} + (a_u - 2q_u \cos \omega t) = 0 \]  \hspace{1cm} \text{Equation 2.4}

\[ a_u = a_x = -a_y = \frac{8zeU}{m\omega^2 r_0^2} \]  \hspace{1cm} \text{Equation 2.5}

\[ q_u = q_x = -q_y = \frac{4zeV}{m\omega^2 r_0^2} \]  \hspace{1cm} \text{Equation 2.6}

In this equation, \( u \) represents the x or y axis, \( a_u \) and \( q_u \) are dimensionless parameters describing the DC and RF fields, respectively (Equation 2.5 and 2.6), and \( r_0 \) is the field radius, which is a constant. An ion will follow a stable trajectory through the quadrupole.
rods as long as its x and y coordinates remain smaller than \( r_0 \). The U/V ratio is adjusted to allow only a narrow \( m/\ell \) range of ions to travel through the quadrupole rods with a stable trajectory, while all other ions are discharged.

2.3.3. Quadrupole time-of-flight (Q/ToF) mass analyzer

A hybrid mass analyzer is formed by coupling different types of mass analyzers to achieve better performance. A quadrupole combined with a ToF analyzer gives the Q/ToF hybrid, in which the two analyzers are placed orthogonally to each other. For MS measurements, the quadrupole is set to RF only mode to transmit all ions to the ToF analyzer. For MS/MS analysis, the quadrupole is supplied with both DC and RF potentials, working as a mass filter to permit the passage of the selected analyte ions only. The ions passing the quadrupole are transferred into a collision cell filled with a collision gas (usually argon) where fragmentation takes place. The intact ions and fragments exiting the collision cell travel into the ToF analyzer, to be separated based on their \( m/\ell \) values.

Q/ToF spectrometers can perform analysis with high mass accuracy (5 to 10 ppm) and resolution (10,000 FWHM, full width at half maximum).

2.4. Detectors

After the ions pass through the mass analyzer, they are converted to electric current, which is proportional to their abundance. There are several types of commercially available detectors, including electron multipliers and electro-optical detectors. The MCP
(microchannel plate) is one of the most commonly used electron multipliers. The MCP detector will be discussed in this dissertation.

A MCP is a plate drilled with parallel cylindrical channels. The channel diameter is in the range of 4 to 25 µm and the center-to-center distance between channels is in the range of 6 to 32 µm; a MCP detector is shown in Figure 2.6. Ions striking the MCP entrance create electrons which, as they travel down the channels, are amplified $10^5$ times. Higher amplifications are possible ($10^8$) by using parallel plates.

![Figure 2.6. Cross-section of a microchannel plate. Reproduced from reference 1 with permission.](image)

2.5. Ion mobility mass spectrometry

Regular mass spectrometry can only separate ions by their mass-to-charge ratio, which does not provide molecular structure information. Ion mobility mass spectrometry (IM-MS) is able to separate gas-phase ions based on their mass, charge and shape, thereby providing both mass and structure information and enriching the analysis ability.
of mass spectrometry. There are primarily three different IM-MS variants, depending on the type of ion mobility spectrometry device attached to the mass spectrometer. The three basic types of ion mobility spectrometry (IMS) include drift-time ion mobility spectrometry (DTIMS), travelling-wave ion mobility spectrometry (TWIMS) and field-asymmetric ion mobility spectrometry (FAIMS), which is also called differential ion mobility spectrometry. Traveling-wave ion mobility mass spectrometry, which was used in this dissertation, will be discussed in detail.

In traditional ion mobility spectrometry (DTIMS), ions are introduced into a drift tube with an applied low electric field and filled with a drift gas (helium or nitrogen) flowing against the ions. The ion velocity in the drift tube is proportional to the electric field, and the proportionality constant is called ion mobility constant ($K$), which is related to the ions' collision cross section (CCS), as described in Equation 2.7:

$$K = \left(\frac{3q}{16N}\right)^{1/2}(\frac{2\pi}{kT})^{1/2}(\frac{M+m}{Mm})^{1/2}(\frac{1}{\Omega})$$

Equation 2.7

where $q$ is the charge of the ion, $N$ is the number density of the gas, $k$ is the Boltzmann’s constant, $T$ is the absolute temperature in the drift tube, $m$ is the mass of the buffer gas, $M$ is the mass of the analyte ion, and $\Omega$ is the CCS of the ion.

Traditional drift-time ion mobility spectrometry is the only IMS technique in which ion collision cross-sections are deduced directly (through Equation 2.7) from the measured drift times.

Traveling-wave ion mobility spectrometry is a modern ion mobility technique. Experiments are performed inside a stacked ring ion guide (SRIG), where a traveling voltage wave is applied. Adjacent electrodes of the SRIG are supplied with radio-frequency voltages of opposite phases, as shown in Figure 2.7. Analyte ions are confined
by the RF field to flow in the center of the TWIMS device. A direct current (DC) voltage pulse is superimposed onto the RF field of an electrode to propel the ions axially; after a certain time, the DC voltage pulse is switched to the adjacent electrode. The DC voltage pulses are applied successively to the electrodes from one end of the SRIG to the other, as described in Figure 2.8. This creates a moving electric voltage (traveling wave) on which the ions can surf through the SRIG. During this process, ions of higher mobility move faster through the SRIG, as they can ride over the traveling waves, resulting in mobility-based separation. The cell housing the SRIG is filled with a buffer gas (nitrogen) flowing in the opposite direction of the traveling wave. Analyte ion separation can be optimized by controlling the speed and magnitude of the traveling wave voltage and the buffer gas flow rate.

Figure 2.7. Schematic diagram of the stack ring ion guide (SRIG). Reproduced from reference 87 with permission.
Due to the constantly changing electric field in TWIMS, there is no mathematical relationship between the measured drift time and the corresponding CCS. Hence, CCSs cannot be determined directly from the experimentally obtained drift times, and drift time calibration using standards with known CCSs is required. Because the TWIMS device is located between Q and ToF mass analyzers (vide infra), it is possible to separate ions formed in the ion source as well as fragments formed in MS/MS experiments.

The calibration curve is created by plotting the corrected CCS ($\Omega'$) of the standards vs. their corrected drift time ($t'_d$) measured under the same conditions as the analyte drift times. The corrected CCS is calculated based on Equation 2.8.
\[ \Omega' = \frac{\Omega}{[ze(m^{-1} + M^{-1})^{1/2}]} \]  

Equation 2.8

where \( z \) is the charge of the ion, \( m \) is the molecular weight of the buffer gas, \( M \) is the molecular weight of the ion, and \( \Omega \) is its published CCS. The corrected drift time \( (t'_d) \) is calculated via Equation 2.9:

\[ t'_d = t_d - \left[ C \frac{m}{z} / 1000 \right] \]  

Equation 2.9

where \( C \) is a correction factor for instrumental flight time offsets, \( t_d \) is the measured drift time of the ion, and \( m/z \) is its mass-to-charge ratio.
3.1. Materials

The materials analyzed in Chapter IV were synthesized by Dr. Yiwen Li in Professor Stephen Z. D. Cheng’s group. The supramolecules discussed in Chapter V and Chapter VI were synthesized by Dr. Anthony Schultz, Dr. Tingzheng Xie, Dr. Shengyun Liao and Mr. James Ludlow in Professor George R. Newkome’s group.

HPLC grade THF, chloroform, acetonitrile, methanol and water are purchased from Sigma-Aldrich (St. Louis, MO).

3.2. Instrumentation

The instruments used in this dissertation will be discussed in detail in the following sections.

3.2.1. MALDI ToF/ToF mass spectrometer

A Bruker MALDI-ToF/ToF UltraFlex III mass spectrometer was used for MALDI-MS analysis. This instrument is equipped with a Nd:YAG laser (355 nm) in the MALDI ion
source and a drift region containing a precursor ion selector (PCIS), LIFT device (used for MS/MS experiments) and linear and reflectron ToF analyzers, as shown in Figure 3.1.

Figure 3.1. Schematic diagram of the Bruker MALDI-ToF/ToF UltraFlex III mass spectrometer. Reproduced from reference 89 with permission.

3.2.2. Synapt HDMS ion mobility mass spectrometer

All ESI and IM-MS experiments were performed with a Waters Synapt HDMS quadrupole/time-of-flight (Q/ToF) mass spectrometer equipped with a z-spray ESI ion source, ion guide, quadrupole analyzer, traveling wave region and time-of-flight tube. A schematic diagram of this instrument is shown Figure 3.2.
Ions are formed in the ESI source and transferred by appropriate potentials into the vacuum system. The Synapt HDMS system uses the traveling wave technique, which was described in Chapter II. The Triwave compartment between the Q and ToF mass analyzers is composed of three traveling wave devices, viz. trap cell, ion mobility cell and transfer cell. The first cell (trap cell) traps ions and releases them periodically into the ion mobility separation cell. The second cell (ion mobility separation cell) separates ions based on their mobility. The third cell (transfer cell) transfers ions into the ToF analyzer for m/z measurement.
CAD (collisionally activated dissociation) fragmentation can be performed in either the trap or the transfer cell, which is filled with Argon (~10^{-3} mbar). For ion mobility analysis, nitrogen gas is admitted in the ion mobility separation cell (~0.5 mbar). In MS/MS experiments, if CAD is performed in the trap cell, the precursor ions are fragmented in this cell and the resulting fragments are separated in the ion mobility cell and then transferred into the ToF analyzer through the transfer cell. The fragment drift times will vary according to their CCSs. Alternatively, CAD may be performed in the transfer cell, after the intact precursor ions have been separated in the ion mobility cell; in this case, precursor and fragment ions will be time-aligned, appearing at the same drift time. CAD experiments in the trap and transfer cells have been conducted in this dissertation to determine the composition(s) and structure(s) of analyte samples.
CHAPTER IV

CHARACTERIZATION OF POSS DIADDUTS BY MASS SPECTROMETRY

4.1. Background

In the past decades, silsesquioxane chemistry, which is based on compounds with the RSiO\(_{3/2}\) repeat unit, has grown increasingly. T8 polyhedral oligomeric silsesquioxanes (POSS) carrying octacyclic inorganic silicon and oxygen frameworks with the formula (RSiO\(_{3/2}\))\(_8\) have been widely used as versatile building blocks for the construction of various macromolecular architectures, polymer cross linking systems and polymer crystals.\(^{91-100}\)

Multi- or mono-functionalization of the POSS cage’s arms allows for its incorporation into organic systems without modification of existing manufacturing processes.\(^{101-104}\) The POSS nanoparticle is a promising, versatile nanobuilding block that can act as a conformationally rigid structural scaffold to carry diverse functionalities. Various functional POSS cages with distinct periphery functionalities, such as carboxylic acids, hydroxyls, polymer chains and liquid crystals have been reported in the literature.\(^{105-110}\) Structural isomers are possible due to the multi-reaction points on the POSS cages. Meanwhile, photo-responsive polymers, especially azobenzene-based polymers, have received considerable attention in the past few decades, because they can be used to form functionalized micelles, vesicles, and thin films.\(^{111-120}\)
Structural isomers of POSS with different substituents (styryl and epoxy phenyl) were investigated by Bowers and co-workers by MALDI-IM-MS. Here, three POSS adduct systems have been examined: 1. the commercially available precursors thiol-POSS and octavinyl-POSS were linked together through thiol-ene “click” chemistry, involving thiohydrothiolation of a C=C bond. Mixing the thiol- and octavinyl-substituted POSS at the 2:1 molar ratio, maximizes the yield of the POSS diadduct VPOSS-(S-BPOSS)<sub>2</sub>. As shown in Scheme 4.1, three isomeric diadducts should exist, containing ortho-, meta-, and para-substituted structures. 2. In a similar POSS system, three isomers should be formed if octaepoxy- and monoamine-substituted POSS are combined in a 1:2 molar ratio (see Scheme 4.2 for structures). 3. Besides such structural isomers of POSS diadducts, spatial POSS isomers also exist. Azobenzene-based interconvertible spatial isomers of POSS adducts (see Scheme 4.4) were also designed and synthesized.

The characterization of such isomers is challenging without separation of the isomeric components regardless of whether NMR, FTIR or X-ray crystallography is used. Chromatographic separation of the isomers may, however, be difficult due to solubility issues, permanent retention on the stationary phase or insufficient dispersion. Conventional mass spectrometry with matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI) also cannot distinguish these isomers even at high mass resolution because their m/z ratios are identical. Hence, analytical methods are desired that would be capable of characterizing the raw synthetic mixture in addition to bearing two critical features, impurities tolerance and the ability to discriminate isomers. Ion mobility mass spectrometry (IM-MS) would be particularly suitable for the
characterization of these POSS adduct systems due to its insensitivity to impurities, dispersive nature, and high sensitivity.

