Charge Detection Mass Spectrometry

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Introduction

Mass spectrometry is a very important tool in physical, analytical, and biophysical chemistry. Recently, it has been used in proteomics[1-5], enzyme studies[6, 7], and many other biological studies[8-13]. Electrospray ionization combined with time-of-flight (TOF) mass spectrometry is of particular interest because of its ability to ionize large non-covalent complexes without destroying their structure.[1, 3, 9, 10, 13, 14] One of the main limitations of conventional TOF mass spectrometers, however, is the detections of very large complexes. The detectors used in conventional TOF mass spectrometers measure ions from their collisions with the detector. Thus, a very large ion requires a large amount of acceleration and a large number of charges. Distinguishing the charge states of these molecules can become extremely difficult, if not impossible.

A few different methods have been developed to solve this dilemma. One such method involves cryogenic niobium superconducting tunnel detectors.[15-18] This method uses a niobium superconducting detector to measure the phonon excitations cause by particle collisions. Its advantage is that it shows 100% efficiency, even for very high mass, slow moving ions. However, cryogenic detectors are expensive and complicated. There are also problems with the manufacturing of the niobium plates that limit the size of these detectors. Currently, the detectors have effective detection areas of only a few millimeters.

Benner and coworkers have developed a much simpler, less expensive method for the detection of large ions in TOF mass spectrometers. This method is called charge

detection mass spectrometry, and uses a small steel tube to measure the image charge from an ion passing through its center.[19-21] When a strongly charged ion passes through the center of the steel tube, it creates an image charge on the tube that is exactly opposite to the one on the ion. This charge can then be amplified and measured though low noise electronics techniques. This approach has the advantage of being simple and cheap to produce. Its drawback is that the electronic noise is very difficult to control. In order for this method to be useful for mass spectrometry of viruses or nanomaterials on the gigadalton size scale, the noise in the measurement needs to be low enough for the determination of the charge to be within +/- ½ of the charge on a single electron. Our group has designed and built a charge detection TOF mass spectrometer based on the design of Benner and coworkers, and we are currently preparing to do a study on the physics and dynamics of electrospray droplets.

Experimental Techniques and Design

Our initial design for the detector tube and ion mirrors was based upon simulations done in Simion. This program computes the flight paths of ions through different electric fields, and is very useful in designing any device meant to steer and focus ions. Figure 1 shows the detector design, including the ion mirrors that have yet to be implemented.
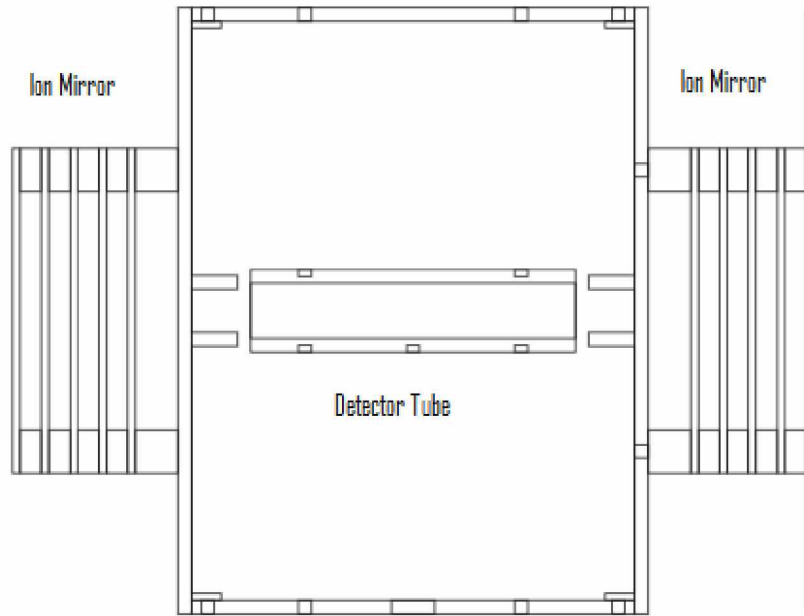
Figure 1: Cut-away view of the detector and ion mirrors.

