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RELATIONSHIP BETWEEN CROSS SECTION MEASUREMENTS AND UNDERSTANDING RADIATION INDUCED DAMAGE TO BIOMOLECULES

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Relationship between Cross Section Measurements and Understanding Radiation Induced Damage to Biomolecules*

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Abstract:

Experimental research performed at the Pacific Northwest Laboratory relating to energy deposition by energetic charged particles is described. How cross section data obtained from gaseous- and condensed-phase studies are related to understanding damage to biomolecules is discussed. Studies to date stress the need for information about energy deposition in individual interactions and show that multiple ionization may play a very significant role in biological damage. Current efforts to relate this gas-phase information to condensed-phase processes and biologically relevant targets are outlined.

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Introduction:

Energetic charged particles traversing matter interact with individual atoms and molecules primarily via ionizing collisions. These interactions deposit a portion of the particle's kinetic energy in localized, nanometer-size, sites along the charged particle trajectory. Electrons liberated in these collisions also interact with the media, thereby distributing the deposited energy over a larger volume. In the case of biological tissue, these energy deposition and redistribution processes are the initial links of a long chain of events that can ultimately result in cell mutation or death. Therefore, measurements of ionization probabilities and a basic understanding of the types and relative importance of ionizing interactions that occur have direct impact on understanding biological damage resulting from irradiation of tissue.

As an example, it is known that in simple systems, e.g., dry enzymes, a single ionization event can produce a biological effect. On the other hand, significant biological effects in mammalian cells are thought to require a double strand break in DNA. Double strand breaks can be conceived as resulting from two independent ionization events (produced by different charged particle tracks) separated by a few base pairs or from one "multiple" ionization event produced by a single track. Modeling the effectiveness of each method requires knowledge of individual ionizing interactions. Cross section and the local density of ionization information may also be essential for the understanding of biological repair mechanisms. Most double strand breaks are repaired but in certain cases the damage is so severe that it is not repaired properly. Cross section data have shown that both the number of radiation chemistry products formed and the ionic species can differ radically for different charged particle interactions. It is logical to assume that the chemical processes leading to damage or repair depend on what ionic species are formed and how these species interact with their neighbors.

Clearly, the severity of damage should depend on the amount of energy deposited within a small volume. To address these questions our experimental physics studies at the Pacific Northwest Laboratory are designed to a) understand the basic mechanisms of energy deposition by charged particle impact and b) to relate these basic mechanisms to damage of biologically relevant molecules. Cross section data derived from these experiments form the basis for modeling energy deposition and damage incurred in biological tissue. Our studies concentrate on damage induced by proton and alpha particles having energies below 1 and 6 MeV respectively since these ionizing particles constitute major environmental risk concerns. In this paper we briefly discuss our present status at understanding basic energy deposition mechanisms and our current efforts to provide additional information relating to biomolecular damage.

Gas-phase Measurements:

Energy deposition is a stochastic process. Therefore knowledge of individual ionizing interactions is essential; average occurrences are only useful for confirmational purposes. To study individual interactions, gas-phase experiments are generally required. Much of this work has recently been reviewed by Toburen (1). To summarize, these studies have shown that the doubly, and singly, differential electron emission cross sections resulting from energetic protons impact on large molecular targets can be expected to scale with the number of loosely bound target electrons and that for proton and alpha particle impact, the differential cross sections scale as $(z/v)^2$, where z and v are the projectile nuclear charge and velocity, respectively. Thus, until recently it was felt that energy deposition for fast proton and alpha particle impact energy deposition was fairly well understood and that the only problems were for heavier projectiles or targets where ionization probabilities become large and multiple ionization of the target increases dramatically. However, two recent experiments (2,3) have shown that multiple ionization plays an extremely important role for energetic electron emission resulting from proton and helium ion impact. This aspect certainly needs to be investigated for biologically relevant molecules since these data undermine commonly accepted notions about the clustering of damage in the medium.

Most investigations to date have concentrated on proton, or fully stripped ion, impact. However, as the projectile deposits energy in the media, it slows and begins to capture electrons from the media. Subsequent collisions can ionize these electrons or capture additional electrons. If the ion trajectory through the media is sufficiently long, the projectile will eventually be completely neutralized and will ultimately stop. These charge transfer processes become significant for projectile energies in the vicinity of the peak in the stopping power curve and tend to dominate for lower energies. For 6 MeV alpha particles, this region corresponds to approximately 20% of the total energy deposition and, hence, interactions involving partially dressed projectiles can be extremely important in biological damage.