4.2. Characterization of POSS diadduct isomers

The thiol-ene POSS diadducts will be characterized first, as their isomers are stable and do not interconvert under the analysis conditions.

4.2.1. Sample preparation

Di-functional POSS was synthesized in Professor Stephen Z. D. Cheng’s laboratory. Two equivalents of functionalized thiol-POSS were mixed with octavinyl-POSS in THF in the presence of 2 mol% of 2,2-dimethoxy-2-phenylacetophenone (DMPA, photoinitiator) at an octavinyl-POSS concentration of 10 mg/mL and the mixture was irradiated under a UV 365 nm lamp at room temperature for about 30 min. The crude reaction product was directly analyzed by MALDI and ESI-TWIM-MS without purification.

MALDI-ToF mass spectra were acquired on a Bruker Ultraflex-III ToF/ToF mass spectrometer (Bruker Daltonics, Inc., Billerica, MA) equipped with a Nd:YAG laser (355 nm). All spectra were measured in positive reflectron mode. The instrument was calibrated externally with polystyrene or PMMA standards at the molecular weight under consideration. *Trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile* (DCTB, Santa Cruz Biotechnology, Inc., >99%), which was used as matrix, was dissolved in CHCl₃ at a concentration of 20 mg/mL. Sodium trifluoroacetate (NaTFA),
which was used as cationizing salt, was dissolved in MeOH/CHCl₃ (v/v = 1/3) at a concentration of 10 mg/mL. All the samples were dissolved in CHCl₃ at 10 mg/mL. Matrix and cationizing salt were mixed in a ratio of 10/1 (v/v). The sample preparation involved depositing 0.5 μL of matrix and salt mixture on the wells of a 384-well ground-steel plate, waiting for the spots to dry, depositing 0.5 μL of sample on the matrix spot, and adding another 0.5 μL of matrix and salt mixture on the dry sample spot. After evaporation of the solvent, the target plate was inserted into the MALDI source. The laser was adjusted and attenuated to minimize undesired polymer fragmentation and to optimize the peak intensity.

Both ESI-MS and ESI-TWIM-MS experiments were performed on a Waters Synapt HDMS quadrupole/time-of-flight (Q/ToF) mass spectrometer (Waters, Milford, MA). The tri-wave region of this instrument, located between the Q and ToF mass analyzers, contains three confined regions in the order trap cell (closest to Q), TWIM cell, and transfer cell (closest to ToF). Either the trap or the transfer cell can be used for MS/MS studies via collisionally activated dissociation (CAD), as described in Chapter III. The following ESI and TWIM parameters were selected: ESI capillary voltage: 3.5 kV; sample cone voltage: 35 V; extraction cone voltage: 3.2 V; desolvation gas flow: 500 L/h (N₂); trap collision energy (CE): 6 eV; transfer CE: 4 eV; trap gas flow: 1.5 mL/min (Ar); ion-mobility cell gas flow: 22.7 mL/min (N₂); sample flow rate: 5μl min⁻¹; source temperature: 120 °C; desolvation temperature: 150 °C; IM traveling-wave height: 10 V; and IM traveling-wave velocity: 350 m/s. The sprayed solution was prepared by dissolving the sample (0.3 mg) in a mixture of CHCl₃/MeOH (1mL; 1/1, v/v) with 3%
(v/v) NaTFA (1 mg/mL, dissolved in MeOH). Data analyses were conducted using the MassLynx 4.1 and DriftScope 2.1 programs provided by Waters.

The drift timescale of the TWIM-MS experiments was converted to a collision cross-section scale, following the calibration procedure described in Chapter II. Briefly, the corrected collision cross-sections of the molecular ions of polyalanine and one synthetic peptide, obtained from published work, were plotted against the corrected drift times (arrival times) of the corresponding molecular ions measured in TWIM-MS experiments at the same traveling-wave velocity, traveling-wave height and ion-mobility gas flow settings, viz. 350 m/s, 10 V and 22.7 mL/min. All charge states observed for the calibrants were used in the construction of the curve.

Energy minimization of the diadduct isomers was conducted with the Materials Studio version 4.2 program, using the Anneal and Geometry Optimization tasks in the Forcite module (Accelrys Software, Inc.). An initially energy-minimized oxidized difunctional POSS (see Results and discussion) was subjected to 400 anneal cycles with initial and mid-cycle temperatures of 50 and 1400 K, twenty heating ramps per cycle, one thousand dynamics steps per ramp, and one dynamics step per femtosecond. A constant volume/constant energy (NVE) ensemble was used; the geometry was optimized after each cycle. All geometry optimizations used a universal force field with atom-based summation and cubic spline truncation for both the electrostatic and van der Waals parameters. For each diadduct isomer, 400 candidate structures were generated for the calculation of collision cross-sections.

4.2.2. Results and discussion

The crude reaction product VPOSS-(S-BPOSS)$_2$ was directly analyzed by MALDI-MS and ESI-MS. In the molecular weight region of singly charged VPOSS-(S-BPOSS)$_2$ (see Figure 4.1), native and oxidized states of the diadduct were observed, corresponding to [M + Na]$^+$, [M + O + Na]$^+$, [M + 2O + Na]$^+$, and [M + 3O + Na]$^+$, with the major form being [M + 2O + Na]$^+$. Figure 4.2, which includes the entire MALDI mass spectrum, further affirms that the thiol-ene reaction also produced the monoadduct VPOSS-S-BPOSS, both in native and oxidized states. The thiol-ene reaction products contain thioether bonds, which can easily be oxidized to sulfoxide and/or sulfone functionalities by solvents and/or during MS analysis. In the M + O product, which gives rise to the peak [M + O + Na]$^+$, one thioether group was oxidized to sulfoxide; in the M + 3O product, which gives rise to the peak [M + 3O + Na]$^+$, both thioether groups were
oxidized, one to sulfoxide and one to sulfone. The composition of the major product, M + 2O, which gives rise to the abundant \([M + 2O + Na]^+\) peak, is however ambiguous. Either one thioether group was oxidized to sulfone or both thioether groups were oxidized to sulfoxide.

![Figure 4.1](image.png)

**Figure 4.1.** Zoomed-in (a) MALDI-ToF and (b) ESI mass spectra of VPOSS-(S-BPOSS)$_2$. NaTFA served as cationizing agent. The peaks marked by arrows correspond to monoisotopic \([M + Na]^+\), \([M + O +Na]^+\), \([M + 2O + Na]^+\), and \([M + 3O + Na]^+\).
Figure 4.2. Zoomed-in MALDI-ToF mass spectra of the thiol-ene crude product: a) monoadduct region; b) diadduct region; the insets show the full mass spectrum. NaTFA served as cationizing agent. In the monoadduct region, the ions at $m/z$ 1545.32, 1561.33, and 1577.33 correspond to $[M + Na]^+$, $[M + O + Na]^+$, and $[M + 2O + Na]^+$, respectively. The minor peak ($m/z$ 1551.31) correspond to the reaction product of the impurity in VPOSS (VPOSS - CH=CH$_2$ + OH) with thiol-POSS.
Figure 4.3. ESI mass spectrum of VPOSS-(S-BPOSS)$_2$; the zoomed-in view provided in the inset shows the isotope pattern of the doubly charged [M + 2O + 2Na]$^{2+}$ ion. NaTFA served as cationizing agent.

In order to confirm the structure of the major product ion [M + 2O + Na]$^+$, the ESI-MS/MS spectrum of the corresponding doubly charged ion at m/z 1247 was acquired by collisionally activated dissociation (CAD). Although [M + 2O + 2Na]$^{2+}$ was lower in intensity than [M + 2O + Na]$^+$ (Figure 4.3), the doubly charged ion fragmented more efficiently, leading to an MS/MS spectrum with significantly better signal-to-noise ratio than the singly charged ion. As shown in Figures 4.4 and 4.5, there are two major fragments in MS/MS spectrum, m/z 930 and m/z 1563, both with a singly Na$^+$ charge, formed by charge separation. As the collision energy increased, the intensity of those fragments increased, while that of the parent ion peak m/z 1247 decreased. The m/z 930 and 1563 fragments are singly oxidized (see structure in Figure 4.4), providing strong evidence that the M+2O product contained two sulfoxide groups, not one sulfone group.
Figure 4.4. ESI-MS/MS spectrum of \([M + 2O + 2Na]^{2+}\) (m/z 1247) from the oxidized diadduct VPOSS-(S-BPOSS)$_2$, acquired by CAD with Ar in the trap cell at a collision energy of 50 eV. Fragmentation occurs by homolytic bond cleavages (as shown in above structure) as well as by concomitant H rearrangements which create fragments that are 1 Da lower in mass than the radical ions.
Figure 4.5. ESI-MS/MS spectra of the doubly charged ion at $m/z$ 1246 from oxidized VPOSS-(S-BPOSS)$_2$, acquired by CAD (Ar) in the trap cell at collision energies in the 10-60 eV range.
Figure 4.6. Two-dimensional IM-MS plot (relative intensity vs. drift time) of VPOSS-(S-BPOSS)₂ ions at \( m/z \) 1247. (a) IM separation was performed using a traveling wave velocity of 350 m/s and a traveling wave height of 10 V. (b) Isotopic patterns of the IM-MS peaks at 4.96, 5.87, and 6.59 ms.
The ESI-TWIM-MS experiments were also conducted on the doubly charged molecular ions of VPOSS-(S-BPOSS)$_2$ at $m/z$ 1247, which led to resolved isomeric components after ion mobility separation. As mentioned above, these ions have the composition of $[M + 2O + 2Na]^{2+}$ and originate from products in which the thioether groups have been oxidized to sulfoxide groups. Three distinct species were successfully isolated from the $[M + 2O + 2Na]^{2+}$ ions due to their unique ion mobilities which resulted in three separate peaks with drift time maxima at 4.96, 5.87, and 6.59 ms (Figure 4.6a). The isotope patterns in the mass spectra extracted from each peak (Figure 4.6b) reveal the presence of only doubly charged ions, having isotope spacing ($\Delta m$) of 0.5 Da. Considering that the more compact molecular ions usually drift faster while more expanded ions drift slower in ion mobility experiments, these three species probably belong to ortho-, meta-, and para-isomers of the diadducts. In order to confirm this tentative assignment, molecular modeling with the Materials Studio program was performed to calculate the sizes of these isomers for comparison with their IM-MS characteristics.

Using the Anneal and Geometry Optimization tasks in the Forcite module, 400 energy-minimized structures were generated for each isomer through an annealing process in order to calculate the average collision cross-section (CCS) of each isomer (Figure 4.7). CCS values were obtained from the optimized structures with the MOBCAL algorithm, which permits CCS computation by the projection approximation, trajectory, and exact hard sphere scattering methods. The projection approximation model tends to underestimate the cross-sections, especially of large and non globular ions, because it does not treat scattering phenomena properly and neglects long-range interactions.
between the ions and the buffer gas. Both the trajectory and exact hard sphere scattering methods account for the details of the scattering process. Additionally, the trajectory method also considers long-range ion/gas interactions. Thus, the more rigorous and time-consuming trajectory method was utilized to calculate the cross-sections of the 400 candidate structures for each isomer.

![Figure 4.7](image)

**Figure 4.7.** Representative energy-minimized structure for the *ortho-, meta-,* and *para-*isomer of the oxidized diadduct VPOSS-(S-BPOSS)₂ ([M + 2O]) obtained by molecular modeling. The carbon atoms are grey, the silicon atoms are yellow, the oxygen atoms are red, and the sulfur atoms are blue.

Plots of the resulting CCS values of the 400 structures against the corresponding relative energies are shown in Figure 4.8, in order to identify all possible molecular conformations for each isomer. A representative structure of each isomer is given in Figure 4.7; Na⁺ was not included in the molecular simulations since cationizing metal ions usually have no significant effect on conformations and, hence, the average CCS
values of very large molecules, such as the POSS-based giant molecules studied.\textsuperscript{34} The average CCS values of each isomer were calculated to be 453 Å\textsuperscript{2} for the ortho-diadduct, 457 Å\textsuperscript{2} for the meta-diadduct, and 462 Å\textsuperscript{2} for the para-diadduct (see Table 4.1).