The small cylinder in the center is the detector tube. It is suspended by PEEK supports

that fit into the larger shielding cylinder. The detector tube itself is 1.5" long, with an

internal diameter of 6.5mm. The shielding cylinder has a diameter of about 2.8" and a

length of about 2 inches. This larger cylinder keeps the electric fields generated by the ion

mirror lenses from reaching the detector tube and affecting our charge detection

measurements. The detector cylinder is attached through a small wire to an Amptek®

A250 charge sensitive pre-amplifier. This changes the measurement of charge on the

detector tube into a voltage that is then fed into an Ortec® model 572 differentiating

amplifier with gain up to x1000. The signal is then fed out to either an oscilloscope, or

our computer with an AlazarTech® ATS460 125 MS/s, 14 bit waveform digitizer for

analysis. With a low enough noise level, the amplitude of our signal can be directly

correlated with the charge on the ion. Also, the length of time that the charge is displayed

on the tube can be used to determine the speed of the ion, which in turn can be related to the M/Z ratio.[19]

Though designed, the ion mirrors have not yet been implemented. The mirrors would work as follows: The ions would fly in through the first set of lenses, which would be set to ground, and into the detector tube. The second set of lenses would already be set to their potentials ranging from -50 volts DC to +300 volts DC. When a charge is first measured on the tube, the front set of lenses would switch to the potentials that match those in the second set of lenses. By the time the ion turns around in the second mirror, the first will be switched on, capturing a single ion. Figure 2 shows the Simion simulation of an ion flying into our detector and being captured by the ion mirrors.
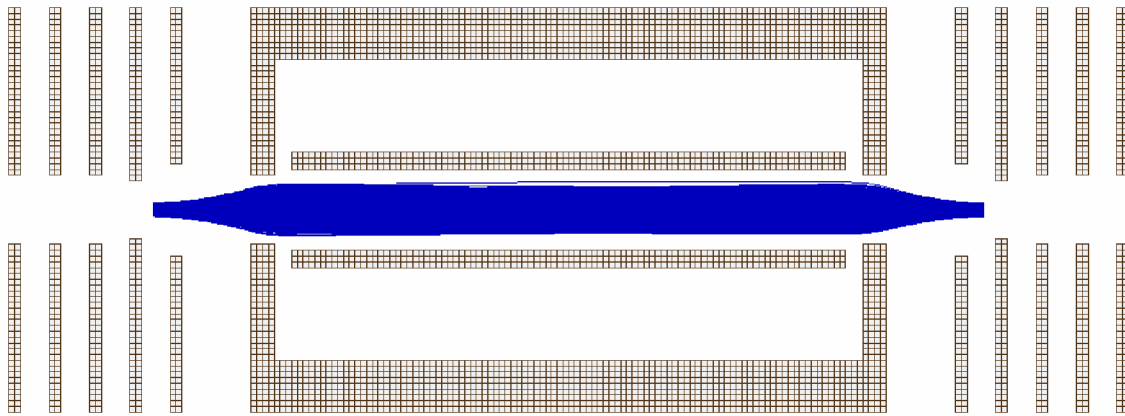

Figure 2: Simion simulation of the ion mirrors and our detector tube.

By reflecting a single ion through our detector tube many times, we can take many measurements of the charge. These measurements can then be averaged to further reduce the noise levels of the measurements. Our simulations show an ion with a mass of 1

megadalton and 1000 charges reflecting a few thousand times through the detector. The simulated trap is able to trap ions within a 2-mm window in the center of the detector.