One of our methods of investigating dressed ion impact is to examine how electrons bound to the projectile influence the ionization probabilities. One aspect is that bound projectile electrons partially screen the projectile nuclear charge. We have investigated this screening as a function of electron emission angle and energy and projectile charge state by assuming that the target ionization cross sections scale quadratically with an effective charge, Z_{eff}. Fig. 1 provides some results for several nitrogen projectiles. Since Z_{eff} is a projectile dependent quantity, in principle determining Z_{eff} for one target should be sufficient. However, again multiple ionization plays a significant role. See Fig. 2. Thus extending information derived for one target to all targets may not be possible.

To summarize, modeling energy deposition requires knowledge of individual ionizing and charge transferring interactions. This knowledge can only be obtained from gas-phase investigations. These data have indicated the importance of multiple ionization and imply that currently used modeling assumptions of outer shell ionization only leading to single electron emission are incorrect. The data also emphasize the importance of charge transferring collisions in the vicinity of the stopping power maximum which existing models tend to ignore.

Condensed-phase Measurements:

Gas-phase data provide detailed information about individual ionizing interactions; but, are these data appropriate for modeling interactions occurring in condensed-phase media? To address this question we are measuring the electron emission, differential in emission energy and angle, from solid targets. This is being done in conjunction with collaborators from the Gesellshaft für Strahlenshutz, Neuherberg, Germany, and the Institut für Kernphysik, Universität Frankfurt, Germany. Initially we are investigating electron emission when thin carbon foils (5 - 40 $\mu g/cm^2$) are bombarded with 1-2 MeV protons. Results obtained a year ago (see Fig. 3) demonstrated that the low energy electron yields are angle dependent but implied that these data are extremely sensitive to surface impurities. We have recently improved our base vacuum and have used low energy ions to sputter clean the target immediately prior to irradiation. Preliminary results indicate that the sensitivity of the low energy electron emission that we previously observed resulted not from the buildup of surface impurities but rather from changing bulk temperatures due to our attempts to clean the foils by baking. This is being investigated further.

The purpose of these condensed-phase data are to test the validity of track structure models based on gas-phase cross sections. In order to provide information about biomolecular targets, the next stage of the experiment is to cool the target to approximately 20 °K or less and to freeze known monolayers of large molecules on the surface. See Fig. 4. Several possibilities then exist. For example, if the substrate is a high Z material and the monolayers are comprised of low Z atoms, then the substrate acts primarily as a "source" of electrons and the monolayers moderate the spectral features due to their "transport" properties. On the other hand, if the substrate is low Z relative to the monolayers, the measured electron emission is a convolution of electron production and transport by the monolayers.

Measurements of this type can provide useful information about interactions occurring in condensed-phase media but care must be taken since the observed emission is a convolution of production and transport probabilities as well as surface conditions. Also the types of targets that can be investigated are somewhat limited by their electrical properties and by vacuum constraints.

Ionization and Dissociation of Molecules:

A third category of studies that provide information about energy deposition and repair processes involves measuring cross sections for ionization and dissociation of molecular targets. As stated above, these data not only provide information about the energy deposition process but may provide crucial information relevant to damage and repair mechanisms.

Several years ago we investigated ionization and dissociation of simple molecules. This was done using time-of-flight spectroscopic methods to determine which ionic species were formed. However, since another of our primary concerns was absolute cross sections for forming these species, our apparatus was designed with limited mass resolution. This restricted our studies to simple molecules. An additional problem with these studies concerns multiple ionization processes. Studies performed with atomic targets (4) have shown that multiple ionization can be rather important for proton and helium ion impact. However, our experimental methods and molecular targets studied were inappropriate for investigating multiple ionization leading to the production of two charged fragments.

The major information derived from these studies, however, concerns the fragmentation pattern for different projectiles and for ionization versus charge transferring collisions. Some of these data are shown in Fig. 5. These data again demonstrate that energy deposition by energetic charged particles is a stochastic process and that information about average effects is of little or no value for modeling purposes.