Experimental CCSs of the three molibility-separated isomers (Figure 4.6a) were also derived from the corresponding drift times. For this, the drift time scale of the traveling wave ion mobility device was calibrated using polyalanine oligomers and one synthetic peptide (TRQARRNAAAWRERQR) as standards (Figure 4.9). The published CCS values of the calibrant ions are listed in Tables 4.2 and 4.3, together with their drift times, measured in this study.
Figure 4.8. Calculated collision cross-sections vs. the corresponding relative energies for the energy-minimized structures of the three different oxidized VPOSS-(S-BPOSS)$_2$ isomers.
Table 4.1. Theoretical and experimental CCS of VPOSS-(S-BPOSS)$_2$ isomers.

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Drift time (ms)</th>
<th>CCS$_{\text{calcld}}$ (Å$^2$)</th>
<th>CCS$_{\text{exp}}$ (Å$^2$)</th>
<th>Relative yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>ortho-</td>
<td>4.96</td>
<td>453</td>
<td>443</td>
<td>21%</td>
</tr>
<tr>
<td>meta-</td>
<td>5.87</td>
<td>457</td>
<td>468</td>
<td>20%</td>
</tr>
<tr>
<td>para-</td>
<td>6.59</td>
<td>462</td>
<td>486</td>
<td>59%</td>
</tr>
</tbody>
</table>

Figure 4.9. Calibration curve obtained by plotting the corrected published cross sections vs. the corresponding corrected drift times of the [M + H]$^+$ ions from polyalanine oligomers (14-19 residues) and [M + nH]$^{n+}$ (n = 2-4) ions from the synthetic peptide TRQARRNAAAAWRERQR.$^{121,122}$
Table 4.2. Published CCSs and experimental drift times of polyalanine [M + H]^+ ions.

<table>
<thead>
<tr>
<th>n</th>
<th>m/z</th>
<th>Drift time (ms)</th>
<th>CCS (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>374.256</td>
<td>1.44</td>
<td>115.0</td>
</tr>
<tr>
<td>6</td>
<td>445.295</td>
<td>1.81</td>
<td>127.0</td>
</tr>
<tr>
<td>7</td>
<td>516.333</td>
<td>2.26</td>
<td>140.5</td>
</tr>
<tr>
<td>8</td>
<td>587.381</td>
<td>5.71</td>
<td>155.5</td>
</tr>
<tr>
<td>9</td>
<td>658.432</td>
<td>3.25</td>
<td>167.9</td>
</tr>
<tr>
<td>10</td>
<td>729.479</td>
<td>3.61</td>
<td>179.3</td>
</tr>
<tr>
<td>11</td>
<td>800.528</td>
<td>4.15</td>
<td>192.2</td>
</tr>
<tr>
<td>12</td>
<td>871.573</td>
<td>4.60</td>
<td>202.4</td>
</tr>
<tr>
<td>13</td>
<td>942.623</td>
<td>5.14</td>
<td>214.9</td>
</tr>
<tr>
<td>14</td>
<td>1013.666</td>
<td>5.69</td>
<td>224.9</td>
</tr>
<tr>
<td>15</td>
<td>1084.719</td>
<td>6.23</td>
<td>235.2</td>
</tr>
<tr>
<td>16</td>
<td>1155.765</td>
<td>6.77</td>
<td>247.4</td>
</tr>
<tr>
<td>17</td>
<td>1226.808</td>
<td>7.31</td>
<td>256.2</td>
</tr>
<tr>
<td>18</td>
<td>1297.848</td>
<td>7.85</td>
<td>265.0</td>
</tr>
<tr>
<td>19</td>
<td>1368.901</td>
<td>8.39</td>
<td>276.3</td>
</tr>
</tbody>
</table>
Table 4.3. Published CCSs and experimental drift times of the [M + nH]^{n+} (n=2-4) ions from the synthetic peptide calibrant.

<table>
<thead>
<tr>
<th>z</th>
<th>m/z</th>
<th>Drift time (ms)</th>
<th>CCS (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2+</td>
<td>1049.5</td>
<td>3.43</td>
<td>373</td>
</tr>
<tr>
<td>3+</td>
<td>699.6</td>
<td>1.44</td>
<td>387</td>
</tr>
<tr>
<td>4+</td>
<td>525.2</td>
<td>1.17</td>
<td>462</td>
</tr>
</tbody>
</table>

Both the order CCS\(_{\text{ortho.}}\) < CCS\(_{\text{meta.}}\) < CCS\(_{\text{para.}}\) and the CCS values predicted computationally are qualitatively and quantitatively in good agreement with the experimental results, confirming that the isomers with drift times of 4.96 ms, 5.87 ms, and 6.59 ms result from the ortho-, meta-, and para-isomers, respectively.

Based on the areas of the three distinct peaks observed after TWIM separation, the relative composition of the three isomers in the mixture could be estimated: para-compound (~59%), meta-compound (~20%), and ortho-compound (~21%). The majority of the diadducts has a para- structure, while the meta- and ortho-compounds are minor products. This result suggests that there is significant steric hindrance when a large thiol ligand approaches the POSS tethered with one bulky substituent from the ortho- and meta-positions during the thiol-ene reaction. The para-position is the most favored site with minimal influence from the existing substituent. In contrast, such selectivity is not observed when small functional thiols are used.
4.3. Probing condensation kinetics and isomer structures in the epoxy-amino POSS system

Epoxy-POSS and amino-POSS are widely applied to form cross-linked polymer networks. Surprisingly, the detailed structure of the linker has remained unknown. In this work, epoxy-amine POSS monoadduct and diadducts were synthesized and analyzed by mass spectrometry. The method developed for the characterization of isomeric thiol-ene POSS systems was also applied to the analysis of epoxy-amino POSS isomers.

4.3.1. Sample preparation

Solutions of Glycidyl POSS (Epoxy POSS) and Aminopropyl Isobutyl POSS (Amino POSS) in iPrOH/THF (1:1) were mixed in the stoichiometric ratio 1:2 and stirred at 80 °C for 1.5 hr. The reactants are shown in Figure 4.10. The crude product was directly subjected to MALDI and ESI mass spectrometry analysis.

4.3.2. Results and discussion

The structures of epoxy-POSS and amino-POSS were shown in Figure 4.10. Both samples were used as bought; epoxy-POSS was specified as a mixture of cages (T8, T10, 12), and amino-POSS as a monodisperse compound. The chemical formula of epoxy-POSS is (C₆H₁₁O₂)ₙ(SiO₁.₅)ₙ, n=8, 10, and 12 for cage sized T8, T10, and T12, respectively. This is confirmed by the MALDI-ToF mass spectrum, shown in Figure 4.11, in which the major peaks at m/z 1359.5, 1693.7, and 2027.8 correspond to [T8 + Na]⁺, [T10 + Na]⁺, and [T12 + Na]⁺, respectively. The minor peaks m/z 1535.6, 1549.6, 1585.6,
1883.8, and 1919.9 are deficient cages, which are missing [RSi + H] (144 Da) or [RSi + O - H] (158 Da) units and contain uncondensed OH groups (+ H₂O), possible structures are shown in Figure 4.12 for a T8 cage. The MALDI mass spectrum of amino-POSS, shown in Figure 4.13, contains a minor peak at m/z 874.4 corresponding to [T8 + H]⁺. Although this POSS was supplied as a monodisperse compound, the MALDI mass spectrum suggests that larger, deficient cages are also present in the sample. For example, the peaks at m/z 1224.7 and 1056.6 are consistent with [T12 - SiR’]⁺ and [T12 - (SiR’)₃ + 2H]⁺ respectively.

Figure 4.10. Structures of (a-c) epoxy-POSS (T8, T10, T12) and (d) amino-POSS.
Figure 4.11. MALDI-ToF mass spectrum of epoxy-POSS.

Figure 4.12. Possible structures of deficient epoxy-POSS (exemplified for a T8 cage).
Scheme 4.2. Synthetic route for the epoxy-POSS/amino-POSS diadduct. The meta isomer is shown, but ortho and para isomers would also be formed.

Figure 4.13. MALDI-ToF mass spectrum of amino-POSS.
Figure 4.14. MALDI-ToF and ESI mass spectra of the crude product from the reaction of epoxy-POSS (E) with two equivalents of amino-POSS (A).

Epoxy-POSS was reacted with two equivalents of amino-POSS (1:2 molar ratio), see Scheme 4.2, and the crude product was subjected to mass spectrometry analysis, as shown in Figure 4.14. The three major peaks observed in both the MALDI-ToF and ESI mass spectra correspond to the adducts of two amino-POSS molecules (T8A) to the T8, T10, and T12 cages of epoxy-POSS (TXE, X= 8, 10, 12). The minor peaks are products from the reaction of amino-POSS with impurities in the epoxy-POSS sample. The doubly protonated [T8E + 2T8A] adduct, viz. the ion [T8E + 2T8A + 2H]2+ at m/z 1544.84 was selected for traveling wave ion mobility analysis. This ion should be a mixture of ortho-, meta, and para isomers of the diadduct due to the cubic symmetry of POSS.
Figure 4.15. TWIM-MS analysis of $[T_8^E + 2T_8^A + 2H]^{2+}$ ($m/z$ 1544). The traveling wave velocity and traveling wave height were 350 m/s and 10 V, respectively.

Figure 4.16. Energy minimized structure of the para-isomer of the epoxy-amino POSS diadduct. Significant folding is possible due to the long spacers between the POSS cages.

As shown in Figure 4.15, only two peaks are observed after IM separation, having drift time distributions with maxima at 5.59 and 7.58 ms. The isotope patterns in the mass spectra extracted from these peaks indicate that they arise from triply and doubly charged ions, respectively. These charge states, combined with the $m/z$ value (1544), identify the
ions giving rise to these peaks as $[T_{12}^{E} + 2T_{8}^{A} + 3H]^{3+}$ and $[T_{8}^{E} + 2T_{8}^{A} + 2H]^{2+}$, respectively, which means that the isomers of the diadduct of epoxy-POSS with amine-POSS, $[T_{8}^{E} + 2T_{8}^{A}]$, cannot be separated with the currently available ESI-TWIM-MS technology. The failure to separate the isomers of $[T_{8}^{E} + 2T_{8}^{A}]$ suggests that their structures have quite similar collision cross-sections. The spacers between the POSS cages of the epoxy-amine system are considerably longer (11 atoms) than those in the thiol-ene system (6 atoms). Folded conformations can more easily be formed with the epoxy-amine system (Figure 4.16), which would compromise the ability to separate ortho-, meta-, and para-isomers by making their collision cross-sections more similar with each other.

Figure 4.17. ESI-MS/MS spectrum of $[T_{8}^{E} + T_{8}^{A} + H]^{+}$ ion ($m/z$ 2211.2), acquired by CAD (Ar) in the trap cell at a collision energy of 35 eV.
Figure 4.17. Fragmentation efficiency vs. laboratory collision energy for the [T8\textsuperscript{E} + T8\textsuperscript{A} + H]\textsuperscript{+} ion (m/z 2211) of the epoxy-aminO POSS monoadcut.

The POSS cages in the epoxy-aminO POSS diadduct are covalently linked. For information on the intrinsic stability of such linkers, the singly protonated monoadadduct [T8\textsuperscript{E} + T8\textsuperscript{A} + H]\textsuperscript{+} was examined by MS/MS as a function of collision energy. A representative MS/MS spectrum is shown in Figure 4.17, while Figure 4.18 shows the fragmentation efficiency curve obtained by plotting relative fragment ion intensity vs. collision energy. Even at the highest collision energy used (60 eV), some monoadadduct survives undissociated (~ 5%), affirming the high stability of the linker units.
laboratory-frame collision energy \((E_{lab})\) can be converted into a center-of-mass collision energy \((E_{cm})\) via the equation

\[
E_{cm} = zE_{lab}m/(m+M)
\]

where \(M\) and \(m\) are the masses of the ion and of the collision gas atoms, respectively, and \(z\) is the ion charge state. \(E_{cm}\) values can be used to appraise intrinsic stabilities. Based on the data in Figure 4.18, \(E_{cm}\) values > 1.06 eV are needed for complete dissociation of the monoadduct.