Currently, the detector does not have the mirror lenses mounted to it. We have a single lens with a 5mm diameter hole in the center kept at ground mounted to the front of the shielding cylinder and a lens without a hole in the center directly behind the shielding cylinder. These two lenses are connected to a picoammeter in order to aid in aligning the ion beam. The current is measured on the front and rear lens to determine whether or not ions are flying through the detector. This allows us to adjust the electrospray conditions to obtain a stable ion current through our detector.
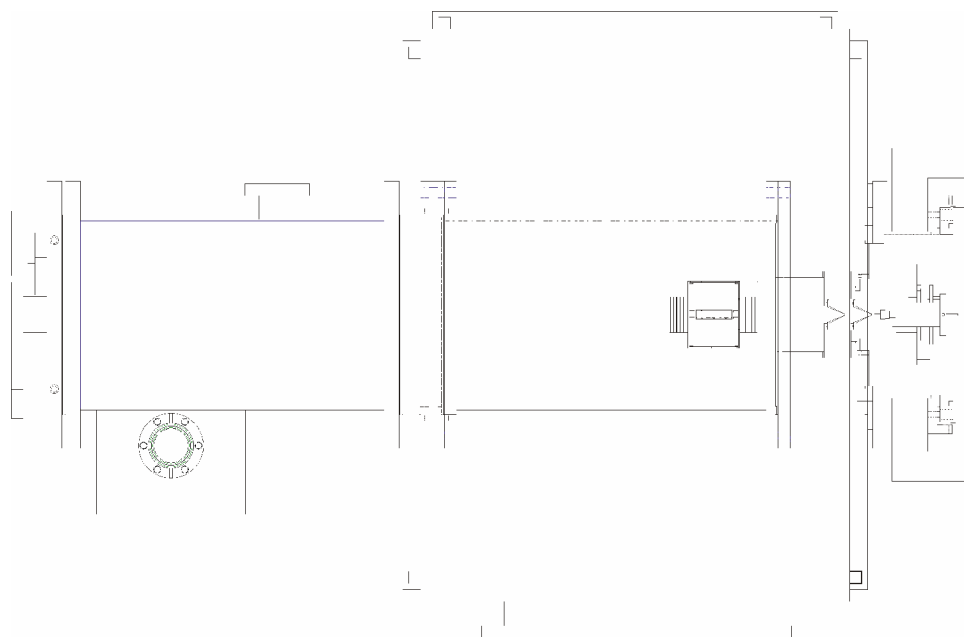

Figure 3: A schematic of our instrument. Ions fly from right to left.

Our instrument has three chambers which are differentially pumped by diffusion pumps. The electrospray ionization generally occurs from an electrospray needle at +4,500 volts DC. The ions then flow through a heated capillary tube into the first region

pumped to about .4 torr. The ions then pass through a skimmer into a region pumped to around $1x10^{-5}$ torr. Finally, they pass through another skimmer into the final region held at around $1x10^{-6}$ torr that contains the detector. Our setup allows us to place potentials on both of the skimmers and on the capillary tube; however we are currently running with those held at ground. The system is designed to be able to accelerate ions through the detector, as shown in Figure 4 which is a three dimensional view of the potentials for acceleration and reflection.
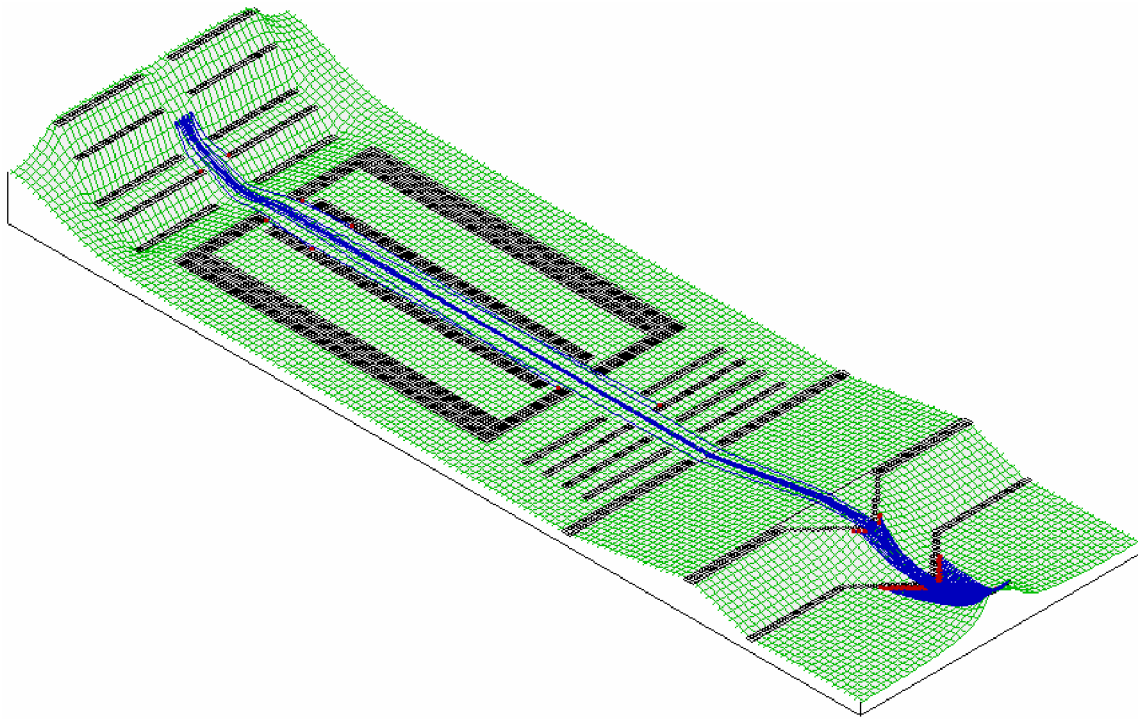
Figure 4: A three dimension Simion representation of the electric potentials in our acceleration region and detector.