Recently we have initiated new studies involving molecular fragmentation processes. The primary purpose of these new studies is to investigate direct, radiation-induced, fragmentation processes for macrobiomolecular targets-specifically for DNA segments. This is being done by using electrospray ionization techniques to form a beam of isolated DNA molecules. By injecting this DNA beam into an ion cyclotron resonance cell, the charge to mass ratio can be extremely well determined. A schematic diagram of the apparatus is shown in Fig. 6. Electrons injected into the cell can ionize and fragment these trapped molecules and the fragmentation pattern can be investigated.

By this method, direct, radiation-induced DNA damage can be investigated without the interference of indirect, media-related processes. However, a unique feature of using the electrospray method is that some control of the number of solvent molecules attached to the DNA ions is possible. By varying the degree of solvation, we hope to provide information about how the media influences DNA damage.

Summary:

In summary, we have described our efforts at the Pacific Northwest Laboratory to provide information about energy deposition by energetic charged particles and how these information can be related to understanding damage to biomolecules. From a physics viewpoint, our studies stress the need for information about individual ionizing interactions and that multiple ionization may play a very significant role in biological damage. Since information about individual interactions is derived from gas-phase studies, our current efforts are related to establishing a connection between gas- and condensed-phase processes.

1. LH Toburen, Physical and Chemical Mechanisms in Molecular Radiation Biology, ed. by WA Glass and MN Varma, Plenum Press, New York, 51-97, 1991.

2. D Manzey, Diplomarbeit der J W Goethe Universität Frankfurt, 1991.

3. Y-S Chung, Ph D Thesis, University of Nebraska, 1993.

4. RD DuBois, LH Toburen and ME Rudd, Phys Rev A 29, 70-6, 1984.

Figure Captions:

Figure 1 The effective charges as a function of electron emission energy and angle $Z_{eff}(\epsilon,\Theta)$, for 100 keV/amu nitrogen ions colliding with water molecules. The effective charges are determined from $[d^2\sigma(\epsilon,\Theta)_{\text{Nitrogen ions}} / d^2\sigma(\epsilon,\Theta)_{\text{protons}}]^{½}$ where $d^2\sigma(\epsilon,\Theta)$ are the doubly differential cross sections for electron emission.

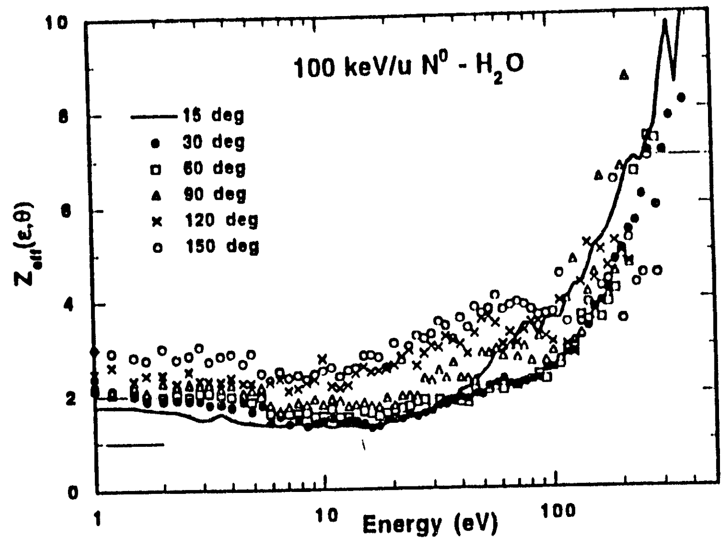
Figure 2 The fraction of electrons produced due to single ionization of neon by helium ion impact. E/M is the impact energy divided by the projectile mass.

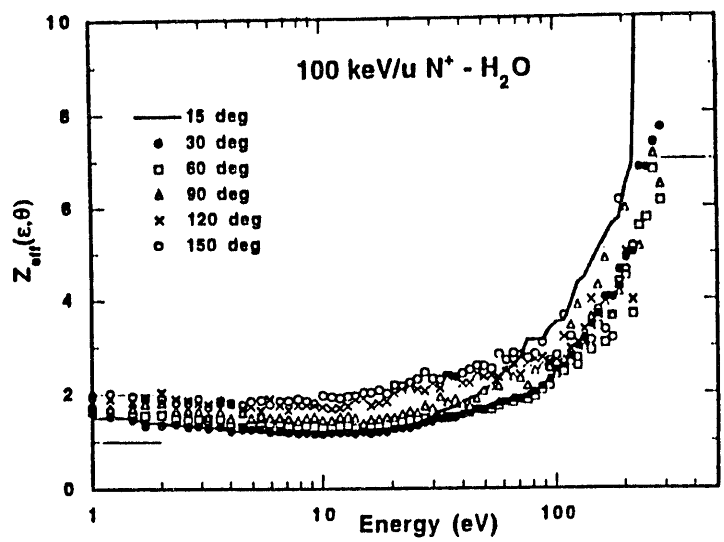
Figure 3 Doubly differential electron yields resulting from 2 MeV proton impact on thin carbon foils. The changing yields shown for the 135° spectrum were originally thought to result from the buildup of surface contamination from the base vacuum constituents but are now thought to indicate changing foil temperatures. See text for details.