4.4. Characterization of photoresponsive \emph{cis/trans} isomerization in azo-POSS

Azobenzene is photoresponsive to UV/Vis light. Its structures changes from \emph{trans} to \emph{cis} under UV light, and returns from \emph{cis} back to \emph{trans} under visible light, as shown in Scheme 4.3. A new method probing this isomerization \emph{in situ} has been developed and will be exemplified on a POSS-substituted azobenzene.

\[\begin{align*}
\text{trans-azobenzene} & \xrightarrow{h\nu} \text{cis-azobenzene} \\
\text{trans-azobenzene} & \xrightarrow{h\nu' \text{ or heat}} \text{cis-azobenzene}
\end{align*}\]

Scheme 4.3. Photoresponsive property of azobenzene.
4.4.1. Sample preparation

Aminopropyl Isobutyl POSS (amine-POSS, HybridPlastics) was reacted with two equivalents of azobenzene-4,4'-dicarbonyl dichloride (Aldrich) in CHCl₃ (Scheme 4.4). The crude product was isolated by solvent evaporation and subjected to ESI-MS analysis. The synthesis was performed by the group of Professor Stephen Z.D. Cheng.

4.4.2. Results and discussion

The crude product was subjected to mass spectrometry analysis. The crude product was dissolved in CHCl₃/MeOH (1/1, v/v) at the concentration of ~0.5 mg/mL; 1% NaTFA (1 mg/mL in MeOH) was added to this solution to aid the ionization process.

![Scheme 4.4. Synthetic route to azo-POSS. Triethylamine was used as catalyst.](image-url)
Figure 4.19. ESI mass spectrum of the azo-POSS diadduct. T8A is the amino-POSS starting material. The ions containing a 42-Da moiety presumably originate from a T8A impurity that is N-propylated.

The ESI mass spectrum of the product (Figure 4.19) contains a singly charged ion at \( m/z \) 2003 and a doubly charged ion at \( m/z \) 1013, corresponding to \([M + Na]^+\) and \([M + 2Na]^{2+}\) of the azo-POSS diadduct, respectively. Singly and doubly protonated forms of these ions are also present in the spectrum. Impurities are detected at \( m/z \) 874, 916, and 938, resulting from unreacted amino-POSS and from a propylated contaminant in the amino-POSS.

The singly charged ion at \( m/z \) 2003 was selected for TWIM-MS analysis (Figure 4.20). Only one peak was observed after IM separation, at a drift time (t\(_d\)) of 11.54 ms, indicating the presence of only one isomeric structure in the product (Figure 4.21, bottom trace). However, after the sample solution was placed under UV light (355 nm) in a dark room for 2 hours, a second peak at \( t_d = 10.13 \) ms was observed (Figure 4.21), and as the exposure under UV light increased, the intensity of the latter peak increased, indicating
the formation of another isomeric structure. After treating the sample under UV light in the dark room for 2.5 hours, visible light was applied; 10 min under visible light later, the peak at $t_d = 10.13$ ms decreased, and after 14 hours under visible light, this peak disappeared completely. The transformation process (trans to cis) is summarized in Figure 4.21. The thermodynamically more stable trans isomer should travel more slowly through the IM region ($t_d = 11.67$ ms) than the cis diadduct ($t_d = 10.13$ ms) because of its more extended geometry (cf. Scheme 4.3).

Figure 4.20. ESI mass spectrum of mass-selected $m/z$ 2003 (isolated azo-POSS).

Figure 4.21. ESI-TWIM-MS drift time distributions ("drift time chromatograms"). The traveling wave velocity and traveling wave height were 350 m/s and 13 V, respectively.
In order to confirm that the two peaks observed at \( t_d = 10.13 \) and 11.67 ms originate from the same compound, their mass spectra were extracted from the drift time chromatogram. These are identical within experimental error (Figure 4.22) and their \( m/z \) values and isotope patterns indeed match those expected for the sodiated azo-POSS diadduct.

Figure 4.22. Mass spectra extracted from the signals at 10.13 and 11.67 ms in the drift time chromatogram of sodiated azo-POSS.

A UV absorption experiment was also performed to confirm that the sample investigated by TWIM-MS contained the azo functional group. The crude product showed an absorption band around 350 nm (Figure 4.23), which is in compliance with the TWIM-MS experimental data.

The TWIM-MS data are consistent with the peaks observed at \( t_d = 10.12 \) and 11.67 ms corresponding to \textit{cis}-azo-POSS, and \textit{trans}-azo-POSS, respectively. In order to
substantiate this conclusion, molecular modeling was performed to address the sizes of these isomers.

Using the Anneal and Geometry Optimization tasks in the Forcite module, 150 energy-minimized structures were generated for each isomer through an annealing process and their collision cross-sections (CCSs) were calculated by the trajectory method available in the MOBCAL program.

Plots of the resulting CCS values against the corresponding potential energies are shown in Figures 4.24 and 4.25. Representative 3-D structures of cis and trans isomer are given in Figure 4.26; Na\(^+\) was not included in the molecular simulations since cationizing metal ions usually have no significant effect on overall sizes of large, relatively rigid systems, such as POSS-based giant molecules. The average CCS value of all 150 candidate structures for each isomer was calculated to be 405 Å\(^2\) for the trans-azo-POSS diadduct and 382 Å\(^2\) for the cis-azo-POSS diadduct (see Table 4.4). The cis-isomer is predicted to have a smaller CCS, as expected from its more compact architecture (cf. Figure 4.26). Hence, the cis-isomer should drift faster through the IM cell, in full agreement with the earlier tentative conclusion that the TWIM-MS peaks with \(t_d = 10.13\) ms and 11.67 ms arise from cis-azo-POSS and trans-azo-POSS, respectively.

It is interesting to note that the average total potential energies of these isomers follow the order that trans \(<\) cis, which is in accordance with the observation that energy must be supplied via UV light to convert the more stable trans-azobenzene into the less stable cis-azobenzene moiety.
Figure 4.23. UV absorption spectrum of the azo-POSS diadduct solution examined by ESI-TWIM-MS.

Figure 4.24. Calculated collision cross-section vs. the corresponding total potential energy for 150 energy-minimized structures of trans-azo-POSS diadduct.
Figure 4.2. Calculated collision cross-section vs. the corresponding total potential energy for the energy-minimized structures of cis-azo-POSS diadduct.

Table 4.4. Theoretical CCS of trans- and cis-azo-POSS isomer.

<table>
<thead>
<tr>
<th>Isomer</th>
<th>CCS$_{\text{calc}}$ (Å$^2$)</th>
<th>Energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-</td>
<td>405</td>
<td>-39</td>
</tr>
<tr>
<td>cis-</td>
<td>382</td>
<td>-46</td>
</tr>
</tbody>
</table>
Figure 4.26. Representative energy-minimized structures for the a.) \textit{trans} and b.) \textit{cis} isomer of the azo-POSS diadduct obtained by molecular modeling. The carbon atoms are grey, the silicon atoms are yellow, the oxygen atoms are red, and the nitrogen atoms are blue, the hydrogen atoms are omitted.

4.5. Conclusions

Thiol-POSS and octavinyl POSS were reacted \textit{via} thiol-ene “click” chemistry to build a tri-POSS system composed of \textit{ortho}, \textit{meta}, and \textit{para} isomers. These three isomers cannot generally be purified or separated by traditional chromatography methods, posing significant challenges to the characterization of this tri-POSS system by techniques probing average structures, such as NMR, FTIR, and/or X-ray spectroscopy. Here, the constituents of this system were isolated and conclusively identified using TWIM-MS. This method blends gas-phase chromatographic dispersion with composition and structure determination by MS, allowing one to probe individual mixture constituents selected from the raw product by their size, charge, and shape. Molecular modeling
provided additional help by measuring the shapes (collision cross-sections) of isomeric architectures to predict the order of their drift times through the ion mobility region for comparison with the experimentally observed drift time orders. All together, it was found that these isomers drifted out in the order ortho, meta, and para with the abundance ratio of approximately 1/1/3 (o/m/p). TWIM-MS unambiguously differentiated ortho, meta, and para isomers and quantitatively revealed the relative ratio of these isomers in a straightforward manner.

The ESI-TWIM-MS methodology was also applied to characterize isomers in other POSS diadduct systems. Although isomeric compositions were separated and quantified for the thiol-ene POSS diadduct system (vide supra), isomers of the epoxy-amine POSS system, which contained a much longer spacer chain between the POSS cubic cages, could not be separated. On the other hand, an azobenzene functionalized diadduct that could undergo trans/cis isomerization could be successfully characterized, and the isomerization could be monitored in situ by TWIM-MS, as the sample was exposed to UV vs. visible light, which induced trans to cis vs. cis to trans rearrangement, respectively, in the azobenzene unit.
CHAPTER V

EFFECT OF COUNTERIONS ON THE COLLISION CROSS-SECTIONS OF SELF-ASSEMBLED METALLO-TRIANGLES

5.1. Background

Self-assembly procedures are widely used to form 2D and 3D supra-macromolecular structures with a variety of geometrical shapes, including dimers, triangles, squares, pentagons, and hexagons. Triangular metallomacrocycles, which have potential applications in energy storage devices and nanomachinery, can be constructed by metal complexation of ligands with a 60° angle between their binding sites. In the resulting supramolecular complexes, the positive charges provided by the metal ions are balanced by negative and usually singly charged counterions.

Electrospray ionization mass spectrometry (ESI-MS) allows for a straightforward characterization of the stoichiometries of supramolecular macrocycles. Generally, ESI produces a distribution of charge states, arising from losses of different numbers of counterions. Travelling wave ion mobility mass spectrometry (TWIM-MS) can be used to distinguish isomeric structures and determine the shapes of the resulting supramolecular cations. The collision cross-sections (CCSs) of the supramolecular ions can be derived from drift times measured by TWIM-MS; both are affected by the mass, charge state, and shape of the ions. The CCSs of possible structures
can also be calculated by molecular modeling.\textsuperscript{125} Comparing experimental and calculated CCSs allows one to determine conclusively the molecular architecture of the species examined.

The effect of the type and number of counterions on the CCSs of metallomacrocycles has not been evaluated yet. Counterions have been generally omitted in calculations of CCSs under the assumption that their relatively small size does not alter measurably the supramolecular architecture.\textsuperscript{126} Whether these anions influence the size and shape of the supramolecular assembly due to electrostatic interactions with the positive metal ions is examined here for all charge states of a triangular complex containing three metal (II) cations and different counterions. Experimental CCSs were acquired by TWIM-MS experiments and compared with theoretical values obtained from computationally optimized structures that included the counterions. Both the projection approximation (PA) and the trajectory method (TM) were utilized to calculate CCSs from the simulated structures.

5.2. Materials and methods

The bisterpyridine ligand used was synthesized via Suzuki coupling of 1,2-dibromo-4,5-dimethoxybenzene and terpyridinylphenylboronic acid by Dr. Anthony Schultz in the laboratory of Professor George R. Newkome. The ligand was purified by either column chromatography or recrystallization and complexed in a 1:1 molar ratio with various Zn salts to yield triangular metallomacrocycles with different counterions.\textsuperscript{125} The sample with PF\textsubscript{6} counterions was formed by precipitation with NH\textsubscript{4}PF\textsubscript{6} of the complex generated
using Zn(NO$_3$)$_2$. A complex with Cd$^{2+}$ ions was also prepared using subsequent precipitation with NH$_4$PF$_6$.

ESI-MS and ESI-TWIM-MS experiments were performed on a Waters Synapt HDMS quadrupole/time-of-flight (Q/ToF) mass spectrometer (Waters, Milford, MA). The tri-wave region of this instrument, located between the Q and ToF mass analyzers, contains three confined regions in the order trap cell (closest to Q), TWIM cell, and transfer cell (closest to ToF). Either the trap or the transfer cell can be used for MS/MS studies via collisionally activated dissociation (CAD). The following ESI and TWIM parameters were selected: ESI capillary voltage, 3.5 kV; sample cone voltage, 35 V; extraction cone voltage, 3.2 V; desolvation gas flow, 500 L/h (N$_2$); trap collision energy (CE), 6 eV; transfer CE, 4 eV; trap gas flow, 1.5 mL/min (Ar); IM gas flow, 22.7 mL/min (N$_2$); sample flow rate, 5 μL/min; source temperature, 30 °C; desolvation temperature, 40 °C; traveling wave velocity, 350 m/s; traveling wave height, 7.5 V or 6.5 V. The sprayed solutions of metallo-triangles were prepared by dissolving ~0.1 mg of sample in 1 mL of CHCl$_3$/MeOH (v/v, 50/50) or ACN/MeOH (v/v, 50/50). Data analysis was conducted with the MassLynx 4.1 and DriftScope 2.1 programs of Waters.