In order to reduce the electronic noise on the detector, a room-sized doubly-shielded faraday cage has been built to house the instrument. A special ground that is only connected to our instrument goes from the cage to earth ground. This cage is made

mostly from 1/8" aluminum plating, with the upper half of two of the walls made from copper screen. The cage has two shells that are isolated from each other and connected to their own grounds. In the rare occasion that electric signal leaks through the outer shell, the inner shell on the ultra-clean ground will continue to provide additional shielding. All of our mechanical pumps have been placed outside of the cage. They use specially designed shielded feed-throughs and PVC piping to pump down the chamber inside the cage. The computer we have inside the cage with the data processing programs and data capturing equipment has been extensively modified to reduce its noise output. We use an LCD (liquid crystal display) monitor to avoid the noise that a CRT (cathode ray tube) monitor outputs, and we have replaced the extremely noisy switching power supply with a linear power supply. As a result, the computer puts out considerably less noise than before. The noise being produced by the computer has not been characterized currently, but by visual inspection of the oscilloscope, it has made an easily noticeable difference. Through the use of this cage, the picoammeter has a noise level of about 10 femtoamps.

A program has also been written in C++ to control the data acquisition card. This program is able to capture a few hundred waveforms in a minute or two while ions are flying through our detector. It stores this data as a comma-delimited text file that can easily be imported into a variety of plotting software for data analysis. Computer software to aid in data sorting and analysis is currently under design.

Results and Discussion

Very recently, this system has been used to collect waveforms for electrosprayed droplets of water, methanol, and mixtures of the two. These droplets contain around 25,000 charges each, and move very slowly, about 380 meters per second. They have a variety of sizes and charges, though, as one would expect. As stated previously, the ion mirrors have not yet been implemented, so the droplets pass through our detector just once. Figure 4 shows a representative "normal" waveform created by electrospraying plain MeOH.
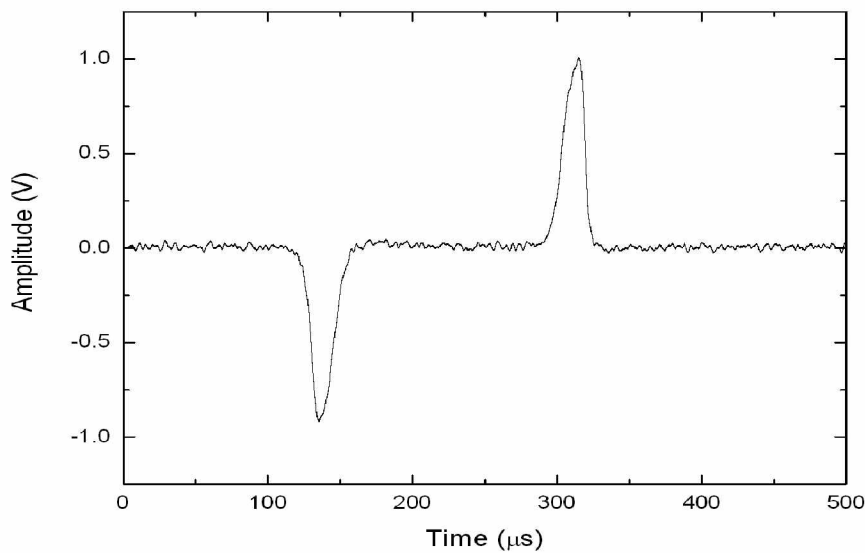

Figure 4: A representative "normal" waveform captured from MeOH.

The waveform that is output by the preamplifier is actually a square wave corresponding to the negative image charge created on the tube by the positive ions. The signal presented is the amplified, differentiated signal that comes out of the amplifier. The

first, negative peak corresponds to a positive ion entering the tube, and the second, positive peak corresponds to the exit of the ion from the tube. The signal is differentiated primarily to make the signal easier to trigger on and to make the time measurement more accurate.