Figure 4 A schematic diagram of future studies designed to investigate electron production and transport in molecular monolayers.

Figure 5 Ionic species formed via ionization and dissociation of molecular nitrogen by helium ion impact. The relative importance of each specie is shown to strongly depend on whether an ionizing or charge transferring collision occurs. An impact energy dependence is also evident.

Figure 6 Schematic diagram of electrospray ionization source and ion cyclotron resonance cell used to investigate fragmentation of biomacromolecules.


100 keV/u N^0 - H_2O
15 deg
30 deg
60 deg
90 deg
120 deg
150 deg
$Z_{eff}(\varepsilon,\theta)$
Energy (eV)
0
2
4
6
8
10
1
10
100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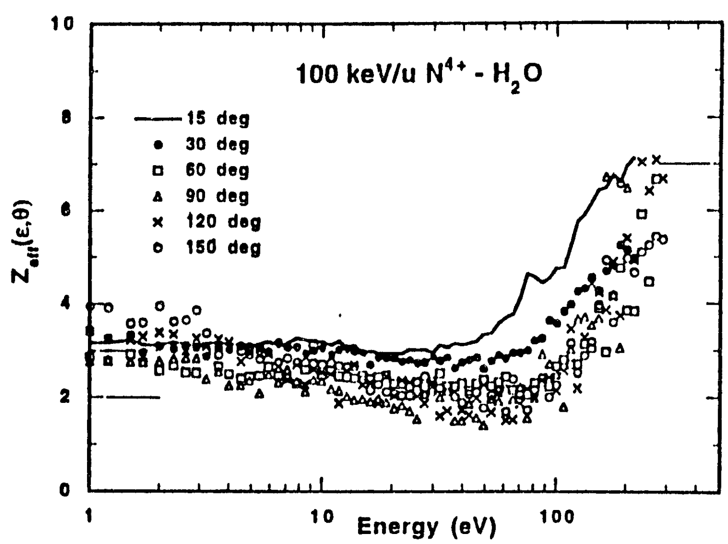
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15 deg
30 deg
60 deg
90 deg
120 deg
150 deg
$Z_{eff}(\varepsilon,\theta)$
Energy (eV)
0
2
4
6
8
10
1
10
100