Poly(methyl methacrylate) (PMMA), purchased from Sigma/Aldrich, and the peptide TRQARRNAAAAWRERQR, prepared by solid-state synthesis in Professor Matthew Becker’s laboratory (University of Akron), were used as calibrants for the determination of CCSs. The PMMA solution was prepared by dissolving ~0.3 mg sample in 1 mL CHCl$_3$/MeOH (v/v, 50/50) containing 1% (v/v) NaTFA solution (1 mg/mL in MeOH). The peptide solution sprayed was prepared in H$_2$O/ACN v/v, 50/50) at a concentration of 0.01 mg/mL. The CCSs of sodiated PMMA n-mers were provided by
Professor Philippe Dugourd (University of Lyon, France). The calibration curve was constructed by plotting the corrected collision cross-sections of PMMA and the synthesized peptide ions against the corresponding corrected drift times (arrival times) measured in our experiments.

Molecular modeling of the triangular metallomacrocycles was performed by the Materials Studio program (version 4.2), using the Anneal and Geometry Optimization tasks in the Forcite module (Accelrys Software, Inc.). Initially energy-minimized structures were built by 300 anneal cycles with initial and mid-cycle temperatures of 50 and 1400 K, twenty heating ramps per cycle, one thousand dynamics steps per ramp, and one dynamics step per femtosecond. A constant volume/constant energy (NVE) ensemble was used and the geometry was optimized after each cycle, utilizing a universal force field with atom-based summation and cubic spline truncation for both the electrostatic and van der Waals parameters. 300 Energy-minimized structures were considered for the calculation of theoretical collision cross-sections using the projection approximation (PA) or the trajectory method (TM) of the MOBCAL program. In the PA method, the ion is modeled by a collection of overlapping hard spheres with radii equal to hard sphere collision distances, and the calculated CCS is an average of projection areas from all possible collision geometries. In the trajectory method, ions are considered as a collection of atoms, and the long-range interactions between the analyte ions and buffer gas are taken into account. The trajectory method is time consuming, but it is considered as a more reliable method for large molecules.
5.3. Results and discussion

ESI mass spectra were acquired for all triangular complexes. All showed very similar characteristics, which will be exemplified with the complex containing Zn$^{2+}$ and PF$^{-}$ counterions whose structures is depicted in Figure 5.1. As documented by Figure 5.2, a series of charge states is detected by ESI-MS, ranging from 2+ to 6+. The minor distribution at $m/z$ 503.1 (5+), 665.3 (4+), 935.5 (3+), and 1497.0 (2+) originates from complexes whose counterions were not completely exchanged with PF$_6^-$ (see section 5.2), and they still contained one NO$_3^-$. These minor byproducts do not affect the IM-MS measurements.

![Figure 5.1. Triangular Zn(II)-bisterpyridine complex with 6+ charges and six PF$_6^-$ counterions.](image)
Figure 5.2. ESI mass spectrum of the triangular Zn(II)-bisterpyridine complex with PF$_6^-$ counterions.

All ions formed by ESI were analyzed by IM-MS and the drift time chromatogram obtained this way is illustrated in Figure 5.3. All charge states observed in the ESI mass spectrum give distinct, well separated peaks in this IM chromatogram, with relative intensities that are in accordance with those in the ESI spectrum. The small peak at $t_d=2.89$ ms arises from the minor product at $m/z$ 503.1 (5+) with one NO$_3^-$ counterion.

Drift time chromatograms were measured for all complexes examined. The drift times of the different charge states observed are listed in Table 5.1.
Figure 5.3. Drift time distribution ("drift time chromatogram") of the ions formed by ESI of the triangular Zn(II)-bisterpyridine complex with PF$_6^-$ counterions. IM separation was effected using a traveling wave height of 7.5 V.

Collision cross-sections are physical properties that are characteristic of the corresponding supramolecular structures. PMMA oligomers and a peptide were used as standards of known CCS to create a calibration curve for the conversion of the experimentally determined drift times into experimental CCS values. It should be mentioned at this point that ions with $t_d < 2$ ms do not spend sufficient time inside the TWIM cell for optimum separation and derivation of the correct CCS. The residence time in the TWIM cell can be increased by lowering the traveling wave height; this improves the separation of the high charge states, but prevent the slower moving low charge states from exiting the TWIM cell within the duty cycle of the TWIM-MS experiment. To
resolve these problems, the CCSs for charge states 2+ to 4+ were determined from $t_d$ values measured at traveling wave height of 7.5 V; whereas, the CCSs for charge states 5+ and 6+ were determined from TWIM-MS measurements at a traveling wave height of 6.5 V. Separate calibration curves were set up for the traveling wave heights of 7.5 V and 6.5 V (Figure 5.4). Based on these curves, experimental CCS values were derived, which are included in Table 5.1. The CCS values of the PMMA calibrant ions are listed in Table 5.2. The CCSs calculated from energy-minimized structures by the trajectory method and projection approximation are also presented in Table 5.1. Representative structures of the Zn(II)-bisterpyridine triangles with PF$_6^-$ counterions are shown in Figure 5.5.

The CCSs of the Cd(II) and Zn(II) complexes with PF$_6^-$ as counterion are very similar (within 5% for all charge states) despite the significantly different ion radii of Zn(II) and Cd (II), which are 0.74 and 0.95 pm, respectively, for hexadentate coordination. Hence, the overall sizes of the complexes are rather dictated by the ligand, and possibly the counterions, but are less sensitive to the identity of the metal. Therefore, the counterion effect was examined only for Zn(II).
Figure 5.4. Calibration plot of corrected cross section vs. effective drift time, obtained using sodiated PMMA n-mers (Table 5.2) and protonated ions of the peptide TRQARRNAAAAWRERQR. Drift times were measured at a traveling wave velocity of 350 m/s and a traveling wave height of 7.5 V. A similar calibration plot was constructed for the measurement performed at a traveling wave height of 6.5 V; the calibration equation in this case was $y = 451.34x^{0.4435}$, $R^2 = 0.9821$. 

\[
y = 451.34x^{0.4435} \\
R^2 = 0.9821
\]
Table 5.1. Collision cross-sections (CCSs) of different charge states of triangular metallomacrocycles with various counterions.

<table>
<thead>
<tr>
<th>Charge State</th>
<th>Drift time\textsuperscript{a} (ms)</th>
<th>CCS\textsubscript{exp} (Å\textsuperscript{2})</th>
<th>CCS\textsubscript{theory} (Å\textsuperscript{2}) (TM)</th>
<th>Error</th>
<th>CCS\textsubscript{theory} (Å\textsuperscript{2}) (PA)</th>
<th>Error\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn/PF\textsubscript{6}\textsuperscript{−}</td>
<td>2+</td>
<td>14.53</td>
<td>545.9</td>
<td>561.9</td>
<td>2.8%</td>
<td>502.1</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>6.14</td>
<td>537.1</td>
<td>555.4</td>
<td>3.3%</td>
<td>498.5</td>
</tr>
<tr>
<td></td>
<td>4+</td>
<td>3.61</td>
<td>537.0</td>
<td>551.6</td>
<td>2.6%</td>
<td>498.2</td>
</tr>
<tr>
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<td>5+</td>
<td>3.25</td>
<td>530.5</td>
<td>565.7</td>
<td>6.2%</td>
<td>511.4</td>
</tr>
<tr>
<td></td>
<td>6+</td>
<td>2.25</td>
<td>504.4</td>
<td>555.4</td>
<td>9.2%</td>
<td>507.5</td>
</tr>
<tr>
<td>Zn/BF\textsubscript{4}\textsuperscript{−}</td>
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<td>13.63</td>
<td>529.9</td>
<td>588.4</td>
<td>9.9%</td>
<td>524.7</td>
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<td>537.3</td>
<td>577.4</td>
<td>6.9%</td>
<td>515.6</td>
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<td></td>
<td>5+</td>
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<td>540.5</td>
<td>564.7</td>
<td>4.3%</td>
<td>511.5</td>
</tr>
<tr>
<td></td>
<td>6+</td>
<td>2.35</td>
<td>504.4</td>
<td>555.4</td>
<td>9.2%</td>
<td>507.5</td>
</tr>
<tr>
<td>Zn/NO\textsubscript{3}\textsuperscript{−}</td>
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<td>10.74</td>
<td>472.9</td>
<td>594.8</td>
<td>20.5%</td>
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</tr>
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<td>5.33</td>
<td>499.2</td>
<td>575.4</td>
<td>13.2%</td>
<td>513.7</td>
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<tr>
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<td>4+</td>
<td>3.52</td>
<td>529.5</td>
<td>576.7</td>
<td>8.2%</td>
<td>517.5</td>
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<tr>
<td></td>
<td>5+</td>
<td>4.06</td>
<td>588.3</td>
<td>571.2</td>
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<td>519.3</td>
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<td>Zn/OTf\textsuperscript{−}</td>
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<td>610.8</td>
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<td>550.7</td>
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<td>4+</td>
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<td>537.0</td>
<td>617.6</td>
<td>13.1%</td>
<td>564.8</td>
</tr>
<tr>
<td></td>
<td>5+</td>
<td>3.34</td>
<td>540.4</td>
<td>588.0</td>
<td>8.1%</td>
<td>538.4</td>
</tr>
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<td>504.4</td>
<td>555.4</td>
<td>9.2%</td>
<td>507.5</td>
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<td>Zn/F\textsuperscript{−}</td>
<td>2+</td>
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<td>443.2</td>
<td>569.5</td>
<td>22.2%</td>
<td>525.3</td>
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<td></td>
<td>3+</td>
<td>4.33</td>
<td>446.3</td>
<td>558.0</td>
<td>20.0%</td>
<td>510.5</td>
</tr>
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<td>4+</td>
<td>3.25</td>
<td>505.2</td>
<td>562.4</td>
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</tr>
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<td>5+</td>
<td>3.25</td>
<td>530.8</td>
<td>556.7</td>
<td>4.7%</td>
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<td>2.44</td>
<td>519.3</td>
<td>555.4</td>
<td>6.5%</td>
<td>507.5</td>
</tr>
<tr>
<td>Cd/PF\textsubscript{6}\textsuperscript{−}</td>
<td>2+</td>
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<td>545.7</td>
<td>581.1</td>
<td>6.1%</td>
<td>520.4</td>
</tr>
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<td>536.9</td>
<td>576.9</td>
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<td>559.3</td>
<td>576.8</td>
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<td>518.4</td>
</tr>
<tr>
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<td>6+</td>
<td>2.44</td>
<td>518.8</td>
<td>565.1</td>
<td>8.2%</td>
<td>515.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}. Measured at a traveling wave height of 7.5 V for charge states 2+, 3+, and 4+; and at a traveling wave height of 6.5 V for charge states 5+ and 6+.

\textsuperscript{b}. Agreement between experimental and calculated CCS.
Figure 5.5. Typical energy-minimized structures of the triangular Zn(II)-bisterpyridine complex with PF$_6^-$ counterion.

Table 5.2. Reported collision cross-sections of sodiated PMMA n-mers and drift times measured in this study by TWIM-MS at traveling wave height 6.5 V and 7.5 V.

<table>
<thead>
<tr>
<th>n*</th>
<th>Charge State</th>
<th>m/z</th>
<th>CCS (Å$^2$)</th>
<th>Drift Time (ms) (6.5 V)</th>
<th>Drift Time (ms) (7.5 V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>2</td>
<td>1074</td>
<td>396</td>
<td>8.84</td>
<td>7.49</td>
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<tr>
<td>23</td>
<td>2</td>
<td>1174</td>
<td>430</td>
<td>10.38</td>
<td>8.57</td>
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<tr>
<td>25</td>
<td>2</td>
<td>1274</td>
<td>461</td>
<td>11.73</td>
<td>9.66</td>
</tr>
<tr>
<td>27</td>
<td>2</td>
<td>1374</td>
<td>466</td>
<td>13.27</td>
<td>10.65</td>
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<tr>
<td>29</td>
<td>2</td>
<td>1474</td>
<td>499</td>
<td>14.26</td>
<td>11.55</td>
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<tr>
<td>28</td>
<td>3</td>
<td>957</td>
<td>488</td>
<td>7.67</td>
<td>5.69</td>
</tr>
<tr>
<td>29</td>
<td>3</td>
<td>990</td>
<td>514</td>
<td>8.03</td>
<td>6.04</td>
</tr>
<tr>
<td>31</td>
<td>3</td>
<td>1057</td>
<td>533</td>
<td>8.21</td>
<td>6.50</td>
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<tr>
<td>35</td>
<td>3</td>
<td>1190</td>
<td>579.5</td>
<td>8.66</td>
<td>7.31</td>
</tr>
<tr>
<td>38</td>
<td>3</td>
<td>1290</td>
<td>610</td>
<td>9.03</td>
<td>8.12</td>
</tr>
<tr>
<td>40</td>
<td>3</td>
<td>1357</td>
<td>610</td>
<td>9.48</td>
<td>7.85</td>
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<td>1457</td>
<td>664</td>
<td>10.38</td>
<td>8.66</td>
</tr>
<tr>
<td>46</td>
<td>3</td>
<td>1557</td>
<td>681</td>
<td>11.46</td>
<td>9.39</td>
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</tbody>
</table>
In general, the complexes with PF$_6^-$ show the least difference between experimental and theoretical CCSs. The agreement between experiment and theory is not universally better with the more rigorous TM algorithm. Some of the experimental CCSs agree well with the less accurate PA method. Evidence has been presented that TM may overestimate CCSs;\textsuperscript{128} whereas, PA tends to underestimate them, which could be the case also in this study.