Figure 5 shows a waveform created while spraying MeOH that contains three droplets flying through the detector at once. Waveforms such as this are interesting to us, because some of them may contain information about the dynamics of droplets colliding with each other or breaking apart mid-flight.
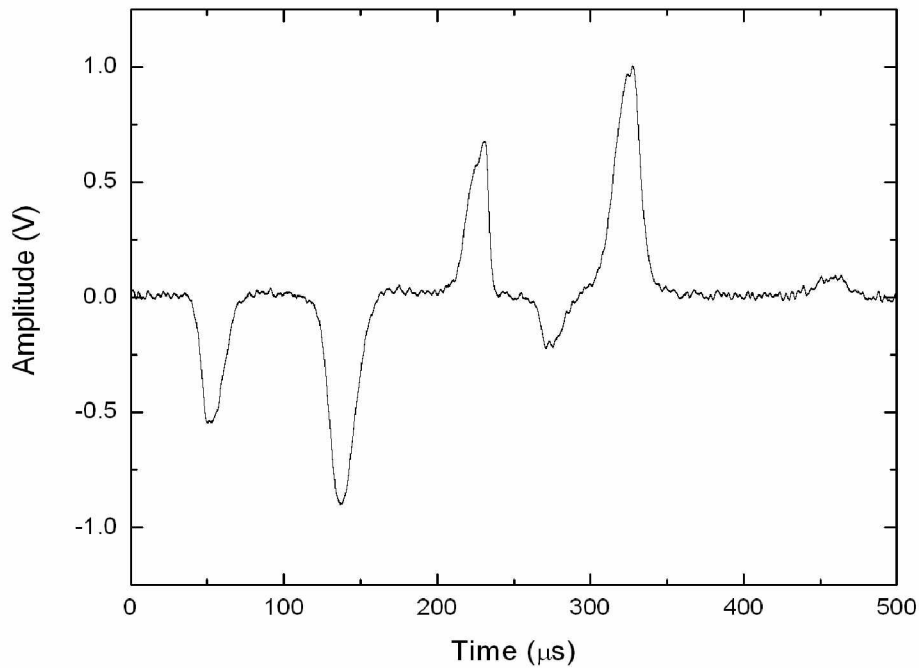

Figure 5: A waveform with three MeOH droplets flying through the detector.

We are currently focusing on developing software that will distinguish between the "normal" waveform involving a single droplet entering and leaving the detector and the waveforms that contain multiple droplets and droplets that fragment. Distinguishing between the two types of waveforms with programming will allow us to easily extract data and create spectra from the "normal" waveforms and allow us to gather all of the waveforms with other interesting behavior for more analysis. The software design is still in its infancy, so the methods for differentiating between the two types of signals have not yet been decided upon. Preliminarily, the data we would like to be able to extract and compile from the "normal" waveforms is the amplitudes of their peaks (Z) and the distance between the peaks (M/Z). This data can be extracted from all of the "normal" waveforms from a single run and combined to create a mass spectrum. The system will be as automated as possible, allowing for a large amount of data to be collected and processed in a relatively short amount of time.

Future Direction

Currently, a detector identical to the one in use and an acceleration/deceleration flight tube are being constructed. These used in conjunction with the original detector will allow us to do comprehensive studies on the physics and dynamics of electrospray droplets in flight in a TOF mass spectrometer. The instrument, when configured for this study, will work as follows. The ions will be sent through the first detector as described above. Then, they will pass into our acceleration/deceleration flight tube that consists of 24 lenses that ramp from ground to -5000 volts DC and back to ground again. Then, the ions travel