Fraction of Electrons Due to Single Ionization of Neon

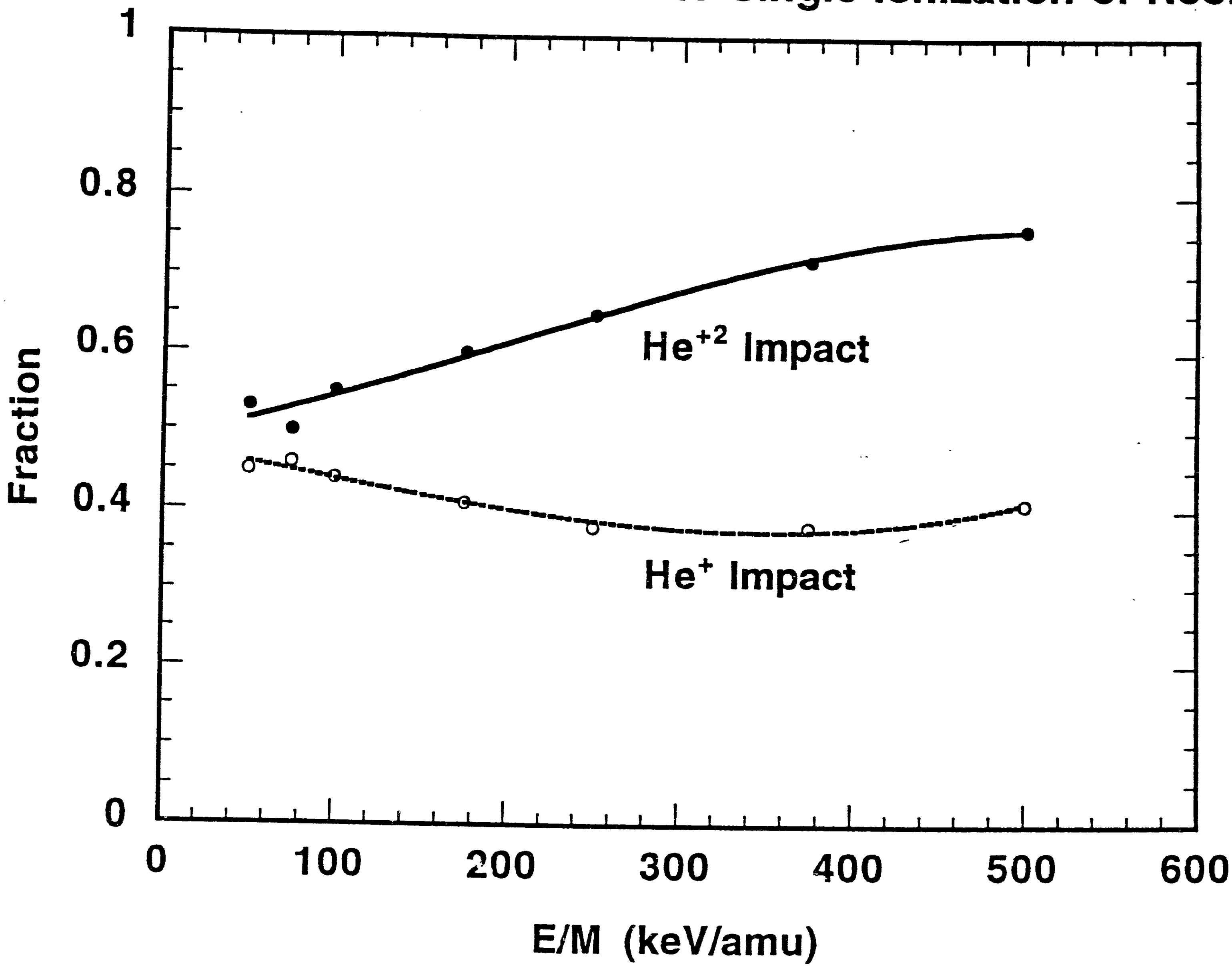