The agreement between measured and calculated CCS, by either TJ or the PA method, is within \( \leq 10\% \) for all charge states that contain the symmetric counterions PF$_6^-$ or BF$_4^-$. The discrepancy between theory and experiment is poorer with the larger CF$_3$SO$_3^-$ (OTf$^-$) and the smaller F$^-$ and NO$_3^-$ counterions, especially for the lowest detectable charge state (2$^+$). It is possible that low-energy structures with four of these counterions were missed in the geometry optimization step.

Generally, the assemblies with 3-4 charges show very similar or indistinguishable collision cross-sections, both in the experiments and calculations, independent of the counterion. Such insensitivity of CCS on select charge states has also been observed for many systems investigated by IM-MS.\textsuperscript{37,42} Nevertheless, the present study demonstrates that CCS variations over the entire charge state distribution observed after ESI are likely. Adding the first counterion to the bare metallomacrocycle consistently increases the CCS (Table 5.1), although further additions may increase, decrease, or cause no significant change to the CCS, depending on how the additional anions affect the size and shape of the assembly. The added mass may cause an increase in size and CCS; however, attractive interactions between the counterions and the positively charged metal centers may also increase the compactness and decrease the size of the supramolecular complex,
resulting in a decrease in CCS. Contribution upon counterion addition is most
pronounced with the smaller $F^-$ and $NO_3^-$ anions, which cause minimal size increase and
can come close to the positively charged sites to develop strong ion-ion interactions.

In several of the IM-MS studies, supramolecular structures were confirmed by
comparison of the average experimental collision cross-section over all charge states
probed by IM-MS with the CCS calculated for the counterion-free assembly.\textsuperscript{37,38,125, 126,}
\textsuperscript{129-132} For the triangular Zn(II) complexes shown in Figure 5.5, the average experimental
collision cross-sections of all charge states are 531, 530, 522, 529, and 489 Å$^2$ for $PF_6^-$,
$BF_4^-$, $NO_3^-$, $CF_3SO_3^-$, and $F^-$ counterions, respectively. With the exception of $F^-$ anions,
these values lie within ~6\% of the CCS computed for the bare organometallic component,
\textit{viz.} 556 Å$^2$. The similarity validates the use of this approach (with counterions other than
$F^-$) for the characterization of supramolecular architectures.

5.4. Conclusions

In conclusion, TWIM-MS and molecular modeling were combined to measure the
effect of counterions on supramolecular architectures. The best agreement between
calculated and measured collision cross-sections was found for $PF_6^-$ and $BF_4^-$ anions,
which also showed the smallest spread in CCS among different charge states. The ease of
forming $PF_6^-$ salts of metallo-supramolecular complexes (\textit{viz.} by precipitation with
$NH_4PF_6$), and the robustness of $PF_6^-$ salts upon ESI, and for smaller complexes even
with MALDI, makes this anion the best choice for samples prepared for mass
spectrometry analysis.
CHAPTER VI

CHARACTERIZATION OF ISOMERS IN SELF-ASSEMBLED METALLO-SUPRAMOLECULES

In this chapter, IM-MS was combined with gradient tandem mass spectrometry (gMS^2) to monitor the structures and intrinsic stabilities of metallo-supramolecules, including a terpyridine-based tetramer, bisterpyridyl o-carborane based suprametallomacrocycles,\textsuperscript{131} and a tristerpyridine-based nanosphere.\textsuperscript{132} Isomeric architectures were detected and characterized by their shapes/sizes (as revealed by collision cross-sections) and their thermal stabilities (as revealed by fragmentation characteristics).

6.1. Materials and methods

Bisterpyridyl o-carborane based suprametallomacrocycles were synthesized by James Ludlow, and the terpyridine-based tetramer and tristerpyridine-based nanosphere by Dr. Tingzheng Xie and Dr. Shengyun Liao in Professor George R. Newkome’s group.

ESI-MS and ESI-TWIM-MS experiments were performed on a Waters Synapt HDMS quadrupole/time-of-flight (Q/ToF) mass spectrometer (Waters, Milford, MA).
The tri-wave region of this instrument, located between the Q and ToF mass analyzers, contains three confined regions in the order trap cell (closest to Q), TWIM cell, and transfer cell (closest to ToF). Either the trap or the transfer cell can be used for MS/MS studies via collisionally activated dissociation (CAD). The following ESI and TWIM parameters were selected: ESI capillary voltage, 3.5 kV; sample cone voltage, 35 V; extraction cone voltage, 3.2 V; desolvation gas flow, 500 L/h (N\textsubscript{2}); trap collision energy (CE), 6 eV; transfer CE, 4 eV; trap gas flow, 1.5 mL/min (Ar); IM gas flow, 22.7 mL/min (N\textsubscript{2}); sample flow rate, 5 μL/min; source temperature, 30 °C; desolvation temperature, 40 °C; traveling wave velocity, 350 m/s; traveling wave height, 7.5 V or 8.5 V.

The sprayed solutions of metallo-triangles were prepared by dissolving ~0.1 mg of sample in 1 mL of CHCl\textsubscript{3}/MeOH (v/v, 50/50) or ACN/MeOH(v/v, 50/50). The sprayed protein standards were prepared by dissolving ~0.01 mg sample in 1 mL ACN/H\textsubscript{2}O (v/v, 50/50) containing 0.1% (v/v) FA (formic acid) solution. The data analysis was conducted with the MassLynx 4.1 and DriftScope 2.1 programs of Waters.

Ubiquitin (bovine red blood cells) and cytochrome C (horse heart) ions with established collision cross-sections served as standards for the calibration of the drift time scale in TWIM-MS experiments. The calibration curve was constructed by plotting the corrected collision cross-sections of the protein ions against the corresponding corrected drift times (arrival times) measured by TWIM-MS experiments in this study.

Molecular modeling of the supramolecules and their fragments was performed by the Materials Studio program (version 4.2). Initially energy-minimized structures were built by 300 anneal cycles with initial and mid-cycle temperatures of 300 and 1400 K, twenty heating ramps per cycle, one thousand dynamics steps per ramp, and one
dynamics step per femtosecond. A constant volume/constant energy (NVE) ensemble was used and the geometry was optimized after each cycle. Geometry optimization utilized a universal force field with atom-based summation and cubic spline truncation for both the electrostatic and van der Waals parameters. 100 or 300 energy-minimized structures were selected for the calculation of theoretical collision cross-sections using the MOBCAL program, which permits CCS calculations by the projection approximation (PA), trajectory (TJ), and exact hard sphere scattering (EHSS) methods.

6.2. Conformers of terpyridine-based tetramer

Scheme 6.1. Synthetic pathway to the tetrameric Ru(II)-bisterpyridine assembly studied.

Scheme 6.2. Proposed conformers of the tetramer.
The synthetic pathway to the terpyridine-based tetramer is succinctly described in Scheme 6.1; Ru(II) served as metal ion and Cl\(^{-}\) as the counterion. Due to the flexible structure of the tetramer, which is an open system, two types of conformers are possible, having compact and extended architecture which, for brevity, are designated as the cyclic and linear structure, respectively, as shown in Scheme 6.2.

A series of continuous charge states (6+, 5+, 4+, 3+) of the tetramer are observed in the ESI mass spectrum (shown in Figure 6.1a), consistent with a single stoichiometry for the analyzed sample. For further characterization, ESI-TWIM-MS (ESI-MS coupled with traveling–wave ion mobility spectrometry) was employed to probe the structure of the tetramer. As shown in Figure 6.1b, all charge states (6+, 5+, 4+, 3+) are separated into two bands with distinct drift times after passing through the IM cell, indicating that two structures of the tetramer exist.

The TWIM bands of the 5+ charge state at \(m/z\) 790.2 in Figure 6.1b were selected and exported into the drift time distribution (drift time chromatogram) shown in Figure 6.2a, which shows the 2D bands in the form of 1D signals, centering at the drift time of 5.32 and 6.77 ms. The mass spectra extracted from each signal (Figure 6.2b) confirm that the ions drifting at 5.32 and 6.77 ms have the same \(m/z\) and isotope pattern, providing evidence that these ions are conformers or isomers.
Figure 6.1. a) ESI mass spectrum and b) ESI-TWIM-MS plot of the tetrameric Ru(II)-bisterpyridine complex studied (Scheme 6.2). The charge states of intact assemblies are marked. The traveling wave velocity and traveling wave height were 350 m/s and 8.5 V, respectively.
Figure 6.2. a) Two-dimensional ESI-TWIM-MS plot (bottom) and drift time distribution (drift time chromatogram, top) for $m/z$ 790.2 (5+) from tetrameric complex; b) isotope patterns in the mass spectra extracted from the signals at 5.32 and 6.77 ms. The traveling wave velocity and traveling wave height were 350 m/s and 8.5 V, respectively.
Figure 6.3. Two-dimensional ESI-TWIM-MS$^2$ plot (bottom) of the IM-separated components of $m/z$ 790.2 (5+) from the tetrameric complex studied, acquired by TWIM separation of $m/z$ 790.2 followed by CAD (Ar) in the transfer cell at a collision energy of 60 eV and ToF mass analysis. The traveling wave velocity and traveling wave height were 350 m/s and 8.5 V, respectively. The top trace shows the combined drift time distributions of parent ion plus fragments for each separated component. The mass spectra extracted from each of the two signals are depicted in Figure 6.4.
TWIM separation was coupled with tandem mass spectrometry (MS/MS or MS²) to further interrogate the two tetramer structures detected by TWIM-MS. The ESI-TWIM-MS² experiment performed involved ion mobility separation of m/z 790.2 and CAD of the individual components in the transfer cell, followed by ToF mass analysis of the CAD products. In such measurements, fragments from the same parent ion will appear at essentially the same drift time (Figure 6.3). Such a procedure offers an efficient means to characterize isomers or conformers. The ESI-TWIM-MS² spectra of the two components of m/z 790.2 show similar fragment patterns (Figure 6.4), differing only in relative intensities; such behavior is indicative of complexes with the same molecular composition but different connectivities or architectures. For the supramolecular complex investigated, isomers, which would differ in their covalent bond frame, are difficult to envision. The presence of different conformers, which differ in geometrical shape, is the most likely scenario. A plausible fragmentation pathway is described in Scheme 6.3: the parent ion m/z 790.2 (5+) loses HCl to form the fragment m/z 782.8 (5+); or one terpyridine ligand (MW = 903 Da) to yield the ion at m/z 609.6 (5+). Similarly, disassembly through competitive and consecutive dissociations accounts for the trimeric fragment ion at m/z 728.0 (4+), dimeric fragment ions at m/z 669.2 (3+) and m/z 953.4 (2+), and monomeric fragment ion at m/z 1003.3 (1+).
Figure 6.4. ESI-TWIM-MS\textsuperscript{2} mass spectra of the separated components of $m/z$ 790.2 containing the parent ion and fragments drifting together (aligned) at a.) 6.68 ms and b.) 5.14 ms. CAD took place in the transfer cell at a collision energy of 60 eV.
Scheme 6.3. Fragmentation pathway of the \textit{m/z} 790.3 ion (5+) from the tetrameric complex.
Figure 6.5. Plot of collision cross-section (CCS) vs. potential energy for 300 energy-minimized candidate structures of the tetramer, generated by annealing simulations. CCSs were calculated by the PA method using the MOBCAL program. Most structures can be grouped into two families of conformers, having compact/folded ("cyclic") or extended ("linear") architecture (in oval or circle), respectively. No counterions were included in the simulations.
Figure 6.6. Calibration curve obtained by plotting the corrected published collision cross-section vs. the corrected drift time for different charge states of ubiquitin (bovine red blood cells) and the synthetic peptide TRQARRNAAAWRERQR. Drift times were measured at a traveling wave velocity of 350 m/s and a traveling wave height of 7.5 V.