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through the second detector for another charge-detection measurement identical to the first.
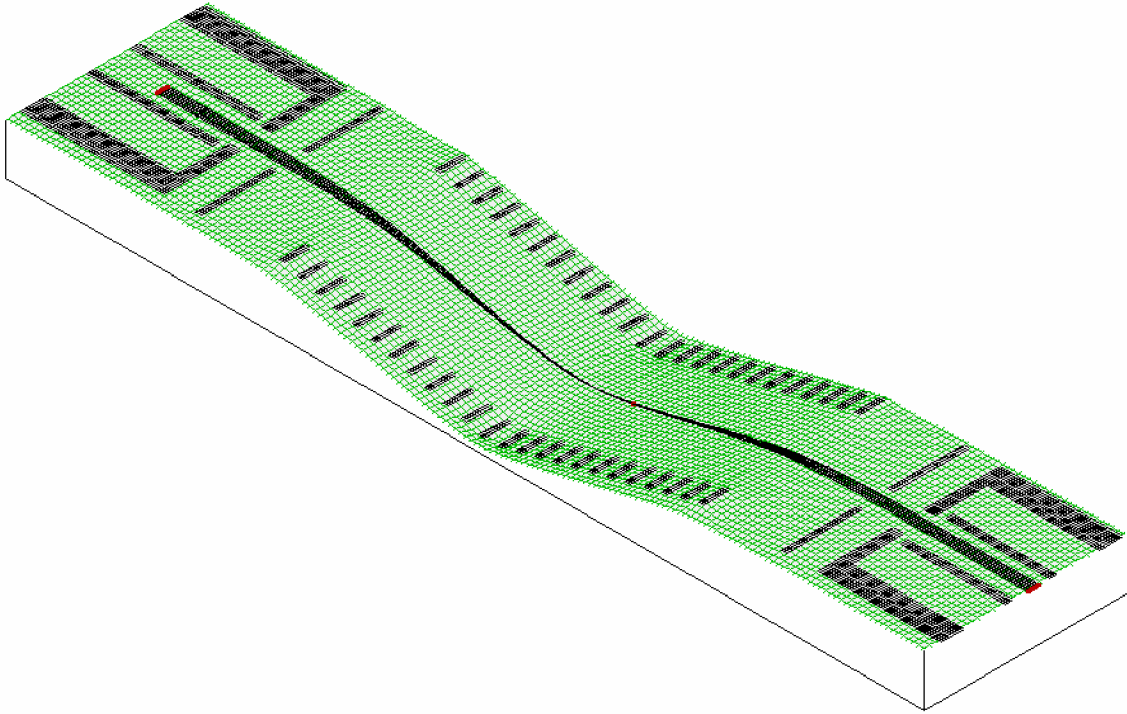

Figure 6: A three dimensional representation of the Acceleration/Deceleration flight tube with twin charge detectors.

Figure 6 is a Simion simulation of this detector setup. This will give us three different M/Z measurements and two separate Z measurements. We can measure M/Z and Z with both of the detectors, and the time it takes the ions to travel between the two detectors will provide another M/Z measurement. This setup will allow us to get obtain more accurate measurements for the droplets, and we will be able to see what happens to the droplets over a longer distance by comparing their signature in the first detector with the one in the second detector. As far as we know, this type of study has not been done on electrospray droplets previously, and it may elicit previously unknown properties of electrospray apparatuses.

Also, further reductions in noise will be attempted. A cooling mechanism for our FET on the A250 pre-amplifier will, along with various upgrades to the A250 chip itself will be designed and implemented helping us approach the noise levels we need for measurements on individual viruses or protein complexes. The ion mirror switching device will be designed and built to allow us to trap individual ions in one of our detectors to average out the noise. Finally, as stated previously, further automation of all of the data acquisition and analysis will allow us to collect data more efficiently.

Once the instrument is operating on a noise scale that is low enough for us to differentiate between single charges, we plan to do studies on intact viruses and/or large nanostructures. There have been a few studies done on intact viruses[9, 12], however this method should allow mass spectrometry to be done on intact viruses more readily and accurately than ever before. Studies on the identification, fragmentation, and self-assembly of viruses are all promising for possible future ventures. Mass spectrometric characterization of large nanostructures will also be available to us. We will be able to characterize through mass spectrometry nanostructures that were previously too large to probe. This could aid in future attempts to make macromolecular machines or other nanostructures.

Acknowledgments:

References

1.   Ashcroft, A. E., Recent developments in electrospray ionisation mass
spectrometry: noncovalently bound protein complexes. *Natural Product Reports*
2005, 22, (4), 452-464.

2.   Katta, V.; Chait, B. T., Conformational-Changes in Proteins Probed by Hydrogen-
Exchange Electrospray-Ionization Mass-Spectrometry. *Rapid Communications in
Mass Spectrometry* **1991,** 5, (4), 214-217.

3.   Loo, J. A., Observation of Large Subunit Protein Complexes by Electrospray-
Ionization Mass-Spectrometry. *Journal of Mass Spectrometry* **1995,** 30, (1), 180-
183.

4.   Rostom, A. A.; Robinson, C. V., Detection of the intact GroEL chaperonin
assembly by mass spectrometry. *Journal of the American Chemical Society* **1999,**
121, (19), 4718-4719.

5.   Thomas, J. J.; Bakhtiar, R.; Siuzdak, G., Mass spectrometry in viral proteomics.
Accounts of Chemical Research **2000,** 33, (3), 179-187.