Fig 2

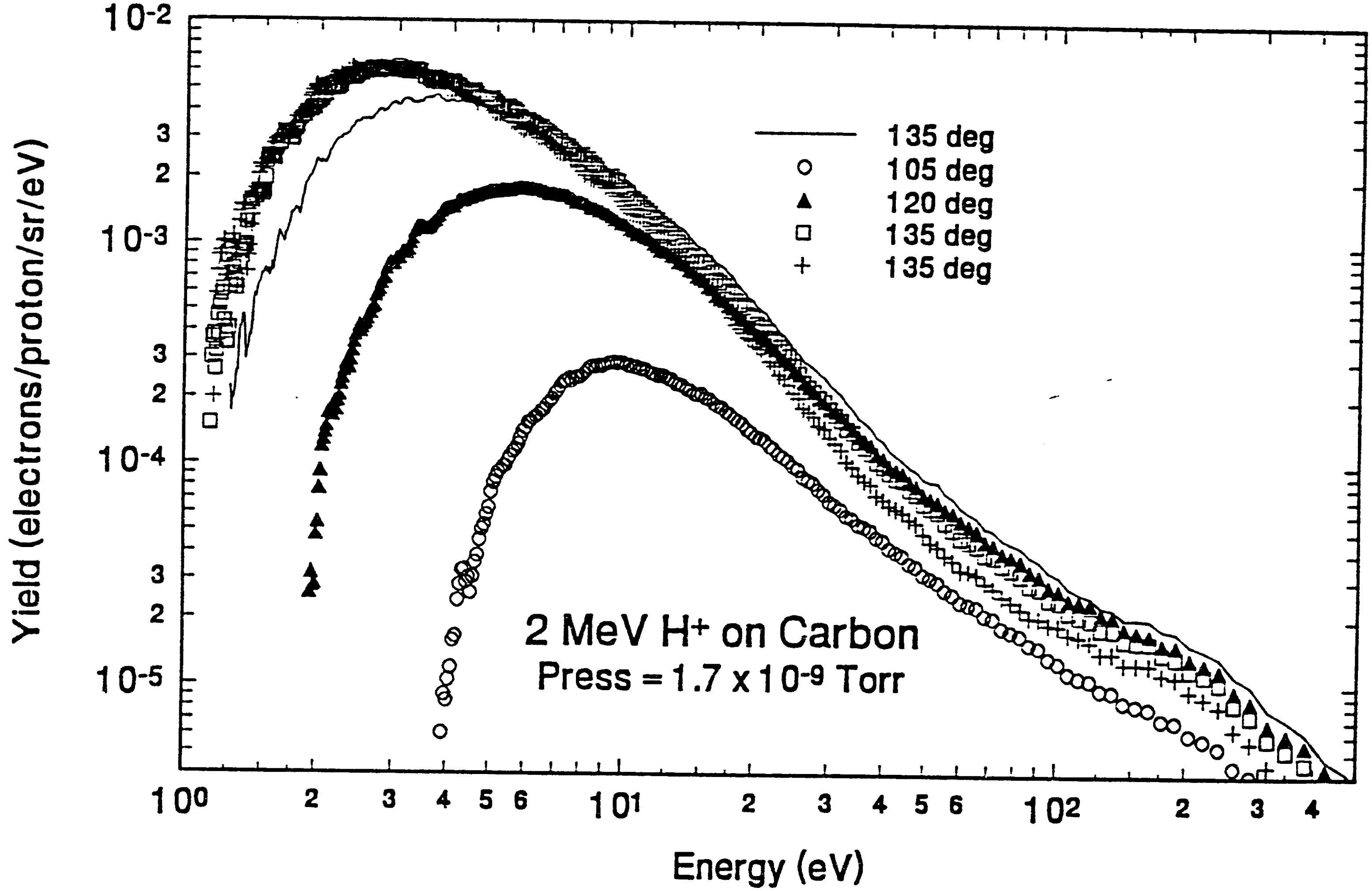
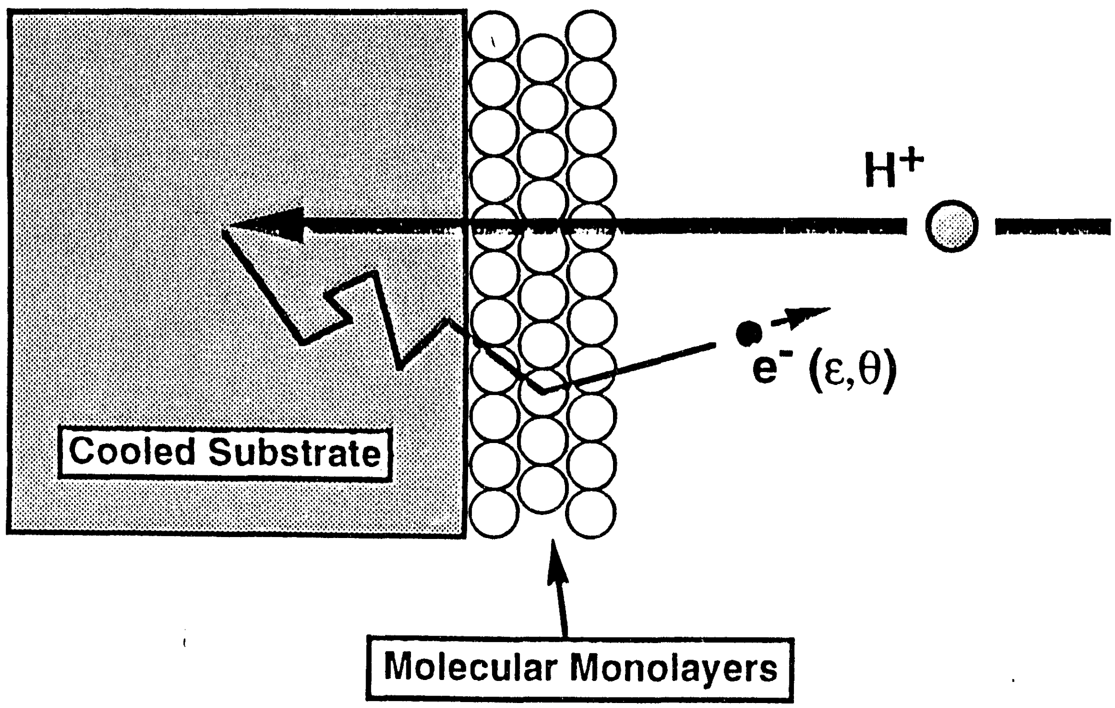