Table 6.1. Drift times and collision cross-sections of the cyclic and linear conformers of the tetrameric Ru(II)-bisterpyridine complex.

<table>
<thead>
<tr>
<th>Charges</th>
<th>Cyclic Drift time* (ms)</th>
<th>CCS$_{exp}$ (Å$^2$)</th>
<th>Linear Drift time* (ms)</th>
<th>CCS$_{exp}$ (Å$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+</td>
<td>7.40</td>
<td>601</td>
<td>10.92</td>
<td>731</td>
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<tr>
<td>4+</td>
<td>6.50</td>
<td>750</td>
<td>7.76</td>
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<td>5+</td>
<td>6.95</td>
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<td>8.84</td>
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</tr>
<tr>
<td>6+</td>
<td>4.78</td>
<td>955</td>
<td>5.78</td>
<td>1059</td>
</tr>
</tbody>
</table>

Average exp. 819 927

CCS$_{theory}$ 861 980

* ESI-TWIM-MS was performed at a traveling wave velocity of 350 m/s and a traveling wave height of 7.5 V.
Molecular modeling was employed for further insight about the architectures of the detected conformers. Theoretical CCSs were calculated by the projection approximation (PA) for 300 energy-minimized structures obtained by molecular mechanics/dynamics simulations. The PA algorithm was used (instead of the more rigorous trajectory method) because of the relatively small size of the complexes. A plot of the computed CCSs vs. the potential energies of the 300 candidate structures is shown Figure 6.5. Most of the 300 structures can be grouped into two conformer families based on the CCS distribution, corresponding to a cyclic and a linear architecture. For the cyclic species, there is a mixture of macrocycle-like and twisted compact structures, with an average CCS estimated at 861 Å² (cf. Table 6.1). For the linear species, most optimized structures have extended conformation, and the average CCS obtained by the PA method is 980 Å².

Experimental CCSs were also determined after calibration of the drift time scale with appropriate standards (Figure 6.6). As discussed above, all charge states observed upon ESI are composed of two conformers. The corresponding CCSs, derived from the calibration plot in Figure 6.6, are included in Table 6.1. A strong dependence of the collision cross-section on the number of Cl⁻ counterion is clearly evident. For both conformers, the CCS increases slightly after addition of the first Cl⁻ anion (i.e. from 6+ to 5+) and then decreases substantially after further Cl⁻ additions, probably because the formation of more folded, globular (3D) architectures with smaller sizes.

Due to the large variation of CCS with charge state, average experimental CCSs do not provide any meaningful information. It is noteworthy, however, that the CCSs in charge state 6+, which does not bear any Cl⁻ anions, are very similar (within ~10%) with the CCSs calculated for the counterion-free conformers, corroborating that the
conformers predicted by theory (Figure 6.5) are reasonable representatives of the species observed in the TWIM-MS experiments.

6.3. Structural analysis of o-carborane based suprametallomacrocycles

Bisterpyridyl o-carborane possesses an angle of ca. 53° between the terpyridyl substituents, which is similar to the 60° angle in bisterpyridine ligands that self-assemble to form cyclic structures. Examples of possible macrocycles that can be prepared from the carborane ligand are shown in Scheme 6.4. The carborane complexes investigated in this dissertation were synthesized by Mr. James Ludlow in Professor George R. Newkome’s group. Two systems were characterized, a labile assembly with the <tpy-Zn II -tpy> connectivity and a non-labile assembly with the <tpy-Fe II -tpy> connectivity. All counterions were PF 6 −. The complexes synthesized could be a mixture, including dimer, trimer (triangle) and tetramer constructs.

![Scheme 6.4. Structures of o-carborane based suprametallomacrocycles, containing dimer (MC2), triangle (MC3) and tetramer (MC4) stoichiometries.](image)

131,135
The labile system, which was formed by the reaction of Zn(II) with an equal molar amount of the o-carborane bisterpyridine ligand shown in Scheme 6.4, showed a concentration dependence during ESI-MS analysis, as described in Figure 6.7. At a high concentration of the Zn\textsuperscript{II}-ligand product, viz. 0.6 mg/mL (Figure 6.7a), a mixture of complexes were observed, which were identified by the following ions: \textit{m/z} 512.1 (4+), \textit{m/z} 731.2 (3+), and \textit{m/z} 1169.3 (2+) originating from the dimer (ZnC2); \textit{m/z} 512.1 (6+), \textit{m/z} 643.4 (5+), \textit{m/z} 841.0 (4+) originating from the trimer (ZnC3); and \textit{m/z} 1607.6 (3+) originating from the tetramer (ZnC4). However, when the concentration was decreased (0.02 mg/mL), only the ZnC2 complex was observed upon ESI-MS (Figure 6.7b), indicating that the stoichiometrically different complexes were in thermodynamic equilibrium.

Figure 6.7. ESI mass spectra of the zinc complexes (ZnC2, ZnC3 and ZnC4) at the concentration of a) 0.6 mg/mL and b) 0.02 mg/mL; the compositions of the various ions observed are marked by ovals (dimer), triangles (trimer), and diamonds (tetramer).
Figure 6.8. ESI mass spectrum of the complex formed with Fe(II); the ions arising from the FeC2 (dimer), FeC3 (trimer) and FeC4 (tetramer) are marked by ovals, triangles, and diamonds, respectively.

For the iron complexes, ESI-MS of the crude reaction mixture revealed the formation of a variety of cyclic species: primarily the dimer, trimer, and tetramer (Figure 6.8), which were identified by the following ions: \( m/z \) 507.1 (6+), \( m/z \) 638.0 (5+), \( m/z \) 833.7 (4+), and \( m/z \) 1060.3 (3+) contain the mass of the trimer (FeC3) and, hence, were assigned to this composition; \( m/z \) 724.9 (3+) was assigned to the dimer (FeC2); and \( m/z \) 600.8 was assigned to the tetramer (FeC4). The most prominent species in the spectrum is the trimer (FeC3), appearing in charge states 3+ through 6+. The dimer and trimer could be separated by column chromatography because of the high stability of Fe\(^{II}\)-coordinative bonds.\(^{131}\) The ESI mass spectra of the separated complexes showed a series of
contiguous charge states of either FeC2 (Figure 6.9a) or FeC3 (Figure 6.9b), but no cross-contaminations, affirming the successful isolation of single stoichiometries.

Figure 6.9. ESI mass spectra of a). FeC2 and b). FeC3, acquired after chromatographic separation of these complexes.
Figure 6.10. Two-dimensional ESI-TWIM-gMS$^2$ plots of $m/z$ 507.7 (4+) from the FeC2 dimer (left), acquired by CAD (Ar) in the trap cell at collision energies in the 6-65 eV range, at a traveling wave velocity of 350 m/s and a traveling wave height of 7.5 V. The TWIM device separates the complex FeC2, which drifts at 3.34 ms, from its reaction products which drift at 4.96 ms. The corresponding drift time distributions are shown at right.
In order to analyze the intrinsic stability and reactivity of the iron complexes, the ESI-TWIM-MS was combined with gradient tandem mass spectrometry (gMS\(^2\)) to examine the gas-phase chemistry of FeC\(_2\) in more detail. For the Fe(II) dimer FeC\(_2\), CAD was performed on \(m/z\) 507.7 (4+), as shown in Figure 6.10. When the trap collision energy was set at 6 eV, barely any dissociation occurred and the parent ion peak \(m/z\) 507.7 (4+) appeared at a drift time of 3.34 ms. As the trap collision energy increased, the intensity of the parent ion decreased, while the intensity of CAD products drifting at about 4.96 ms was increasing. At even higher collision energies, product ions appeared at about 5.69 ms. The mass spectrum extracted from the 5.69 ms peak showed \(m/z\) 507.7 (2+) and \(m/z\) 523.2 (2+), which were assigned to a fragment composed of one ligand and one Fe(II) ion and its methanol adduct. The 4+ complex ions of FeC\(_2\) dissociated completely when the trap voltage reached 60 V, which corresponds to a center-of-mass collision energy (\(E_{cm}\)) of 4.64 eV.

The results at 40 eV for \(m/z\) 507.7 (4+) from FeC\(_2\) (TWIM-gMS\(^2\) plot and drift time distribution) are reproduced in Figure 6.11a, and the mass spectra extracted from the mobility separated peaks are depicted in Figure 6.11b. The latter spectra attest that the peak at 3.34 ms contains the quadruply charged (cyclic) parent ion \(m/z\) 507.7. On the other hand, the peak at 4.96 ms, which appears to be composite, contains \(m/z\) 507.7 as well as \(m/z\) 515.4 (both 4+), which were attributed to the ring-opened FeC\(_2\) and its methanol adduct. As the FeC\(_2\) dimer dissociates into an unfolded structure, the bonding between Fe(II) and one terpyridine ligand is destroyed. Methanol can now attach to the free Fe (II) ion, resulting in the larger fragment at \(m/z\) 515.4 (4+). The drift time of the
unfolded products is significantly larger than that of the intact FeC2 dimer, in agreement with their more extended (linear) architecture as compared with the cyclic FeC2.

The reactions discussed occur in the trap cell of the Synapt Q/ToF mass spectrometer (cf. Figure 3.2). Methanol is added to the sprayed sample to improve ionization, but it is pumped away when the droplet aerosol enters the vacuum system of the mass spectrometer. The MeOH in the trap cell is attributed to ions composed of a carborane ligand ionized by CH$_3$OH$_2^+$ and Na$^+$ whose combined nominal mass (56 Da) is identical with that of Fe. These ions are transmitted together with the FeC2 precursor ions (m/z 507.7) into the trap cell where, due to their week bonding dissociate completely even at the lowest collision energy of the trap cell (6 eV); CH$_3$OH molecules formed in this process render should be a reasonable explanation for the methanol adduct of FeC2.

Figure 6.11. a) Two-dimensional ESI-TWIM-gMS$^2$ plot for m/z 507.7 (4+) from the dimer FeC2 at the collision energy of 40 eV; b) mass spectra extracted from the drift time chromatogram peaks at 3.34 ms and 4.96 ms.
6.4. Characterization of a 3-D self-assembled nanosphere

The Ru(II) based, metallo-nanosphere studied was prepared from tristerpyridine ligands and Ru(II) metal ions mixed in the molar ration 4:6. The synthetic route was designed and implemented by Tingzheng Xie and Shengyun Liao in Professor George R. Newkome's laboratory. The raw product was purified by column chromatography using silica as the stationary phase and a mixture of acetonitrile, saturated aqueous KNO₃, and water (20:1:1, v/v/v) at the mobile phase. The purified product was isolated as the nitrate salt (NO₃⁻ counterions). Because of the high symmetry of the 3D nanosphere structure, it is difficult to derive structural details on the complex by regular ¹H NMR spectroscopy. For such symmetrical supramolecules, ESI-MS and ESI-TWIM-MS have become an important analytical tool for complementary insight about their stoichiometry, as revealed by their mass, as well as about their 3D size/shape and connectivity, as revealed by their TWIM-MS and gMS² characteristics.
Scheme 6.5. Synthetic pathway to the Ru(II)-based metallo-nanosphere analyzed, which was synthesized using Ru(DMSO)$_4$Cl$_2$ and the tristerpyridine ligand shown. Reproduced from reference 132 with permission.
Figure 6.12. 1D ESI-MS spectrum of the nanosphere complex (NO$_3^-$ counterions); the zoomed-in view in the inset shows the region of 10+ charged ions.

The ESI mass spectrum of the nanosphere complex (Figure 6.12) with nitrate anions supports the expected stoichiometry of L$_4$Ru$_6$ (L = tristerpyridine ligand) by revealing a series of dominant peaks at $m/z = 460.0, 507.5, 564.4, 634.0, 720.8, 832.8, 982.2$ and $1191.0$ corresponding to the intact assembly in charge states 12+ to 5+, respectively. The zoomed-in view in the inset of Figure 6.12 shows the $m/z$ region of 10+ ions which carry two counterions. The minor peaks at $m/z 558.0$ (10+) and $m/z 561.8$ (10+) are the result of incomplete counterion exchange, based on observed and theoretical isotope patterns; $m/z 558.0$ corresponds to nanosphere complex with two Cl$^-$ anions ($C_{348}H_{228}N_{36}Ru_6Cl_2$), and $m/z 561.8$ corresponds to nanosphere complex with one Cl$^-$ and one NO$_3^-$ anion ($C_{348}H_{228}N_{36}Ru_6NO_3Cl$), while the major ion at $m/z 564.4$ corresponds to nanosphere complex with two NO$_3^-$ anions ($C_{348}H_{228}N_{36}Ru_6N_2O_6$).
Figure 6.1. a) ESI-MS spectrum of nanosphere complex with the 12+ charged ion's isotope, b) two-dimensional ESI-TWIM-MS plot of nanosphere complex \((m/z \text{ vs. drift time})\) with the charge states of intact assemblies marked. Ion mobility separation was effected using a traveling wave height of 7.5 V and a traveling wave velocity of 350 m/s.