6.   Bothner, B.; Chavez, R.; Wei, J.; Strupp, C.; Phung, Q.; Schneemann, A.; Siuzdak,
G., Monitoring enzyme catalysis with mass spectrometry. *Journal of Biological
Chemistry* **2000,** 275, (18), 13455-13459.

7.   Ganem, B.; Li, Y. T.; Henion, J. D., Observation of Noncovalent Enzyme
Substrate and Enzyme Product Complexes by Ion-Spray Mass-Spectrometry.
Journal of the American Chemical Society **1991,** 113, (20), 7818-7819.

8.   Banoub, J. H.; Newton, R. P.; Esmans, E.; Ewing, D. F.; Mackenzie, G., Recent
developments in mass spectrometry for the characterization of nucleosides,
nucleotides, oligonucleotides, and nucleic acids. *Chemical Reviews* **2005,** 105, (5),
1869-1915.

9.   Bothner, B.; Siuzdak, G., Electrospray ionization of a whole virus: Analyzing
mass, structure, and viability. *Chembiochem* **2004,** 5, (3), 258-260.

10.   Griffiths, W. J.; Jonsson, A. P.; Liu, S. Y.; Rai, D. K.; Wang, Y. Q., Electrospray
and tandem mass spectrometry in biochemistry. *Biochemical Journal* **2001,** 355,
545-561.

11. Schalley, C. A., Supramolecular chemistry goes gas phase: the mass spectrometric examination of noncovalent interactions in host-guest chemistry and molecular recognition. *International Journal of Mass Spectrometry* **2000,** 194, (1), 11-39.

12. Thomas, J. J.; Bothner, B.; Traina, J.; Benner, W. H.; Siuzdak, G., Electrospray ion mobility spectrometry of intact viruses. *Spectroscopy-an International Journal* **2004,** 18, (1), 31-36.

13. Tito, M. A.; Tars, K.; Valegard, K.; Hajdu, J.; Robinson, C. V., Electrospray time-of-flight mass spectrometry of the intact MS2 virus capsid. *Journal of the American Chemical Society* **2000,** 122, (14), 3550-3551.

14. Loo, J. A., Electrospray ionization mass spectrometry: a technology for studying noncovalent macromolecular complexes. *International Journal of Mass Spectrometry* **2000,** 200, (1-3), 175-186.

15. Benner, W. H.; Horn, D. M.; Jaklevic, J. M.; Frank, M.; Mears, C.; Labov, S.; Barfknecht, A. T., Simultaneous measurement of flight time and energy of large matrix-assisted laser desorption ionization ions with a superconducting tunnel junction detector. *Journal of the American Society for Mass Spectrometry* **1997,** 8, (10), 1094-1102.

16. Frank, M.; Labov, S. E.; Westmacott, G.; Benner, W. H., Energy-sensitive cryogenic detectors for high-mass biomolecule mass spectrometry. *Mass Spectrometry Reviews* **1999,** 18, (3-4), 155-186.

17. Frank, M.; Mears, C. A.; Labov, S. E.; Benner, W. H.; Horn, D.; Jaklevic, J. M.; Barfknecht, A. T., High-efficiency detection of 66000 Da protein molecules using a cryogenic detector in a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer. *Rapid Communications in Mass Spectrometry* **1996,** 10, (15), 1946-1950.

18. Westmacott, G.; Zhong, F.; Frank, M.; Friedrich, S.; Labov, S. E.; Benner, W. H., Investigating ion-surface collisions with a niobium superconducting tunnel junction detector in a time-of-flight mass spectrometer. *Rapid Communications in Mass Spectrometry* **2000,** 14, (7), 600-607.

19. Benner, W. H., A gated electrostatic ion trap to repetitiously measure the charge and m/z of large electrospray ions. *Analytical Chemistry* **1997,** 69, (20), 4162-4168.

20. Schultz, J. C.; Hack, C. A.; Benner, W. H., Mass determination of megadalton-DNA electrospray ions using charge detection mass spectrometry. *Journal of the American Society for Mass Spectrometry* **1998,** 9, (4), 305-313.

21.   Schultz, J. C.; Hack, C. A.; Benner, W. H., Polymerase chain reaction products analyzed by charge detection mass spectrometry. *Rapid Communications in Mass Spectrometry* **1999,** 13, (1), 15-20.