Yield (electrons/proton/sr/eV)
Energy (eV)
135 deg
105 deg
120 deg
135 deg
135 deg
2 MeV H+ on Carbon
Press = 1.7 x 10-9 Torr

Fig 3

Condensed Phase Effects: Future Plans



Dissociation of N_2

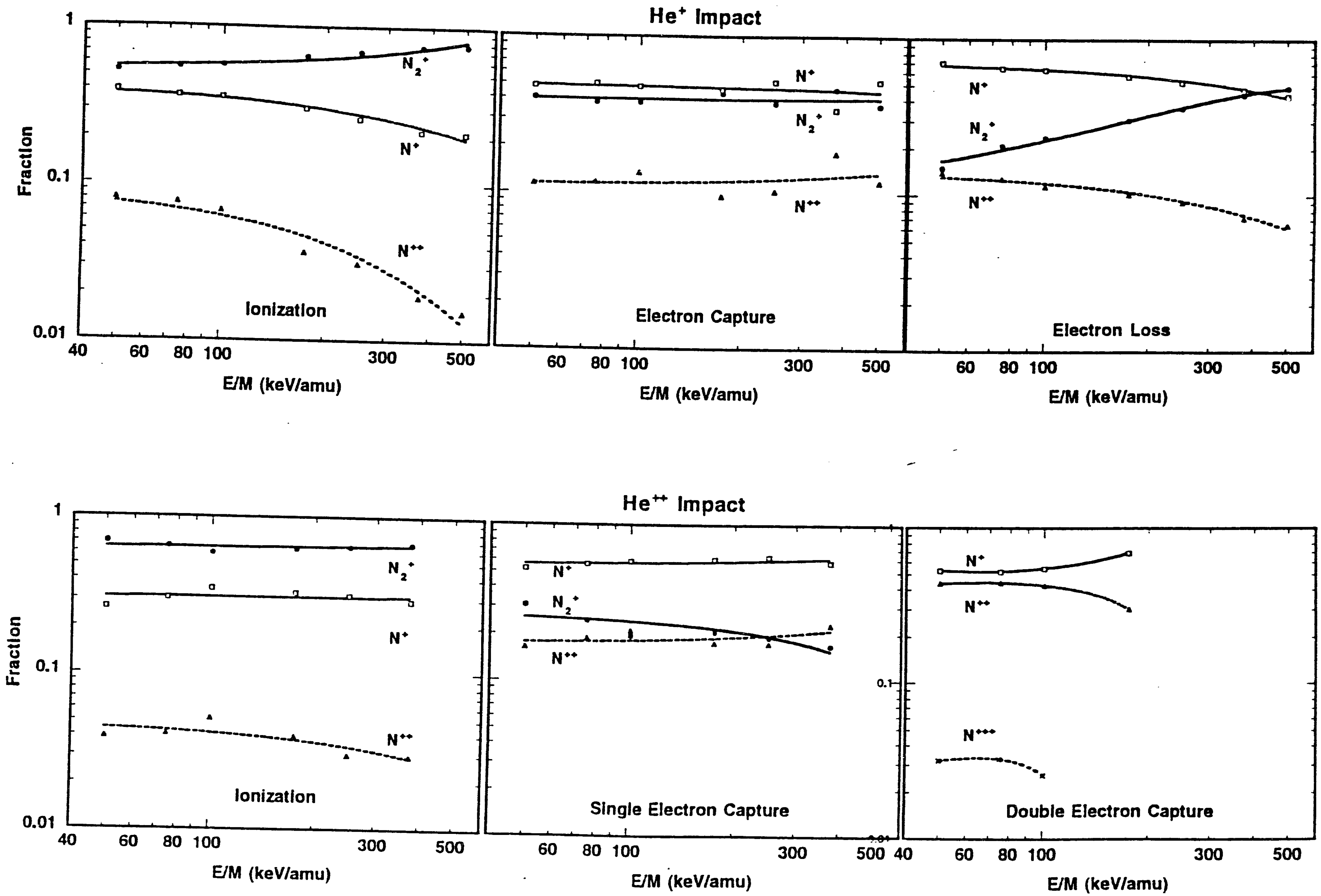
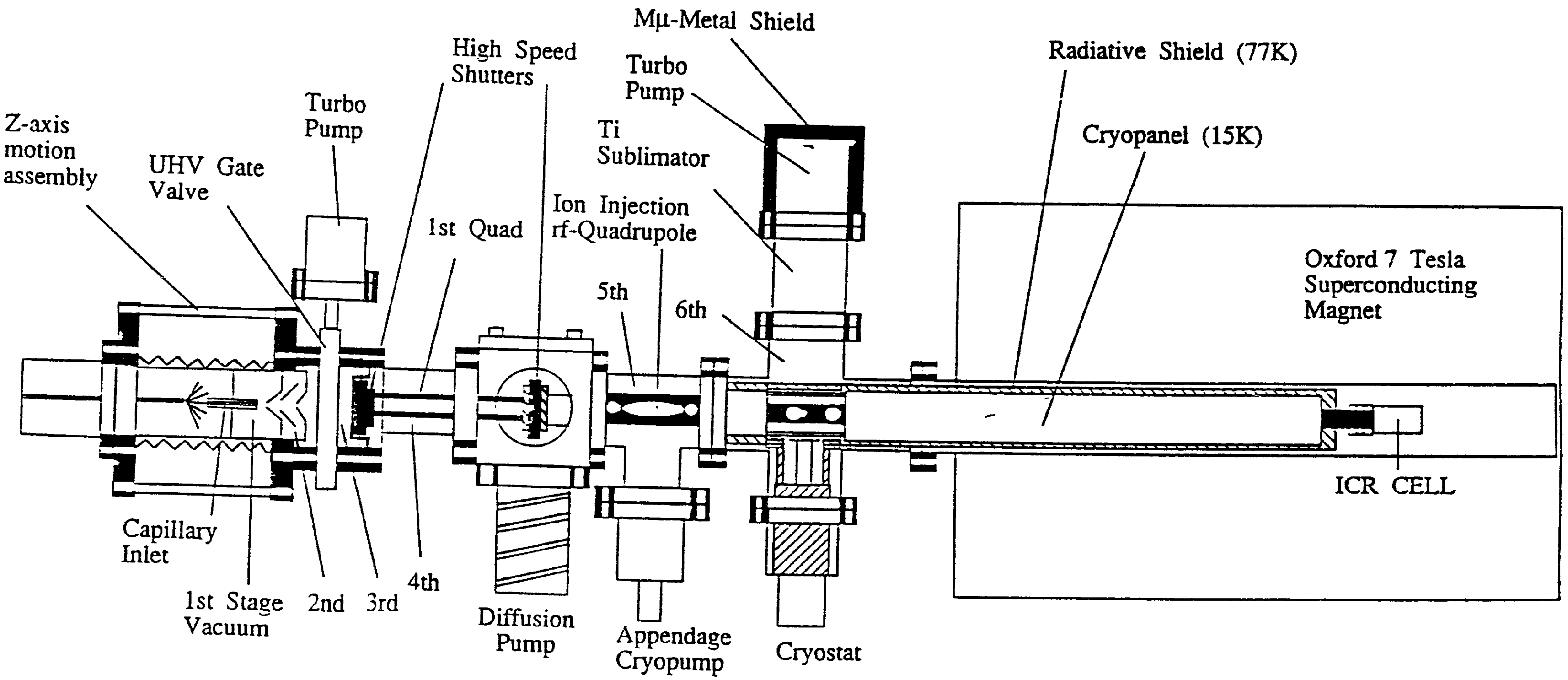


Fig 5



Mμ-Metal Shield
High Speed
Shutters
Turbo
Pump
Radiative Shield (77K)
Turbo
Pump
Ti
Sublimator
Cryopanel (15K)
Z-axis
motion
assembly
UHV Gate
Valve
Ion Injection
rf-Quadrupole
1st Quad
Oxford 7 Tesla
Superconducting
Magnet
5th
6th
ICR CELL
Capillary
Inlet
1st Stage
Vacuum
2nd
3rd
4th
Diffusion
Pump
Appendage
Cryopump
Cryostat

Fig. 6

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