ESI-TWIM-MS was employed to confirm the structure of the nanosphere complex. Cations with charge states 12+ to 6+ all showed single and narrow bands on the two-dimensional TWIM-MS plot (Figure 6.13b), clearly indicating a single structure for the complex.
Figure 6.14. Two-dimensional ESI-TWIM-gMS$^2$ plot of m/z 1191.0 (5+) from the nanosphere complex (left), acquired by CAD (Ar) in the trap cell at collision energies in the 10-55 eV range, followed by TWIM separation, at a traveling wave velocity of 350 m/s and a traveling wave height of 7.5 V, and subsequent ToF mass analysis. The corresponding integrated drift time distributions (drift time chromatograms) are also shown (right). The precursor ion gives rise to a signal at 6.86 ms (see top trace). The TWIM device separates the fragments of the nanosphere complex into mainly two drift time regions centering at about 6.8 ms and 9.0 ms. The fragments at the longer drift time originate from unfolded structures (see text).
The stability and unimolecular reactivity of the nanosphere complex were examined by ESI-TWIM-gMS^2 (Figure 6.14). The 5+ charge state with seven NO_3^- counterions (m/z 1191.0) was isolated and subjected to CAD prior to ion mobility separation at trap collision energies ranging from 10 to 55 eV. As the trap collision energy was increased, fragments appeared and the intensity of the precursor ion decreased as those of the fragment peaks increased. When the trap collision energy reached 52 eV, the 5+ ion (m/z 1191.0) of the nanosphere complex completely disappeared, yielding several fragments by the losses of Ru(NO_3)_2 units and H_2O moieties.

The 2D ESI-TWIM-gMS^2 plot and drift time chromatogram of the nanosphere 5+ ion at a trap collision energy of 55 eV are reproduced in Figure 6.15a. At this collision energy, only fragments are observed (vide supra), and those with 5+ charges are separated mainly into two drift time regions, giving rise to the peaks centering at 6.77 and 9.03 ms. The mass spectra extracted from these peaks are shown in Figure 6.15a. Based on m/z values and isotope patterns, the fragment peaks were assigned as follows: m/z 1100.8 (5+) originating from loss of two Ru(NO_3)_2 units (C_{348}H_{228}N_{36}Ru_{4}N_{3}O_{9}); m/z 1097.0 (5+), m/z 1093.5 (5+), m/z 1090.2 (5+) and m/z 1086.8 (5+), all resulting from successive H_2O losses after two Ru(NO_3)_2 units were lost.

Very similar mass spectra and isotope patterns are observed from the families of fragments with drift time distribution centering at 6.77 ms and 9.03 ms. This finding indicates the existence of isomeric structures for the thermally activated nanosphere complex. The fragments drifting faster (6.77 ms) most probably originate from a folded (compact), nanosphere-like structure with broken coordinative bonds, whereas the
fragments drifting more slowly (9.03 ms) most likely originate from an unfolded (less compact) structure of the nanosphere with broken coordinative bonds.

Figure 6.15. a) Two-dimensional ESI-TWIM-gMS$^2$ plot (bottom) and drift time chromatogram (top) for $m/z$ 1191.0 (5+) from the nanosphere complex at the trap collision energy of 55 eV; b) extracted mass spectra from the drift time chromatogram peaks at 6.77 ms (top) and 9.03 ms (bottom).
Figure 6.16. Calibration curve constructed from corrected drift times against corrected published cross sections for the multiply charged ions arising from ubiquitin (bovine red blood cells) and cytochrome C (horse heart).\textsuperscript{142,143} Drift times were measured at a traveling wave velocity of 350 m/s and a traveling wave height of 7.5 V.

For further information on the nanosphere architecture, collision cross-sections (CCSs) were deduced from the drift times in the TWIM-MS experiments (using the calibration curve in Figure 6.16) for comparison with theoretical values predicted by molecular modeling. Experimental collision cross-sections for the intact nanosphere in charge states 5+ to 12+ are listed in Table 6.2; all lie within a relatively narrow window, viz. $956 \pm 40 \text{ Å}^2$ (mean and standard deviation, respectively), attesting that adding or removing NO$_3^-$ counterions does not change appreciably the supramolecular structure and consistent with the nanosphere having a rigid and shape-persistent architecture. The
average experimental CCS of all charge states examined (956.0 Å²) agrees well with the theoretically predicted CCS for the counterion-free complex (947.6 Å²), which was calculated from the corresponding energy-minimized structure (Figure 6.17) using the trajectory method that rigorously considers the collision process between ions and the buffer gas in the ion mobility region.

Table 6.2. Drift times and collision cross-sections for the nanosphere complex

<table>
<thead>
<tr>
<th>Charges</th>
<th>Drift time (ms)</th>
<th>Collision Cross Sections (Å²)</th>
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<tr>
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</tr>
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<td>11+</td>
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</tr>
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</tr>
<tr>
<td>theoretical</td>
<td>/</td>
<td>947</td>
</tr>
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</table>

a). This value may be inaccurate because of the low drift time in the TWIM cell (see Chapter V).

b). standard deviation = 40 Å². Omitting the inaccurate value leads to 964 ± 35 Å².
Experimental collision cross-sections were also derived for the fragment families from 5+ nanosphere complexes drifting at 6.77 ms and 9.03 ms (Figure 6.15). These are reported in Table 6.3. As mentioned before, the fragments with 5+ charges drifting at these times originate from intermediates that lost two Ru(NO$_3$)$_2$ units. Consequently, theoretical CCSs were obtained by modeling counterion-free fragments that have lost two Ru$^{2+}$ ions, either from adjacent sites (structure #1) or geometrically opposite sites (structure #2). Representative energy-minimized structures for fragments #1 and #2 are shown in Figure 6.17. Structure #2 is more extended and, thus, has larger CCS than structure #1, which is more compact due to the loss of two adjacent ruthenium ions. Computationally predicted and measured collision cross-sections follow the same order. Further, the differences between theoretical and experimental collision cross-section for the same fragment are less than ~10%, independent of whether the theoretical CCSs were calculated by the PA (projection approximation) or TM method (trajectory) (Figures 6.18 and 6.19). The trajectory is believed to be more reliable for large systems, such as the nanosphere, because it considers more rigorously the collision process between ions and the buffer gas in the ion mobility region, but cases have been reported in which it overestimates experimental CCSs. The theoretically predicted fragmentation intermediates #1 and #2 reconcile satisfactorily the fragment families observed in the TWIM-gMS$^2$ experiments. The fragment families drifting at ~6.77 ms must arise through the more compact (folded) intermediate #1, and those drifting at ~9.03 ms must arise through the more extended (unfolded) intermediate #2.
Table 6.3. Drift times and collision cross-sections for the nanosphere fragment families with 5+ charges observed upon CAD of the intact complex with 5+ charges (cf. Figure 6.15).

<table>
<thead>
<tr>
<th>Structure #</th>
<th>Drift time (ms)</th>
<th>CCS\textsubscript{theory} (Å$^2$)</th>
<th>CCS\textsubscript{theory} (Å$^2$)</th>
<th>CCS\textsubscript{exp} (Å$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TM</td>
<td>PA</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.77</td>
<td>1029</td>
<td>912</td>
<td>947</td>
</tr>
<tr>
<td>2</td>
<td>9.03</td>
<td>1043</td>
<td>921</td>
<td>1099</td>
</tr>
</tbody>
</table>

Figure 6.17. Representative energy-minimized structures a). the intact nanosphere complex, b). fragment #1 arising by loss of two adjacent Ru(II) ions, and c). fragment #2 arising by loss of two Ru(II) ions in opposite positions, all obtained by molecular mechanics/dynamics modeling. The carbon atoms are grey, the hydrogen atoms are white, the nitrogen atoms are blue, and the ruthenium atoms are red.
Figure 6.18. Calculated collision cross-sections vs. the corresponding potential energies for the energy-minimized structures of nanosphere complex fragment #1, observed at a drift time of 6.77 ms and originating from the loss of two opposite Ru(NO$_3$)$_2$ units. The average CCS of all 100 candidate structures are a). 912 Å$^2$ and b). 1029 Å$^2$. 
Figure 6.19. Calculated collision cross-sections vs. the corresponding potential energies for the energy-minimized structures of nanosphere complex fragment #2, observed at a drift time of 9.03 ms and originating from the loss of two adjacent Ru(NO₃)₂ units. The average CCS of all 100 candidate structures are a). 921 Å² and b). 1043 Å².
6.5. Conclusions

An analytical approach combing ESI-MS, ESI-TWIM-MS and ESI-TWIM-gMS along with molecular modeling was developed and applied to the analysis of metallo-supramolecules, including a terpyridine-based tetramer, bisterpyridyl o-carborane based suprametallomacrocycles, and a tristerpyridine-based nanosphere.

The structures of tetrameric terpyridine-based conformers were characterized by ion mobility mass spectrometry and further confirmed by molecular modeling. The stoichiometry and architecture of the bisterpyridyl o-carborane based suprametallomacrocycles studied were found to be concentration dependent for the labile Zn(II) complex system, but not for the more stable Fe(II) complex. The structure and stability of a 3D nanosphere complex were also elucidated. Isomeric architectures were detected in the dissociation of thermally excited nanosphere assemblies.

ESI-MS coupled with ion mobility mass spectrometry and tandem mass spectrometry is proven to be an efficient method for the characterization of supramolecules, which are challenging to analyze by traditional analytical methods, including X-ray diffraction and NMR, because of the difficulties in purifying or crystallizing the sample or because the particular symmetry of the sample makes it difficult to detect structural details from averaged structures.
CHAPTER VII

SUMMARY

The work reported in this dissertation has demonstrated that mass spectrometry is a powerful and efficient analytical method for a variety of synthetic materials, ranging from small organic molecules to polymers and self-assembled supramolecules. Single-stage mass spectrometry provides molecular weight and, hence, composition (stoichiometry) details about the analyte. Tandem mass spectrometry (MS/MS) is used to characterize the fragments from precursor ions, which provide information about the sequence of chemical bonding (connectivity) of the analyte. Meanwhile, mass spectrometry combined with ion mobility spectrometry show great promise in separations of analyte architectures, especially of supramolecular isomers, which are difficult to purify or crystallize for identification by traditional methods that probe average properties.

Chapter IV in this dissertation illustrated the applicability of ion mobility mass spectrometry to the analysis of isomeric nanoparticle adducts. The chemical structure of a polyhedral oligomeric silsesquioxane (POSS) diadduct synthesized by thiol-ene chemistry was determined by tandem mass spectrometry, proving unequivocally that the thioether bond is oxidized readily to sulfoxide but not sulfone moieties. Three isomers of the POSS diadduct system were successfully separated and quantified by ESI-TWIM-
MS. Molecular modeling further confirmed that the architectures detected were *ortho*, *meta*, and *para* isomers. Further, the light-induced isomerization of a photo-responsive POSS adduct containing a functionalized azobenzene group could be monitored *in situ* by ion mobility mass spectrometry in the presence of impurities/byproducts due to MS's dispersive nature.

Chapter V investigated the counterion effects on the CCSs of a supramolecular complex. Counterions have been traditionally omitted in calculations of collision cross-sections, but these ions may affect the CCS due to interactions with the metal ion and ligands. A simple triangular metallomacrocycyle with a series of counterions was analyzed by ion mobility mass spectrometry and molecular modeling. Counterions with small and symmetric structures, such as PF₆⁻, BF₄⁻, and NO₃⁻ showed the best agreement between average experimental CCS of different charge states and calculated CCS of the counterion-free complex.

Finally, in Chapter VI, a methodology coupling ESI-TWIM-MS with gradient tandem mass spectrometry (gMS²) was designed and applied to the analysis of various metallo-supramolecules. ESI-TWIM-gMS² was used to monitor the structures and intrinsic stabilities of such systems. Conformers of intact terpyridine tetramers could be distinguished. Conformers of fragmentation intermediates were detected for supramacrocycles and a 3D nanosphere structure for the first time. Intact and fragmenting architectures were simulated by molecular modeling for further confirmation of structure and intrinsic reactivity upon thermal excitation.
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APPENDIX

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